PREPARATION AND PHARMACOLOGICAL EVALUATION OF METFORMIN LOADED MICROSPHERES

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Kolkata, India
Year 2022

1. Title of the thesis

"Preparation and pharmacological evaluation of Metformin loaded microspheres"

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3. List of Publications

i) Biplab Kuamar Chakra, S. Karan, Biswajit Das, Souvik Debnath, T. Chatterjee 'A controlled release microsphere formulation of an antidiabetic drug and characterization of the microsphere, International Journal of Pharmacy and Pharmaceutical Science, Vol 10, Issue 10, pp 30-38, 2018, DOI: http://dx.doi.org/10.22159/ijpps.2018v10i10.27541

ii) Biplab Kumar Chakra, Souvik Debnath , Avinaba Mukherjee , Ketousetou Kuotsu, Mayur Chakraborty and Tapan Kumar Chatterjee , Potent Anti-Diabetic Activity of Polymeric Microsphere Formulated Metformin on Streptozotocin-Induced Diabetic Rat Model.(2022).Int. J. Life Sci. Pharma Res.12(2), 68-81 P 68-81http://dx.doi.org/ijlpr 2022; doi 10.22376/ijpbs/lpr.2022.12.2.

iii) Biswajit Ruhidas, Rajat Ray, Debojyoti Naskar, Biplab Kumar Chakra, Tapan Kumar Chatterjee , Pharmacological and toxicological investigations of etodolac loaded gum katira microsphere prepared by W/O/W emulsion solvent evaporation technique in rats.Brazilian Journa of Pharmaceutical Science . Vol 53, n 04, https://doi.org/10.1590/s2175-97902017000400212

iv) Chakra, Biplab Kumar and Dr.Sunil Kumar Mandaland Dr. Tapan Kumar Chatterjee. "ROLE OF ALKALINE PHOSPHATASE ENZYME IN THE FERMENTATIVE PRODUCTION OF ERYTHROMYCIN ANTIBIOTIC AND ITS INCUBATION PERIOD." *International journal of pharma and bio sciences* (2015)

v) S. Karan , H. Chowdhury , B.K. Chakra, T.K. Chatterjee, ' Polymeric Microsphere Formulation for Colon Targeted Delivery of 5-Flurouracil using biocompatible natural Gum Katira " Asian Pacific Journal of cancer Prevention , Vol 20; Issue ; pp 253-266, 2019. DOI:10.31557.APJCP.2019.20.7.2181

4. List of patents

Nil

5. List of presentation in national/international/conference/workshops

i. Chakra BK, Karan S, Das B Debnath S, Chatterjee TK, "Reduction of Manufacturing Time of Metformin loaded W1/O/W2 Emulsion microsphere by a combination of polymers : A novel Approach" National Seminar on

"Pharmacy & Healthcare: Traditonal Knowledge to Modern Techniques" Jadavpur University.

ii. Biplab Kumar Chakra , Biswajit Das Saumen Karan , Saswata Banerjee, Debjyoti Naskar, Biswarup Sen , Dr Tapan Kumar Chatterjee, "A Report: Fermentative production of erythromycin and it's relationship with alkaline phosphatase" national seminar on "Clinical Pharmacology- Bech Beside" Clinical Research Centre, JADAVPUR University in association with AMRI Hospitals, Kolkata 2014

“Statement of Originality”

I Biplab Kumar Chakra registered on 21.08.2017 do here by declare that this thesis entitled “Preparation and pharmacological evaluation of Metformin loaded microsphere” contains literature survey and original research work done by the undersigned candidate as part of Doctoral studies.

All information in this thesis have been obtained and presented in accordance with existing academic rules and ethical conduct. I declare that, as required by these rules and conduct, I havefullycitedandreferredallmaterialsandresultsthatarenotoriginaltothis work.

I also declare that I have checked this thesis as per the “Policy on Anti Plagiarism, Jadavpur University, 2019”, and the level of similarity as checked by iThenticate software is 6 %.

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CERTIFICATE FROM THE SUPERVISOR

This is to certify that the thesis entitled "Preparation and pharmacological evaluation of Metformin loaded microspheres" submitted by Shri Biplab Kumar Chakra , who got his name registered on 21.08.2017 for the award of Ph.D.(Pharmacy) degree of Jadavpur University is absolutely based upon his own work under the supervision of Prof.(D r) T a p a n K u m a r C h a t t e r j e e and that neither his thesis nor any part of the thesis has been submitted for any degree/diploma or any other academic award anywhere before.

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Signature of the Supervisor
And date with Office Seal

ACKNOWLEDGEMENT

This investigation was carried out under the direct supervision of Prof. (Dr.) Tapan Kumar Chatterjee, Former Professor , Division of Pharmacology , Department of Pharmaceutical Technology, Jadavpur University.

I take this opportunity to express my sincere gratitude to him for the guidance, constant encouragement and inspiration he provided me during the tenure of my work. I appreciate fully all his contributions and encouragement with new ideas to show me ways of success. It's a matter of great pleasure as he provided the necessary infrastructure and framework for the research . under his guidance I learned a lot to overcome all the difficulties in the research work. Despite his busy schedule , he reviewed and corrected my research papers and provided necessary information to complete the thesis . I will follow and respectfully all his directions regarding research. I was highly influenced by his extremely enthusiastic nature of encouragement.

I remain deeply indebted to Prof Sonmoy Karmakar Head of the Department of Pharmaceuticals Technology , Jadavpur University , Prof L.K. Ghosh, Prof Amalesh Samanta, Prof. T. Bera, Prof. T.K. Maity and prof B.Mukherjee (Ex head of the Departmnet of Pharmaceutical Technology , JADAVPUR University) for providing me necessary encouragement and help to carry out my research work.

My sincere regards and gratitude to Prof B. Sa and Dr K Kuotsu, department of Pharmaceutical Technology , Jadavpur University , for giving valuable advice.

I must render my deep gratitude to Prof Sunil Kumar Mandal and Dr. Sujata Ghosh Dastidar

I will always remember the help and co-operation of my lab mates (Dr. Saumen Karan, Dr. Souvik Debnath, Sri Saswata Banerjee and Shri Debojyoti Naskar) to carry out the research work.

I also remember the help of Dr. Biswajit Das, Dr. Arijit Guha, Dr. Nityananda Sahoo, Dr. Avinaba Mukherjee for their sincere help and inspiration.

I remember the help of Samrat Chakraborty, Mayur Chakraborty , Sonjit Roy, Avisek Mandal , Protik Chakraborty, Suvankar Saha is Ashiqul Haque for their kind help and inspiration.
I would like to provide special thanks to all staffs of the departmental library, instrumentation labs and all the other of Departmnet of Pharmaceutical Technology , Jadavpur University, Kolkata.
I would like to express gratitude and sincere thanks to all my respected teachers and staffs of departments of Pharmaceuticals Technology , Jadavpur University , Kolkata for their help and encouragement to conduct the research work.
I am extreamly thankful to all my teachers in life.
I would like to thank all those in different countries who silently helped me for the research work.
From the inner core of my heart I express my deepest condolence and love to my parents.
I also thank to all my friends and family members for their sincere help and co-operation and feeling extremely indebted to the departed soul of my parents who always bless me from the heaven.

Biplab Kumar Chakra
Index No.- D- 7/E/576/17 Dated 21.08.2017
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July 2022

List of Abbreviations

5 –ASA	-	5Aminosaliculic acid
AKT	-	Protein kinase B
Amp	-	Adinosin monophosphate
Ampk	-	Amp activated protein kinase
ATP	-	Adinosin tryphosphate
CDK	-	Cyclin dependent kinase
Chk 23	-	Serine threonine protein kinase
CREB	-	cAmp response element binding protein
Cyp 3A 4/5	-	Cytochrome P450 3A4/5
DCM	-	Dichloromethane
DEE	-	Drug Entrapment Efficiency
DM	-	Diabetes Mellitus
DNA	-	de oxo ribonucleic acid
DPP4	-	Dipeptidyl peptidase-4
DPPC	-	Dipalmityle phosphatidyl chloride
EAC	-	Elhrich’s Ascites carcinoma
EDS	-	Energy dispersive X-Ray spectrometry
FESEM	-	Field Emmission scanning electron microscopy
FTIR	-	Fourier Transformed infrared Spectroscopy
G.I.T	-	Gastro intestinal tract
GLP	-	Good laboratory practice
GLUT	-	Glucose transporter
GUV	-	Giant Vesicles

HPLC	-	High Performance Liquid Chromatography
HPMC	-	Hydroxy propyl methyl cellulose
IDDM	-	Insulin dependent diabetes mellitus
NIDDM	-	Non Insulin dependent diabetes mellitus
O/W/O	-	Oil in water in oil
OVV	-	Oligo vesicular vesicles
PEG	-	Poly Ethelene Glycol
PEG	-	Poly ethylene glycol
PHA	-	Poly hydroxyl alkanoates
PLA	-	Poly lactic acid
PLGA	-	Poly lactoglycolic acid
PMMA	-	Poly methyl methaerylic acid.
PPARG	-	Peroxisome proliferator activated receptor genome.
PPARY	-	Peroxisome proliferator activated receptor
ROS	-	Reactive oxygen species
RPM	-	Revolution per minute
SEM	-	Scanning electron microscopy
SUV	-	Small unilamellar vesicles
TEM	-	Transmission Electron microscopy
USL	-	Ultra small unilamellar vesicles
VLDL	-	Very low density lipoprotein
W/O/W	-	Water in oil in water
XRD	-	X Ray Crystallography

ABSTRACT

Scientific world is nothing but a vast world of thinking . When this thinking goes in a proper direction it becomes idea. This philosophical ideas come real and true when it is supported by facts, experiments and reality . All the research institutions in te world are involved in developing the drugs and medicine to get some better approach from the existing one.

Ds mellitus is a life threatening disease. The main problem of this disease is it destroys various parts of the body slowly. So it is required to control the blood sugar level always inside the blood. Continuous release of drugs from the dosage form is required to achieve proper therapeutic conc in the blood . controlled release drug delivery is an effective approach towards this goal.

Here we have prepared microspheres of metformin. We have used guar gum as a matrix building material. Double emulsion solvent evaporation technique was utilized for the preparation of the microsphere. Small spherical microspheres was produced with size range of µm. The characterization of the microsphere was done with FTIR , XRD, studies , SEM was done for the particle size and appearance.

The antidiabetic study was performed with swiss albino rats . it was found that the formulation provide better antidiabetic profile than the standard (powder) drug molecule used in the market. The formulated also provided better HDL, LDL, VLDL level and also the urea and creatinine content is under control.

We saw its antitumor activity was also increased . to check the functionality of the Guar Gum microsphere cell cytotoxicity assay using DAP I staining (flurocent microscopy study) and in vivo anticancer study of EAC bearing mice was also performed. Histological studies of the liver and kidney tissues was also done to compare the toxic side effects . it was also tested for the cell morphology atudies of the EAC cell. At the same time the functionality of the microsphere was also determined by using MIT on different cell and the anticancer activity was found by sarcoma 180 bearing mice model.

The formulation provides a suitable release pattern . the best optimized formulation showing a regular release with 12 hours in vitro studies . release curves show the release pattern is continuous and following controlled release pattern . DHP1 results showed that the blank microsphere was having no cytotoxicity on MDA-MB-231 breast cancer cell lines and that shows that the guar gum microspere as a carrier of the drug having no toxic side effects.

Here it was found that the drug treated groups showed various prominent features of apoptosis such as chromatin condensation , weight gaining of micenuclear fragmentation and condensation.

A more constant and significant decrease in EAC liquid tumor and increased life span was observed with the treated mice. It was found that the rate of variation of cell morphology was more in the metformin loaded microsphere than the powder. From the facts received of the hematological and biochemical parameters it can be guessed that the metformin loaded microsphere is better tolerated that the marketed metformin powder.

The histopathological studies also found that the controlled release formulation is having continuous and slow release pattern.

So from the above findings it can be said that the microsphere formulated of metformin with guar gum is showing continuous release and better compliance in antidiabetic and anticancer studies.

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21. Fig. 9: This picture exhibits the oxidative-stress dependent apoptotic effect by the metformin loaded microsphere on in-vivo EAC induced swiss albino mice model. (A) This picture exhibits the Metformin loaded microsphere-induced ROS generation. In-vitro DCFH-DA ROS fluorescence intensity of EAC solid tumor cells on different concentration drug treatment. (B) This metformin loaded microsphere-induced apoptosis through the loss of MMP potential Δψm. In-vitro mitochondrial membrane potential Δψm assay of EAC solid tumor cells on different concentration drug treatment. (C) This metformin loaded microsphere-induced apoptosis. In-vivo caspase-3 assay of EAC solid tumor on different concentration drug loaded microsphere treatment. (D) metformin loaded microsphereinduced apoptosis through p53 up-regulation. In-vitro p53 assay of EAC solid tumor cells on different concentration drug loaded microsphere treatment. (E) metformin loaded microsphere-induced apoptosis. In-vitro PARP assay of EAC solid tumor cells on different concentration drug loaded microsphere treatment. Values are mean ± SD with $n= 3$. ***$P<0.001$, **$P<0.01$, *$P<0.05$

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22. Fig. 10: Metformin loaded microsphere nanoparticle drug therapy modulate immune checkpoint and activates the antitumor immune response. Immune microenvironment remodulation within EAC solid tumor after formulated drug therapy compare with free drug and EAC control group. CD4+ T-cells;(B) CD8+T-cells;(C) CD40+ cells; proinflammatory cytokines (D) IFNγ, (E) TNF-α, (H) IL-10 and (I) IL-17; (G) CD86+ cells;(F) NK cells;(J) Bcells. Significant statistical differences analysiswas assessed using *t*-test to determine differences within groups and between groups, respectively. Results are presented as mean (SD).

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Chapter -1
Introduction

1. Introduction-

Targeted delivery is an important route of drug administration as it provides the drug in the effected area instantly. Continuous supply of medicine in a regular fashion is an essential requirement for the targeted drug delivery approach. An initial bolus dose followed by continuous maintaining dose in a regular fashion is an essential requirement for targeted drug delivery system. There are so many forms are available for this targeted drug delivery system like liposomes, microspheres, nanoparticles, hydrogels etc. The polymeric microspheres are successful candidates proceeding the drug in the proper site for their action. The drug is embedded in the polymeric matrix and is released slowly in a sustained manner. The therapeutic agent is mixed in the biodegradable matrix in a profound manner so that the drug will be distributed or released in the targeted area.[1]

In the microsphere matrix the existence of gum is an important factor for its effectivity [2]. Guar gum is proven to be an effective gum in the matrix as it has been used in the food industries from times immemorial. There are a big numbers of gums available in the market as xanthan gum, dextran, chitosan, guar gum, lechitin locust bean gum etc are used for the preparation of the microsphere.[3]The drug is entrapped in the gummy matrix and released slowly from the matrix in the targeted area but continuously to proceed a constant pattern of release through 24 hrs and thus build the effective therapeutic concentration. Microspheres are used to treat various diseases like cancer, diabetes, pain and inflammation, arthritis etc. In the present work microsphere of metformin was produced. As we know diabetes is a drastically deadly disease and basically arises as a result of metabolic disorder. Carbohydrate foods taken

into our body is broken down into simple sugars by the process of metabolism[4]. This process of break down into simple form is required for the energy generation. Insulin has a big role for the entry of glucose in the cell. Insulin helps our cells to accept glucose and so the cells get energy from glucose in the cell. Insulin helps our cells to accept glucose and so the cells get energy from glucose. This is a complex mechanism of releasing insulin in the blood. After eating food threshold makes the secretion of insulin from pancreas.[5]This insulin helps in pushing the blood glucose into the cell and thus lowers the blood sugar. This ultimately helps in controlling the blood sugar level of the body.[6]

Diabetes mellitus is a dangerous disease which is nothing but existence of glucose cells in the blood. This is due to the fact that-

i. There may be lack of production of insulin in the pancreas due to various reasons.
ii. The body may not produce insulin and may lead to type-1 diabetes mellitus.
iii. There may be existence of insulin but that is not absorbed by the receptor cells.

Chronic hyperglycemia is an immediate result of disorder of carbohydrate metabolism . As blood sugar level goes higher it comes out from the body with urine. Medically this stage is known as glycosuria. So although blood contains a big number of glucose the cells do not receive it and so their essential energy and growth is hampered. So in a long run the hyperglycemic patients face many difficulties like destruction of so many organs in the body. Diabetes mellitus can destroy so many parts of the body . It may produce various types of symptoms like blurred vision, thirst, polyuria, weight loss etc. Ketoacidosis may appear as a serious problem in case of long term presence of diabetes mellitus. Diabetic retinopathy is a serious problem in case of long term presence of

diabetes mellitus. Diabetic retinopathy is a serious problem which arises in a long term presence of diabetes mellitus.[7]

Diabetic nephropathy is also an associated problem in long term presence of diabetes mellitus in the human body. Now a days kidney damage is very common due to the long term diabetic effect. At the same time people with diabetes mellitus are also associated with upcoming symptoms of cardiovascular fatalities. Cardiovascular and cerebral attacks are regular outbreaks from diabetes mellitus.

The type-1 or insulin dependent hyperglycemia (IDDM) due to absence of insulin secreted by pancreas. This is mainly due to the beta cell destruction in the islets of langerhans. Insulin is very much required for the survival of human being and thus it prevents the occurrence of ketoacidosis coma and death. So, for those patients suffering from type 1 diabetes mellitus artificial insulin prepared by recombinant DNA technology is mandatory.[8]The type 2 diabetes mellitus is a specific form of diabetes mellitus an age-related factor where with the age the release of insulin from the islets of langerhans is decreased. This type of diabetes can be controlled by the use of anti diabetic drug, physical exercise and following of proper diet chart.

The treatment procedure followed for the type 2 diabetes mellitus is frequently distributed amongst is the medicines used by the practitioners.

The sulfonylurea group of oral antidiabetic containing big number of oral antidiabetic drugs such as glimepride, glyburide, tolbutamide, chlorpropamide, tolazamide, acetoheximide etc. They are useful antidiabetic drug and are used vigorously by the patients. They generally act by stimulating the pancreas to secrete the hormone insulin by blocking the K^+ channels in the beta cells. It is having so many side effects like hyperinsulinemia, hyperhyperglycemia, weight gain etc[9].

Another class of antidiabetic drug are known as biguanides, metformin, phenformin, etc. are the representatives of this groups. Metformin reduces the amount of prepared glucose by blocking gluconeogenesis. It also increases the uptake of glucose in muscles and its utilization. General side effect of the medicine is anorexia, lacticacidosis etc. Another group of medicine is meglitinides such as repeglinide, nateglinide etc.

They also can initiate insulin secretion by influencing and antagonising K^+ channel in beta cell. The general side effect is hypoglycemia and weight gain etc.

Another group of antidiabetic drugs are thiazolidinediones such as rosglitazone, pyoglitazone etc. They are also group of drugs which can increase the insulin secretion by activation of PPAR-g receptors.

They are having common side effects like fluid retention and weight gain etc[10].

The alpha glucoside inhibitors such as acarbore and migital are the drugs which can lower the hepatic glucose production and also it can make a decrease of absorption of glucose. The common side effect of this drug is flatulence abdominal blockage etc.

Apart from that there are another group of drugs known as DPP4 inhibitors for the example sitagliptine and vildagliptine. They basically increase the release of GLP - 1 and GLP. The untoward reaction of this drugs leading to acute pancreatitis, severe hypertensive reactions and upper respiratory tract infection.

The thiazolidinedione group of drug consists of rosiglitazone and pioglitazone . They generally stimulate the PPAR-G receptors. Their side effects are mainly include increase in weight, fluid accumulation and increase risk of bladder cancer.

The meglitinides group of drugs known as rapeglinide, nateglinide etc. work as blocking the K^{++} channel in the beta cell. The popular side effect is increase in weight and hypoglycemia etc.[11]

The alpha glucosidase inhibitors are known as acorbose and migitol. They can reduce the production of glucose in liver.

The absorption of glucose is also hampered by the use of the drug. The popular side effects are abdominal pain, gas formation etc.

Novel drug delivery system is a big development in modern Pharmaceutical drug delivery system and more practical approach in day to day life. Microspheres are coming under novel drug delivery System as they supply the drug in the target area following targeted drug delivery System. The microsphere drug delivery system is a modern drug delivery system to supply the drug to the target area in a desired concentration. There are free flowing powders and for their preparation various techniques are adopted. They are small particles in the micrometre range and they can deliver the drug in the target area with the proper and fixed dose. They are having a big surface to volume ratio as compared to the other dosage forms. There are so many advantages of microsphere drug delivery system.[12]

(a) Advantages

i) The bioavailability of the product is increased in a big amount.

ii) The patient safety and compliance is increased in a big amount.

iii) Target site drug delivery is achieved by this drug delivery system with suitable means so as to produce proper amount of drug in the targeted area.

iv) It helps in reducing the effectivity of the core in comparison to outside environment.

v) The reactivity of the particle depends on the size, surface charge, and surface hydrophilicity of the microspheres. These particular parameters are important for working of microspheres at the original site of action.

vi) The rate of evaporation of the volatile core material is also decreased.

vii) It helps in changing the liquids into the solid form and also reduces or abolish totally the bitter taste.

viii) The gastrointestinal disturbance is protected by the effectivity of the drug.

ix) It is found that due to the coating of polymer across the matrix and the polymer dissolves slowly in the gastrointestinal tract so it helps in protecting the gastrointestinal tract from toxic side effects of the drug.

x) There is a big advantage of biodegradable microsphere. for their implantation in the target site and the removal from there does not require any surgical follow up.

xi) The microspheres provide controlled release drug delivery system and this is very much active in providing toxic side effect free environment inside the body.

Comparison of the microsphere:-

There are a big number of materials are utilised for the preparation of microsphere.[13]

It is a combination of various materials in the dosage form to prepare a microsphere. They may contain various materials like-

i. The active ingredients

ii. Various Pharmaceutical additives and diluents.

iii. Surface active agents and polymers.

iv. Gum for the preparation of the matrix.

v. Suitable polymers are required.
vi. In some preparations the drug release enhancers are also used to increase the rate of drug release.

1.1 Selection of the vehicles-

There may be aqueous or non aqueous vehicle used for the preparation of the microspheres. There are so many polymers used for the preparation of microsphere. Some of them are natural polymers whereas the others are synthetic Polymers. Natural Polymers may be of three different categories like- proteins, carbohydrates and chemically modified carbohydrates.[14]

The proteins are the albumins, gelatin and collagen. The carbohydrates are available in the form of agarose, carrageenan, chitosan and starch. the chemically modified carbohydrates are also available like polydextrance and Polystarch etc.[15]

The synthetic Polymers are also available and there may be two types of synthetic polymers non Biodegradable and biodegradable polymers.[16] The non biodegradable polymers are like polymethyl methacrylate (PMMA), acrolein and epoxy polymer. The example of biodegradable polymers can be found like lactides, glycolides and their copolymers polyalkyl cyano acrylates etc.[17]

The coating material used may be of various types like inert polymers, plasticizer, colouring agent and also found like gelatin gum arabica, methylcellulose, beeswax, carnuba wax etc. [18]

The preparation of microsphere can be found in various techniques available:

The techniques can be found in so many ways like single emulsion, double emulsion and polymerization techniques.[19]

Other different manufacturing techniques are also available to prepare and formulate the microspheres. phase separation and coalervation, spray drying,

and congealing, solvent extraction etc.[30]The characterization of the microsphere depend on so many factors there maybe morphological characters like scanning electron microscopy (SEM), particle size analysis, swelling studies, density determination, isoelectric point, entrapment efficiency and angle of repose etc.

There are so many in vitro methods available for this system. There are so many methods and techniques available for the preparation of microsphere.

1.1.1. Single emulsion technique

Microsphere of carbohydrate protein etc are prepared by this technique there are the natural Polymers like carbohydrate and protein and they are dissolved dispersed in water followed by oil for preparation of microsphere by the single emulsion technique crosslinking agent are also used to prepare the microsphere successfully.

Double emulsion technique

This is a very important process for the preparation of microspheres. Multiple emulsion solvent evaporation technique is followed generally to prepare the microspheres.

Gums are used for the preparation of matrix of the microsphere. Generally Polymers are also used for preparation of the microspheres. There may be both natural or synthetic type of polymers. This method is applied for the preparation of microspheres of water soluble drugs. generally the drug in embedded in the matrix with the gum and releases slowly and continuously showing a continuous dissolution profile for a longer period of time. The release profile of the drug shows a continuous release pattern for a longer period of time and follow up prolonged-release pattern. This type of drug delivery comes under Novel drug delivery System and modern day microspheres are helpful in delivering the drug at proper target so that also known as targeted drug delivery system. First of all to prepare microsphere microemulsion is to be produced.

The micro emulsion maybe of two types W/O/W and O/W/O type depending on the method of preparation of the microspheres. Generally homozenizer are used for the preparation of microspheres. At first the primary emulsion is prepared. Matrix containing the drug is injected into the organic phase with the help of the needles. Once the primary emulsion is produced it is transferred into the continuous phase with the help of syringe.

Solvent evaporation from the continuous phase is generally found for the preparation of the microsphere and after drying at the airy atmosphere the microspheres are collected and used for study purpose.

1.1.2. Polymerization

Another process of preparation of the microsphere is known as process of polymerization which can be divided into two parts also and they are normal polymerization and interfacial polymerization.

The normal polymerization process involves various techniques for the preparation of microspheres.

There may be different techniques for the polymerization processes which can be classified as bulk suspension, precipitation, emulsion and micellar polymerization processes.

the bulk polymerization process is an unique one in which a monomer or a mixture of monomers and it is attached with a catalyst is heated to start polymerization. These polymers are utilised to prepare microspheres . The important factor in prepairing the medicine is drug loading and this can be achieved by the process of polymerization.

The suspension polymerization is an important process of preparation of microspheres. Preparation of dispersion is required as droplet can be prepared to

form droplet dispersion. The monomer or mixture of monomers are heated to prepare a continuous aqueous phase.

Suspension polymerization is another class of polymerization. a dispersion is to be prepared. This can be prepared from the droplets. Monomers or a mixture of monomers can be heated to form a droplet dispersion.

Emulsion polymerization also found to take place. It is a particular type of preparation and the initiators shifts to the surface of the micellar from the aquous phase.

The interfacial polymerization also happens when it seems to take place while it is between two immiscible liquid phases and to prepare a polymer.

1.1.4 Phase separation

This is a particular type of system to prepare microsphere of the water soluble drugs. Here also the active medicament can be introduced into the matrix of the material. The matrix type of preparation of the microsphere is an important technique of preparation of microsphere. This is an effective way of preparation of the microspheres by phase separation technique. This is the technique which is based mainly on polymers solubility in the vehicle.

1.1.5 Spray drying/congelling

It is an important process of preparation of microsphere. atomization is required for the preparation of small droplet and after solvent evaporation microspheres are produced.

1.1.6 Solvent extraction

This is another process of preparation of microspheres. In this process organic phase is removed. This is a very specific process in which water soluble phase is removed by specific technique.

Drug is added directly into the solvent

1.2 Characterization of prepared microsphere:-

This is very important as microspheres are standardized by the process of characterization There are so many methods for characterization of microsphere scanning electron microscopy particle size analysis, swelling index, density determination, isoelectric point,entrapment efficiency, angle of reposeetc.

There are so many methods available for the process

Beaker method, interface diffusion system dissolution apparatus brief discussion on these topics are required as these are the most important manufacturing process followed by various workers

Amongst the morphological behaviour scanning electron microscope is an important parameter as it depicts particle size and shape in an exact size. It is very important to have an idea of all batches of microspheres to get it in a system so that we get an idea of SEM pictures provided by them

1.2.1 Scanning electron microscopy

This is very important to study the shape and surface behaviour of the microspheres Scanning electron microscopes are used for this purpose.As they can get the pictures of the microspheres and its surface generally the microspheres are placed on the sample holder and photographed by the instrument Energy Dispersive x-ray Spectrometry (EDS)The X-ray spectrum of the microspheres is obtained by this process so that we can have an idea of the particle particle behaviour and the semi quantitative analysis is done also.

1.2.2 Particle size analysis

It is done by laser light scattering It is generally done by using dispersion of the microspheres is placed on the dispersion unit and stirring is done

1.2.3 Swelling index

This is an important criteria for the characterization of the microsphere This is done for the characterization of the mucoadhesive microsphere the microspheres

can swell in the appropriate solvent for a particular time range how much it is swollen by changing its volume to be particular range is predicted by difference of weight between the two forms that is original weight and swollen weight. After making wetted with the solvent for a particular time the microspheres were dried with the help of an oven to get into its original form this swelling index can be calculated from the difference of weight

$$\%\text{of swelling} = \frac{w_2 - w_1}{w_1} X\ 100$$

W1= Initial weight

W2= final weight.

1.2.4 Determination of density:-

Generally it is done by the help of a pycnometer.Samples are weighted properly and kept in the pycnometer.It is a complex technique to determine the density of the microsphere the density of the microsphere was determined by the difference of pressure made by intrusion of helium gas into the chamber.This is a big fact that density of the microspheres are determined by this way of using pycnometer as an useful tool for the determination

1.2.5 Isoelectric point

It is a very important parameter for the characterization of the microsphere. The isoelectric point is calculated and detected by micro electrophoresis.The particle movement time of a certain interval is calculated.The electrical mobility of the microspheres helps to standardise the microspheres

1.2.6 Drug entrapment efficiency

It is a very important parameter as directed by drug in entrapment efficiency as per the characterization of the drug in concerned.How much drug is entrapped in the microsphere formulation is estimated by this drug entrapment and efficiency.This particular thing can be calculated by the formula

$$\text{Drug entrapment efficiency} = \frac{Actual\ Content}{Theritical\ content}\ X\ 100$$

1.2.7 Angle of contact

Angle of contact governs the waiting property of the particle.The angle of contact is important as particle- particle interaction is important to prepare the microsphere.The hydrophobic and lycophilic property of the microsphere is characterized by the angle of contact.The thermodynamic property is a very important factor for the characterization of the microsphere It is measured at solid water interface

1.3 In vitro techniques

There are so many in-vitro techniques available for this purpose.The release profile of the drug is a very important factor in pharmaceutical formulation

Release of drugs is a very important parameter for preparation of the microsphere as in case of microspheres the release rate is an important parameter so that the release profile can be estimated.This is a very important feedback for preparation of the microsphere.In vitro release provides the release covers so that we can estimate a different drug release characteristics

1.3.1 Beaker method

In a beaker the solution was kept and stirred for a definite period The medium used was kept in a in the beaker to perform the study.Generally it should be within 50- 500ml it was operated with a stirring speed of 60 to 300 RPM generally.There are so many other methods which can be appreciated in this process

Utilisation of the microsphere in pharmacy

Drug targeting is an important approach for the microsphere so that targeted drug delivery can be achieved

Availability of the drug at the proper site of a measured quantity

Economical approach as per the targeted Drug Delivery is concerned

Targeted drug delivery for the drug like doxorubicin and also an important remedy forleishmanasis.

Can be attached with the monoclonal antibodies for the targeted drug Delivery to be achieved by the ideas of microsphere

Used in various diagnostic kit for the detection of various diseases caused by bacteria virus and fungus

Targeted drug delivery can be achieved by the concept of microsphere like in cases of hepatitis ,influenza diptheriaetc. Diabetes mellitus is a deadly disease and can be controlled by improve improving the oxidative status of the cells. Reactive oxygen species ROS generally in the cells is their natural outcome of the biochemical Pathways which may be controlled by introduction of various antioxidant several vitamins(C,E) etc and also various food items can be involved in building the antioxidant activity so that to induce immunity in The system there are so many vitamins like Vitamin C and Vitamin E is proves to be as useful as a specific antidote for oxidative stress

This has also been seen to reduce the glycosylated hemoglobin in diabetic patient,There are so many plant products which are also available to reduce the oxidative stress and as to provide antioxidant activity in a long run.

Free radicals are very important in in promoting diseases in so many ways diseases like liver cirrhosis, atheriosclerosis, cancer, diabetes and so many other diseases can be initiated by generation of free radicals in the body.Thus the antioxidants are very effective to protect the body against various diseases.Lipids suffer a problem due to peroxidation which ultimately produces destruction in protein structure and also produces diabetes.This has been found that some agents can produce diabetes such as alloxan can be used as and

diabetoegenic agent and it destroy the pancreas, so antioxidant may play a major role in in treating diabetes also[21]

So antioxidant supplements can improve the body's protective mechanism against diabetes. Diabetes mellitus is a complex metabolic disorder the metabolism of carbohydrate fat and protein can be a regular problem in case of diabetes mellitus.This is a basically a deficiency disease caused by the hormone insulin secreted from islets of langerhans.Nowadays this disease diabetes mellitus is spreading in a huge manner for so many reasons in the country. Day by day India is becoming the diabetic capital of the universe[22]. Over 20 million people are now affected by the disease diabetes in India.There is a possibility that it may rise up to 20 million by 2050 Type 1 insulin dependent Diabetes mellitus is found to occur due to the lack of insulin secretion from the pancreas.There may be no production of insulin due to some disease or something like that in pancreas on the other hand the type 2 diabetes mellitus is due to the fact that there is a very less production of insulin in comparison to the the requirement may be due to the aging factor is involved.. In the fact there are so many herbal products also which can be used as antidiabetic remedy Diabetes mellitus is a complex condition and can stay throughout life as it is a metabolic dysfunction[23].As it is a silent killer can be influenced so many parts of the body as there is a big chance of damage by the disease.There may be problems in the retina of the eye mainly due to the hyperglycemia condition and it is known as diabetic retinopathy diabetic nephropathy may be found in kidney and that can be result of hyperglycemia[24]. Apart from that serious heart related disease resulting cardiac ischemia is stroke can be resulted. A large number of people throughout the globe was affected by the disease[25]. A number of almost 171 million people where damaged by the disease it was reported that almost 1.5 million deaths due to the diabetic in 2012[26].

The Type 1 Diabetes mellitus which accounts for almost 5 to 10% of total diabetic patient throughout the globe[27]. This insulin dependent diabetes mellitus popularly known as IDDM is very much seen in in paediatric patient so it is also known as Juvenile diabetes.Another form of diabetes mellitus which is the type 2 diabetes or non insulin dependent diabetes mellitus is found only with aged individual accounts for 90 to 95% cases of diabetic patient.In the in NIDDM patients insulin is not a mandatory one but can be treated with various anti diabetic drugs.

This disease is basically a prominent endocrine disorder that may be finally understood by studying the propagation of the disease.A big number of population may be 5% of the world is suffering from this problem.This big problem can be solved by treating the disease with modern medicine physical exercises controlling diet and curiously improving the the lifestyle of the individual as it is characterized also as a lifestyle disease disorder disease.

Sometimes some abnormal factors also arise with hyperglycemia such ashyperlipidemia hyperinsulinemia, hypertension and all these problems are due to the metabolic disorder associated with carbohydrate fat protein metabolism in the body.We cannot deny the role of reactive oxygen species or ROS generation inside the cells to provide the abnormality like Diabetes mellitus

Stress is a big problem in the modern world.Oxidative stress can lead to to problems like free radical generation in the body which in turn can produce diabetes and its, involvement with serious diseases.Cell and tissues damage is a result of this kind of abnormality which is due to the presence of reactive oxygen species.At the same time antioxidant can play a major role for the control of propagation of diabetes

Pancreatic beta cells are very much influenced by the ROS or reactive oxygen species.This is due to the fact that these cells are not that much experienced to fight with ROS.So damage in the pancreatic beta-cell produced by the ROS is a very important factor to produce diabetes.Again this also supports the fact that various natural antioxidants enriched with vitamin C and E can become helpful for the treatment of diabetes mellitus.On the other hand flavonoids and Vitamin supplements can fight ros to deliver oxidative stress and thus to neutralize the disease diabetes mellitus.In this way various natural products also come into the way for the treatment of diabetes mellitus from times, immemorial.Although the black hood of the disease continuously becoming more dense day by day as there may be a big problem due to the stress and strain related factors being increase day by day by the advancement of the time and the human civilization . This is a big disadvantage of modern day civilization and advancement of technical era. through out the globe , diabetes is taken for granted as seventh amongst the leading causes of death and if it comes with its complications it may be third amongst the serious diseases.[28] Pancreatic regulation of various diseases by producing insulin and glucagon is a major factor for controlling the disease like diabetes mellitus with the effective treatment of diabetes mellitus . It is often found to be a major role of those pancreatic hormones to keep the level of glucose at 70-120 mg/ml. There may be a probable reason for the disease and that may be the ineffectiveness of pancreas turns it into serious complicated disease like diabetes mellitus.[29]

The various methods of treatments were found for diabetes mellitus. As it is a life style disorder so various ways to improve life style can be followed to counteract diabetes mellitus. There may be various methods like physical exercise and morning walk, running etc. can be followed. Apart from that one can go for dieting and medication etc. for controlling the symptoms of diabetes mellitus. Medications like oral antidiabetic drugs and insulin can also be taken

for controlling diabetic mellitus if it is not controlled by physical exercise and dieting.[30 3]

Insulin can produce severe hypoglycemia if the dosage is not controlled properly . This is a major draw back of insulin . There are various oral hypoglycemic groups these can be taken carefully. A targeted drug delivery is suitable means for transporting the drug to the target site. There are so many methods for the targeted drug delivery . Oral drug delivery is most favourable way for the disposal of drugs through the targeted drug delivery. Targeted drug delivery is composed so that continuous and regular supply of medicine will be achieved through out a regular and constant period of time. This may be achieved by several sustained action dosage forms or controlled release drug delivery system. To prolong the release of the drug through a longer period to become prolonged release dosage form period of 24 hours to fulfill the goals of sustained release drug delivery to fulfil the target to continuous supply of drug through a longer period . Again for the preparation of sustained action drug delivery in a particular form sometimes it is required to produce the dosage in a manner to satisfy the requirement in full and so a bolus dose is required for the initial loading dose to maintain the therapeutic concentration. And then a constant supply of medicines required. And this is the concept which reflects the drug to be attained a certain percentage of bolus dose inside the body followed by prolonged drug treatment through out the duration. . This is a reason why controlled release drug deliveries are attained for the particular interest to satisfy the continuous supply of the drug through the duration of therapy. So controlled drug delivery may be a sustained action drug delivery can be achieved by various forms of pharmaceutical preparations like microspheres , nanospheres, liposomes, etc. These are the various dosage form which can help to prepare sustained action and prolonged release dosage forms to achieve the goal of controlled release drug delivery. The microsphere are

prepared by multiple emulsion solvent evaporation technique which enables the preparation of matrix at the liquid phase of the emulsion. The matrix of the microsphere contains the active drug. In W1/O/W2 micro emulsion type the oil phase or organic phase may contain the polymers a very essential one as it can provide stability to the microsphere.On the other hand the w2 phase is the water as the external phase. By this way the microsphere are prepared in a corresponding and complex manner. This is big phenomenon that microspheres are used as an antidote in leading diseases like diabetes, cancer etc. The antidiabetic drug metformin is an age old drug which was introduced in 1950 for the patients of diabetes. [31] But afterwards it has been found that it also having antineoplastic effects in a long run. Several studies are being made to establish relationship in between cancer formation and its destruction by drugs like metformin. Metformin has antineoplastic activity against various type sof cancer cells like prostate[32] [50]., breast[33], colon[34], and glial cancer[35] [8]. This is evident that cancer is a deadly disease which might be cured by surgery, chemotherapy, radiation treatment etc. Gastro intestinal cancer is also a very wide spreading disease through out the universe.

Pancreatic cancer is also a big cause of different pancreas related problems. Gastro intestinal cancer is a very big problem now a days and a leading cause of death world wide. It has been seen that in a recent survey that patients who are suffering from type-2 diabetes mellitus and are taking metformin having low risk of facing pancreatic cancer than those who don’t take metformin [36] [4]Generally gastric cancer studies are fulfilled in various cancer cell lines like MKNI, MKN45 and MKN74 . These ate the perfect cell lines for the in vitro study of gastric cancer cells. The effects of drug in various cancer cell lines establish its effectivity in anticancer drug therapeutics in vitro model. Animal study generally found with mice model to provide the effective dosage dependent antieoplastic activity. So this is a good drug which can cure or

control diabetes as well as cancer . In this manner it can help to reduce the propagation of gastric cancer cells by various apoptotic pathway . There may be inhibition or reduction of cyclin D , cyclin dependent kinase (CDK4) CDK6, etc. this type of angiogenic activity establishes its usage against various cancer cell lines it is a well known drug for its anti metabolic effects especially in glucose and fatty acid metabolism [37, 38,]

Gastric cancer cells are one of the most troubled features of cancer now a days. already we have seen and established a relationship between metformin and gastric cancer cells. It has also been seen that metformin is active against various cancer cell lines in vitro and in vivo assays. The gastric cancer cells can be found as MKN 1, MKN45, and MKN 74 and was performed for the experiment. The in vitro assay of metformin shows its activity against the gastric cancer cell lines in a concentration ranging at 1,5 and ml/lt. It also shows its in vitro antitumor action in lab mice model in a different concentration range. In a study it was seen that metformin changed the phosphorylations of various proteins. the proteins may be AKT, CATENIN, CREB, Chk23[39,] , and CSRC [40] .

It has been seen that there is a major improvement in the number of gastric cancer cells in recent years. This is mainly due to the fact that advancement of modern therapies in cancer chemotherapy is a big factor in treating the cancer patients. [41]

The drug metformin works in metabolism of various carbohydrate and fatty acids in liver. The main function of the drug is to block neoglucogenesis in liver. [42,43,17,18]

Metformin helps in various cancer cell lines to act against cancer. It has been found to be effective in breast glial and prostate cancer cell line also.

Guar gum is a natural gum and it is a polysaccharide which may be obtained from the seeds of cyamompsis tetragonolobus (family- laguminaceae) [44] It is having a big character of swelling from its original form.

It forms a big viscous molecule after swelling . It is having the gelling property which may be responsible for decrease in the release of drug. It is used as the matrix swelling material for various microsphere formulations.[45,46] The polymer

of monosaccharides are the polysaccharides and they can be used for various preparations as a matrix building material. Guar gum is used as a matrix building material in the present study and it has been observed that guar gum can produce a big viscous mass in the matrix and which holds the drug in the microsphere . It is having a good swelling power which can be found for its building material. But its viscosity building property is also a reason for its slow release from the microsphere. Release of the drug from the microsphere is an important criterion . The release of the drug actually shows the effectivity of the formulation as it can be standardized in formulation in the large range of effectivity of the microsphere. Diabetes is a big problem now a days for the human society, throughout the globe. It promotes life threatening diseases.

1.4 Theory of microencapsulation technique

In the year of 1960 we first found the approach of microencapsulation technique. All the time it was found to be formulated on silicone rubber and polyethylene.The major problem was that it was not biodegradable which cause a major problem. Day by day a new concepts came and we got the biodegradable polymers for coating the organic medicinal (Masom et al. 1976).

Microencapsulation is nothing but a specialised coating process in which the size ranges from 1-1000µm. This is a fine mix up in which solid liquid droplets are made by special technical support from the field of emulsion science and polymers.These studies enable us to prepare microcapsules. Which is an advanced coating technique in which solid liquid and gases are quoted by polymeric outer coat and the inner material is released with the proper release profile in a time-dependent manner to ensure a drug delivery approach that is fine sustained or controlled release dosage form. This is the new objective to prepare a prolonged release dosage form in the form of micro capsules so that liquids are converted into solid with the help of knowledge of the emulsion.

a. Advantages:-

2. Absorption of the drug in the gastrointestinal tract is higher as the particle size in the micro range.
3. The drug maintains a continuous release profile being coated with suitable polymers.
4. Different problem related to incompatibility of medicament can be controlled.
5. Prepared microcapsules improve the dosage stability.
6. It can help in the modifying that is of different preparations.

7. The unique characteristic of this method help in using toxic materials too.

b. Disadvantages :-

1. Cannot protect from the side effect of the medicine.
2. The system cannot be used for those drugs having longer half Life.
3. Selection of dosage regimen is critical.
4. Cost effectiveness is also be considered as the instruments are costly.

The medicament is to be ingested and swallowed by the oral route to pass through the gastrointestinal tract. Gastrointestinal tract primarily does three main functions of the body that is secretion, digestion and absorption. It is customary to screen the higher molecular weight particles and medicaments through the gastrointestinal barrier. It only allows for passing into the systematic circulation of nutrients and vitamins. After taking the food the food particles are distributed throughout the body. The gastrointestinal tract is an important part of the body which control the absorption distribution digestion and elimination of non digested food particles. It mainly contains three part namely stomach small intestine and large intestine. The small intestine comprises of three parts Deodinum, jejunam and ileum and it maintains a pH range of 5 to 7.6. And this is the principal site of absorption of drug. The sustained or controlled release dosage forms are prepared targeting this intestinal area. Small intestine contains some unique properties so that it can be taken as an ideal part for the absorption of drug. The properties may be like that the long passing time the permeability is high to the small intestine and transit time is also very high through the small intestine and that is main reason for higher absorption in the gastrointestinal tract. On the other hand stomach maintain ph ranges from 1 to 3 and it is due secretion of hydrochloric acid in the stomach switch on the other hand favours the absorption of acidic drugs .

Again the large intestine contain very small surface area for the absorption of drugs. So it is not favoured as an ideal site for the absorption of drugs. But the poorly absorbed drug may be absorbed as there is a long residence time which is also supported by various observation.

According to various study it has been established that various drugs are absorb in the gastrointestinal tract due to the environment environmental PH the transit time in the g I tract etc. Drug release behaviour depends on so many other factors too which provide basic fundamental for working drug and food particles to be absorbed over the gastrointestinal tract. There are so many other factors also which can influence drug absorption like particle size surface area hydro felicity and Lipo felicity of the drug PH and pk a values etc.

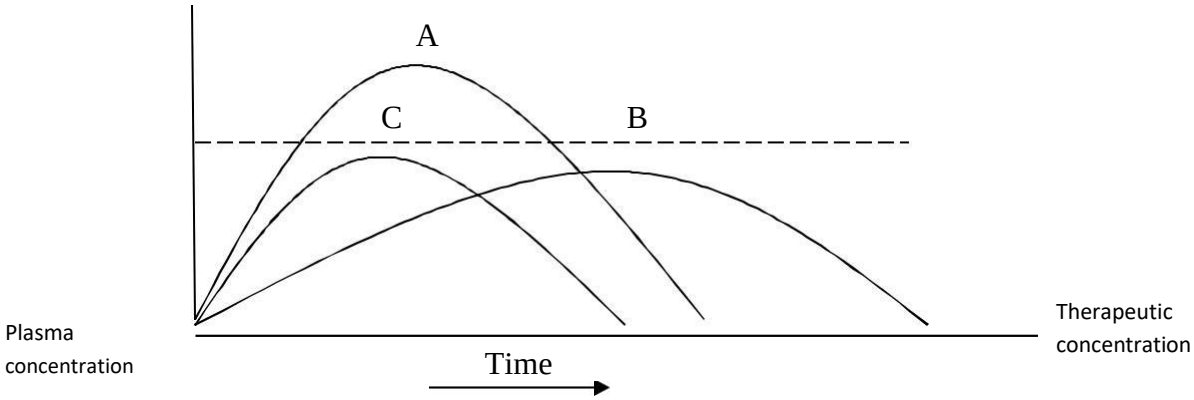
1.5 The concept of microsphere :-

The concept of microsphere arises from the fact that effect of drug should be longer inside the body so that the therapeutic efficacy of the molecule becomes higher. Micro particles are microspheres concept come with a fundamental principle that smaller particles having high surface area could be lightly absorbed in the small intestine. This particular microspheres are having particle size ranging from 1 to 1000 µm. The concept of preparing microsphere is nothing but drug is embedded in the matrix of microspheres are prepared with the concept that there may be a mixture of drug and polymeric materials in a certain proportion to coat the drug effectively. This is also developed with the concept of drug entrapment efficiency drug release and other physicochemical parameters to standardized the preparation. Sustained release drug delivery is an unique technology to provide the active ingredient in the target area for a longer time. So the active ingredient will be available for the longer duration in the site of action. At the same time the concentration of the drugs at the particular site may suddenly increase toxicity problem simultaneously and that is why a continuous release of the drug for a prolonged period is more essential. So this type of drug delivery provides the therapeutic efficacyefficacy of the drug at the desired level and maintains it for a longer duration.

a) Advantages :-

1. Continuous availability of the drug through a longer time.
2. The absorption of the drug is reproducible
3. It provides a desired dosage form with a sustained effect.
4. As a longer action dosage form so frequency of dosing is minimised
5. The half life of the drug is increased so better dosage from for the drugs with short half Life.
6. Periodical constant release of drug does not accumulate high drug concentration in the gastrointestinal tract sources for the gastrointestinal tract.
7. The bioavailability of the drug is increased as the biological half life is extended.
8. One big advantage is that it cannot face the first pass metabolism
9. As microspheres having small size so can be used parenterally also.
10. Dose dumping generally not found with microspheres

The availability of the drug in the biological system is known as bioavailability basically this is denoted as the fraction of the drug that reaches the systematic circulation is known as bioavailability. This particular phenomenon can be represented graphically also(47).

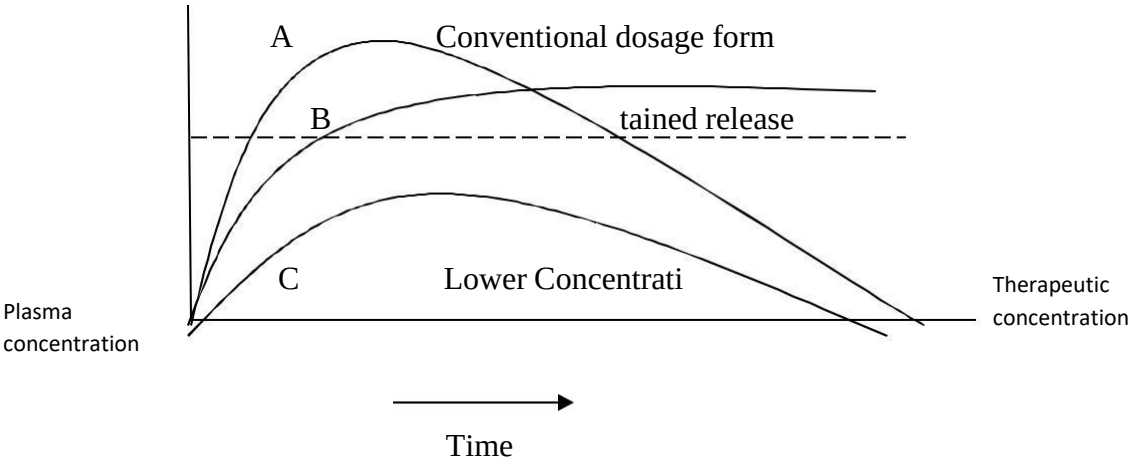


A- Formulation A above the therapeutic concentration level will show therapeutic effect.

B.C- formulation will not show therapeutic effect.

Thus in this way we can get an idea of the dose-response curves in the terms of therapeutic concentration and bioavailability.

The release of drug is an important parameters as it is related to the bioavailability. The plasma concentration of the drug depends on the release of the drug in the intestine. This also happens in case of microspheres. Slow but continuous release to a longer duration can be a stable therapeutic concentration through a longer period. So ultimately drug will be available for a longer period above the therapeutic concentration level for the desired bioavailability for a longer time.



So from the graph of Plasma concentration versus time it has been clearly understood that the sustained release formulation deliver the drug for a longer period above the minimum effective concentration. That is the ultimate aim of the sustained release formulation that it can supply the drug for a longer period at the desired target site. In case of microspheres it is

achieved by formulating the microsphere in a basic conceptual dosage form so that the drug will be released in a continuous manner throughout a longer time. So bioavailability of drug above the therapeutic concentration will be highly achieved and will be maintained for a longer time. The drug release behaviour can be further change and it depends on the polymers used in the polymeric microsphere as well as the drug molecule present in the microsphere. The polymers and the other adding additives constitutes the carrier molecule which controls various properties of the microspheres such as drug absorption metabolism and excretion from the biological system. So this is very important for the therapeutic efficacy of the drug molecule.

The release of the drug from the microsphere is an important phenomenon and it is mainly based on the fact that the drug is embedded in the matrix and that attachment should be curtailed so the drug comes out from the surface of the polymer to enter the biological system. The effectivity of the formulation depends on this particular phenomenon and with time how much drug is released is known as rate of drug release. The rate of drug release mainly depends on the technique of the preparation the polymer used nature and properties of the drug molecule, pH of the biological system therapeutic efficacy of the drug molecule and overall behaviour of the carrier molecule.

1.6 Mechanism of drug delivery :-

Sustained or controlled release formulation are prepared for continuous supply of the drug at the affected site. There may be so many techniques for that and microspheres are very useful for that. There may be so many concept and mechanism for that.

Control of dissolution

Dissolution of drug is a very important phenomenon for the absorption of drugs from the biological systems. By retarding the process of dissolution the rate of absorption can be delayed which may be a factor for the sustained release for formulations. Coating over the formulation can be a better idea for this kind of medication.

According to Noyes Whitney equations-

$= K_D A (C_s - C) = D_A (C_s - C) / \Delta h$

A= Surface Area, D= Diffusion Co-Efficient

Δh= diffusion layer thickness

$C_s - C$ = Difference in the concentration

In this particular equation it has been found that surface area of a particle is a very important parameter for the dissolution of the dosage form.

There is another one equation for dissolution known as Hoffenberg

$$M_t / M\alpha = 1 - [\, 1 - \frac{Kot}{Coa} \,]^{n}$$

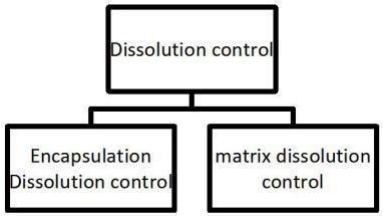
M_t $M\alpha$ are two parameters which denote the concentration of active ingredient

n = 1 (slab)

= 2 (cylinder)

= 3 (Spherical particle)

Again there are two types of dissolution control products.



In case of encapsulation dissolution control the coating materials should be chosen in a proper way so that the rate of dissolution in a controlled manner depending upon the coating material.

The matrix dissolution control is a complex system in which the matrix controls the dissolution process periodically releasing the drugs from the matrix and it depends on the gum concentration nature of The matrix, dissolution control is a complex system in which the matrix controls The dissolution process periodically releasing the drug from the matrix and it depends on the gum concentration nature of gum solubility of the drug candidate and nature of the polymer to coat over the microsphere. Nature and pH of The dissolution medium is equally important for the release of the drug from the matrix.

Diffusion controlled release system

Diffusion depends upon the difference of concentration across the membrane. It is governed by the Ficks law

$$J = -D \frac{dc}{dx}$$

J= flux of drug towards decreasing contraction through the membrane.

D= constant of diffusion

dc/dx= minute change in contraction (c) with distance (x)

in the steady state

J is the amount of drug released.

At the same time rate of drug release can be given by

Dm/df= ADK ΔC/L

A= Surface area (dosage form)

D= Coefficient of diffusion

K= Particle of drug (polymeric membrane and the drug core)

L= Path length difference

ΔC = small change in concentration through the

membrane This is the equation which satisfies the zero

order release.

The matrix device of microsphere-

The active ingredient is distributed through out the matrix homogeniously and diffuses to the medium and diffuses to the medium . this is a process of availability of the drug from the outer to the inner surface. This is a system of diffusion or sustained mediated release.

Higuchi equation can be expressed as

$$Q= [D. \epsilon/T (2A - \epsilon Cs). Cst]^{1/2}$$

Q= Quantity of released drug per unit area.

D= co efficient of diffusion of the drug in the medium.

ϵ = matrix porosity

T= matrix tortuosity

Cs= Drug concentration in the polymeric matrix (g/ml)

And the equation becomes

$$Q= K.t^{½}$$

Where K= an arbitarary constant

This model of drug release by higuchi can be controlled by some factors like drug in polymer matrix , porosity and the drug solubility.

Diffusion and dissolution control system of prolonged action dosage forms-

This is the system in which drug is embedded in the matrix and released slowly with the passage of time.

The release of release of drug can be expressed as followed:-

$$Q= AD(C_1-C_2) /L$$

Q= Rate of drug release

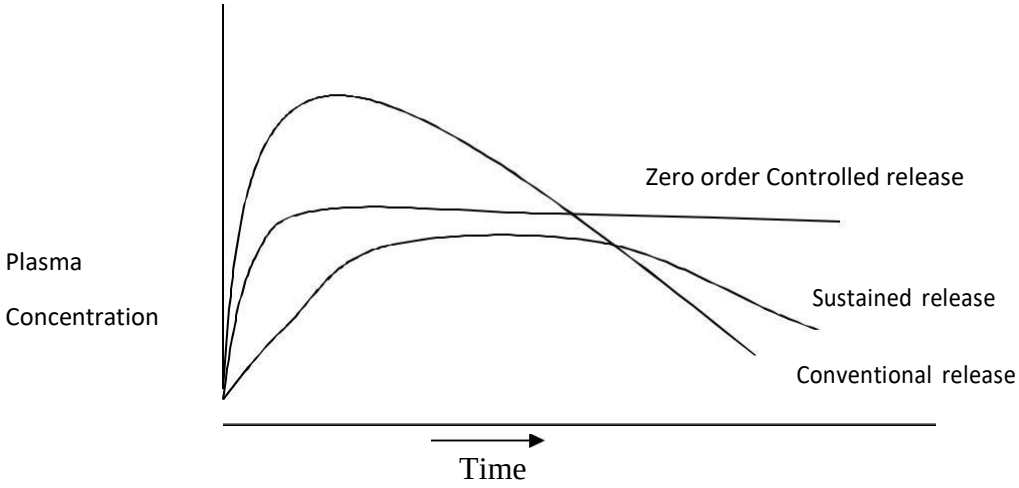
A= surface area

D= coefficient of diffusion of the molecule

L= diffusion path length

C_1= Dug concentration in the core

C_2= Drug concentration in the dissolution media.



1.7 Mathematical interpretation

The in vitro drug release from microsphere depend on various mathematical models of drug release behaviour from various Kinetic models that as is zero order first order equation, Koresmeyer and peppes equation and hixon– crocaells cube root of time equation.

First order equation 2

Higuchi's square root of time equation 3.

Korsmeyer and peppas equation 4

Hixon- crowells cube roof time equation 5

$$C=K0t \text{ -------} (1)$$

Where K0 = zero order rate constant and that can be fund in conc/time where 't' is time.

$$Log= Log\ C0- K_1t/\ 2.03\text{----------}(2)$$

When C0 = initial concentration of the molecule of drug and K1= First order constant.

$$Q= K_n\sqrt{t}$$

K_n is the constant density design variables of the system.

$M_t/M_\alpha= K.t^n$

M_t/M_α is fractional solute release at time 't'.

K=Constant (structural and geometric characteristic of drugrelease device)

Table: list of generally used mathematical model

Sl no.	Kinetic Model	Mechanism of drug release
1	Zero Order	Homogeneous dissolution
2	First order	Coated dosage form or membrane controlled
3	Higuchi square root of time	Model is diffusion controlled. The drug candidate is in the medium.
4	Korsmeyer peppas	Diffusion controlled.
5	Hixon-Crowell cube root	Water soluble drugs in the matrix. The rate of release is limited by the dissolution rate of the drug.
6	Baker-Lonsolale	Uniform dispersion of the drug in the matrix (W1/O/W2 emulsion technique)
7	Hopffenberg	The device are surface eroding

1.8 General concept

Some fundamental concept should be there for the preparation of polymeric microspheres so that microspheres preparation can be controlled. The physicochemical characters are also be considered.

Core of the microsphere and coating polymers

Polymers are essential part of the preparation of microsphere due to their physical activity and usefulness as a coating material around the drug.

This actually a big Discovery with the help of technologies related to the development of polymers and polymer Sciences.

Bioadhesive drug delivery is an important factor in Novel Drug Delivery System as it is an approach towards the preparation of suitable dosage form in the laboratory. It depends on the pharmaceutical technologist how the Polymers can be used in the way of preparing the microspheres.

Polymers should have some qualities like biocompatibility different physical chemical properties and it should be compatible with the ingredient of the microspheres and obviously with the drug candidate. Several test are performed to get its safety profile

There are different other properties such as degradation by hydrolysis and swelling properties of the polymer. There are different surface properties such as hydrofilicity smoothness and surface energy which may control the biocompatibility of tissue and blood.

There are different other characteristics like bulk properties and molecular weight and solubility should be considered.

There are different other structural properties also of the matrix such as porosity of the molecule and surface texture and morphology of the molecule.

These area the factors with critically determine the mass transfer through the matrix in the form of water and the drug candidate.

At the same time as we know that polymers can degrade as it is the natural property of the polymer and there are other properties like erosion which is controlled by the dissolution and other characteristics like diffusion etc.

The properties of the polymer such as erosion can be described in different manners such as surface and bulk erosion. Surface erosion is nothing but a physical phenomenon where the rate of erosion might be higher than the rate of water entry into the material constituted by the polymer and it is the primary requirement for the kinetics of the drug release following zero order in case of bulk erosion the situation is different and the rate of water permeation may be quicker than that of the surface erosion and from the polymer. Total degradation or

destruction of the matrix total release of the drug molecule from the core of microscope takes place. Generally this kind of technology followed by the polymer used in the formulation. There may be a different concept for the use of the drug and polymer and sometime a mixture of the two polymers and also used.

The natural Polymers are having and unique properties that it can be degraded in the system. The efficacy of the natural polymer can be increased by the addition of the chemical groups such as ester, ortho ester, anhydride and carbonate in their chemical structure.

The core is made up of Gums to prepare the metric microsphere and in this manner the sustained release formulation is prepared.

All these polysaccharides from the herbal origin are very much important as they can provide a big functional and fundamental performance due to their various working procedures. In this microsphere formulation guar gum is used as a gumy material to build up the matrix. Guar gum is used from times immemorial as a viscosity builder in various food preparations and pharmaceuticals.

In this formulation of microsphere ethyl cellulose is used as a polymer to prepare the dosage form. Chemical ethyl cellulose is an analogue of cellulose was some glucose unit are being changed chemically at its -OH side Chain and being replaced by ethyl Ether group.

Ethyl cellulose mainly introduced in the development of the formulation as a coating agent. According to FDA it is a safe molecule to be used in medicine and food industries. The unique property of ethyl cellulose is its insolubility in water but highly soluble in organic solvent.

Now this is used in microsphere preparation so that it can form the primary emulsion in the solvent evaporation multiple emulsion technique where dichloromethane is used as a solvent for the non aqueous phase. This technique can be used for the preparation of microsphere and here W1/O/W2 type of multiple emulsion is formed in the microsphere.

1.9 Solvent evaporation (multiple emulsion) technique :-

The evaporation of solvent is required for the preparation of microsphere in most of the cases. It was discovered in the year of 1970 last part and develop to make it better in 1978 - 1980. This is an unique preparation when a drug is dissolved or made soluble to form an uniform dispersion with the help of gum.

The use of polymer is essential as it can coat the material in the nonaquous Phase to make the primary emulsion. The drug is embedded in the matrix where it is fixed with the gum used to prepare the core of the microsphere matrix and this solution is introduced dropwise into the non aqueous phase with stirring so that the primary emulsion is formed and after formation of the primary emulsion it is added dropwise to the external aquas phase with continuous stirring to prepare the multiple emulsion and with vigorous stirring the solvent is evaporated so that multiple emulsion solvent evaporation technique is introduced.

The microsphere preparation is governed by various factors. These factors may be concentration of gum in the matrix amount of solvent in the matrix amount of drug in the matrix non aqueous phase solvent stirring speed of the mechanical stirrer etc.

The stirring speed of the microsphere is an important parameter in the mechanical stirrer as it controls the evaporation of the solvent. Evaporation of the solvent is required very much as it often controls the spherical nature of the microspheres. This particular phenomenon is very much important as microspheres shape and size is important for the preparation of the dosage form.

Constant stirring is a primary Criterion for the preparation of the microsphere in the solvent evaporation technique and that is the difference of other methods i.e. that is spray drying, sonication and homogeniser which is nothing but the regular method of preparation of microsphere. This is the reason behind its nature of constant staring at room temperature is required to prepare the microsphere.

Stirring speed is very important parameter for the preparation of microsphere. As stirring speed is related to the formation of microspheres by the rapid evaporation of the solvent from the aquous dispersion. The size and shape of the microsphere is also related to that.

At the same time the solvent used (dichloromethane, chloroform) etc should be very carefully controlled as they are toxic in high concentration. So a very little amount should be used to prepare the formulation.

Drag entrapment efficiency generally found to be at lower extent in this method. So suitable measures should be adopted to increase the drug entrapment efficiency.

Solvent is an important factor for the preparation of the microsphere. A good solvent generally differs from the internal Phase to the external face very quickly and efficiently so

the choice of the appropriate solvent is an important parameter for the selection of a solvent. Ethyl cellulose is an important polymer for the preparation of the microsphere and dichloromethane can be a good solvent for polymer like ethyl cellulose. The advantage of solvent dichloromethane is due to that it is having very low water solubility and that is the reason why it is used with dichloromethane.

At the same time the boiling point is also very less with the use of this solvent di chloromethane microsphere formation becomes easier and we get microspheres in multiple emulsion solvent evaporation technique with the use of dichloromethane as a solvent in the non aqueous phase of the multiple emulsion.

Important factor for the preparation of formulation:-

Absorption of drug

The efficacy of medicine totally depends on its absorption at the biological system which determines its bioavailability and therapeutic effect in the clinical system. Release of the drug should be a continuous process and that can potentiate the absorption of drug. In sustained release drug delivery the rate of release is a slower process but a continuous one and therapeutic efficacy will be there in a longer and continuous duration as the drug is also absorbed continuously in the system

1.10 Half life of a drug :-

This is generally meant for the time taken by the drug to be half of its original concentration. So there may be drug with short biological half life and those are having long half life. So there must be a system for the increase of short half life and sustained release drug and that is achieved by sustained release drug. So the medicament with short half-Life would be a fine candidate to be increased by their half Life by this technique. But the drugs having longer half life more than 8 hour may not be found suitable for the purpose.

1.11 Drug metabolism :-

Another one very important factor for the sustained release medication is its metabolism in the gastrointestinal tract. The drug which readily metabolised in the small intestine can not act in a sustained release medicament as it is not available for a longer time in the system.

1.12 Physicochemical factors :-

There are some physicochemical factors which may act as the basic parameters for the formulation of sustained release medicaments

Drug solubility (aquous)-

Very important factor as it determines the miscibility of the drug molecule in aqueous or non-aqueous system.

This is basically a parameter which determine which section of drug can be found in the portal circulation. Mainly the drugs are medicines coming into fluid part of the gastrointestinal tract is called the permeability of drug

These drug which do not have that much of water solubility and also having very slow rate of dissolution can be given to the sustained action drug delivery. In this type of drug delivery system it is customary to see the dissolution of drug Through the matrix is an important factor. and again the main driving factor is the amount of drug present in the polymeric matrix.

The concentration of drug will be less for weakly soluble drugs

The drugs which are highly water soluble and having very high dissolution rate is often very tough to decrease the the dissolution rate and slow absorption rate. The water solubility of the weak acids and weak bases generally based on the Pka of the material and ph of The dissolution medium

The ph partition theory describe that the absorption of weakly acidic drugs non ionized form will be high in stomach. The absorption of weakly basic drug ionized form will be poor. The solubility which is pH dependent can create another type of problem in the physiological range.

Again the pH partition hypothesis says that in stomach the absorption of weakly acidic drug (non ionized) form is high and that of the weekly base drug (ionized form) is poor.

It is a bigger problem while the pH dependent solubility is a bigger problem as different pH found throughout the gastrointestinal tract and that is why different dissolution rate will be found.

Again there is an accessibility through the biopharmaceutical classification system which may include some major particles such as a solubility, dissolution and gastrointestinal permeability.

1.13 Partition coefficient

It is generally understood by the fact that part of drug partitioned or got passed into the oil phase and also to the subsequent Aquous phase and the ratio of that is expressed as partition coefficient

i.e. k=

where, C_o- Equilibrium concentration of all form of the drug in organic phase at equilibrium.

C_w- Equilibrium concentration of all form of the drug in acquous phase.

It generally indicates the permeation of the drugs through the biological membrane and also the diffusion of the drug through the matrix membrane in composite system of oil water interface. The drug should diffuse through a variety of biological membrane and that membranes are nothing but the biological barriers. Show the partition Coefficient should be higher of the drug to act as a sustained release formulation.

1.14 Diffusibility and size of the molecule

The molecules should go through various types of membranes. It is the property of the drug in an prolonged release drug delivery system that it should pass through different kinds of membrane. The capacity of the drug molecule to pass through different kinds of polymeric matrix is a function of its diffusibility (diffusion coefficient) and is also function of its molecular size (molecular weight, M) $\log D = -S_V \log V + K_V$

$$= - S_M \log M + K_m$$

Where V= molecular volume . S_V, S_M, K_V, K_m = constant

Again D can be related to shape and size of the cavities of polymer at the same time the shape and size of the drug molecule.

The diffusion coefficient be is basically related to shape and size of the cavities of a polymer at the same time the shape and size of the drug molecule.

And also there is a difference between the diffusion coefficient of different molecules. The drugs which are having molecular wait more than 500 Dalton their diffusion coefficient is very less.

1.15 Stability of the drug :-

The most considerable thing in the stability of the drug is its various properties like base hydrolysis enzyme matic degradation and also various factors like metabolism in the gastrointestinal tract at the time when the drug is used through the oral route.

Again the relative bioavailability can be improved and that may be due to the release of the drug in specific areas of gastrointestinal tract. There are many drugs which may be unstable in the stomach in that case they should be released in the intestine whereas drugs that are not suitable in the intestine they can be released in the stomach. So it is not evident to find stability problem with sustained release dosage form as it can deliver its content uniformly.

1.16 PKa and ionization constant of the drug:-

The acidity and the basacityof the medium is understood by the pk values of the medium. The activity of the drug is found to be at its peak when it is in unionized form in the unionised form of drug molecule is absorbed better than that of the ionized form.

The acidic drugs are having the peak a ranges in 3.0- 7.5 and that of the basic drug is 7.0-11.0

Physicochemical properties of microsphere influenced by the parameters :-

The most important parameter is stirring speed as it controls the formation of the microsphere. In case of solvent evaporation technique the speed of stirring is a key factor as it controls the rate of evaporation of the solvent from the bulk phase. Again for the preparation of the primary emulsion the speed of rotation of the magnetic stirrer is an important Criterion to prepare the W/O primary emulsion. In the composite system of multiple emulsion of W1/O/W2 type, the preparation of primary emulsion is an important factor as it controls the formation of microsphere.

1.17 The gradient of concentration between the phases:-

In case of preparation of microsphere the concentration of solvent in above two phase is an important criterion. There may be a change in concentration gradient from one phase to another phase so there may be a shiftment of the solvent.

Again the water from the w phase when shifts into the W_2 phase the size of the microsphere may be smaller one. In that case the drug entrapment may reduce.

The introduction of the buffer in the internal phase may potentiate the shiftment of water from the external phase to the internal phase opposite to the concentration gradient.

By adding some salt in the outer phase may produce stronger polymer matrix in the inner face.

Again salt in the outer phase that is in the external Aquous phase may create another problem of less solubility of organic solvent and the polymer may produce some precipitation on the microsphere surface.

1.18 The internal phase volume and temperatures :-

The internal phase volume is an important factor in the formulation of the microsphere.

If the solution in the internal phase is more viscous then there may be production of larger particles and matrix may be porous in nature.

Again if there is an increase in the volume of external phase and the pH is decreased there may be increase in particle size and drug entrapment efficiency.

1.19 Solvent :-

Proper solvent is an important Criterion for the preparation of the drug loaded microsphere.

In case of multiple emulsion solvent evaporation technique the solvent may be aquous in nature and can produce W1/O/W2 type of emulsion system.

In this type of preparation as the solvent is water in the internal phase the drug is embedded in the matrix with the help of gum.

This type of matrix microsphere is very common to see as there is release of drug from the core matrix is low but the continuous for longer duration producing sustained action dosage formulation.

In the external phase water may be the solvent and Rapid evaporation of the solvent from the bulk phase may be produce porous microsphere.

1.20 Temperature of the system :-

The system temperature is very important Criterion for the preparation of the microsphere. If the temperature in the external phase is higher then there maybe rapid evaporation of the solvent and microspheres will be produced in quicker time. But the walls of the microsphere may be porous in nature due to rapid evaporation of the solvent.

Cancer is a big problem and it produces uncontrolled and massive growth of cells and tissues of a particular part of the body surface which may lead to metastasis of the cancer from one part to the another. The world cancer report of 2014 reflect that it is one of the great cause of death worldwide and if it is primarily detected surgery can be done to remove the cancer cells from the body. Surgery is a great method to remove the cancer cells from the body system whereas the metastases can be blocked by additional entry of drugs are medicines into the system that is chemotherapy should be done to prevent metastasis. DNA replication is a major cause of uncontrolled growth of the cells as it produces a big number of cells at a time causing massive production of malignant cells to produce cancer and that's why the anticancer drug usually target the DNA replication(48,49).

The main operational area of the anticancer drug is to treat the DNA replication are in a biosynthesis or maybe the protein biosynthesis and obviously the biosynthesis of DNA(50). There may be a few drugs working on the hormone or the supply of nutrients to treat the cancer cells(51).

The entry of the anticancer drug into the cancer cell depends on the cell definition that is at the stage of the mitosis where the anticancer drug target their action. The anticancer drug may be of both types i.e. they may be hydrophilic(52)(53). Their chemical moiety can be hydrophobic(54). It is evident that serious side effect may arise with the use of anticancer drug due to the cytotoxic effect of the anticancer drug (55).

So it is evident for a medicine to be prescribed and used for a local application or there may be targeted delivery as it can produce cytotoxicity. Targeted drug delivery can be achieved by selection of suitable vehicle and appropriate route of administration of the anticancer drugs so that active ingredients at the property site of action of the disease.

1.21 Route of Administration:

There may be various route of administration for the use of the medicine in the patients.

1.21.1 Intravenous use:

The best route of using medicines for a quicker effect is the intravenous route as the drug enters the vein immediately producing immediate effect of the drug in the system. But the anticancer drug also can be delivered by this route for the immediate effect although there may be possible side effects which may be a problem for using this route in a long run.

There may be various carriers for the intravenous drug delivery in a selective approach nanotubes(56), nanoparticles(57), vesicles(58), an emulsions(59). Although this route is not deliberately supported by the patients as these may be disliking due to the injectable route it may produce sieve problems also may be due to the blood related infections and thrombosis(60)

1.21.2 Oral tract:

The oral rout of drug administration is most convenient and suitable for the oral use of drugs as it is easy to swallow the medicine and get into the body systems through proper systems of enteric administration. Now this is a route of safe medication as there is no chance of infection through the needles as needles may contaminate a person if it is not made sterile before use. At the same time repeated use of the medicine can be done safely as drugs can be taken in the oral route through mouth and easy to use for the paediatric and the older particles too.

And generally it is delivered through various formulations like emulsion(61) microspheres(62), Liposomes(63) and nano particles(64).

1.21.3 Transdermal route of drug delivery:

The medicines are introduced into the body through the topical route or skin as it can deliver the drug in to the target area. But the limitations of this approach is that it can not deliver the drugs properly into the internal sites of drug dispersing. Although it may be effecve for the upper or outer surface of the body environment and this can be done by nano emulsions(65), emulsions and gels(66).

Ionophoresis is an alternative method of drug administration where the ionic drugs is penetrated through the skin and it is done by lowvoltage of continous constant current(67).

Hydrophilic drugs sometimes used with higher voltage and this method is known as electroporation(68). In head and neck department of cancer chemotherapy this method is widely used(69).

1.22 Drug carriers for antitumor delivery:

1.22.1 Main objectives:

Drug delivery to the desired site is completed by the useful administration of drug carriers which deliver the drugs properly to the desired site of action.. while the drug molecule is delivered to the proper site of action in a proper amount the efficiency will also be good. This is the way where there will be minimization of side effects as there is no extra delivery of the medicine to the target site. This is an important feature of the targeted delivery now a days (70,71).

This is an approach which basicallytriggers the administration of the drug at the particular site for a longer purpose. Continuous release of drug at a proper time

interval in the target site is a big therapeutic out come in the targeted drug delivery reducing the risk of adverse drug reactions or any toxicological outcome(70,71).

The efficiency of the drug carrier depends upon releasing the drug at the proper site of action. The cells must provide entry of the drug molecule for its action and i.e. the role of carriers to present the drug at the proper site of action(70-72)

1.22.2. Hydrogel:

These are basically used in the cancer treatment as carriers of anticancer drug delivery.

It can be said that they are complex network of polymers or may be thermoset gels having high affinity for water or aquous systems. The crosslinking is an important factor for the preparation of the hydrogel as they can move in to formation of a sponge like polymeric structure which is very important for a complex network. So it works like a storage body and releases the drug in a continuous fashion regularly in a specific release pattern.

And in the treatment of cancer it is seen to find use of hydrogel of metformin and 5-fluro uracil in colon cancer.

1.22.3 Polyhydroxy carbolic acid:

These are biodegradable polymers used in various kind of anticancer drug treatment carriers. PLA and PLGA are successfully used as polymers where PLA resembles Poly lactiglycolic acids(72). This type of drug carriers is also involved to make a steady state of concentration in the blood stream of anti cancer drugs. Now a days polyhydroxy carboxylic acid(PHA) of medium chain moiety found to produce better result(73).

The microsphere preparations with coated polymers may be a suitable example for this kind of medication used in the cerebral tumors. The coating material

selection depends specifically on drug it self as it can develop the release of drug in the proper at the target site of release and thus we see chitosan can be effective in 5-Fu loaded microparticles(74). Whereas polyethyline glycol can be used as a coating material for taxol containing drugs(75)

1.22.4 Nanotubes-

These are typical examples of formulations producing the drug at the target site in the anticancer medication and the internal volume will be filled with the drug. Inside the nanotube the drug and medicamets should be completely filled to provide the drug molecule in the target site. There are existence of single walled nanotubes(76). And it may be made of carbon, boron carbide, boron nitride and silicon and there may be multi walled carbon nanotubes(77). These are made of carbon nanotubes and are of unique feature showing its activity in the aquous media(78).

Organic nanotubes (79) can be used to encapsulate a poorly water soluble drug such as hydrocortisone whereas 5-Fu nanotubes can be made by TiO_2/Zns (80).

1.22.5 Nanoparticles –

From the name it is evident that the particles will be of small to smaller size. Size may be in the size range of 1-100 nm and mostly of spherical sizes and shapes . various types of nanoparticles can be seen in the preparation of novel drug delivery, , may be solid-lipid-nanoparticles (81) and polymeric nanoparticles (82).

Use of both natural and synthetic polymers can be done to prepare the nano particles. Cellulose , chitosan etc. may be the examples of the natural polymers whereas acrylic polymers, polymethyle methacrylate and budroyit can be found as synthetic polymers. The main objective of coating a drug by the polymer is to prevent the degradation or release of the drug in the stomach and to release the drug in the farthest part of the intestine i.e. the ileum and so the nano approach

of drug delivery is very important for oral sustained release dosage forms in the novel drug delivery.

At the same time we have seen various formulations using paclitaxel as a core drug for the anticancer drug delivery in nanoparticular form to treat various diseases like ovarian, breast, lung, pancreatic and colon cancers. Paclitaxel is a fine drug in anticancer drug delivery and becomes finer while it is in the nanoparticular dosage form to obtain specific result in drug delivery (83). While a hydrophilic drug 5-Fu is used typically in a dosage form of nanoparticles using chitosan as a polymeric material . The efficiency of these dosage form is that it can release the drug in alkaline pH in the intestine very effectively whereas resist the release acidic pH of the stomach (84). Now a days we have seen the use of more efficient polymer to coat the nanoparticles known as graphene or its oxide known as graphene oxide (85). The main boding graphene oxine in the intermolecular chain may be due to the π- π bonding amongst its molecular chain (86).

There are newer approaches coming to develop the molecular structure of graphene oxide so that it can be used effectively as a polymer in nanoparticular delivery (87).

The combination of cyclodextrin with graphene oxide to align in the molecular structure can be a newer approach to achieve a promising drug delivery (88). Functionalized grapherene sheets crosslinking can be prepared to achieve nano formulations (89). Mainly loading of the drug can be increased by the increasing use of graphene oxide with supramolecular sheets of cyclodextrins adjusting in molecular level crosslinking to achieve the goal of the therapeutic level novel drug delivery approach.

1.23 Introduction of concept of vesicles and liposomes-

Vesicle is a improved technology which encloses aquous phases. The water soluble drugs may be incorporated for encapsulation, on the other hand oil soluble drugs also may be introduced in the central core of hydrophobic material (90,91). So it can be said that vesicles can be used in both hydrophilic and hydrophobic ingredients . they are utlised in all the preparations like medical preparations as chemical and personal care products. It is also seen that vesicles can be used for drug delivery in cellular exchange process by membrane fusion and separation (92,93).

Vesicles are found in various ways according to size and lamellarity. Generally vesicles are found to be of round shape. There are various types of vesicles found in these filed like ultra small unilamellar vesicle (USUL) and its diameter may be 5-10 nm (94) . The size of small unilamellar vesicle (SUV) is larger its size may vary from 8-40 nm (89). Generally more that 20-50 SUV are used for the preparation of intravenous injection (103).

And it should be within the size range of 100 nm (100) and that may be assumed by checking the internal dimension of circulatory system may be of seven thousand nano meter (99).

The blood cells having diameter of 8000 nm generally pass though these capillaries, It has been seen that vesicles are less flexible (95). And its diameter may be less that 100 nm (96).

The vesicles are found to contain only one bilayer and they are prepared generally by the solvent injection method (97), and may be they can be prepared by the sonication or it can be due to the non availability of the large vesicle precursors.

The large unilamellar vesicles or LW are having a diameter which may not be more than 100 nm or might be 01 µm (98). The size range can be extended upto

a size of several μm (99). Solvent injection method is an useful method which may contain ether or ethanol. There may be larger sizes seen in the M1Vs(96,98) or may be of similar sizes that of Luv but they are generally found to contain two or more concentric bilayers(100).

It is found that thin film hydration(96) may be very effective(96) or there may be followed freeze drying technique of the SUV dipersions in the aquous solutions obtain encapsulation(96). There may be some biggest vesicles which are also known as giant vesicles(GUV) and that may contain a size range of 5-100 μm(101) although there may be a category of oligovesicular vesicles(OVV) which may contain small vesicles and that may be inside big vesicles(102), and it is made to provide effective circulation inside the blood stream. It may be seen also various names for this particular form of dosage form(99) (vesicles).

A general existence of liposomes are found to be used in nano-particular dosage forms of novel drug delivery category. These are made of phospholipids(96,97,103) and binds effectively various anticancer drugs found to be encapsulated including taxels. There may be existence of niosomes which may contain non toxic Surfactants(104) and catanoic Surfactants also can be take part in the formation of vesicles. It is seen that these cationic Surfactants or “catansomy” are made of using anionic and cataionic surfactants in an unimolar ratio(105).

At the same time we can also find ethnosomes which may be made of phospholipid vesicular systems utilizing ethanol in higher concentrations and that may be of 20-50 at %(106).

Characterization:

1.23.1 Physio - chemical characterization:

Particle size is understood and determined by various methods and may be by dynamic light scattering(107). Scanning electron microscopy or SEM studies can be applied for the determination of the particle size at the microparticle level to understand and study the microparticular drug delivery.

At the same time field emission scanning electron microscopy(FESEM) and transmission electron micrscopy (TEM) (108) can be used to determine the nanoparticular drug delivery.

Liposomes or other vesicular system & can are understandable by using CRYOTEM(109) for the determination of the particle size.

Complex systems are composed of various formulations which may be stabilizing the development of the charged particles in the system. These charged particles determined by the zeta potential in the system. If the zeta potential in the system is lower than the possibility of flocculation or coagulation can be found. At the same time with higher and higher zeta potential results less chance of coagulating or flocculation providing stability to the systems. 40 mv to 60 mv zeta potential shows good stability in the system where as more than 60 mv shows good stability in the system(110,111). But if the zeta potential is within the range of 0-5 mv positive or negative, there is a chance of rapid flocculation or coagulation in the system.

Moderate stability may be found within the range of 30 mv to 100 mv where as 10 to 30 mv zeta potential shows very low stability.

1.23.2 Drug entrapment efficiency (DEE)

The drug loading mechanism is an important criterion as it predicts the amount of drug to be entrapped in the formulation. Various spectrophotometric methods were utilised for the determination of drug content or to calculate the percentage of drug entrapped in the preparation.

The methods are generally found to utilise for DEE various instruments like U-V spectroscopy (112), fluorescence spectroscopy (113) HPLC(114) and a combination of HPLC with mass spectroscopy (106).

The drug entrapment efficiency also renders the free drug calculation which was not entrapped in the formulation.

This can be followed by ultrafiltration or centrifugal filter devices (107). At the same time some other techniques can be applied (108).

By the help of suitable calibration curves we can find the specific amount of supernatant and sediment in the preparation.

The drug entrapment or drug encapsulation efficiency is nothing but the amount of drug i.e. entrapped inside the drug carrier. The amount of drug entrapped can be varied with the method of preparation and the technique followed to prepare the formulation.

It has been also observed that 5-Fu encapsulation can be better if it is done by DPPC or, Dipalmitoyl phosphatidyl chloride than with ethanolic injection method to be entrapped in the vesicles (115).

The encapsulation efficiency can be increased and that is due to the inclusion of DPPC without cholesterol and that was also in a modified reserve phase evaporation also(116). At the same time the use of a different surface active system can increase the % EE to about 80%(117).while it has been found that if

we use polymeric nanoparticles prepared from triphosphate crosslinked chitosan may be left effective then than the vesicular system (118,119) .

1.23.3 Drug release

The release profile of the drug is a very important criterion as it directly relates the bioavailability of the drug and from that therapeutic efficacy is also concerned. The important factor of concern is the dialysis bag method to study the in vitro drug release of the formulation.

The drug while encapsulated placed inside the dialysis bag in the mouling water at 37°c which reflects the body temperature. The concentration of the drug is checked time to time may be within 1 hour interval to study the release behavior of the drug. There are so many differences in the dialysis bag and that is to study the diffusion cells, ultra centrifugation and ultrafiltration approaches (120,123)

As cancer is a deadly disease and the malignant cells can make uncontrolled cell proliferation so it becomes the most common cause of death . According to a study report published by GLOBOCON 2018 SK1) a huge number of cancer affected people can be seen whereas can cancer deaths (probably 9.5 millions) can be happened.

Lung cancer is very dangerous and found very frequent as the habit of smoking is increasing day by day. The percentage (11.6%) of lung cancer patients increasing day by day.

Other cancers are also very dangerous like breast cancer (11.6%), prostate cancer, (7.1%), colorectal cancer (6.1%) etc. (SK-2) etc.

1.23.4 Historical fact

The word cancer came from the term karkinos and it was used by Hippocrates (460-370 BC) . Karkinos is a greek word and the word cancer came from this. It was seen in the mummies of ancient Egypt the existence of human bone cancer and the ancient manuscripts also showed the fact around 1600 B.C.

There was a proof of existence of breast cancer at 1500 B.C. in Egypt. It was also known at that time that there was no proper treatment for cancer and only palliative treatment (SK-3) was available. The oldest cancer patient was reported by H. Seherg et al. (SK-3) in the year 2015. Above all there was no proper destination or line of treatment for the cancer patient. And Paul Elrich (SK-5) in the early 1900 showed the way of cancer treatment by preparing some animal models and used some chemicals to register first chemo Therapy in the Globe. He outline dthe anticancer activity of arsenic in the year 1908. He was also founder of some alkylating agents and aniline dye for the anticancer activity.

There are three major ways to treat the disease cancer and they are surgery, radiation and chemotherapy. Mixed chemotherapy i.e. composition of two anticancer drugs in the dosage form could be a great achievement for the anticancer chemotherapy.

1.23.5 Unnatural growth-

Cancer is nothing but the unnatural growth of cell within a certain area. The cancer cells can multiply abruptly without following any system. This is known as malignancy and malignant tumors are very easy to detect as they metastalize without any system. Mutation can block the cancer cells as it shows on genomic variants. Cancer cells live in the genome.

Generally cancer is found in the form of solid tumor. Liquid tumors are also found and they do not form solid tumors except in case of leukemia.

1.23.6 Different cellular reasons

There are different cells and tissues inside the body. The four different type sof cells are found generally known as a) Epithelial tissue, b) connective tissue, c) Muscle Tissue, and d) Nerve Tissue .

There are various reasons may be therefore the cells proliferation and angiogenesis can be there. There are different cell cycles to promote this kind of cellular angiogenesis and various phases of cell division may involve.

Conclusion

Targeted drug delivery is an important system for administration of drugs into the proper site of action. This is the major system containing various drug carriers to satisfy drug delivery into the targeted site. Microspheres is a major tool to satisfy the availability of the drug into the proper site of action and providing a sustained release dosage form vesicular drug delivery system is an important mode of approach for the targeted drug delivery systems. Nano particles are important candidates for the drug delivery research and nano particles with graphene oxide may be an important formulation to achieve targeted drug delivery at the proper site.

With the advancement of novel drug delivery various approaches have established their presence. Microspheres are important tool to deliver drugs at the target site in case of diabetes and cancer patients. In chronic cases regular supply of drugs is required to maintain a steady state of the drugs in the blood system.

Diabetes mellitus type-II is an important disease as it can produce so many diseases like diabetic nephropathy, diabetic retinopathy, hyperglycemia and stroke. So regular supply of medicine is required in the blood circulation to control the blood sugar level in the body.

Cancer patients also suffer from a regular problem of drug availability at the site of cancer cells as it can control the growth of cancer cells which may be a big problem if not treated earlier. In that case of typical problem of malignancy targeted drug delivery in the form of microspheres may be an effective tool for the anticancer drug delivery. Metformin a first line antidiabetic drug a first choice in case of type-II diabetes mellitus can be prepared in the form of microspheres to achieve the goal of sustained release drug delivery.

Even in case of cancer microsphere of metformin showing good results to promote apoptosis in the EAC and MDA-MB- breast cancer cell lines which is discussed in the later chapters.

Chapter -2
Literature Review

Contents

2 Literature review

Diabetes is a life threatening disease and precursor of so many diseases. Metformin is an effective drug to cure diabetes by reducing hyper glycemia in a standard dosage regimen.

But the drug metformin is counterproductive as it can produce severe side effects like lactic acidosis. This fact encourage me to prepare microsphere of metformin to release in intestine to control its G.I side effects. In relation to that I started searching literature so as to bind the drug in gum. I found no research paper using guar gum to bind metformin in a gummy matrix of guar gum. Different methods have studied so a s to prepare a microsphere of metformin using guar gum.

2.1 CHAURASIA et al:-

Guar gum microsphere by the emulsification method using glutaraldehyde as a crosslinking agent. SEM studies done for the proper surface morphological studies. The microspheres of methotrexate was having drug entrapment efficiency of 75.7%.

The invitro drug release mechanism was done by US Pharmacopoeia PADDLE TYPE I dissolution testing apparatus at Ph 7.4. different relase pattern was studied by the workers at Ph 7.4 Pbs and the datas were compared in vivo rat coecal medium.[122]

The authors concluded effective delivery of drug particle in the colon targeted delivery of methotrexate with guar gum.

The controlled relase of antihypertensive drug from the interpenetrating network polyvinyl alcohl guar gum hyrospheres.

2.2 Soppirmath K et al:-

Guar gum and acrylmide was grafted and crosslinked by glutaraldehyde in W/O emulsification method.[123]

Verapmil and nifidipine was loaded in the microspheres. The SEM pictures showed spherical microsphere with 391 to 594 µm internal diameter. The

entrapment efficiency (DEE) of the drug was found to vary from 81.1 to 90.0% . The release profile of the microsphere loaded with verapamil and nifidipine was found to vary from 80-85% .
The researchers were very much successful to encapsate the antihypertensive drugs nifidipine and verapamil in the grafted co-polymers. The initial burst release of verapamil further adjusted with continous release of the drug 3-6 hrs.

The nifidipine microspheres showed longer release with continous release profile upto 12 hrs. . the drug showed a zero –order release profile.

2.3 K. Santhi et al:-

The particle size of the beads was found to vary from 1125 to 1208 µm . The drug loading was found to vary from 58 to 68%.[124]
The release profile showed a continuous release of the drug from the microsphere up to 12 hrs. and it may go upto 90%.
The researchers successfully entrapped the drug in the cross-linked guar gum microsphere achieving proper loading and release. Crosslinked guar gum microspheres were prepared with orinidazole as the active indradient. The drug was targeted for colonic delivery.[125]
Emulsification method was followed to prepare the micropsheres. Glutaraldehyde was used as a crosslinking agent.
Researchers found good results in the in vitro drug release studies as well as in the rat coceal contents.

2.4 Kumaresh S. Soppimath et al:-

The microsphere of nifidipine was prepared with polyvinyl alcohol guar gum microspheres crosslinked with glutaraldehyde.
The SEM studies showed particle size to be 300 µm.[126] The release studies showed continuous release of the drug.

2.5 Praveen V. Kajjari et al:-

Ciprofloxacin as an active ingradient present in the microsphere. Chitosan was blended with acrylamide grafted with guar gum. Ciprofloxacin is an antibiotic drug

and having its half life is 4 hrs. The drug entrapment efficiency (DEE) was found to be 74%.
The FTIR studies of the microsphere were performed and it showed no interaction amongst the chemical components. It also confirmed the grafting reaction and chemical stability of ciprofloxacin.[127]

The XRD studies showed the existence of polymorphs to provide excellent . documentation of grafting Differential scanning calorimetry (DSC) also the confirmed the presence of drug with proper molecular shifting in the microsphere.
Dissolution profile of the drug was studied in both Ph 1.2 and 7.4 media showed good continuous in vitro release profile of the drug following Korsmeyer papers and the value of n was between 0.19 and 0.33

2.6 Sharma et al

Ph sensitive methacrylic acid(MAC) and biodegradable guar gum was grafted to prepare an effective grafted polymer. The crosslinked microspheres were prepared following W/O emulsion process. It was cross linked by glutaraldehyde.[128]
Ftir showed no interaction amongst the components of the microsphers . The release profile was diffusion type and the value of n was 0.8782.
Guar gum microsphere of the drug mebeverine hydrochloride was done and its characterization was completed.
Different drug to polymer ratio proceeded different types of micropsheres specific drug loading and relase profile.
The drug loading varied from 49% to 37.54 %.
The drug entrapment efficiency (DEE) varied from 75.5 to 85.5 5
Particle size of the drug varied from 82.53 to 81.23 µm.

The researchers found good efficiency in the crosslinked guar gum microspheres.

2.7 K.Santh et al-

Nifidipine was trapped by the micropsheres in the ionotropic technique.
Ionotropic gelatin techniques was followed to prepared guar gum microspheres.

The average particle size of the beads were ranging from 1208 to 1125 µm.
The drug loading in the microspheres were ranging from 58 to 68 %.[129]
Cumulative % of release of the drug was shown to found 91 to 100%.

2.8 Dinesh Kaushik et al

The colon targeted microspheres of 5asa(5- Amino salicylic acid) was prepared by emulsion polymerization technique 5 asa is a drug from cohn's disease, ulcerative colitis etc.
The microspheres were prepared by ionotropic gelation techniques. Guar gum was used as a polymer in castor oil solvent and microsphers were prepared by ionotropic gelation technique. Span 80 was used a a stabilizer . individual mixers prepared of 5 amino salicylic acid and guar gum was prepared in fixed proportions to prepare the microsphere.
SEM studies showed the existence of spherical particles in the microspheres the size range was varied in the particles from 128.32 µm to 162.45µm.
The drug entrapment was also good. The DEE (5) was also found to vary from 65.12% to 82.39.
The FTIR pictures showed there was no interaction between ingradients of the microsphere (Guar gum microsphere).
The XRD picture of the 5 ASA microsphere showed that there was existence of polymorphic form to ensure the presence of gum in the microspheres. The crystalline structure was also profound in the picture. The release profile of the drug was also satisfactory.[130]
The researchers found it safe and convenient to prepare microspheres of 5-ASA.

Formation and characterization of calcium chloride guar gum microspheres of theophylline:-

Guar gum microspheres were prepared of theophylline. The method of preparation of guar gum microspheres was emulsification and solvent evaporation.
The entrapment efficiency (DEE) of the coated drugs was found to vary from 60.42 to 71.46 %

Previous works on solvent evaporation techniques

2.9 Prasanth et al:-

Salbutamal sulphate was encapsulated by solvent evaporation method. The drug has a half life of 4-6 hrs. The polymer used in the formulation were HPMC and carbopol.[131]

The particle size prepared microspheres were ranging from 110 to 224 µm.

The drug entrapment efficiency (DEE) of the prepared microspheres was varying from 71.7 to 79.7%

The drug release was found to follow a zero order rate for the optimized formulation. The release showed a continuous rate over 12 hrs.

2.10 Asif et al:-

The active ingradient contained in the microsphere was losartan. The elimination half life of the drug losartan was found to be 1.5-2 hrs. So it should be used to prepare a microsphere formulation to extend its release profile. Ethyle cellulose was used as polymer and methanol as a solvent

The dispersion medium was made of light liquid paraffin having 0.1 % span 80 as a stabilizer. Microspheres were prepared by solvent evaporation method.

The SEM studies showed spherical microspheres particle size ranging from 18.04 to 86.83 on an average. [132]

The drug entrapment efficiency (DEE) was found to be 73.15 to 91.32 %

It showed continuous release profile of the drug for 12 hrs. It followed zero order kinetics for most of the formulations. It showed continuous release profile of the drug for 12 hrs.

2.11 Patel et al:-

Emulsion solvent evaporation technique was followed to prepared metformin loaded microsphere. The polymer used in this work was celluslose. Different formulations were prepared by different drug polymer ratio.[133]

SEM studies of prepared microspheres showed spherical particles of little bit rough surface microspheres.

The drug encapsulation efficiency of the prepared microspheres (DEE%) was found to vary from 95.00 to 97.31%.

The XRD studies of the drug particle showed clean pictures of sharp peaks in the pure drug amorphous nature of peaks were found with microspheres having ethyl cellulose as a polymer.
The polymeric micropsphere of the drug showed a complex nature of peaks with sharp peaks of metformin and existence of polymorphs as the drug dispersed into the microspheres.
The release studies show continuous release profile upto 12 hrs
The researchers found it safe and efficient to prepare microspheres with loaded drug metformin.

2.12 Garud et al:-

Drug loaded microspheres were prepared by non aquous solvent evaporation method. The drug loaded microspheres metformin was prepared by using various polymers such as ethyl cellulose hydroxyl propyl methyl cellulos(HPMC), carbopol 934P, and chitosan.[134]

The SEM studies of the microspheres showed spherical structure of the microsphere with rough surface.
The drug entrapment (DEE) was found to vary from 61% to 85% approximately.
The average particle size of the microsphere was found to vary from 352 to 458 µm.
The drug release profile showed a continuous release upto 12 hrs. It was seen that with the increased stirring speed the rate of release of the drug was also increased.
The researchers found it successful to encapsulate the drug metformin in various polymers and to study the profile of the microsphere made.

Solvent evaporation technique-
Preparation of floating microspheres of metfomin –

2.13 Mishra et al:-

The microspheres of metformin was prepared by emulsion solvent evaporation technique.
Ethyl cellulose, HPMC etc. were used as polymer system for the microspheres .
The polymer first dipped into acetone and then slowly transferred into 200 ml of liquid paraffin. [135]

Continuous stirring of the preparation was made for the complete evaporation of the solvent.
SEM studies showed spherical particles with smooth and rough surface.

The release profile of the microspheres showed a continuous release of the drug up to 12 hrs.
The researchers felt successful in preparing floating microspheres of metformin hydrochloride.

Studies on floating microspheres of metformin HCl-

2.14 Dubey et al

The floating microspheres were prepared by using HPMC and Eudragit RS100 polymers.
The microspheres showed a zero-order release profile.
The microspheres were prepared by non-aquous solvent evaporation method.[136]

A mixture of polymers like Eudragit rs 100 and HPMC was used in varying concentrations to prepare different batches of the microspheres SEM studies showed different spherical particles in the pictures.

The in- vitro release studies was conducted in USP XXIII basket type dissolution apparatus. Both Ph 1.2 and 6.8 buffers were used for the dissolution studies of the microspheres. The dissolution studies showed continuous release profile up to 12 hrs resulting zero-order release.

The researchers finally prepared the micro-adhesive microspheres of floating metformin.

Floating microspheres of metformin
Microspheres of different antidiabetic drug
Microspheres prepared from different gums

2.15 Ratan Parkhi et al:-

Floating microspheres of the drug metformin HCl was prepared by using different polymers like ethyl cellulose, HPMC etc. non- aquous solvent diffusion method was followed to prepare the microspheres
Drug and polymers were used in different ratios to prepare different formulations.[137]
The drug entrapment efficiency(DEE%) of the drug varied from 78.2%-99% approximately.
The comparative study of metformin microspheres with the pure metformin and other polymers were done. It was found that there was no inter-relation between the polymers with the pure drug in the FTIR analysis
The SEM studies showed existence of spherical particle sand the size ranges varied from 470-511µm.
The release profile of the prepared microsphere show continuous release upto 12 hrs. in a controlled release manner.

2.16 Deb J et al

The microspheres were prepared by emulsion solvent evaporation technique. Carbopol was used as a polymeric carrier. Carbopol polymer use as a carrier of the drug metformin were as HPMC was also used as a polymer in the formulation.
Carbopol polymeric solution of the distilled water was charged with the drug metformin HCl and uniform mixing was done. The microsphere was prepared following multiple emulsion solvent evaporation technique. The collected microspheres were used for different characterization procedures.

The scanning electron microscopic pictures (SEM) showed spherical particles of rough surface and irregular shape. The particle size of the microspheres vary from 289.9 to 435.1µm.[138]

The animal study of the prepared microspheres also supported the efficiency of metformin loaded microspheres.

Previous works on microspheres prepapred from antidiabetic drugs

2.17 Kohli et al:-

Repaglinide microspheres were prepared by solvent diffusion evaporation technique. The emulsifier used in the formulation was poly vinyl alcohol (PVA) .

Repaglinide and ethy cellulose mixing was done with certain ratios. Poly ethylene glycol (PEG) surfactant modified the system. Stirring was done in propeller agitator for the solvent evaporation.
Microsphers were prepared, washed and collected for further use.

The FTIR studies of prepared microspheres were done.[139]
When there was a comparative study between the FTIR structure of the pure drug ethyl cellulose and microspheres of the antidiiabetic drug clear distinctions were found.

Sharp peaks of the drug Repaglinide showed due to its organic crystalline structure inside the molecule.

Ethyl cellulose showed its peaks of the polymer.

In the formulation studies of FTIR there was no interaction between the drug and polymer showing peaks in different wavelengths.

The mean particle size of the prepared microspheres were found to vary from 187.12 to 243.41µm.
The SEM studies of the prepared microspheres showed spherical structures of un-uniform appearance.
Various histopathological studies were carried out to find its applicability.

The researchers were successful in preparing microspheres of repaglinide and its characterization.

2.18 M.Santosh Kumar et al:-

Gliclazide is known drug for diabetes. This works on type-II non- insulin dependent diabetic mellitus.

Microspheres were prepared with sodium alginate and gum konda gogu. The microspheres were prepared by orifice-ionic gelation method and emulsion gelation technique.

Gliclazide microspheres were prepared by mixing the drug in a solution of sodium alginate and the gum konda Gogu. The solution then dipped into the soln of calcium chloride to prepared microspheres. [140]

The emulsion gelation method was also followed to prepare the microsphere. Drug sodium alginate and the gum Konda Gogu was mixed in different ratios to prepare the microspheres. Span-80 was also used as a stabilizer.

Particle size analysis by SEM studies were done. FTIR and XRD pictures were taken to confirm the homogeneity of the microspheres.

2.19 Nayak et al:-

Drug microspheres preparations were done by inotropic gelation method. Sodium alginate and isapagula was used as natural polymers in the preparation . The drug and polymer was mixed in different concentrations . Various formulation were prepared by changing the ratios of drug and polymer.

The drug entrapment efficiency(DEE) of the prepared microsphere were founary from 50.5% to 78.9% .[141]

Where as average particle size of the prepared microsphere were found to vary from 33.4µm to 1249.8µm. The sem picture microsphere confirmed its spherical shape. With uneven outer surface. In vitro release pattern of the drug show continuous release of the drug I the controlled release manner of the 12 hours.

Solvent Evaporation technique

The poor drug in the matrix was salbutamol sulphate (SS). The drug was loaded in the microsphere with the help of two polymers mainly hydroxyl propyl methyl cellulose (HPMC) and can be polymers.

Same studies of the prepared microsphere revealed spherical particles with rough surface. The particle size ranges between uo± 0.02 and 183±0.02µm .

The drug entrapment (DEE) was calculated for different batches of the microspheres and it was varying roughly from 68% to 74%.

2.20 N.Yuksel Dt Baykara et al:-

The authors prepared polymeric microspheres with the principle drug chosen as nicardipine hydrodileride. This particle drug was being prepared into the microspheres following solvent evaporation technique. The polymer used in this method was Eudragit RS and Sucrose stearate was used in varying concentrations to prepare the microsphere and sucrose steanate was added in the formulation as a droplet stabilizer . It was found that with the increase in concentration in sucrose stearate the porosity in the surface of the microsphere was increased.[142]

2.21 Vandana Singh et al:-

Ranitidine hydrochloride was prepared in the microsphere form as a drug particle. The microspheres were prepared by modified solvent evaporation technique. Eudragit was used as a polymer to prepare the microsphere . Eudragit Ripo was used a polymer for the preparation.

SEM studies revealed that the mean particle size of the spherical microspheres varied between 247± 13µm to 286±16µm.[143]

In vitro release studies of the drug showed continuous release of the drug in a controlled sustained manner to provide an uniform dosage form.

2.22 Jithan Aukunuru et al:-

Metformin hydrochloride microspheres were prepared by solvent evaporation method. A W1/O/W2 multiple emulsion process was followed to encapsulate the microsphere. The particle size distribution of the prepared microspheres was found to be lying in the rang of 52.33±to 124-56±3.78.

The drug entrapment efficiency was also upto 72.5 ±0.56 incase of metformin HCL as per the results and the lower limit was 13.2±0.56. So the DEE values was in the range between 72.5±0.35 to 13.2±0.56.

The authors prepared glipizide microsphere which were having entrapment efficiency 6.12±0.51 to 21.12±0.37.

2.23 Naveen chella et al:-

Drug loaded ethyl cellulose microspheres were prepared and ethyl cellulose was chosen as a polymer for the microsphere preparation. The microspheres were prepared following W/O/O technique.

The solvents followed for the preparation of the microsphere was DCM/ethanol system. The formulation was enriched by the introduction of span -80 as a surface stabilizer where as n-hexane was an added agent for the formulation as to solidify the microsphere .

The entrapment efficiency of the prepared microsphere was found to be I the range of 28.6±1.8(%) to 51.2±0.53(%).[144]

The release profile of the prepared microspheres was found to follow controlled release mechanism releasing the drug in na controlled manner upto 12 hrs.

Study about Gums and guar gum

2.24 Dinesh Kaushik et al:-

A colon targeted microsphere having 5-aminosalicylic acid as the active ingredient was formulated. The microspheres were prepared following emulsion polymerization technique. The drug is used in colon disease ulteractive colitis although it is basically an anti

The entrapment efficiency of guar gum loaded microspheres were found to be in the range of 65.12±0.85 to 83.38 ±4.89

The SEM studies of the prepared microspheres showed spherical particles. The particles were found to show smooth surface.

XRD pictures of the prepared microsphere showed no interaction between ingredients.

The release study of the prepared microspheres showed continuous release through 12 hours and a sharp controlled release pattern of the drug release.

The mean particle size of the prepared microspheres 150.32±11.8µm.[145]

2.25 Somdas Roy et al:

The Oral antidiabetic drug glipizide was prepared in the form of microspheres.

This is used as an antidote for type-II diabetics mellitus and is proposed for non-insulin dependend diabetis mellitus. The microspheres were prepared by emulsion cross linking method. In this preparation polyvinyl alcohl and glutaraldehyde was added as coating polymer and cross linking agent.

The SEM studies reveal spherical particles with more or less smooth outer surface indicating homogeneity of the microspheres.[146]

The average particle size of the prepared microspheres vary from 962.10µm to 1000.92 µm.

The drug encapsulation efficiency of the prepared microspheres were found vary between 84.64 to 94.40%. the drug dissolution studies found to follow continous release pattern through 12 hrs and controlled release manner.

2.26 Literature Survey on Diabetes.

Diabetes is a deadly disease whch is mainly a metabolic disorder medicinally known as hyperglycemia , hyperlipemia, negative nitrogen balance,glycosuria etc.

It may be classified in two types- 1. Insulin dependent diabetes mellitus. (IDDM) or type -1 and Non – Insulin dependent diabetics mellitus NIDDM or type-2

In case of Insuling dependent diabeties mellitus may be found as a result of an auto immune disorder so that the B- cells of islets of langerhance got destroyed and so it cannot produce insulin at all/ or in a very small amount in the body. As a result of that the patient suffers fro, diabetes mellitus from his early childhood days and should be treated with insulin injections as there is no other alternative.This kind of childhood victims of the deadly disease of diabetes mellitus are very unfortunate people as they have to survive with the insulin injection as a basic hypoglycaemic agent.

But incase of non insulin dependent or type-1 diabetes mellitus the patient does not suffer from childhood days. This is due to assign problem when the pancreas itself produce lwer amount of Insulin which may be insufficient for the conversion of glucose into glycogen. This is a big unfortunate as there may be damage in the B –cells o itself of Langenhance so that it can be produce sufficient amount of Insulin. With the age of the person it becomes acute as various complication start with the ageing factor.

To control this problem one can follow physical excercise and diet control as the preliminary remedy of the disease on the other hand some other faults also to be considered such as control in taking carbohydrate containing diet and also to take drugs and medicines that are applicable in type-2 diabetes mellitus.

Insulin is having a major role in the ocurrance of diabetes mellitus. Glucose is converted to glucose in liver and muscles due to the help of the enzyme glucose – 6- phosphate which is potentiated by glucokinase activity is stimulated by insulin. Insulin also promotes the action of glycogen synthatase so that the transformation from glucose to glycogen takes place in the muscles and liver

Insulin helps to reduce neoglucogenesis in liver so that production of glucose in liver is reduced in the absence of insulin, protein-amino acid-glucose transformation takes place in liver. Insulin inhibit this syntheses of glucose from proteins by lowering the syntheses of the enzyme phosphoenol pyruvate carboxykinese

Insulin having its receptor in cells which are tyrosine kineses.G –protein coupled insulin receptor are responsible for non-complience of insulin in the cellular receptor side so that insulin becomes inactive in the body and so on which may lead to inactivity of insulin in the cell surface leading to increased concentration of blood glucose in the body that may be a probable cause of type-II diabetes mellitus.

Insulin is a hormone secreted by Beta cells in pancreas and this particular fact was observed and reported by two eminent scienticist Banting and Best in 1921.

As it is a protein so it is having polypeptide changes in its structure. After proper structural illucidation it was found that the insulin is a protein having to amino acid chain connected by two disulfide bonds.

There are so many insulin preparation are available in the market. They can be used in diabetes mellitus.

For type-II diabetes mellitus different medications are available

Classification of Anti-diabetic Drug

A. Oral hypoglycaemic drug;

1. Sulfonyleneas (k_{ATP} Channel Blockers)

First generation- Tolbutamide

Second generation-Glibenclamide, Glipizide,Glicazide,Glimepride

2. Meglitidine /Phenyl alanin analogues, Repaglinide, Nateglinide
3. Glucagon like peptide receptor-I(GLP-I) agonist liraglutide.
4. Dipeptidine peptidase -4(DPP-4), inhibitors –sitagliptin, bildagliptin, saxagliptin, alogliptin

B. Overcome insulin resistance

1. Biguanides(AMP activator) metformin
2. Thiazoldindiones (PPARY activator), pioglitazone

C. Miscellaneous anti diabetic drug

1. A glucosidase inhibitors acrabose miglitol, voglibose
2. Amylin analogues pramlinitide
3. Dopamine D_2 receptor agonist bromocriptine
4. Sodium glucose co transport -2 (SGLT-2) inhibitors Dapagliflozine

The sulfolylurea are useful type-II diabetes mellitus and the second the generation sulfonylurea are available only at the present days. They act by blocking the k+ ATP channels and thus insulin is released from b-cells of pancreas as aside effect it can produce hypoglycemia and sometimes some hypersensitive reactios like rashes , pupurea transient leucopenia etc. the sus can produce hypoglycemia.

The D-Phenylalanine mainly analogous mainly pepaglinide and nataglinide are available in the market. They also act by blocking k+ATP channels and thus promotes insulin release from the pancreas. The insulin release is promt and sustaining.

The D-PP 4 inhabitants like sitagliptine and lildogliptine act by promoting the release of GLP-1 enzyme from the gut and they produce insulin release. Sitagliptine is relative blocker of peptidyl tranferase -4 (DPP-4) inhibit and thus potentiates the action of insulin. Vildagliptin binds with the enzyme DPP-4 forming covalent bond formation.

Amongst the biguanides (pHenformin & metformin) . metformin is accepted as a first line of therapy against DM Tye-2, it works on suppressing hepaptic neoglucgenesis and reducing glucose output from liver. It also potentiates liberation of glycogen in muscle . The action of metformin is stayed dependent on AMPK i.e. AMP Dependent Protien Kinase. It also helps in peripheral glucose utilization. So as to provide control in one blood glucose concentration. Both the biguanide class of antidiabetic drugs i.e Phenformin and metformin were introduced in 1950s of which phenformin was banned at 2003 due to reported

severe lactic acidosis side effects are drizziness, nausea, metallic taste abdominal pain etc. lactic acidosis is not that much severe.

Diabetis mellitus is a common disease now a days which is basically a number of syndrome like hyperglycemia, lipid metabolism affects , diffects in carbohydrate and protein metabolism. 147

As diabetis mellitus is a disease in which there should be a continuous presence of the drug in the blood circulation to lower the concentration of glucose 148

Now a days the tendency of preparing controlled release sustained release preparation is increasing to provide sufficient amount of drug in the blood stream to maintain a continuous concentration of drugs in the blood stream. So various methods and preparations are followed to provide the effective therapeutic concentration and for a longer time.149

Microspheres are available now a days to accomplish the goal by providing control release of the active ingredient for longer time. They produce control of delay release of the medicament and alters the surface properties of the particle and thus an unique one. 150

The drug is embedded in the matrix in the microsphere formulation and it is released in the controlled manner in the intestine so that can be flown regularly to the systematic circulation. The release of the drug will be in control manner with continuous release pattern to provide the effective therapic concentration for a longer time.151

There are so many antidiabetic drugs are available in the market. Most of them are being worked on to prepare in the form of microsphere. A big number of preparations are being reported in the form of microspheres.

An effective antidiabetic drug like glipizide is available in the market to treat type-2 diabetic mellitus.

The biological half life of the drug is 3.4 ± 0.7 hrs. And so 2-3 dozes of the drug is required to maintain the therapeutic concentration. The general effective doses of the drug is 2.5 -10 mg per day.152

In this context glipizide microspheres are reported as a novel antidiabetic formulation of the prolonged ques.

153-161

The thiazolidinediones are another group of antidiabetic drugs. Pioglitazone is available now a days in the market where as Rosglitazones is banned in india as it is having greater risk in cardiovascular disease with increase in myocardial infection. Heart attack etc.

They are selective against of nuclear peroxisome proliferator activated receptor(PPARY). Which is expressed mainly in fat cell. They enhance the blood GLUT-4 expression and translocation. And glucose entry in muscle and fat is accelerated. Pyoglitazone reduce both the level of glucose in blood and level.[162-189]

The type-1 i.e. insulin dependent diabetis mellius is totally controlled by insulin hormone secreted by beta cell islets of langarhanse.

Insulin is a big polypeptide and is contains two polypeptide chains. It contains 51 amino acids in its chemical structures which comprise of A chain of 21 amino acids and B chain containing 30 amino acids both the chains are connected by disulfide bonds. Secretion of insulin from the pancreas is a continuous process and it is influenced by several factors (1) chemical (2) hormonal, (3) neural.

Chemical factors include release of insulin from the B-cells of islets of langherhance which is prompted by entry of glucose. Thus activation of channels induce inhibition of ATP sensitive k+ channels and depolarizes the B-cells partially. Thus intracellular Ca^{++} availability is increased and insulin released from the expocytic cells.

Some hormones like growth hormones and corticosteroids also influence insulin release. Thyroxin also plays an important role to release insulin from the pancreas. Prostaglandens, somatostatin and glycogen also play major role in controlling insulin release.

Neutral control of insulin release due to cholinergic and adrenergic stimulation found to occur.

1. There are so many functions which are done by insulin in cells it helps in entry of glucose in the cells.

There are so many chemicals /factors whictransport glucose into the cells such as GLUT-4 and GLUT-1. Glucose transporter glycoprotein which help in transportation of glucose molecules in the cells. This GLUT-4 & GLUT-1 functions is mostly controlled by insulin.

2. Insulin is the key factor of hormone in glycogenesis .i.e. transformation of glucose into glycogen. In this process of transformation from glucose to glycogen insulin potentiate the action of so many enzymes. The formation of Glucose 6-Phosphate to glucose is promoted by production of glucokinensis enzyme wih the help of insulin. It also helps glycogenesis in liver, muscle and fat by promoting

the action of glygen synthase are also inhibit phosphorylase to block glycogenolysis in liver.

3. One of the major fuctions of insulin is it controls neoglycogenesisi in liver.with increased concentration of insulin it helps in lower production of phosphoenol pyruvate carboxykinase. Thus with increased concentration of insulin it can resist neoglycogenesis i.e. production of glucose from amino acids/lipids.
4. In diabetes patients generally lipid profile is also high and ketonuria i.e. ketone bodies in urine results. As in insulin deficiency lipid molecules are broken down into PFA and trigly. The FTA –ACETSLCOA- KETONEMIA-METONEMIA.
5. Very low density lipoprotiens (VLOL) concentration is minimized by the actn of insulin. It promotes the action of vascular endothelial lipoprotein lipase and thus VLOL clear is increased from the body.
6. Insulin helps in the transformation of blocks breakdown of protein. In insulin deficiency rapid breakdown of proteins take place.

i. Mechanism of action of Insulin:-

The action of insulin depends mainly of its receptor binding activity in the cell membrane. Insulin binds with tyrosinekinase receptor (receptor tyrosine kinase RTK) in the cell membrane.

The receptor tyrosinekinase contain two subjects of each. The -2 α with insulin.

The β-sulrunits are active for its tyrosine protein kinase activity.

α- submits bind the insulin hormone which promotes the β-submits for phosphorylation and it phosphorylates molecules to expose its catalytic to LRS1 AND LRS2 i.e. Insulin receptor substrate proteins.

Insulin promotes glycose passage through the cell membrane by ATP dependent translocation of GLUT-4. The genes producing GLUT-4 one over expressed to produce more glut-4 by the action of Insulin.

When given orally insulin is destroyed in the G.I.T. That is the major drawback in the insulin therapy so that it should be taken in the injectables only. Insulin is mainly metabolizes in the liver.

There are various types of insulin preparation available in the market. Regular insulin soluble insulin and this insulin preparation is stabilize by zinc.

In this preparation the insulin molecules form hexamers with zinc ions so that after S.c. injection insulin is obtained from the preparation. The pharmacological action of the medicine stays upto 8 hrs whereas it starts its action after 2-3 hrs of administration.

The injection is given to the patient generally 0.5-1hrs before a meal.

The main drawback of this type of preparation is mainly its slow onset of action is a problem for the patient as the insulin is released from the hexagonal attachment which takes time. Regular injection intake is also a big problem as it is painful. To overcome this problem long acting insulin preparations are developed.

ii. LENETE INSULINE (Insulin Zinc Suspension)

Generally it is found to provide a mixture of two types of insulin preparations the suspension of insulin zinc with larger and smaller particle of which the larger particles are long acting and the smaller particles are short acting. The mixture in 7:3 ratio is intermediate acting and is known as lente insulin.

iii. Isophane (neutral protamine Hagedom or NPH) Insulin:

Protamine insulin preparation are available with intermediate duration of action. Human insulin are recent modifications of insulin injection to be taken by subcutaneous route. Recombinant DNA is proinsulin recombinant bacterial is the technique to produce the insulin having same amino acid sequence as that of human insulin. In the current times generally porcine and bovine insulin is not found in US market but some European countries and some asian countries including India produce porchine or bovine insulin. Although most of the physician now a days prefer human insulin as an antidote to diabetes mellitus. Human insulin are having faster subcutaneous absorption rate.

There are so many insulin analogues produced by changing the position of amino acids in their chemical structures. Amino acids of the carboxy terminal at the B28 and R29 is positioned reversed to produced insulin lispro an analogue of insulin.

This is a modified preparation. This insulin lispro in which containing great contrast over the glycemic index at the lunch or dinner post pandinal hypoglycaemic is also very less, HbA_{1C} control is also achieved by the preparation useing 2-3 times a day of the insulin lispro injections.

There may a change can be made by replacing the B28 position of human insulin i.e. proline is replaced by aspartic acid. The preparation known as insulin aspertate having same pharmacological action like insulin lispro.

Insulin glangine is another one insulin analogue with longer action. Here is an addition of 2 arginine residue at the b chain in their carboxy terminal and in the A chain also there glycine is exchange by asperagine at A21. This preparation can control glycemic index at meal time but effective in lowering hyperglycemia at bed time. It is a larger action insulin so once daily dosage regimen can be sufficient for its action. It is a depart forming preparation from which insulin is released slowing in the blood stream.

This was until 1921 when two scientist benting and Best first demanded that the extract of beef pancreas can lower the blood sugar level pancreatectomized dogs.

The effective treatment through insulin made a great relief to the diabetic patients and the clinicians were satisfied with the treatment with insulin.

The secretion of insulin from B cells if islets of langerhance depends on the presence of the other hormone. The existence of glucagon is also a major factor for the secretion of insulin.

Although pancreasproduce both the hormone insulin and glucagon both act in conperative manner. Glucagon produce and increase in that secretion of insulin from the b cells islets of langerhance. On the other hand insulin opproses the secretion of the glucagon from the lpha cells of the pancreas. There is another one hormone mane as somatostatin which is secreted from the b cells of the pancreas produce a negative effect on the secretion of insulin and glucagon from the pancreas.

Somatostatins along with insulin is effective in type -1 i.e. insulin dependent diabetes mellitus. Feling et al proposed that hyperglycemia induced by somatostatin is only due to the fact that it opperess carbohydrate absorption.

In the year of 1889 two eminent scientists named as mering and minkowski found that there is a relationship between insulin release and pancreas. They got the impression from the lab animals devoid of pancreas.

Later in 1921 two scientists Benting and Best demanded that the extract of beef pancreas can lower the blood sugar level of pancreatactomized dogs.

The insulin preparation was found to be in the crystalline form b Abel in the year of 1926.

The structure and chemistry of the insulin molecule was described by Sanger and co-workers in 1950.

The chemical mstructure of insulin molecule showed two polypeptide chains. A chains and B chains. The A chains was having 21 amino acids while B chains was having 30 amino acids. In between the two chains two disulfide bridges were present. The insuli n obtained from pork and cow are similar to human insulin. The insulin from the pancreas of pork and that from the human pancreas differ only the fact that it ts heaving of c terminals alanin in the b chain whereas the human insulin contains a C terminal threonine. They are very similar in chemical structure and so the porcine insulin can be converted to human insulin and can be used for human diabetic patient. The bovine insulin and the human insulin are of almost similar in shape and chemical stgructure.

There is a difference 8^{th} and 10^{th} position of the chemical structure. Alanine is there in place of threonine whereas valine is there in place of isoleucine in beef insulin.

The x-ray crystallographic studies show the chemical structure of insulin is a small globular protein. It can be crystallized at neutral ph also.

Insulin although found to be stored in the form of Hexamer in the granules but its active form is dimer. Insulin was chemically synthesised also. The fact of synthesis of insulin was done by 3 groups of eminent scientists in thne year 1960. They tried to synthesised the chemical structure of insulin by synthesising the A chain and b chain separately. It was a laborious workand very time taking also. The process involved more than 200 steps and several years required.

But solid state synthesis discovered by morifield and margline is not that much time taking and the product can be obtained within some days.

The problem with the synthesis of insulin in laboratory was due to it two disulfide bond so it was problem regarding the positioning of the disulfide bond in the 3 divisional network of the molecule although it can be solved by reversible cross linking of the disulfide bonds.

Again some scientist first formulated the disulfide bond between the a and b chain. Then the other parts were formed and the fragments of the a and b chain properly formed and inserted into it.

Human insulin produce in this technique is identical to the natural human insulin.

Although it was a good technique of synthesising insulin chemically in the laboratory but the biochemically engineer insulin produced by recombinant DNA

technology was most favoured for the production of the insulin in the day to day basis of the common people.

Gene mediated synthesis of insulin was started with Ulrich and co worked in 1970 by grafting the DNAs from the rat two bacterial plasmid.

Dr Alibert and co- workers also followed the same principle of DNA grafting and synthesis proinsulin from bacterial plasmid.

The fundamental basis of genetic technology produce huge momentum in the recombinant DNA technology to produce bio engineered insulin i.e human insuslin.

The activity of insulin mostly depend on on its attachment with the receptor. There are so many receptor binding region in the age Chain and B chain of the insulin. So one can try to produce some developments in the receptor binding site so that more absorption can occur.

Insulin basically comes in the form of Pro insulin fast to prepare insulin on the ribosome of Endoplasmic reticulum. The human insulin gene is mapped in in the chromosome [21]

On the other hand it can be found that this Pro insulin may be originated from preproinsulin which is a big protein in the Golgi apparatus the proinsulin is found to be insulin with the addition of secretory granules. Inside the cytosol the insulin molecules get enriched.

It is important to know that carbohydrate lipids and protein metabolism is controlled by insulin. In fact insulin plays a major role in controlling Diabetes mellitus in human. It is a fat studied by so many of workers that insulin helps in absorption of glucose by changing the membrane potential. The action of insulin is quite Complex inside the body. Some studies revealed that glucagon can increase the concentration of cyclic a m p inside the cell. From this finding it can be clearly assume that insulin will decrease the cyclic a m p level inside the cell. This is so thought because insulin is having the opposite action of glucagon.

The receptor of insulin is very special and specific. It has been seen that these receptor are selective only for the specific hormone that is insulin.

The receptor binding of insulin is not a simple thing. It requires a number of different factors. The concentration of insulin preparation is an important factor for

its absorbence. If there is a good level of Binding in the site there may be a decrease in the absorbence of insulin. The degradation of insulin only possible if it is properly bound in the site.

Insulin effect are very specific and distinct on the carbohydrate protein and fat metabolism. For the proper metabolism of insulin are involved is that of liver. The effect of insulin are destroyed by the action of glucagon and epinephrine.

The most fundamental and important function of glucose may be its ability to produce glycogen from glucose. Glucose is transported from the blood into the tissues. Absorption of glucose in the liver is followed by facilitated diffusion mechanism. At the same time as we all know that the main source of energy for brain is glucose. The availability of glucose in brain also does not require the presence of insulin.

Metabolism of glucose present in the cell can be found in many ways. It can be found to be oxidized by the emp pathway and that the kreb cycle produce energy. On the other hand it can enter into the hexose monophosphate shunt to get the reduced cofactors so that the biochemical reactions can be carried on otherwise the fate of glucose to be stored in muscles as glycogen fore refuse afterwards by glycogenolysis.

Insulin plays the key role in information of glycogen by the process of glycogen in muscles And Labour while it also blocked glycogenoanalysis. The obvious effect of insulin is counter act glycogenolysis formation of glucose producing A reduction in the hepatic glucose

One of the major important factors regulating secretion of insulin is the presence of some amino acids as arginine and leucine. Different fatty acid the parasympathetic nerves nervous system and incretins.Incretins are the major factors such as glp 1 and glp. Among the drug sulphonyl urea class of the drug release insulin.

Metformin is a drug originated from the biguanide group of drug found from the French plant Galgeaofficinalis.

The main physiological function of the drug metformin can be classified

Lower glucose production by gluconeogenesis

In skeletal muscles they increased the glucose uptake and its usage.

Lowest carbohydrate absorption

Fatty acid oxidation is also increased.

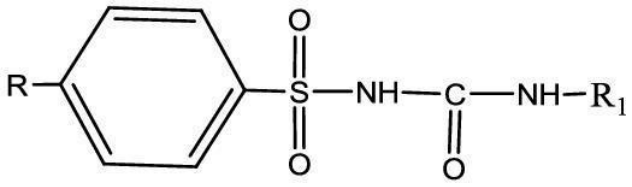
It is having a major beneficial it lowers the concentration of the LDL and vldl in the blood circulation.[190-212]

Insulin helps in lipogenesis that is it helps in production of fatty acid and triglycerides from carbohydrate precursors. Insulin also helps in maintaining the concentration of the lipid by blocking its breakdown. Thus Insulin plays a major role in maintaining the concentration of free fatty acids in the plasma and energy storage is also maintained. Insulin helps in controlling the concentration of ketone bodies in in the plasma. In severe hypoglycemia Ketone bodies concentration is increased in the plasma. Diabetic acidosis is a major factor which may be found in case of diabetic ketoacidosis.

There are so many drugs and pharmaceuticals available in the market to control this hypoglycemia.

iv. Sulphonyl urea

Sulphonyl urea are very effective in controlling diabetes there may be a large number of sulphonyl urea is available to control the disease.



R,R_1 – Alkyl Groups

Generally they work by decreasing blood glucose concentration of the patients. They are effective against type 2 diabetes Whereas they show no effect against type 1 diabetic patient. Now a days first generation sulphonyl ureas are not in use only the second-generation sulphonyl ureas are used.

The sulphonyl urea are known to help in releasing insulin from the pancreas. It follows the mechanism depicted in figure 19.6 the drug release insulin at any concentration of glucose and can produce severe hypoglycemia.

It is found that which group of drug are very finely absorbed through the oral route and more than 90% of it was bound to plasma protein.

There are so many adverse effect found from the drug. Hypoglycemia may be found in majority of cases. The patient starts gaining weight after the use of these sulphonyl urea group of drug. There may be existence of rashes and purpurea,phyto sensitivity etc found.

Repaglinide is a drug used in the oral route and it is mainly used to control mil time glucose outburst. Although it is not a drug from the group of sulphonyl urea but they bind to the sulphonyl urea receptor to produce their hypoglycemic action. After being attached to the base you are sulphonyl urea receptor that can produce closure of a atp-dependent channels or more precisely the channels which are atp-dependent k Channel are depolarised producing a positive trust for the insulin release.

This particular drug Repaglinide is very quickly absorbed in the bloodstream to produce immediate lowering of glucose level. This is the reason of its use as an meal time antidiabetic. This drug is having a short half life inside the body and so it can produce severe hypoglycemia. This drug is very selective drug for post padinal hyperglycemia. In cases of patients with liver disease this drug should be avoided.

It can produce more liver hypoglycemia then Repaglinide. Generally used for meal time hyperglycemia and should be taken 10 minutes before the meal. It does not work on fasting blood glucose levels. Generally hypoglycemia is not a very common occurrence like sulphonyl urea. It produces some unwanted side effect also those maybe nausea.

v. Dpp 4 inhibitors

When there is Glucose level in blood is increased glp 1 always potentiates the release of insulin from Beta cells of pancreas and help in a lot in antidiabetic

mechanism. On the other hand It blocked glucagon release from the pancreatic beta cell.

The enzyme dpp4 or dipeptidyl peptidase4 generally destroys the glp 1 which is a basic problem for the diabetic. The drug in the class of dpp 4 inhibitors that play a major role in controlling diabetes. There are three main members of this group of drug of dpp 4 inhibitors. They may be sitagliptinevildagluptine, saxagliptine

Sitagliptin is the most ancient of all the dpp 4 inhibitors and is very much effective in the controlling diabetes. This drug is a having an unique action in inhibiting DPP4 enzyme. This is basically a competitive and selective DPP4 blocker. That's why this action of blocking the DPP4 enzyme GLPI activity is increased and ultimately insulin production is increased.

It can decrease the sugar level of the diabetic patient and that is almost comparable with metformin. The drug can be used in combination with other sulphonyl urea. Sitagliptin metformin is very finely administered in the anti diabetic medications.

When it is administered in the oral route it is observed the very well in the guard and it is also metabolized in the liver in a very minute amount and also is excreted through the urine. It is generally unchanged and it is half life is almost 12 hours

The dose of sitagliptin maybe reduced in case of renal impairment although in case of hepatic disorder this dose reduction is not required. This drug sitagliptin is very well tolerated in the body. The side effect of the drug are nothing but nausea loose stools headache allergic reaction edema etc.

The second drug in its group of dpp 4 inhibitor is known as vildagliptin. This drug work by making covalent bond with the enzyme DPP4. It is a short acting drug which is having a very short life to 24 hours inside the body.

The dose of the drug may be one tablet per day or twice tablet per day as per the requirement.

Another one drug is this group is saxagliptin. Which also worked in the same mechanism of action that of vildagliptin. The drug basically works in the mechanism by making a covalent bond with the enzyme dpp4. The effects of the medicine passes for 24 hours and it is having a plasma half life of 24 hours.

The metabolism of the drug is by cytochrome P450 3A4/5 (CYP3A4/5) and it can produce an metabolite which is having a half life of 3.27 hours.

Saxagliptin is having a general dosage schedule of 5 mg.

There are biguanide class of drug like metformin and phenformin.

Those were introduced in 1950

Lactic acidosis is a common side effect of biguanides. The drug phenformin was banned due to this effect. All the metformin does not produce lactic acidosis in a normal produres of day to day life of the diabetic patients.

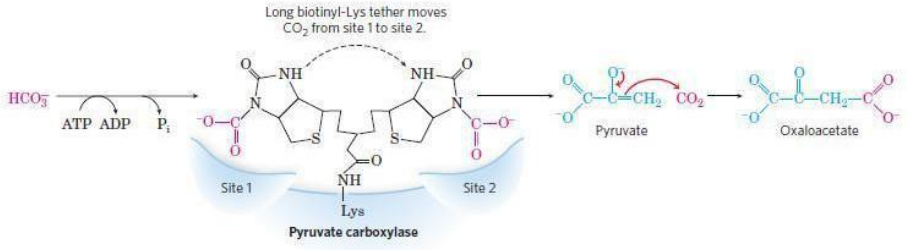
It is generally found to produce no hypoglycemia to the patient who are not suffering from diabetes. This is the peculiarity of the medicine that it can rebate the patient of diabetes only for its hypoglycemic action.

Another one important Criterion of the action of metformin is that it does not stimulate the pancreas to release insulin as it acts in the different biochemical pathway it can also improve the lipids profile in type 2 diabetic.

This is also a matter of great concern that although metformin does not induce insulin release from the pancreas presence of insulin is very much required for its action. Metformin can not work on animals those do not contain pancreas revealed by a study report of animal survey.

The mechanism of action of metformin can be summarise like this

The drug metformin is having any unique mechanism of action of blocking the hepatic glucose in Asus and does it can decrease the glucose production in liver. In diabetes mellitus this is a very big factor how to control the blood sugar level by making use of many processes. New glucose Genesis is a process of producing glucose from other sources than glycogen.



Recently it has been found that it can simulate the enzyme AMPK i.e. AMP dependent protein kinase enzyme and which is a big factor for the action of metformin. Generally pyruvate carboxylase kinase enzyme promotes the formation of Malicacetate some pyruvate and this oxaloacetate breakdown by Pep carboxykinase to rotate the neogenesis pathway to form glucose. But metformin can simulate the enzyme known as AMPK which can block the neo glucogenic pathway.

The factor for working of the particular drug metformin lies on the fact that it can help in formation of glycogen from glucose. Net glycogen can be stored in the muscle and be deposited these as this fact of glucose into glycogen is very much done by the process of glycogenesis.213-231

Insulin also help in glucose utilisation peripherally that is in the peripheral glucose utilisation. This is nothing but the fact that it can block the mitochondrial respiratory chain so that peripheral glucose utilisation can be increased. The rate of absorption of glucose in the intestine also can be reduced or inhibited by the action of insulin.

Metformin do not produce very serious side effects generally. Although gastric disturbance anorexia abdominal pain etc are found with this particular drug metformin. This drug is not found suitable for the people who are alcohol takers. This is because alcohol can produce acidosis in a normal force of oxidation that is metabolism inside the body. Metformin can promote or increase this acidosis as it can produce lactic acidosis as a side effect. Metformin Jeevan cannot be used if there is any symptom of cardiovascular problems like failure of heart etc. Serious serious cardiovascular problems can be more fatal if metformin is used along with stroke or heart failure. In hypertensive state obviously it should not be used and also in patients who are suffering from any urinary problem for liver disorder.

Metformin poisoning can be increased if there is presence of drug like as Cephalexin produce competition for absorption from the particular site. So as there will be competition inhibition in absorption so excretion of metformin will be delayed which may produce toxicity.232-256

Nowadays the use of metformin as a first line of therapy in type 2 Diabetes mellitus is very finally established. It is a drug of choice for type 2 Diabetes

mellitus singly or in conjugation with any other sulphonyl urea. Sometimes Sometimesdpp 4 inhibitors like sitig Lipton can also be combined with metformin to get a better result. So with all these factors available metformin can be counted as an ideal drug to be used in case of type 2 Diabetes mellitus.

Metformin is an agent which can produce loss of weight in so many diabetic patient so to say it is an effective anti dose for a the fatty patients of diabetic.

The thiozolidinediones are effective drugs for type-2 diabetes mellitus although there usefulness are now a days in a problem because of its so many hazardous side effects. Like its probable carcinogenecity. Rasiglitazone is a drug of this group which is withdrawn fromthe market and it was banned due to its possible side effects like stroke, heart failure, CHF and death.

These drugs are used as against for a very important mechanism of the body system. i.e. (PPARY) the expression of which generally observed in the adipose tissues. Although it is found to be expressed in other tissue and muscle cells also. The occurance of gluconeogenesis in liver is also found to be very less or rather it can produce an obstacle for hepatic neoglucogenesis. This group of drug and medicine can change insulin resistance by promoting the expression of GLUT-4 and its translocation. In the muscles and fat entry of glucose is increased.

There is a major impact of the drug in the lipid profile of the diabetic patients. It can increase the level of HDL cholesterol in the blood when the concentration of the LDL-Chl the body removes almost same. This provides a relief to the diabetic patients who are also suffering from problems is the lipid profile. At the same time the drug can reduce the concentration of serum triglyceride in the blood. This is a major advantage for the diabetic patients suffering from lipid profile too.

Generally it is found that pioglitazones I pity finally adjusted with the body. Although some side effects like headache, mild anemia also reported. Sometimes problems in the liver is also found. It should not be used with patient off liver disorders and also in chronic heart failure.

It is another drug found to work for that type 2 diabetes mellitusbut not in type 1 diabetes mellitus. This is found only in the cases where the patient is responded to this drug. Generally it is found that this drug can reduce the blood glucose and HB

and it does not increase the level of insulin in the blood. It can be used alone without combining with any other antidiabetic drug if the diabetic level is not that much high. There are some drugs which can inhibit the carbohydrate digestion process at the last step by blocking the enzyme namely alpha-glucosidase. As the digestion of carbohydrate is hampered so its absorption will also be less in the body that is the level of blood sugar in the circulating blood is also less. That is a big thing in controlling the blood sugar level in the body. But one major problem with this type of drug is that there may be major in digestion problem with the carbohydrate food which is in turn may produce side effects like in the digestion of carbohydrate food and and popularity of the medicine. It reduces the HB level in the blood and thus it act as antihyperglycemic but it is not hypoglycemic drugs.

The usual dose of the drug is 50-100 mg

Dopamine D2 agonist drugs like bromocryptine can be used as a mild antidiabetic drug. It is a drug which can act on the hypothalamus to control the release of dopamine and thus IT Act against resistance against insulin.

Thus in a manner it can produce mind antidiabetic effect ranging from 0.8 mg 4.8 mg od and can be used for glycaemic control with other antidiabetic drugs like sulphonyl urea or metformin.

This is a doubtful case to say whether cardiovascular mortality is increase with metformin or sulphonyl urea or not because some workers of USA demanded that cardiovascular mortality is increased with metformin and sulphonyl urea where as the scientist of the UK demanded that it may not increase the death rate.

Metformin also is a very important drug which can make a control on the macrovascular complications.257-271

The most important factor in the antidiabetic mechanism is found in the efficacy of the antidiabetic drug that insulin and the sulphonyl urea as are very much effective in reducing the so-called microvascular complications like diabetic retinopathy diabetic nephropathy and neuropathy etc but they cannot cure thebut they cannot cure the macrovascular complications like coronary artery dosage stroke a peculiarity of the fat is found with the drug metformin that it can control both microvascular and macrovascular complications. The effectivity of metformin

in controlling the microvascular complications as well as the risk of death. Generally most of the antidiabetic drug are very much effective in controlling the symptoms of excessive blood sugar in the body and also the condition of glycosuria.

Utilisation of glucose inside the body

Generally glucose is utilised in three process inside the body. If one represent 8 schematically it can be showed by the figures showed below

Basically glucose can be produced and stored in the form of glycogen and starch in muscle and liver tissues. It can reduce its carbon skeleton by following the metabolic pathway of pentose phosphate pathway to form ribose 5 phosphate. It can again follow the glycolytic pathway to produce energy. This is the fate of glucose probably the normal biochemical mechanism of degradation of glucose in various forms.

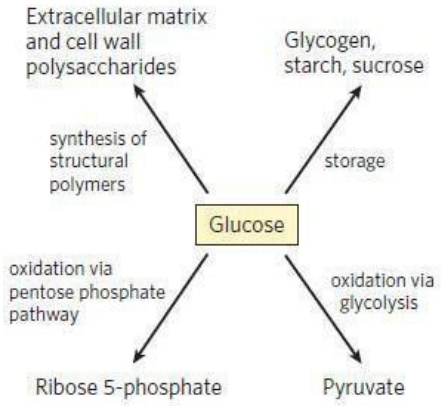


Fig: 1 Utilisation of glucose

Generally in vertebrates and some microorganisms glycogen is formed as a storage form of glucose.

This is a big thing because the body mechanism of glucose is controlled by this fact. Starch is the storage form of glucose in plant materials. The main function of glycogen to be stored in liver is nothing but to produce energy for metabolism inside the body. Glycogen stored in the hepatic tissues provides a big function for the body as they can be used at the time of fast. Regular when glucose is not available from food intake then liver glycogen can be broken down to provide

glucose. Generally liver glycogen is broken down in 12 to 24 hours. The process of formation of glycogen from glucose in the body tissues can be summarised in the following manner.

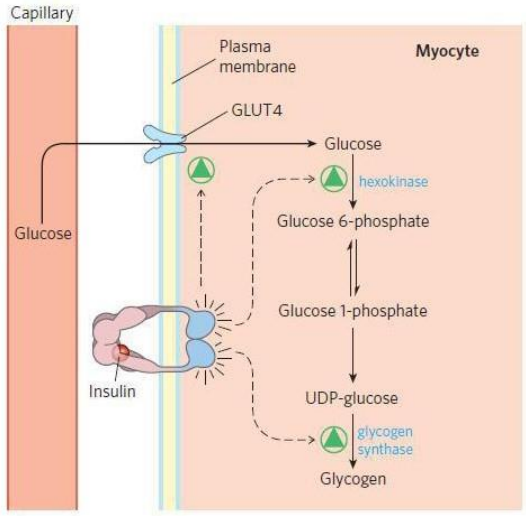


Fig: 2 Role of insulin to form glycogen from glucose

From this figure it can be very finely assumed how insulin plays a vital role in the formation of glycogen from glucose molecules.

Nowthis insulin secreted from beta cells is a major player in the management of diabetes in the human body. So these biochemical cycles like glycolysis where glucose is converted into glycogen having big arose in controlling the Deadly disease.272-281

Another one very important concept comes from the utilisation of glucose in the alanine cycle which takes place in between muscle and liver. This is basically formation of glucose in liver from different biochemical chain from amino acids. As we all know that protein breakdown inside the human body to form its simplest unit known as amino acid which in turn produces ammonium ions in the muscle cells.

Glucose converted into pyruvate in the muscle cells by the process of glycolysis. Pyruvate is converted into alanine in the muscles by the action of the enzyme

alanine aminotransferase. Ammonium Ion produced from the proteins also helps to convert pyruvate into alanine by transferring the Alpha amino acid of glutamate into the molecule of Alpha ketoglutarate. The α ketoglutarate in so formed in the muscle enters the blood stream to produce alone in the blood and the alanine enters the liver. Here in the liver alanine again is converted into pyruvate by the help of the enzyme alanine aminotransferase which produces α ketoglutarate to glutarate.

Priteins and fat in the liver molecules is converted into glucose by the process of neoglucogenesis.

Some drugs like biguanides block this step of formation of glucose from pyruvate. Metformin blocks the formation of glucose in liver from pyruvate. Glucose that produced in the cycle in liver is transported to the muscles to complete the cycle.

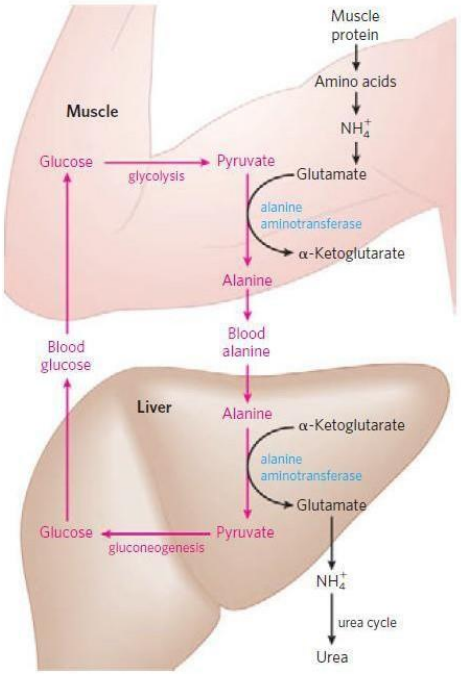


Fig: 3 Glucose Alanin cycle

So this particular system is very important in converting the urea cycle with the glucose alanine cycle to form glucose from pyruvate and its transport to muscle cells in glucose form. So by controlling these particular steps of formation of glucose we can control diabetes type 2.[282-285]

In fact glucose as we can see that is changed into glycogen by the process of glycogenesis can be converted to lactate in the process of glycolysis to produce ATP in the muscles when it is required. Thus muscles contraction is fulfilled by the glycolysis producing energy ATP to contract the muscle. Again this ATP is produced by heavy activity like muscle glycogen to elect following glycolysis. Again by the process of light activity fatty acid ketone body and blood glucose also produced ATP.

Some amount of lactate produced in the muscle transported in the blood for blood lactate and that blood lactate in the liver to produce glucose by the process of neoglucogenesis.

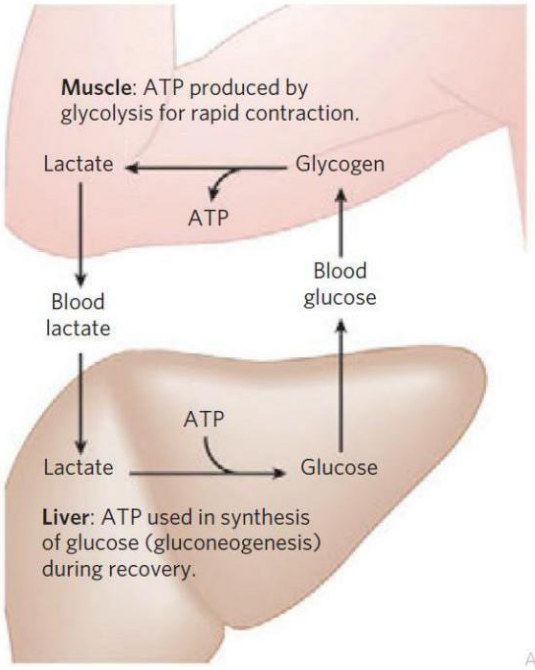
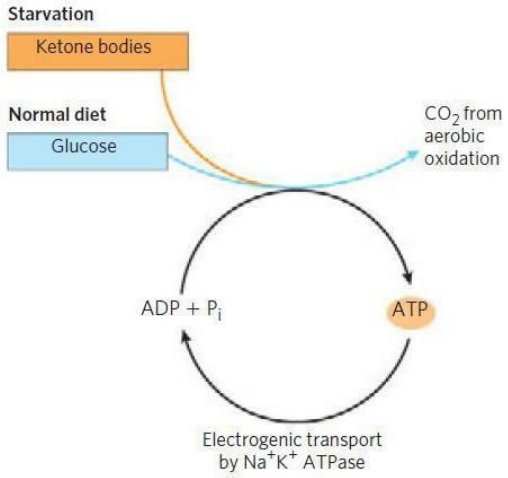


Fig: 4 Metabolic cooperation between skeletal muscle and liver

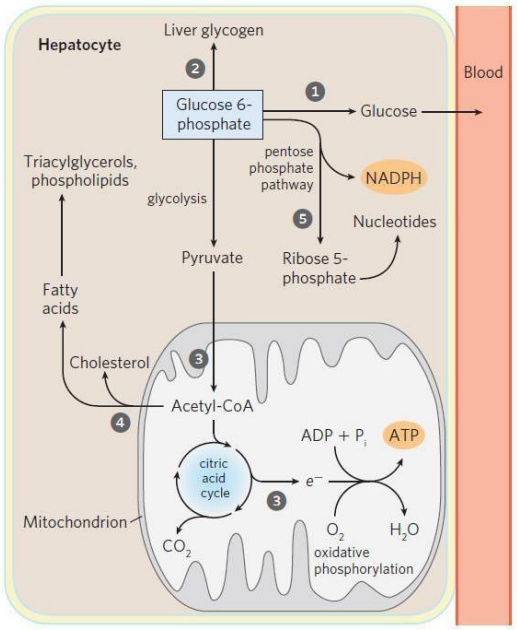
Thus glycogen and lactate conversion takes place between muscle and liver for utilisation of glucose to produce ATP required for muscle contraction.



Even in this Cori cycle as we see the pathway to produce glucose in the liver with the utilisation of ATP in the liver cell.

The drug metformin can produce a block there in the neoglucogenesis pathway. So that glucose can be not produced. Thus diabetes type 2 can be prevented by the drug metformin. So these biochemical cycles are blocked by the drug metformin.

These cycles are interconnected to each other urea cycle is attached to cyclic acid cycle by arginino succinate shant.



So from glucose glucose 6 phosphate is produced which may produce glycogen to be stored in the muscles and liver. Again this glucose 6-phosphate can produce pyruvate following glycolytic pathway to yeild energy. That pyruvate again enter

into the TCA cycle forming acetyl-coa. TCA cycle again attached with the urea cycle to produce urea.

So in this manner glucose is utilised inside the body in a regular fashion or a systematic manner to yield energy and to carry on the biochemical step take place in the body.

Cancer is a big problem and it produces and controlled and massive growth of cells and tissues of a particular part of the body surface which may lead to metastasis of the cancer from one part to the another. The world cancer report of 2014 reflect that it is one of the great cause of death worldwide and if it is primary detected surgery can be done to remove the cancer cells from the body. Surgery is a great method to remove the cancer cells from the body system whereas the metastases can be blocked by additional entry of drugs are medicines into the system that is chemotherapy should be done to prevent metastasis. DNA replication is a major cause of uncontrolled growth of the cells as it produces a big number of cells at a time causing massive production of malignant cells to produce cancer and that's why the anticancer drug usually target the DNA replication

The main operational area of the anticancer drug is to treat the DNA replication are in a biosynthesis or maybe the protein biosynthesis and obviously the biosynthesis of DNA . There may be a few drugs working on the hormone or the supply of nutrients to treat the cancer cells.

The entry of the anticancer drug into the cancer cell depend on the cell definition that is at the stage of the mitosis where the anticancer drug target their action. The anticancer drug may be of both types i. e. They may be hydrophilic. Their chemical moiety can be hydrophobic . It is evident that serious side effect may arise with the use of anticancer drug due to the cytotoxic effect of the anticancer drug .

So it is evident for a medicine to be prescribed and used for a local application are there may be targeted delivery as it can produce cytotoxicity. Targeted drug delivery can be achieved by selection of suitable vehicle and appropriate route of Administration of the anticancer drugs so that active ingredients at the property site of action of the disease.

Drug carriers for antitumor delivery:

Main objectives:

Drug delivery to the desired site is completed by the useful administration of drug carriers which deliver the drugs properly to the desired site of action. While the drug molecule is delivered to the proper site of action in a proper amount the efficiency will also be good. This is the way where there will be minimization of side effects as there is no extra delivery of the medicine to the target site. This is an important feature of the targeted delivery now a days.

This is an approach which basicallytriggers the administration of the drug at the particular site for a longer purpose. Continuous release of drug at a proper time interval in the target site is a big therapeutic out come in the targeted drug delivery reducing the risk of adverse drug reactions or any toxicological outcome.

The efficiency of the drug carrier depends upon releasing the drug at the proper site of action. The cells must provide entry of the drug molecule for its action and i.e. the role of carriers to present the drug at the proper site of action

Hydrogel:

These are basically used in the cancer treatment as carriers of anticancer drug delivery.

It can be said that they are complex network of polymers or may be thermoset gels having high affinity for water or aquous systems. The crosslinking is an important factor for the preparation of the hydrogel as they can move in to formation of a sponge like polymeric structure which is very important for a complex network. So it works like a storage body and releases the drug in a continuous fashion regularly in a specific release pattern.

And in the treatment of cancer it is seen to find use of hydrogen of metformin and 5-fluro uracil in colon cancer.

Polyhydroxy carbolic acid:

These are biodegradable polymers used in various kind of anticancer drugs treatment carriers. PLA and PLGA are successfully used as polymers where PLA resembles Poly lactiglycolic acids. This type of drug carriers is also involved to make a steady state of concentration in the blood stream of anti cancer drugs. Now a days polyhydroxy carboxylic acid (PHA) of medium chain moiety found to produce better result.

The microsphere preparations with coated polymers may be a suitable example for this kind of medication used in the cerebral tumors. The coating material selection depends specifically on drug it self as it can develop the release of drug in the proper at the target site of release and thus we see chitosan can be effective in 5-Fu loaded microparticles. Whereas polyethyline glycol can be used as a coating material for taxol containing drugs

Nanotubes-

These are typical examples of formulations producing the drug at the target site in the anticancer medication and the internal volume will be filled with the drug. Inside the nanotube the drug and medicamets should be completely filled to provide the drug molecule in the target site. There are existence of single walled nanotubes. And it may be made of carbon, boron carbide, boron nitride and silicon and there may be multi walled carbon nanotubes. These are made of carbon nanotubes and are of unique feature showing its activity in the aquous media.

Organic nanotubes can be used to encapsulate a poorly water soluble drug such as hydrocorlisane whereas 5-Fu nanotubes can be made by TiO_2/Zns.

Nanoparticles –

From the name it is evident that the particles will be of small to smaller size may be in the size range of 1-100 nm and mostly of spherical sizes and shapes. Various types of nanoparticles can be seen in the preparation of novel drug delivery, may be solid-lipid-nanoparticles and polymeric nanoparticles .

Use of both natural synthetic polymers can be done to prepare the nano particles cellulose , chitosan etc. may be the examples of the natural polymers whereas acrylic polymers, polymethyle methacrylate and budroyit can be found as synthetic polymers. The main objective of coating a drug by the polymer is to prevent the degradation or release of the drug in the stomach and to release the drug in the farthest part of the intestine i.e. the ileum and so the nano approach of drug delivery is very important for oral sustained release dosage forms in the novel drug delivery.

At the same time we have seen various formulations using paclitaxel as a core drug for the anticancer drug delivery in nanoparticular form to treat various diseases like ovarian, breast, lung, pancreatic and colon cancers. Paclitaxel is a fine drug in

anticancer drug delivery and becomes finer while it is in the nanoparticular dosage form to obtain specific result in drug deliver . While a hydrophilic drug 5-Fu is used typically in a dosage form of nanoparticles using chitosan as a polymeric material. The efficiency of these dosage form is that it can release the drug in alkaline pH in the intestine very effectively whereas resist the release acidic pH of the stomach. Now a days we have seen the use of more efficient polymer to coat the nanoparticles known as graphene or its oxide known as graphene oxide. The main boding graphene oxine in the intermolecular chain may be due to the π- π bonding amongst its molecular chain.

There are newer approaches coming to develop the molecular structure of graphene oxide so that it can be used effectively as a polymer in nanoparticular delivery.

The combination of cyclodextrin with graphene oxide to align in the molecular structure can be a newer approach to achieve a promising drug delivery[40]. Functionalized grapherene sheets crosslinking can be prepared to achieve nano formulations [41]. Mainly loading of the drug can be increased by the increasing use of graphene oxide with supramolecular sheets of cyclodextrins adjusting in molecular level crosslinking to achieve the goal of the therapeutic level novel drug delivery approach.

Introduction of concept of vesicles and liposomes-

Vesicle is a improved technology which encloses aquous phases. The water soluble drugs may be incorporated for encapsulation, on the other hand oil soluble drugs also may be introduced in the central core of hydrophobic material. So it can be said that vesicles can be used in both hydrophilic and hydrophobic ingredients . They are utlised in all the preparations like medical preparations as chemical and

personal care products. It is also seen that vesicles can be used for drug delivery in cellular exchange process by membrane fusion and separation.

Vesicles are found in various ways according to size and lamellarity. Generally vesicles are found to be of round shape. There are various types of vesicles found in these filed like ultra small unilamellar vesicle (USUL) and its diameter may be 5-10 nm. The size of small unilamellar vesicle (SUV) is largand its size may vary from 8-40 nm (41). Generally more that 20-50 SUV are used for the preparation of intravenous injection.

And it should be within the size range of 100 nm (52) and that may be assumed by checking the diameter of the blood capillary which may be of 7000 nm.

The blood cells having diameter of 8000 nm generally pass though these capillaries, It has been seen that vesicles are less flexible [47]. And its diameter may be less that 100 nm .

The vesicles are found to contain only one bilayer and they are prepared generally by the solvent injection method, and may be they can be prepared by the sonication or it can be due to the non availability of the large vesicle precursors.

The large unilamellar vesicles or LW are having a diameter which may not be more than 100 nm or might be 01 µm . The size range can be extended upto a size of several µm. Solvent injection method is an useful method which may contain either or ethanol[48]. There may be larger sizes seen in the M1Vs or may be of similar sizes that of Luv but they are generally found to contain two or more concentric bilayers.

It is found that thin film hydration may be very effective or there may be followed freeze drying technique of the SUV dipersions in the aquous solutions obtain

encapsulation. There may be some biggest vesicles which are also known as giant vesicles(GUV) and that may contain a size range of 5-100 µm although there m be a category of oligovesicular vesicles(OVV) which may contain small vesicles and that may be inside big vesicles, and it is made to provide effective circulation inside the blood stream. It may be seen also various names for this particular form of dosage form (vesicles).

A general existence of liposomes are found to be used in nano-particular dosage forms of novel drug delivery category. These are made of phospholipids[49,50,56] and binds effectively various anticancer drugs found to be encapsulated including taxels. There may be existence of niosomes which may contain non toxic Surfactants and catanoic surfactants also can be take part in the formation of vesicles. It is seen that these cationic Surfactants or "catansomy" are made of using anionic and cataionic surfactants in an unimolar ratio.

At the same time we can also find ethnosomes which may be made of phospholipid vesicular systems utilizing ethanol in higher concentrations and that may be of 20-50 at %.

Characterization:

Physio - chemical characterization:

Particle size is understood and determined by various methods and may be by dynamic light scattering. Scanning electron microscopy or SEM studies can be applied for the determination of the particle size at the microparticle level to understand and study the microparticular drug delivery.

At the same time field emission scanning electron microscopy(FESEM) and transmission electron micrscopy (TEM) can be used to determine the nanoparticular drug delivery.

Liposomes or other vesicular system & can are under standable by using CRYOTEM for the determination of the particle size.

Complex systems are composed of various formulations which may be stabilizing the development of the changed particles in the system. These charged particles determined by the zeta potential in the system. If the zeta potential in the system is lower than the possibility of flocculation or coagulation can be found. At the same time with higher and higher zeta potential results less chance of coagulating or flocculation providing stability to the systems. 40 mv to 60 mv zeta potential shows good stability in the system where as more than 60 mv shows good stability in the system . But if the zeta potential is within the range of 0-5 mv positive or negative, there is a chance of rapid flocculation or coagulation in the system.

Moderate stability may be found within the range of 30 mv to 100 mv where as 10 to 30 mv zeta potential shows very low stability.

Drug entrapment efficiency (DEE)

The drug loading mechanism is an important criterion as it predicts the amount of drug to be entrapped in the formulation. Various spectrophotometric methods were utilised for the determination of drug content or to calculate the percentage of drug entrapped in the preparation.

The methods are generally found to utilise for DDE various instruments like U-V spectroscopy , fluorescence spectroscopy HPLC and a combination of HPLC with mass spectroscopy .

The drug entrapment efficiency also renders the free drug calculation which was not entrapped in the formulation.

This can be followed by ultrafiltration or centrifugal filter devices . At the same time some other techniques can be applied .

By the help of suitable calibration flows we can find the specific amount of supernatant and sediment in the preparation.

The drug entrapment or drug encapsulation efficiency is nothing but the amount of drug i.e. entrapped inside the drug carrier. The amount of drug entrapped can be varied with the method of preparation and the technique followed to prepare the formulation.

It has been also observed that 5-Fu encapsulation can be better if it is done by DPPC or, Dipalmitoyl phosphahdy chloride than with ethanolic injection method to be entrapped in the vesicles .

The encapsulation efficiency can be increased and that is due to the inclusion of DPPC without cholesterol and that was also in a modified reserve phase evaporation also. At the same time the use of a different surface active system can increase the % EE to about 80%.while it has been found that if we use polymeric nanoparticles prepared from triphosphate crosslinked chitosan may be left effective then than the vesicular system .

Drug release

The release profile of the drug is a very important criterion as it directly relates the bioavailability of the drug and from that therapeutic efficacy is also concerned. The important factor of concern is the dialysis bag method to study the in vitro drug release of the formulation.

The drug while encapsulated placed inside the dialysis bag in the mouling water at 37°c which reflects the body temperature. The concentration of the drug is checked time to time may be within 1 hour interval to study the release behavior of the drug . there are so many differences in the dialysis bag and that is to study the diffusion cells, ultra centrifugation and ultrafiltration approaches

As cancer is a deadly disease and the malignant cells can make uncontrolled cell proliferation so I t become the most common cause of death. According to a study report published by GLOBOCON 2018 SK1) a huge number of cancer affected people can be seen whereas can cancer deaths (probably 9. millions) can be happened.

Lung cancer is very dangerous and found very frequent as the habit of smoking is increasing day by day. The percentage (11.6% 0 of lung cancer patients increasing day by day.

Other cancer are also very dangerous like breast cancer (11.6%), prostate cancer, (7.1%), colorectal cancer (6.1%) etc. (SK-2).

Historical fact

The word cancer came from the term karkinos and it was used by Hippocrates (460-370 BC). Karkinos is a greek word and the word cancer came from this. It was seen in the mummies of ancient Egypt the existence of human bone cancer and the ancient manuscripts also showed the fact around 1600 B.C.

There was a proof of existence of breast cancer at 1500 B.C. in Egypt. It was also known at that time that there was no proper treatment for cancer and only palliative treatment (SK-3) was available. The oldest cancer patient was reported by H. Seherg et al. (SK-3) in the year 2015. Above all there was no proper destination or

line of treatment for the cancer patient. And Paul Elrich (SK-5) in the early 1900 showed the way of cancer treatment by preparing some animal models and used some chemicals to register first chemo Therapy in the Globe. He outline dthe anticancer activity of arsenic in the year 1908. He was also founder of some alkylating agents and aniline dye for the anticancer activity.

There are three major ways to treat the disease cancer and they are surgery, radiation and chemotherapy. Mixed chemotherapy i.e. composition of two anticancer drugs in the dosage form could be a great achievement for the anticancer chemotherapy.

Unnatural growth-

Cancer is nothing but the unnatural growth of cell within a certain area. The cancer cells can multiply abruptly without following any system. This is known as malignancy and malignant tumors are very easy to detect as they metastalize without any system. Mutation can block the cancer cells as it shows on genomic variants. Cancer cells live in the genome.

Generally cancer is found in the form of solid tumor. Liquid tumors are also found and they do not form solid tumors except in case of leukemia.

Different cellular reasons

There are different cells and tissues inside the body. The four different type sof cells are found generally known as a) Epithelial tissue, b) connective tissue, c) Muscle Tissue, and d) Nerve Tissue.

There are various reasons may be therefore the cells proliferation and angiogenesis can be there. There are different cell cycles to promote this kind of cellular angiogenesis and various phases of cell division may be involve.

Conclusion

Targeted drug delivery is an important system for administration of drugs into the proper site of action. This is the major system containing various drug carriers to satisfy drug delivery into the targeted site. Microspheres is a major tool to satisfy the availability of the drug into the proper site of action and providing a sustained release dosage form vesicular drug delivery. System is an important mode of approach for the targeted drug delivery systems . nano particles are important candidates for the drug delivery research and nano particles with graphene oxide may be an important formulation to achieve targeted drug delivery at the proper site.

With the advancement of noble drug delivery various approaches have established their presence. Microspheres are important tool to deliver drugs at the target site in case of diabetes and cancer patients. In chronic cases regular supply of drugs is required to maintain a steady state of the drugs in the blood system.

Diabetes mellitus type-II is an important disease as it can produce so many diseases like diabetic nephropathy, diabetic retinopathy, hyperglycemia and stroke. So regular supply of medicine is required in the blood circulation to control the blood sugar level in the body.

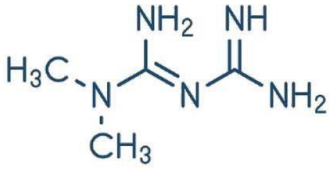
Cancer patients also suffer from a regular problem of drug availability at the site of cancer cells as it can control the growth of cancer cells which may be a big problem if not treated earlier. In that case of typical problem of malignancy

targeted drug delivery in the form of microspheres may be an effective tool for the anticancer drug delivery. Metformin a first line antidiabetic drug a first choice in case of type-II diabetes mellitus can be prepare din the form of microspheres to achieve the goal of sustained release drug delivery.

Even in case of cancer too microsphere of metformin showing good results to promote apoptosis in the EAC and MDA-MD- breast cancer cell lines which is discussed in the later chapters.

Metformin-Drug Profile

Chemical Structure



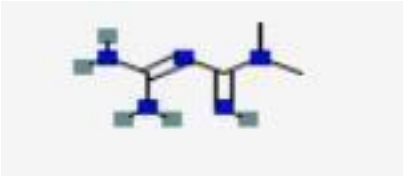
Molecular Formula: $C_4H_{11}N_5$

General name: 1-1- Dimethyl biguanige

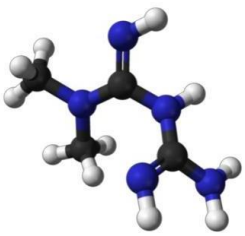
I.U.P.A.C.: N,N-dimethyl imidodicarbonmidic diamide

Molecular Weight: 129.16 g.mol^{-1}

2D Structure:



3D Structure:



Route of administration: By mouth

Bioavalability: 52-60%

Protein binding: Minimal

Elimination half life: 4-8.7 hours

Excretion: urine

Chapter - 3
Aims and objectives

AIMS AND OBJECTIVES

With the passage of time newer techniques evolved in the field of drug delivery. Drug delivery research now a days mostly based on development of novel drug delivery system mostly by newer technologies of sustained or controlled release systems. The development of newer techniques with microsphere and nano particles had opened a big horizon in the field of drug delivery research. As the bioavailability is higher with a particular type of dosage form the newer approaches are well accepted in the big pharma market also, to keep a big influence in the health system of research.

Drug-dosage form relationship based on acceptability in the market is an important phenomenon to design its proper route of administration . Generally oral route is preferred for the commonly used tablets, capsules, syrups and other forms of solid and liquids dosage forms. With the advancements of sustained or controlled release technologies the duration of drug action inside the body is increased; sustained release formulations provide a constant supply of the drug to the target area for longer period with an efficient concentration that makes the acceptance of the product in a longer time with a suitable bio-avaliability. That is the best achievement for preparation of a sustained release dosage form. Microsphers are also very specific formulation as they also provide sustained action for a longer duration.

Antidiabetic drug are very specific as they work as life saving drugs. This particular fact is always valid to prepare a developed product in antidiabetic segment. Moreover the gastric side effect of some of the antidiabetic drug is masked by microsphere preparation as it releases in the small intestine at Ph 6.8 . A microsphere preparation done by W1/O/W2 emulsion solvent evaporation techniques with a matrix prepared by sodium alginate and guar gum in varying ratios can create a suitable dosage form of microsphere for the sustained actions. Sustained release or prolonged release of the drugs helps to rectify frequent dosing of medicines through G.I Tract. The release of the drugs is almost 90% to achieve its concentration upto 100% release so that bio-avaliability will be maximum.

In a nutshell the main objective of the present work areas follows-

1. Identification and selection of the gum to prepare a controlled release formulation.
2. After identification of the gum it is required to collect the gum.

3. The particular drug molecule is to be relected as the present topic is an antidiabetic drugs so a proper antidiabetic drug is to be selected.
4. The drug molecule is to be properly reviewed.
5. Different properties of the drug molecule is to be studied.
6. Selection of the proper process to prepare the drug in a novel dosage form so that it can deliver the drug in adequate amount in a controlled manner at the targeted area.
7. After preparation of the microsphere proper characterization (physio-chemical) should be done.
8. The studies should be verified by pharmacological studies.
   These working schedule will be fulfilled only f it is followed by a proper working schedule.
   1. Pre-formulation plan-
      a) Phosphate buffer (Ph 6.8) preparation.
      b) 0.1(N) HCl solution of Ph 1.2 preparation.
      c) Preparation of standard curve of the drug metformin at Ph 6.8 of phosphate buffer by UV-Spectroscopy.
      d) Standard curve for metformin in 0.01(N) HCl by UV- Visible Spectroscopy.
      e) Standard curve for metformin in distilled water.
      f) Metformin solubility testing in different solvents like PO_4 buffer (Ph- 8.8) .0.01(N) HCl soln., distilled water etc.
9. Various physiochemical properties of meformin is to be studied to cary out the effective determination of the products.

   Those are lambda max, melting point, FITR and XRD studies.

10. The excipients are studied for its application with the drug metformin.
    2) Physiochemical properties of Guar Gum
    Swelling characteristics microbial load study toxicity study of the guar gum was to be checked by suitable methods.
    3) Metformin loaded microsphere prepared by W1/O/W2 solvent evaporation techniques.
    Various characterization procedures of the microspheres were done by SEM, FTIR, XRD studies.
    4) Evaluation of the antidiabetic drug metformin in animal model.

Chapter - 4

Preparation and characterization of microsphere

Contents

Preparation and characterization of microsphere

1. INTRODUCTION

Metformin, a white crystalline, hygroscopic powder of a 1,1-dimethyl biguanide analog used as an antidiabetic drug from times immemorial [286]. It has a scientific name N-N-dimethyl imido di-carbonamide diamide hydrochloride(fig. 1)[287].

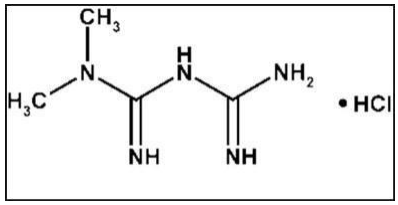


Fig.1:Chemical structure of metformin hydrochloride

It reduces blood sugar by mainly blocking neoglucogenesis rather than increasing the secretion of insulin from the islets of Langerhans in the pancreas [288]. According to some experts, metformin is a drug of choice for newly diagnosed type-2 diabetes and perhaps the best oral hypoglycemic agent proven to decrease cardiovascular mortality [289]. It is also used in polycystic ovary syndrome as it can reduce insulinresistance so as to produce ovulation and pregnancy [290]. The drug generally works by suppressing hepatic neoglucogenesis and decreasing glucose output in the liver. It also increases insulin-mediated glucose disposal in muscle and fat. It increases the GLUT1 transport from intracellular site to the plasma membrane. It also decreases the rate of intestinal absorption of glucose, other hexoses, amino acids, and Vit-B12. It increases peripheral glucose utilization by enhancing anaerobic glycolysis [291]. In diabetic patients, the blood sugar level should be maintained at a certain level for along period. So, a sustained or controlled release dosage formulation will be beneficial for proper controlling of diabetes mellitus.

Microparticle preparation provide longer duration of drug action with minimal gastric

irritation. As microsphere preparations contain the drug embedded in the matrix made of guar gum and sodium alginate,resulting in a continuous slow release through a longer period [292]. Metformin lowers blood glucose levels in the patients, those who do not produce insulin in appreciable quantity to control hyperglycemia [293]. So, the microspheres of metformin will be very helpful to fight against hyperglycemia for a longer duration [294].

Guar gum is used in food and pharmaceutical industries for longertimes [295]. It is a natural nonionic polysaccharide obtained from the seeds of Cyamompsistetra tetragonolobus (Family-Leguminosae). Itis hydrophilic in nature and produces its swelling property in coldwater. Its gelling property is useful in producing a delay in the drug release so a better choice for controlled release formulations [296].

2. MATERIALS AND METHODS

2.1 Materials

Metformin received from stadmed lab . all other ingredients were of laboratory grade, guar gum was produced from Bikaner District of Rajansthan India.

2.2 Animals

Albino rats was used for the animal experiement. All the guideline was followed. Animals were kept in the animals with food and water ad libitium [297].

2.3 Methods Preformulation studies

2.3.1 Preparation of stock solution

stock solution 10 mg of the drug was dissolved in50 ml of distilled water kept in a 100 ml of volumetric flask. Then thesolution wasprepared to100 ml by adding distilled water, and 100µg/mlstocksolutionwas made.

2.3.2 Determination of λ max

10 µg/ml of drug solution was made from the stock solution. The absorbance in the UV

spectrum was found by scanning of a range 200 nm to 400 nm, and maximum absorbance was found at233 nm while distilled water was used as a blank. So the λmax of the sample became 233 nm.

2.3.4 Preparation of standard curve

Seven point calibration curve was drawn by spiking appropriate amounts of the stock solution into the corresponding buffer to obtain a final concentration of 5,10,15,20,25,30, 35 µg/ml for the study. Absorbance was detected at 233 nm, and a calibration curve was made by plotting absorbance against the concentration (fig.2).

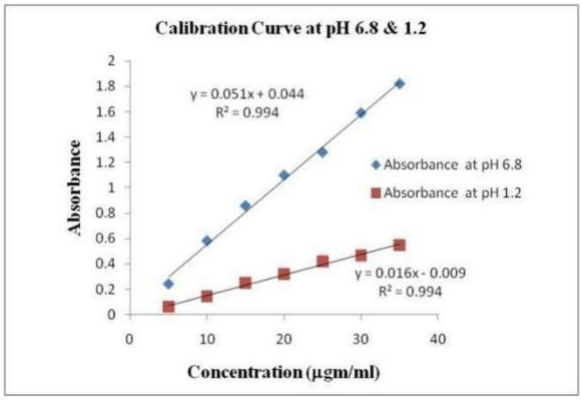


Fig. 2: Seven point calibration curve of metformin at 233 nm wavelength of pH 6.8 and 1.2

2.3.5 Swelling index

1 gm of Guar gum was incorporated in pH 6.8 buffer solution, 10 ml ofdistilled water and 0.1 N HCl solution. Then it was shaken for 10 minin a magnetic stirrer at high speed and allowed to stand for 24 h.Swelling capacity was measured using the formula [298].

$$\% \text{ weight change} = \frac{Xv - Xi}{Xi} \times 100$$

2.3.6 Microbial load study

The pour plate method was used to study the microbial and fungal load of the sample.Two sterilized Petridishes were used, and Soybean-Casein digests agar medium was poured into both thePetri dishes aseptically with fresh guar gum and ten months older sample respectively. After solidification of the media, both the

Petridishes were placed in the incubator at 37˚C for one day. A total number of colonies were counted by the Coulter Counter method. Inthe same way, the total number of the fungal count was done by using potato dextrose agar medium [299].

2.3.7 Toxicity study

The sub acute toxicity study was done on male Westar rats (120-130 g). The solution of guar gum at a dose of 100, 200 and 400mg/kg body weight was administered orally to the three groups of six rats, respectively, for every day 14 d and control received a vehicle of the same volume. The toxic manifestation, i.e. body weight, mortality, and behavioral changes were regularly monitored. After 14 day the surviving animals were fastened overnight and anesthetized with diethyl ether, and the heparinized blood samples were collected for determination of hematologic parameters and animals were sacrificed by euthanasia for the collection of internal organs kidney and liver.Then the collected organs were preserved in 10% formal dehyde solution for histopathological examinations. The research was conducted in accordance with the ethical rules on animal experimentation approved by the Ethical Committee, Department of Pharmaceutical Technology, Jadavpur University (Approval No:147/1999/CPCSEA).

2.3.8 Preparation of microsphere

A small quantity of sodium alginate was dissolved in 5 ml of distilledwater in a small beaker placed on a magnet stirrer. Guar gum wasintroduced into it with continuous stirring. Metformin as a weight of 80 mgwas added to it and stirring was done for almost half an hour by amagnetic stirrer. Then the preparation was taken with a 20 gauge needle. An organic solution was prepared with 1gm of ethyl cellulose and 30 ml of dichloromethane (DCM).Then 300 µl of span
80 was added to this organic solution. A primary emulsion of W/O type was produced. A water phase was prepared with 100 ml of distilled water and 200 µl of tween 80. The preparation was kept under a mechanical stirrer rotating at 780-800RPM. The primary emulsion was then poured into the water phase drop wise by a 16 gauge needle and rotated for 3.5 h through mechanical paddle stirrer. Small spherical microspheres were formed which were filtered, air dried, weight and stored in a

desiccators for future study [300].

3. Characterization of preparedmicrospheres

3.1 PercentageYield

The percentage yield of microsphere was determined by theratio of practical yield and theoretical yield. Practical yield is the weight ofthe microsphere obtained. Theoretical yield is the total weight of the raw materials [301].

$$\text{Percentage of yield} = (\text{Practical yield}/\text{Theoretical}) \times 100$$

3.2 Drug entrapment efficiency

A small amount (40 mg) of prepared microspheres were triturated properly and made into powder form, and then 100 ml of phosphate buffer (pH 6.8) was added to it. This preparation was placed in the magnetic stirrer for 2 h. Filtration of the solution was done by Whatman filter paper. 10 ml of this stock solution was diluted with phosphate buffer(pH-6.8)and analyzed for metformin content at 233 nm [302].

Drug entrapment efficiency (DEE) =Experimental drugcontent / Theoretical drug content × 100

3.3 Particle size distribution and zeta potential

The particle size distribution was analysed from the optical microscopic method. The mean average diameters of the microsphere particles were obtained in the SEM studies. A weighed quantity of the experimental sample was dissolved in Milli-Q water (Milli-Q, Merck Millipore, Billerica, MA, USA) by vortexing and then sonicated and placed in a cuvette for zeta potential measurement [303].

3.4 Scanning electron microscopy (SEM) analysis

Particle size, shape and surface morphology was detected by SEM analysis. SEM was done by CARL ZEISSE VO 18 special edition machine with the platinum coating. The platinum coating was made of QUORUM Q 150 TES machine [304].

3.5 Fourier transform infrared spectroscopy (FTIR)study

FTIR was done on IR-Prestige-21,Shimadzu, Japan [305].

3.6 X-ray diffraction (XRD) studies

The samples of various batches were examined by X-ray diffractionstudies. XRD studies were done by X-ray diffractometer of model noUltima-111, Renuka (Japan), Cu target slide 10 mm. The possible drug-polymer interaction was studied by XRD studies

of the drug and the drug-loaded microsphere [306].

3.7 Drug release study

A drug release study was made in the dissolution test apparatus, LABINDIAD S8000 USP–type2 (paddletype) apparatus calibrated at 37˚C and rotated at 50 RPM. In the dissolution test apparatus initially, 750 mlof acid buffer of pH-1.2 was added, and microspheres of 50 mg wasintroduced into it. Then after 2 h, 150 ml of trisodium orthophosphate buffer solution was added in to the acidic solution o that the mean pH of the solution became 6.8. From the final solution, 5 ml of the sample was withdrawn in every 1 hour, and it was replaced by 5 ml of phosphatebuffer of pH 6.8 every hour. The same thing was done at initial 2 h maintaining pH 1.2. This process was continued for 12 h [22].

4. RESULTS

4.1 Swelling characteristics of the gum

Swelling capacity of guar gum in distilled water, 0.1NHCl and pH 6.8buffers was described fig. **3**. Here the swelling index of the gum was slightly higher in 0.1 NHCl than in buffer 6.8 and distilled water.

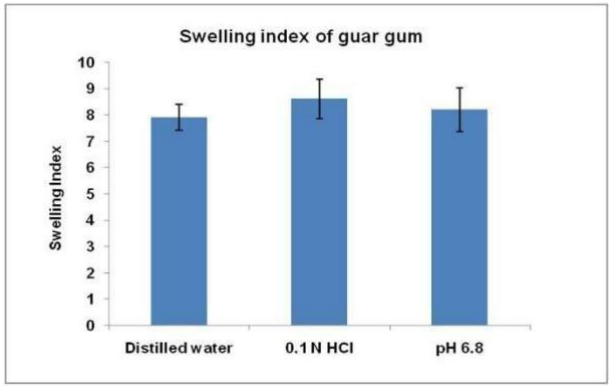


Fig. 3: Represents the swelling characteristic of guar gum at different pH (1.2and6.8). SD; Standard deviationof n=3

4.2 Microbial load

The Microbial count was found to 290 Colony Forming Unit/gram, 265 Colony Forming Unit/gram respectively for the older and fresher sample of guar gum for the older and the fresh sample of guar gum. The fungal count was found to be 75 CFU/gand73CFU/g for the older and the newer sample of gum. Fungal count w found to be 75 CFU/G and 73 CFU/g for the older and newer sample of the gum.

4.3 Toxicity study of guar gum

With the normal range of treatment with guar gum 100,200 and 400 mg/kg bw the RBC count and haemoglobin level was stabilized where as the WBC become slightly down.

the differential count, lymphocytes and monocytes were found to have some changes.

In the histopathological studies on treatment using test compound, the cellular features were found (fig. 4), some irregularities, e. g., deformation in the hepaticartery and irregular bile duct could be found. Along with this, guargum treatment at a dosage of 100, 200 and 400 mg/kg b. w, very least Histopathological evaluation and normal kidney tissue of the rats showed the normal structure of glomerulus encircled by the Bowman's capsule, distal convolutedtubules and proximal convoluted tubules without any swelling alterations (fig. 4). Microsphere treated group showing normal body features.

4.4 Percentage of yield and encapsulation efficiency

The percentage yield and encapsulation efficiency of metformin was changed in all microsphere formulations. The percentage yield of microspheres varied from 42.20±0.83% to 94.84±1.88%. It was also seen that increasing gum academy in internal phase the percentage yield was also increased. Due to the variation of drug and guar gum ratio, formulations were affected in table 2. The loading was increased upto an optimum ratio of drug and guar gum after which an increase in the ratio this decreases the drug loading.

4.5 Average particle size determination and zeta potential

The particle size of the microspheres seen to be increased after a certain size range of 327.08 µm. After this optimum amount(**table 3**), the particle size became larger with the increase in theamount of the guar gum. With the increase in the amount of guargum in the solution resulted in an increase in the viscosity of the solution which resulted bigger particle formation. So after an optimum concentration of guar gum with increasing gum concentration particle size was also increasing.

Table1:influence of guar gum on blood hematological and biochemical parameter

Parameters	Control	Guargum(100mg/kg)	Guargum(200mg/kg)	Guargum(400mg/kg)
Hemoglobin(gm%)	14.41±0.023	14.40±0.034**	14.45±0.026**	14.38±0.011**
RBC(106/µl)	8.64±0.037	8.62±0.089**	8.71±0.201**	8.85±0.056**
WBC(103/µl)	13.73±0.068	13.65±0.048**	13.55±0.075**	14.02±0.022**
Neutrophil(103/µl)	21.77±0.658	22.01±0.358**	21.92±0.661**	22.56±0.454**
Monocyte(103/µl)	2.33±0.347	2.31±0.060**	2.55±0.214**	2.73±0.124**
Lymphocyte(103/µl)	73.56±0.347	73.67±0.627**	73.78±0.653**	72.89±0.583**
Eosinophil(103/µl)	2.35±0.177	2.32±0.748**	2.57±0.432**	2.21±0.654**
Platelets(103/µl)	1231.01±1.066	1238.00±1.811**	1241.00±0.713**	1241.01±1.036**
SGOT(U/l)	88.99±0.323	89.11±0.437**	89.42±0.552**	89.23±0.331**
SGPT(U/l)	32.63±0.541	32.32±0.643**	31.19±0.430**	33.14±0.473**

The each point represents the mean ± SEM. (n= 6 mice per group). **p<0.01 statistically significant when compared with the normal saline group and drug-treated group.

Fig. 4: Histopathological studies, HandE stained sections of liver and kidney slices of healthy rats. Loops without any inflammatory cells as compared to the normal healthy rat (marked by the box).SD; Standard derivationforn=6

Table2: Percentage of yield as well as drug entrapment efficiency of different formulations along with the different drug-gum ratio

Formulationcode	Drug:guar gum	%of yield	%of DEE
F1	1:0.22	42.20±0.83	30±1.45
F2	1:0.37	49.16±1.27	53.1±1.52
F3	1:0.50	49.58±0.63	59.5±1.07
F4	1:0.62	49.65±0.56	66.78±2.1
F5	1:0.75	52.14±0.28	50.15±2.23
F6	1:0.87	89.38±1.32	32.5±1.89
F7	1:1	94.84±1.88	35.75±1.47

SD; Standard deviation
forn = 3

Table3:The average particle size of drug-loaded microspheres with a different drug-gum ratio

Formulation code	Drug:guar gum	Average particle size (µm)	Zeta potential(-mV)
F1	1:0.22	327.08±2.45	11.13±0.11
F2	1:0.37	392.08±1.96	13.21±0.92
F3	1:0.50	397.78±3.61	10.35±0.12
F4	1:0.62	540.42±2.55	9.98±0.23
F5	1:0.75	548.77±1.39	9.32±0.45
F6	1:0.87	553.48±5.38	8.91±1.07
F7	1:1	556.48±4.15	8.21±0.15

SD; Standard deviation forn=3

4.6 Scanning electron microscopy (SEM) study

The SEM was used to detect the particle size distribution, surface morphology, texture and also to find the morphology of the fracture or sectioned surface of the prepared microspheres. The absence of pores in the microspheres was of primary importance for the underlying drug release mechanisms because drug releases through the water-filled cavities were much faster than through dense polymeric matrix network. All microspheres was spherical in shape; smooth surfaced and had

Small pores on the surface. In (fig.**5**) SEM studies showed the spherical surface of the microsphere. The surface of the wall of the microspheres were rough and showing small holes due to the vaporization of the solvent. The surface of drug-loaded microspheres was found to be smooth with small pores whereas the surface of blank microspheres was rough having larger pores. In the study, the microsphere formulation with guar gum prepared having smooth surface showed as low and prolonged drug release profile in comparison to the microspheres formulation without guar gum.

Fig. 5: A-microsphere containing active ingredient. B-The best-optimized formulation (f2) showing smooth texture on the outer surface with a fine round shape in the appearance. Little roughness in the external surface helped its fine attachment with the intestinal lumen. C-Theblankmicrospherewithoutthedrug, having asphericalshapeandroughinappearance. D-As,the concentration ofthegum,increasedinthe internalphasesoitproducedalargersizedue tothe increase inviscosityintheW1phase ofW1/O/W2microemulsion

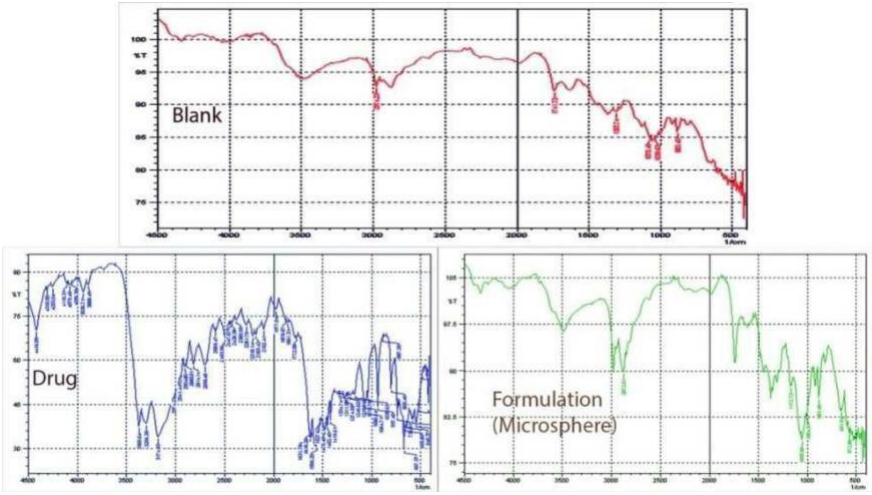


Fig. 6: 1**. Blank-No peak was found at 3172 cm-1 due to N-H stretching, At 1065 cm-1 no peak was found due to C-N stretching, At 1590 cm-1 no peak was found due to N-H bonding of the primary amine group. 2. Drug-At 3172 cm-1 sharp peak due to N-H stretching, At 1065 cm-1 peak was found due to C-N stretching, At 1590 cm-1 a peak was found due to N-H bonding. 3. The formulation-almost similar peak at3172 cm-1 due to N-H stretching, (Microsphere) At 1065 cm-1 a peak was found for C-N stretching, At 1590 cm-1 a little bit similar peakwas foundduetoN-Hbonding**

4.7 Fourier transform infrared spectroscopy (FTIR) study

The entire observed spectrum is presented in fig. **6** where the characteristic peaks of drug-loaded microspheres were compared with that of the standard spectrum of metformin. From the FTIR studies, it was seen that no chemical interaction happened between the drug metformin and guar gum and polymer ethyl cellulose. The absorption peak of the drug found at 3172 cm-1 due to N-H stretching of the primary amine group(-NH2). At 1065 cm-1 due to C-N stretching a peak of metformin wasfound. At 1590 cm-1 at peak due to N-H bending of the primary amine group. The same type of peaks appeared (N-H stretching, C-Nstretching and N-H bending vibrations) in the spectra of guar

gum containing microspheres of metformin. Although, similar characteristic peaks was not found in the blank microsphere formulations.

4.8 Dissolution study

The release profile of microsphere formulations was studied. The release profile in acidic solution was slow than an alkaline solution (pH-6.8) (fig. **8**). The collected drug release data from the dissolution test was evaluated using a number of kinetic models.

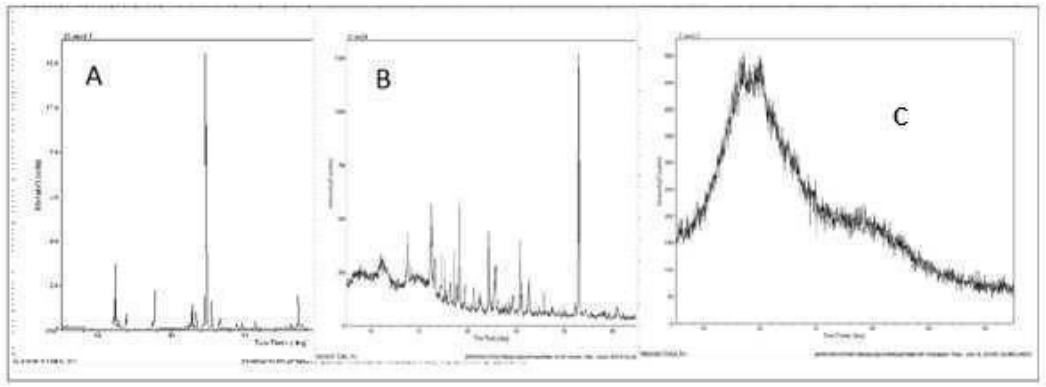


Fig. 7: A-Pure metformin introducing sharp crystalline peaks as there was no existence of polymorphic form. B-Metformin microsphere formulation containing both the drug and the excipients. Due to the presence of the drug crystalline peaks were found, whereas gums andexcipients were responsible for the existence of polymorphic forms in the chromatogram. C-XRD study of gum showing the amorphoustypeofpicturecontainingno crystallinepeaks

4.9 XRD study

Drug-loaded microsphere and guar gum were shown in the fig. **7**. The diffraction pattern of pure metformin and metformin loaded microsphere showed a crystalline peak, but guar gum alone produced anamorphous peak. The result showed that the crystalline peak of metformin and metformin loaded microsphere formulations were almost similar, but peak intensity diminishes in the formulation diffractogram due to dilution of the drug with gum and polymer. So, it can be said that the conducted XRD experiment also resulted

that metformin was compatible with gum andother excipients.

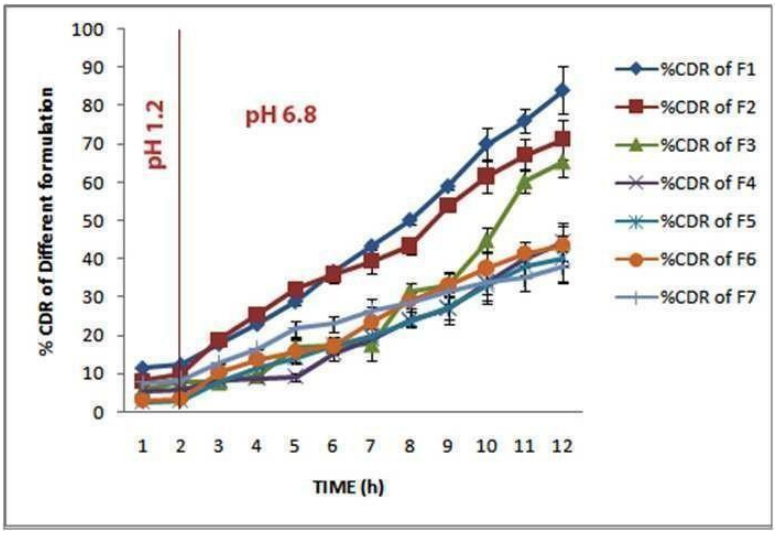


Fig.8:Dissolution profile of F1 toF batches are depicted here in the figure.F1batch is having the lowest concentration of gums showing the highest percentage of drug released (85%). The optimized formulation F2 is showing 72% of drug released in 12 h. With the concentration of the gum release of the drug from the microsphere was retarded. SD; Standard derivation form=3

The best-fitted model was analyzed by comparing the correlation coefficient values of different mathematical models in table 4. Fromthe recorded data the correlation coefficient value of drug loaded microsphere formulation (r2=0.99) was found to be higher and

suitable according to the percentage of yield, the percentage of DEE and particle size than the other drug loaded microsphere formulations. The Zero Order kinetic model was the best satisfactory mathematical model for the microsphere formulation (f2).

Table4: Drug release profile in dissolution media from different drug loaded microsphere formulations

Formulationcode	Zero-ord	er	Firstorde	r	Higuchi		Korsme y	er-peppas		Hixoncr	owell
	K0	R^2_0	K1	R^2_1	Kh	R^2_h	n	Kkp	R^2_{kp}	Khc	R^2_{hc}
F1	6.983	0.9833	-0.064	0.8986	29.39	0.9175	0.8929	0.9008	0.9419	0.211	0.9949

F2	5.90	0.9905	-0.045	0.957	26.87	0.9607	0.941	0.8245	0.9767	1.4521	0.9579
F3	5.423	0.8888	-0.0369	0.8161	20.65	0.7687	0.9646	0.5794	0.8503	0.206	0.9557
F4	3.6936	0.9411	-3.6936	0.9411	16.24	0.8537	0.926	0.5307	0.871	0.1745	0.9804
F5	3.547	0.9885	-0.02	0.9009	16.00	0.9436	1.1988	0.3045	0.9757	0.1873	0.9618
F6	3.924	0.9879	-3.924	0.9879	17.77	0.951	1.18	0.3742	0.9619	0.214	0.9765
F7	2.837	0.9831	-0.0161	0.9917	13.117	0.9855	0.7119	0.8061	0.9702	0.1274	0.9384

From the table, it is found that all the formulations released the drugs up to twelve hours. The best formulation F2 following zero-order drug release kinetics with almost 72% release of the drug

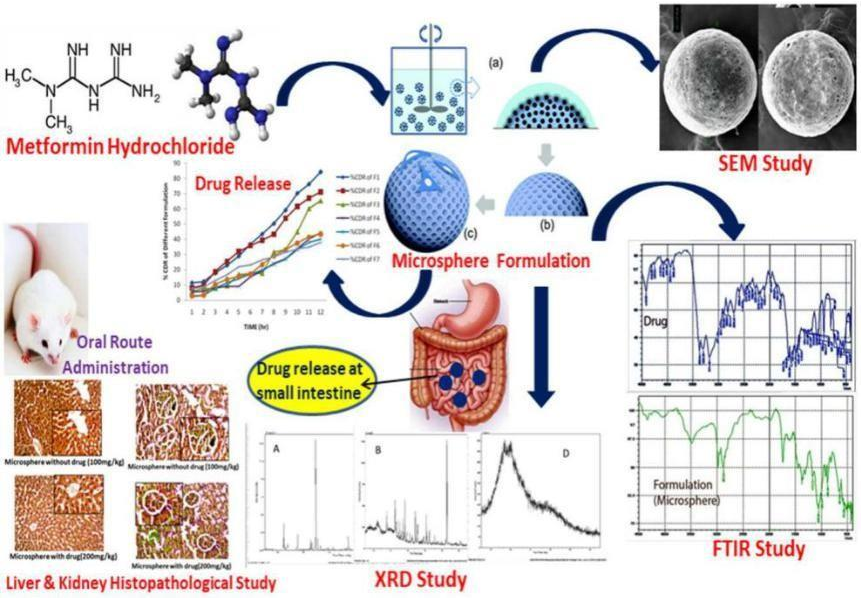


Fig.9Graphical abstract of metformin loaded microsphere

1. DISCUSSION

Guar gum and sodium alginate was used as matrix building materials whereas ethyl cellulose was applied as a coating polymer and these produced metformin loaded microsphere by W1/O/W2 emulsion solvent evaporation technique. Initial experiments resulted that a higher volume of the organic phase and internal aqueous phase as well as processing temperature considerably decreased DEE of the microsphere. If the volume of organic solvent increases, then the time required for vaporization of this solvent will be more which provides

Partitioning between the phases. So the entrapment of the microsphere decreased (66.78to35.75). volume of the external phase may influence the entrapment efficiency [308].

At the time of manufacturing of microsphere the building up of the matrix is an important phenomenon of DEE. This observation found alike with the results of other researchers [309-310]. In this research Drug Entrapment Efficiency also varied due to changes in the ratio of guar gum and these changes gave maximum DEE(66.78%) at a particular ration (drug : guar gum= e. This promoted more partitioning medicament between the phases. So the Drug Entrapment Efficiency of the also the percentage of yield of microcapsule was proportionate with the gum ratio (94.4at1:1drug:guar gum).

Increasing the amount of guar gum increases the size of the microsphere [311].the aqueous phase controls the size of the microsphere . Same result was observed by various workers [312].

Addition of guar gum concentration in the aquous phase changed the shape of microsphere (327.08 µm to 556.48µm). increasing guar gum conc would increase the size of the microsphere

droplets. In addition, an increase in viscosity of W1 phase made the primary W1/O emulsion more viscous and formed larger W1/O/W2 emulsion droplets. So the bigger size of microspheres found (556.48µm).

Table5:Parameter of the release of drug from the metformin loaded microsphere

Formulationcode	t50%(h)	t80%(h)
F1	7.53	11.85
F2	8.38	12.23
F3	10.82	b
F4	a	b
F5	a	b
F6	a	b
F7	a	b

---a Drug release was less than 50% in12h ---b Drug release was less than 80% in12h

The cumulative drug release formulated in varying concentration of gum of thew1 phase was not fast. Exchange of the dissolution medium after 2h with phosphate buffer (pH6.8) enhanced the drug release . At the same time, as the concentration of the gum in the W1phase was increased, cumulative drug release decreased. The mean time for 50% (t 50%) and 80% (t80%) drug release was determined from the cumulative percentage release versus time curves. Here t50% was found to increase from 7.53 h to

10.82 h, and t 80% increased from 16.35h to 11.85h, as the amount of gum in the internal aqueous phase increased that showed the microcapsule had the capability to retard the release. That shows it can be used as a sustained drug delivery formulation.

The higher the conc of gum in the aquous phase , increased the porosity of the wall of the microcapsules and depicted faster drug release [313]. The SEM studies showed small holes which is the result of leakage of water.

The drug release properties of the microspheres showed a regular release pattern of the drugs up to twelve hours. The f2 batch having lowest content of guar gum showing a reasonable release of drug for sustained delivery. But as the guar gum concentration increases the release of the drug becomes lower, possibly due to the more viscous nature in the W1 [315].

2. CONCLUSION

In this research work, guar gum and sodium alginate were used as a matrix building material. Guar gum matrix microsphere of metformin, an anti-diabetic drug, was prepared. Hematological, histopathological and toxicological data represented that guar gum is safe and non toxic. With the increasing guar gum concentration, it was found to entrap more amounts of drugs up to a certain extent, and the release of the drug is also dependent on the concentration of the guar gum in a sustained manner.

The release of the drug from the entire microsphere was shown in drug release curves . from this we establish the microsphere of metformin using guar gum.

having high DEE that may release less amount of drug in stomach minimizing the emergence of gastric adverse effects and at the same time may produce a prolonged release in the intestine to obtain better drug therapy.

Chapter - 5

Preparation of microsphere and its anti diabetic activity

Contents

Preparation of the microsphere and its antidabetic activity

INTRODUCTION

The oral administration route always implies prominence in any therapy due to its well-established advantages[316]. However oral route of administration produces gastric side effects[317]. Instant release of drug do not produce sustained action in the body[318]. To overcome these problems researchers have developed controlled release drug delivery systems. Some sustained release preparations are already available in the market. From the intestine the drug is circulated throughout the body. The extended contact of the microsphere with the absorbing membrane allows an increase in drug bioavailability.[319-321]. For this reason various modified drug delivery systems are formulated,. They are bioadhesive , mucoadhesive systems, colon targeted microparticle system, liposome, nanoparticle and intestinal targeted microsphere.[322].

"A controlled release microsphere formulation of an antidiabetic drug and characterization of the microsphere" we formulated W1/O/W2 emulsion microsphere. The metformin loaded microsphere was prepared by multiple emulsion solvent evaporation technique. Here the article revealed that the increase in gum concentration in the W1 phase, increases viscosity in the W1 phase. This results in an increase in the drag entrapment in the optimum level. It also decreases the release rate. So, it can prolong the action. By using this tool, we can say that metformin-loaded microsphere formulation would be a suitable pharmaceutical formulation for the treatment of diabetic patients in modern drug therapy for its prolonged action. Here we have observed in-vivo antidiabetic effects in Wistar albino rats. Further, we also assayed the hematological and biochemical parameters of the studied animals. Histopathology of the liver, kidney, and pancreas was also tested.

MATERIALS AND METHODS

2.1. Chemicals

Metformin was gifted from Stadmed Pharmaceuticals, Kolkata, India. Ethyl cellulose was purchased from Quest chemicals Kolkata, India. Dichloromethane, tween-80, and span 80 were purchased from Merck India. All other reagents were of analytical grade. Guar gum was collected from the Bikaner district of Rajasthan, India. Streptozotocin (STZ) was purchased from Sigma Aldrich, India.

Formulation Development

2.2.1. *Preparation of stock solution*

Metformin in 50 ml of distilled water kept in a100ml of volumetric flask. The solution was then made to 100ml by adding distilled water. Thus 100 µg/ml stock solution was prepared[2].

Determination of λ max

10µg/ml of drug solution was prepared from the stock solution. The absorbance in the UV spectrum was determined by scanning a range of 200 nm to 400nm. The maximum absorbance was found at 233 nm. Distilled water was used as blank. So, the λ max of the sample was 233 nm.

2.2.3. Preparation of standard curve

Seven-point calibration curve was prepared by spiking appropriate amounts of the stock solution into the corresponding buffer. Absorbance was studied at 233 nm. A calibration curve was prepared by plotting the absorbance against the concentration.

2.2.4. Swelling Index

1gm of guar gum was added in pH 6.8 buffer solution, 10 ml of distilled water, and 01N HCI solution. Then it was shaken for 10 minutes in a magnetic stirrer at high speed. It was allowed to stand for 24 hours. Swelling capacity was measured using formula 1

$$\%\ of\ weight\ change = \frac{Xv - Xi}{Xi} X\ 100$$

Where Xv= final volume
Xi= initial volume

2.2.5. *Preparation of metformin loaded microsphere*

A small amount of sodium alginate was dissolved in 5 ml of distilled water and placed on a magnet stirrer. Guar gum was added to it with continuous stirring. 80 mg of metformin was added to it and stirring was done for almost half an hour by a magnetic stirrer. Then the preparation was taken with a 20-garage needle. An organic solution was prepared with 1 gm of ethyl cellulose and 30 ml of dichloromethane (DCM). Then 300µl of span 80 was added to this organic solution. A primary emulsion of the W/O type was produced2.
A water phase was taken with 100ml of distilled water in a beaker. Tween 80, 200 µL was added into it. The beaker was placed under a magnetic stirrer. The stirrer was rotating at 800 rpm for 3.5 hours. Small, spherical microspheres were filtered and collected. After drying they are stored in desicator.

2.2.6. Characterization of prepared microsphere

Practical yield is the weight of the microsphere obtained. The theoretical yield is the total weight of the raw materials .

$$Percentage\ Yield = \frac{practicalyield}{theoretical\ yieldtheoreticalyield} X\ 100$$

2.2.7. Drug entrapment Efficiency

40 mg of prepared microspheres was correctly triturated and made into powder form. Then 100 ml of phosphate buffer (pH 6.8) was added into it and was subjected to a magnetic stirrer for 2 hours. Filtration of the solution was done by the Whatman filter paper. 10 ml of this stock solution was diluted with phosphate buffer (pH 6.8) and analyzed for metformin content at 233 nm

Drug entrapment efficiency (DEE) $= \frac{Experimental Drug content}{theoretical drug content} X\ 100$

2.2.8. Particle size distribution and zeta potential

The particle size distribution was obtained from the optical microscopic method. The mean average diameters of the microsphere particles was obtained in the SEM studies. A weighed quantity of the experimental sample was dispersed in Milli-Q water (Milli-Q, Merck Millipore, Billerica, MA, USA)by vertexing and then sonicated and placed in a cuvette for zeta potential measurement .

2.2.9. Scanning electron microscopy (SEM) analysis

Particle size, shape, and surface morphology was detected by SEM analysis. SEM was done by CARLZEISS EVO 18 special edition machine with the platinum coating. The platinum coating was done by QUORUM Q150 TES machine.

2.2.10. Fourier Transform Infrared Spectroscopy (FTIR) Study

FTIR was done. It was done on IR-Prestige 21, Shimadzu, Japan

2.2.11. X-ray diffraction (XRD) studies

The samples of various batches were evaluated by X-ray diffraction studies. XRD studies were done by X-ray diffractometer of model no Ulcinia-111,

Renuka (Japan), Cu target slide 10 nm. The possible drug-polymer interaction was detected by XRD studies of the drug and the drug-loaded microspheres.

2.2.12. Drug release study

A drug release study was done in the dissolution test apparatus, LAB INDIA DS 8000 USP -type 2(paddle type) apparatus calibrated at 37°C and rotated at 50 RPM. In the dissolution test apparatus initially 750 ml of acid buffer of pH-1.2 was added. Microspheres of 50 mg was added into it. Then after 2 hours, 150 ml of trisodium orthophosphate buffer solution was added into the acidic solution so that the solution's resultant pH became 6.8. From the final solution, 5ml of the sample was withdrawn every 1 hr, and it was replaced by 5ml ml phosphate buffer of pH 6.8 every hr. The same thing was done at initial 2 hrs maintaining pH 1.2. This process continued for 12 hours[324].

In vivo study of the metformin loaded microsphere

Animals and the maintenance

Studying animals of both sex, 180 to 220 grams for rats (n=6) were done maintaining all the standard protocols.

2.3.2 Incorporation of hyperglycemia

It was done by streptozotocin and it was injected 5mg/k body weight.Animal swere experimental by following orders.

Group-1 Normal rats were treated with distilled water.

Group -2 There were diabetic control rats treated with distilled water.

Group-3 Standard drug metformin was given orally as 80 mg/kg b.w.

Group-4 Metformin loaded microsphere was given orally at a dose of 150 mg/kg. b.w.

Group-5 Metformin loaded microspheres 150 mg/kg. b.w. with thiamine 70 mg/kg b.w. was given orally.

Blood Samples (0.5-0.6 ml) were obtained from the tail vein in cold heparinized tubes at- 0 hr, 1 hr, 3hr, 5 hr, 7 hr to estimate blood glucose level. Thereafter through centrifugation plasma was separated and put at -20°C. The plasma glucose concentration was evaluated by the method of glucose oxidase-peroxides using Span Diagnostic kits.

STATISTICAL ANALYSIS

RESULT AND DISCUSSION

3.1. Formulation Development

Guar gum and sodium alginate were used as matrix building materials, Ethyl cellulose was applied as a coating polymer. This produced metformin loaded microspheres it was found that higher concentration of gum in the matrix reduced the drug entrapment . it is also effected by the higher amount of dichloromethane. As a result the DEE of the microsphere decreased 66.78 to 3.75. this is due to the peculiar behavior of the guar gum also. At the same time the higher concentration of the gum in the w1 phase may produce more thirst on the w2 phase resulting a shift of the drug between the phases resulting decreased DEE. [325-326]. In this research, DEE also varied due to changes in guar gums ratio. These changes gave maximum DEE (66.78 %) at a particular ratio (drug: guar gum = 1:0.62). The percentage of yield of the microsphere was proportional with the gum ratio (94.4 at 1.1 drug: guar gum) (**Table 3**).

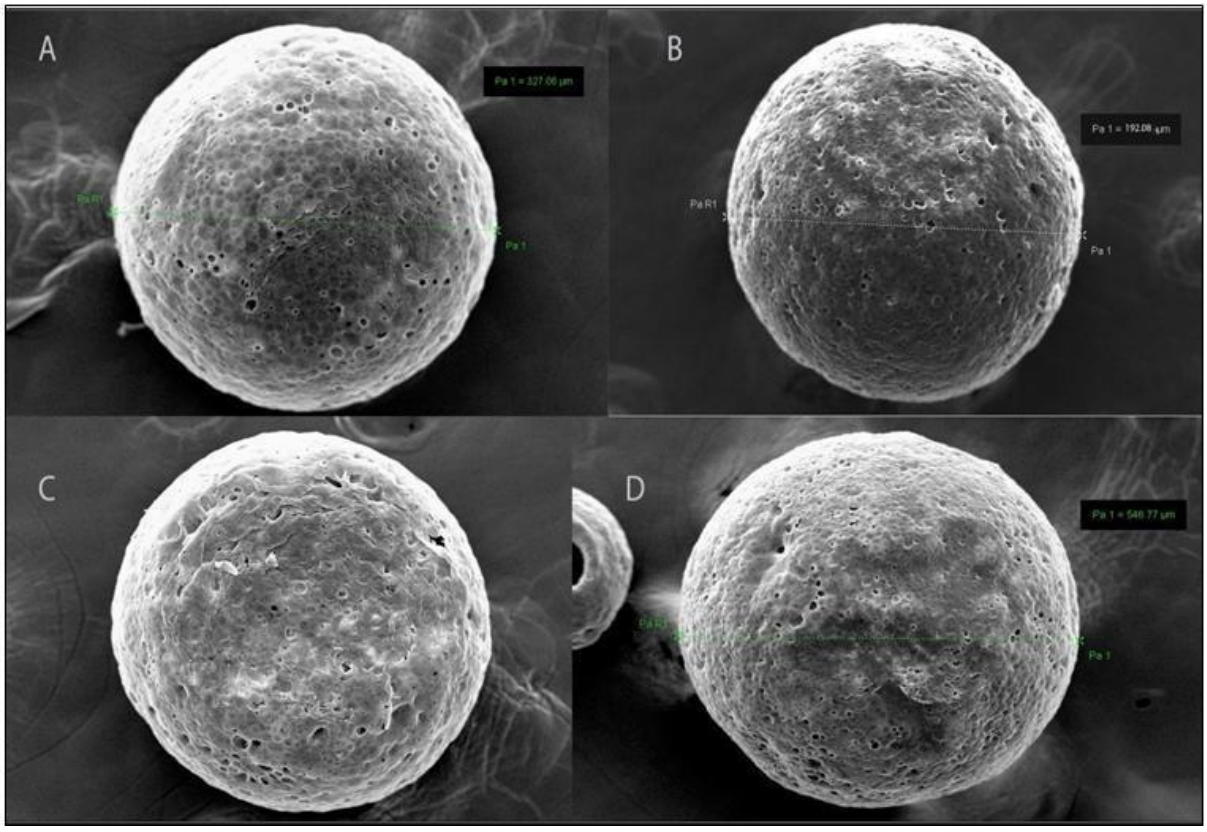


Fig 2: A- Drug-loaded microspheres. B- The best-optimized formulation (f2) showing smooth texture on the outer surface with a fine round shape in the appearance. Little roughness in the external surface helped its fine attachment with the intestinal lumen. C- The blank microsphere without the drug, having a spherical shape and rough in appearance. D-As the concentration of the gum increased in the internal phase so it produced a larger size due to the increase in viscosity in the W1 phase of W1/O/W2 micro emulsion [2].

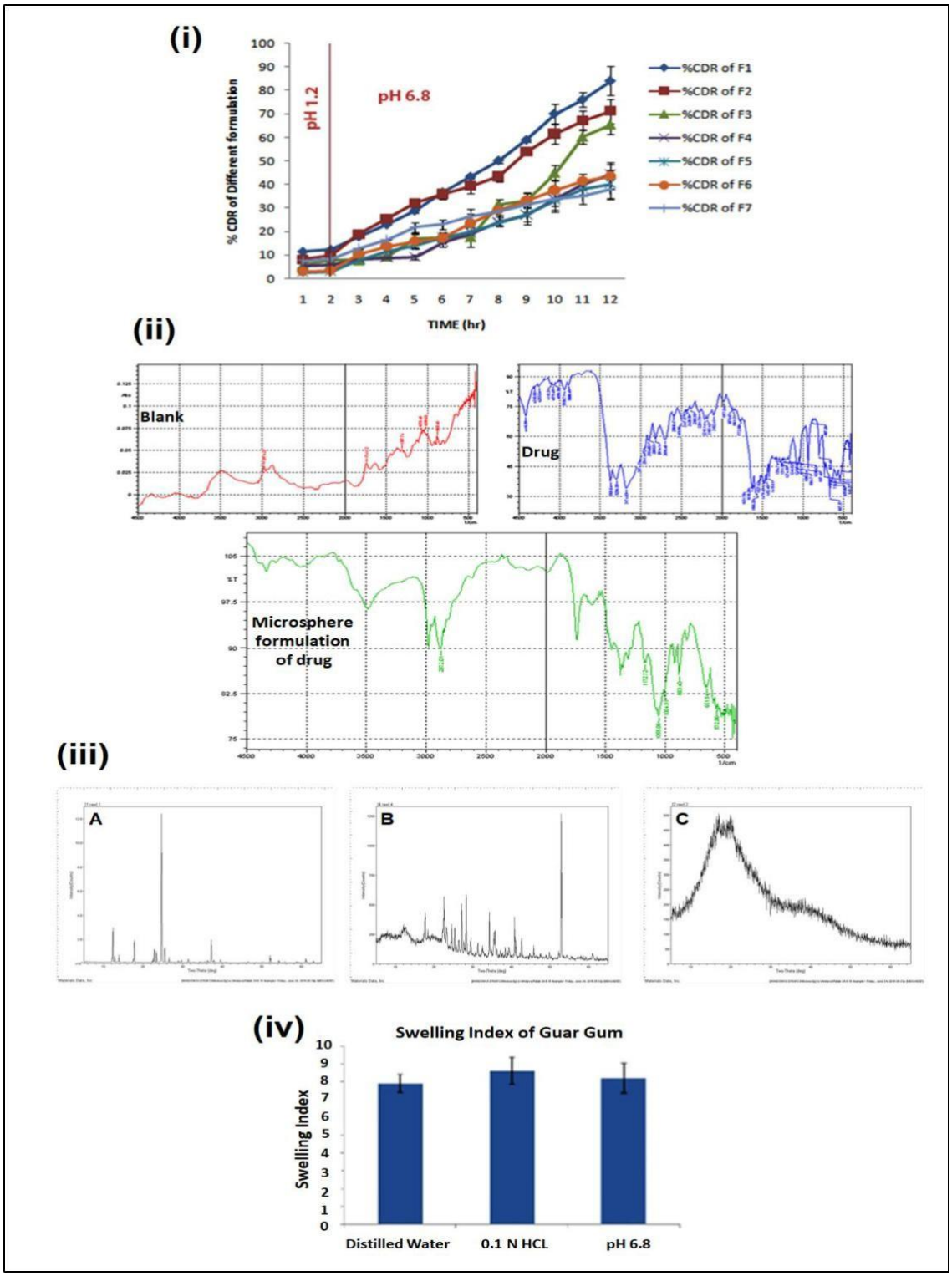


Figure 3: (i) Dissolution profile of F1 to F7 batches are depicted here in the figure. F1 batch having lowest concentration of gum showing highest percentage of drug released (85%). The optimized formulation F2 is showing 72% of drugs released in 12hrs. Fig. (ii) FTIR study of (A) blank microsphere (B) pure metformin and (C) metformin loaded microsphere. Fig. (iii) XRD

study of (A) pure metformin (B) metformin loaded microsphere and (C) gum. Fig (iv) represents the swelling characteristic of guar gum at different pH (1.2 and 6.8). SD; Standard deviation of n=3 [2].

As there was a rise in the concentration of guar gum so there was an increase in the size of the microsphere . 327-328 as guar gum is an extreamly viscous material so it can build the volume . and as there is a big rise in the volume so the size of the microsphere was also increased . thus the addition of guar gum in the W1 phase also affected the matrix microsphere's size. (327.08µm to 556.48 µm) microsphere size was increased due to the addition of guar gum (able -3) guar gum was a viscosity builder for the preparation resulting in a higher size of the microsphere (556.48 µm) (Figure-2) Here we have used acidic Ph (1-2) and alkaline Ph (6.8)to check the release of the drug. The release of the drug was higher in alkaline Ph . this may be due to its solubility.

A cumulative percentage release curve was drawn from the release rate. This showed a zero order release profile of the formulation. The drug was released through twelve hours all these datas are enough to establish its sustained release profile. The higher the volume of the W1 phase more increase the pressure in the barrier. As a result release rate will be higher. [329]

simultaneously water from the external aqueous phase back diffuses into the droplets. The back diffusion was related to the difference in the Osmolarity between the internal and external phases. The greater the back diffusion, the greater is the leakage of water [330], and hence the wall of the microcapsules became more porous providing faster drug release. The microspheres' drug release properties showed a regular release pattern of the drugs for twelve hours. The f2 batch had the lowest guar gum content showing a reasonable release of drug for sustained delivery. However, as the guar gum concentration increases, the drug release becomes lower, possibly due to the more viscous nature in the W1 phase, with the increase in the concentration of guar gum in the W1 phase hindered the release of the drug[331-332]. The resultant formulations were produced by optimizing the process through the W1/O/W2 solvent evaporation technique. The resultant microsphere's diameter was found 392.08. ± 1.96. Here, within the limit of our experimentation, the percentage yield and DEE were 49.16 1.27 and 53.1+1.52, respectively. Moreover, the release of drugs from the optimized microsphere at 12 hrs was found to be 72%. The swelling capacity of the optimized microsphere in distilled water, 0.1NHCl, and pH 6.8 buffers were represented in **Figure 3**. The optimized microsphere resulted in good swelling behavior at pH6.8. The conducted XRD experiment also demonstrated that metformin was compatible with gum and other excipients. The SEM study revealed that the optimized microspheres have a good spherical appearance and anticipated surface morphology[33-334]. The best-fitted model was analyzed by comparing the correlation coefficient values of different mathematical models in **Table 1.** Drug release profile in dissolution media from different drug loaded microsphere formulations. The correlation coefficient value of optimized microsphere formulation (r2=0.99) was found to be higher and suitable than the other drug-loaded microsphere formulations from the recorded data. The Zero order kinetic model was the best satisfactory mathematical model for the microsphere formulation [f2][2].

Table1: Drug release profile in dilution media from different drug loaded microsphere formulation.[2]

Formulation code	Zero order		First order		Higuchi		Korsmeyer –Pepas			Hixon crowell	
	K_0	R^2_0	K_1	R^2_1	K_h	R^2_h	N	K_{kp}	R^2_{KP}	K_{hc}	R^2_{hc}
F1	6.983	0.9833	-0.064	0.8986	29.39	09175	0.8929	0.9008	099419	0.211	0.949
F2	5.90	0.9905	-0.045	0.957	26.87	0.9607	0.941	0.8245	0.9767	1.4521	0.9579
F3	5.423	0.8888	-0.0369	0.8161	20.65	0.7689	0.9646	0.5794	0.8503	0.206	0.9557
F4	3.6936	0.9411	-3.6936	0.9411	16.24	0.8537	0.926	0.5307	0.871	0.1745	0.9804
F5	3.547	0.9885	-0.02	0.9009	16.00	0.9436	1.1988	0.3045	0.9757	0.1873	0.9618
F6	3.924	0.9879	-3.924	0.9879	17.77	0.951	1.18	0.3742	0.9619	0.214	0.9765
F7	2.837	0.9831	-0.0161	0.9917	13.117	0.9855	0.7119	0.8061	0.9702	0.1274	0.9384

Table2: parameters of the release of drug from metformin loaded microsphere [2]

Formulation code	$T_{50\%}$(h)	$T_{80\%}$(h)
F1	**7.53**	**11.85**
F2	**8.38**	**12.23**
F3	**10.82**	---[b]
F4	---[a]	---[b]
F5	---[a]	---[b]
F6	---[a]	---[b]
F7	---[a]	---[b]

---[a] drug release was less than 50% in 12 h. ---[b] drug release was less than 80% in 12 h.

Table3: the average particle size of drug loaded microsphere with a difference drug –gum ratio[2]

Formulation code	Drug: guar gum	% of yield	% of DEE	Average particle size (m)	Zeta potential (-Mv)

F1	1:0.22	42.20±0.83	30±1.45	327.08±2.45	11.13±0.11
F2	1:0.37	49.16±1.27	53.1±1.52	392.08±1.96	13.21±0.92
F3	1:0.50	49.58±0.63	59.5±1.07	397.78±3.61	10.35±0.12
F4	1:0.62	49.65±0.56	66.78±2.1	540.42±2.55	9.98±0.23
F5	1:0.75	52.14±0.28	50.15±2.23	548.77±1.39	9.32±0.45
F6	1:0.87	89.38±1.32	32.5±1.89	553.48±5.38	8.91±1.07
F7	1:1	94.84±1.88	35.75±1.47	556.48±4.15	8.21±0.15

Each point represents the mean ±SEM (n=6). Values are expressed as mean ±SEM mean values are significantly different from each other ($^{**}p<0.01$)

3.2. In-vitro antidiabetic activity

Metformin is a well-known anti-diabetic drug from times immemorial. It reduces blood sugar, and so is important stuff in the anti-diabetic group of drugs. While provided in microsphere form as the spherical shape produces a larger surface area, the probability of adsorption increases bioavailability. At the same time, it provides controlled release of the drug's effect, so the drug's effect is for a longer duration. The gastro-irritant side effect of the drug is countered as the drug is released in the small intestine. All these positive results make someone interested in preparing a microsphere and having a study on it.

Effect of drug loaded microsphere treatment in blood glucose

The blood glucose and lipid-lowering impression of metformin on Streptozotocin-induced diabetic mice was previously well documented [335-336]. In the present investigation the blood glucose level of metformin loaded microsphere and metformin loaded microsphere with thiamine group animals were observed to be 238.14±1.42 mg/dl and 234.25±12.03 mg /dl on the initial treatment (0^{th} day). The significant decline in the blood glucose level of 186.07± 3.51 mg/dL metformin loaded microsphere treated group and 185.0±2.11 mg/dL in metformin loaded microsphere with thiamine-treated animals were seen on the 14th day of oral drug administration. On the 21st day, oral drug administration of metformin loaded microsphere, the notable decline in the blood glucose level to 121.10 ± 5.18 mg/dL. It was 120.02±1.04 mg/dL in metformin loaded microsphere with thiamine-treated group (**Table 4**).

Table4: Effect of metformin loaded microsphere on fasting blood glucose level on streptozotocin induced rats after a single dose (n=6)

Drug	(Mg/kg)	Day 0	Day 7	Day 14	Day 21
Normal Control	-	77.0±1.82	78.25±0.95	77.75±2.21	79.50±2.64
Diabetic control	150	246.25±9.53**	284.0±5.47**	309.50±8.73**	319.75±7.80**

Metformin free drug	80	233.01±12.03**	216.75±8.65**	190.50±4.18**	126.15±4.07**
Metformin loaded microsphere	150	238.04±1.42**	211.50±4.65**	186.07±3.51**	121.10±5.18**
Metformin loaded microsphere & thiamine	150	234.25±12.03**	210.0±4.10**	185.0±2.11**	120.02±1.04**

*Values are expressed as mean ± S.E.M. mean values are significantly different from each other (**P<0.01).*

This propitious remission of diabetic condition was significant with the metformin 80 mg/kg treated group value of 126.15+ 4.07 mg/dL **(Table 4).**Throughout the 21 days study period, the increasing level of blood glucose was seen in diabetic control animals due to the untreated situations. The control group animals displayed no adverse modification. The reverse blood glucose level was observed on the 7th day of the metformin 80 mg/kg treated animal group. (**Table 4)**. It showed the blood glucose level changes in all the experimental animal groups. The treatment of metformin loaded microsphere and metformin loaded microsphere with thiamine restrain the diabetic condition meaningful with the standard metformin 80 mg/kg treated group.

Oral glucose tolerance test (GTT)

In animal experimentation, the GTT is practiced appraising the degree of diabetes and testing the wanted effects of insulin or other drugs on the body's capability in processing glucose. It can also be utilized to recognize the unintended side-effects of medicines intended to treat other unrelated diseases. All the animals were treated with glucose (2gm/kg b.w.). Normal control, diabetic control, metformin powder standard group, metformin microsphere test group and metformin microsphere with thiamine group[337-339].

Table5: Effect of metformin loaded microsphere on blood glucose level of streptozotocin induced diabetic rats.

Drug	mg/kg b.w.	Initial	1Hr	3hr	5 hr	7 hr
Diabetic control	150	220.0 ±1.05	245.2±1.39	262.9±0.75	264.7±0.98	266.2±0.95
Metformin free drug	80	228.4±1.77	175.3±2.18** *	170.0±2.18** *	151.4±1.65** *	131.3±0.60** *
Metformin loaded microsphere	150	226.8±1.55	174.2±2.18** *	160.2±2.18** *	148.3±1.12** *	129.8±1.17** *
Metformin loaded microsphere & thiamine	150	222.6±1.77	169.3±2.18** *	165.0±2.18** *	160.0±4.39** *	130.2±0.60** *

*Data are represented as mean± S.E.M. ***p<0.001 when compared to control. n=6 per group.*

In the normal control rats, there was an almost minimal increase in the blood glucose level. In the diabetic control groups, there was a significant rise in the blood glucose level in 60 min, and then it was sustained at 120 min and 180 min (**Table5**). The standard (metformin powder) group significantly restrain the rise in blood glucose and the same thing found in the test - I (metformin loaded microsphere) and test 2 (metformin leaded microsphere - thiamin) groups. The insulin response curve in type I (absolute insulin deficiency) diabetes describes

the pancreas inability to release insulin in reply to the glucose load. The deficiency of an insulin response, which is accountable for the failure of the diabetic to utilize the added glucose, prolonged hyperglycemia happens. A crucial factor uniting hyperglycemia occurs the overproduction of glucose by the liver. The test dose of glucose stays in effect, added to the already being oversupply of glucose. Because the steady-state level at which the liver stops to supply or withdraw glucose is elevated in diabetes, the liver continues to oversupply glucose, contributing to the tolerance curve's slow return to its original level.

Table 6: Effect of metformin loaded microsphere on blood glucose level of streptozotocin induced diabetic rats.

Drug	(mg/kg b.w.)	Day 1	Day 7	Day 14	Day 21
Normal Control	-	202.1± 1.9	204.83±1.02	210.00±1.05	216.83±1.52
Diabetic control	150	229.2±2.1**	145.00±5.2**	122.33±2.51**	110.31±1.62**
Metformin free drug	80	228.15±1.62**	209.02±1.3**	215.20±2.4**	225.07±3.6**
Metformin loaded microsphere	150	227.25±0.73**	218.66±0.63**	222.83±0.55**	226.56±0.82**
Metformin loaded microsphere & thiamine	150	228.19±0.28**	211.58±0.30**	220.39±0.30**	227.37±1.83**

Values are given in average body weight (g) ±SEM for groups of six animals each. ** *p<0.001.*

Drug induced modulation of hepatic maker (liver function) enzyme and kidney function

Table7: The effect of metformin loaded microspheres on liver function markers in streptozotocin induced diabetic albino rats.

Drug	(mg/kg b.w.)	AST (IU/L)	ALT (IU/L)	LDH (IU/L)
Normal Control	-	14.55±0.63	18.91±0.56	116.16±2.6
Diabetic control	150	17.35±0.15***	33.60±7.75***	314.50±5.9***
Metformin free drug	80	12.70±1.38***	19.62±3.35***	119.38±2.8***
Metformin loaded microsphere	150	12.52±1.24***	19.17±0.66***	117.64±3.4***
Metformin loaded microsphere & thiamine	150	12.10±1.26***	18.30±2.42***	117.15±1.9***

Values are given in average body weight (g) ±SEM for groups of six animals each. *** *p<0.001.*

The metformin-loaded microsphere and metformin-loaded microsphere with thiamine treated group's hepatoprotective enzymes showed the significance with diabetic control group level of enzymes. This also represented the microsphere treated group to give some hepatic protection capability. This may slow the release properties of microsphere at the 21st day of treatment[340-345].

Table 8: The effect of metformin loaded microsphere on kidney function markers in streptozocin induced diabetic albino rats

Drug	(mg/kg b.w.)	Creatinine (mg/dl)	Urea (mg/Dl)
Normal Control	-	0.54±0.3	31.83±2.2
Diabetic control	150	57. ±0.1***	69.5±1.8***
Metformin free drug	80	0.59±0.2***	31.38±3.5***
Metformin loaded microsphere	150	0.56±0.3***	32.19±1.5***
Metformin loaded microsphere & thiamine	150	0.57±0.2***	31.30±2.1***

Values are given as mean ± SEM for groups of six animals each ***$p<0.001$*

The metformin powder treated group and metformin loaded microsphere treated group shows the control level of urea viz., 31.38±3.5. v mg/dL and creatinine 0.59 ± 0.2, 0.56±0.3 mg/dL are (*$p<0.05$) significant with the normal control group 31.83± 2.2, 0.54 ±0.3 mg/dl **(Table 8).** The increased urea and creatinine levels in the diabetic control group shows adverse damage to the organs. The formulated drug have potentially recovered the kidney and liver from the damaged condition. However, the retrieval of liver and kidney marker enzymes and kidney function indicators shows the treated formulation can control the diabetic condition[346-351].

Effect of total cholesterol and triglyceride

Table 9: Effect of metformin loaded microsphere on serum on total lipid profile in streptozotocin induced diabetic albino rats

Drug	(mg/kg b.w.)	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
Normal Control	-	145.36±3.2	86.83±5.5	3.83±2.5	91.32±1.2
Diabetic control	150	271.16±10.5**	200.83±1.6**	20.05±1.9**	189±12.4**
Metformin free drug	80	148.65±5.6**	90.21±2.9**	36.63±2.1**	93.65±3.6**
Metformin loaded microsphere	150	145.61±3.6**	88.12±5.1**	36.47±2.6**	95.21±3.7**
Metformin loaded microsphere & thiamine	150	145.18±2.9**	87.20±5.2**	34.17±2.5**	93.54±3.8**

Values are given as mean ± SEM for groups of six animals each ***$p<0.01$*.

Histopathology of liver, kidney and pancreas

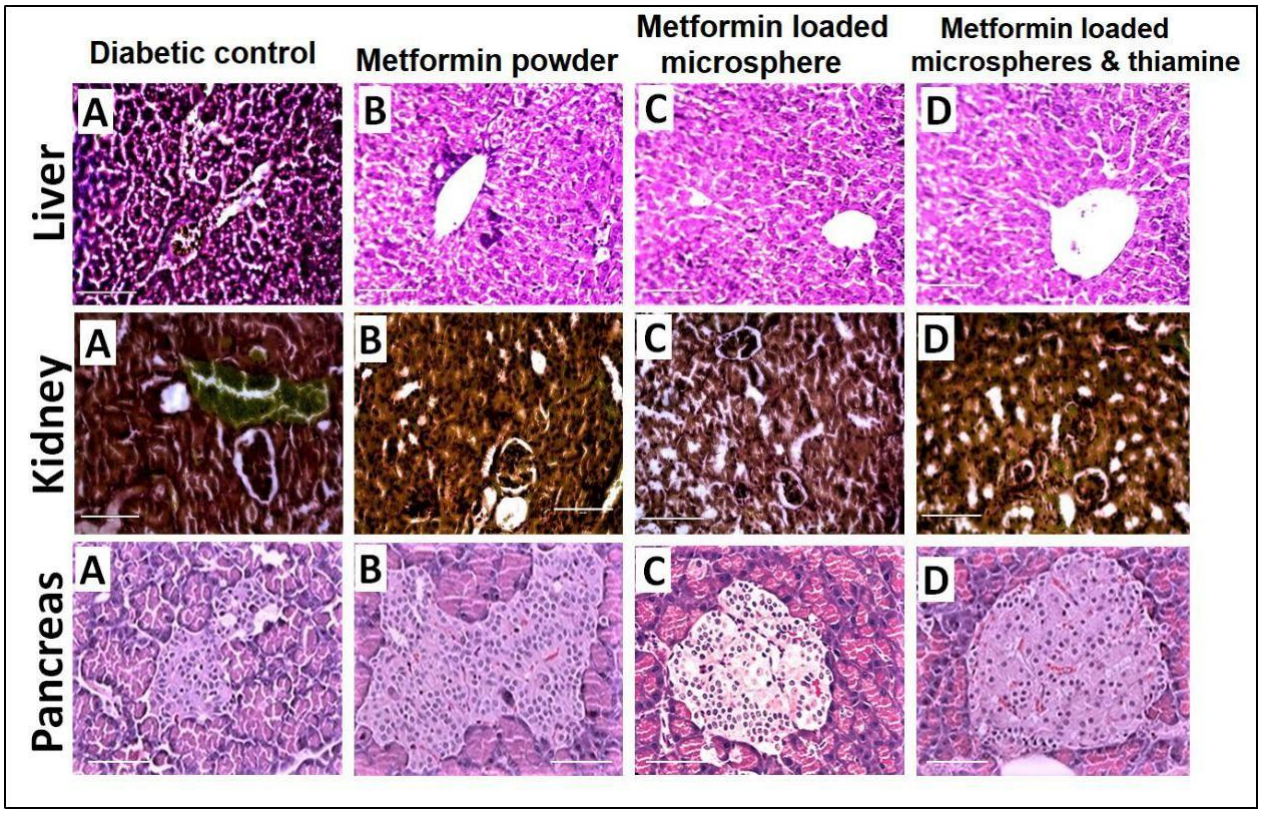


Fig 4:H & E stained section of liver, kidney and pancreas of treated rats showing cell morphology of groups (A) diabetic control (B) metformin powder (C) metformin loaded microsphere and (D) metformin loaded microsphere with thiamine.

The histology of pancreas cells was 10X if the light microscope **(Figure 4).** The clumping structures in the Islets of Langerhans was seen. The acne beta cell structure was shrunken, necrotic lobular and improper arrangements observed in the diabetic control group. The 21 days oral drug-treated group exhibits the proliferation of islets of Langerhans with recovered lobular cell arrangements observed in the metformin-loaded microsphere group metformin-loaded microsphere with thiamine treated groups.

Gluconeogenic enzymes in the liver

The increased level of glucose-6-Phosphatase in the liver 11.02±1.09 observed in the diabetic control group **(Table 10)** and the succinate dehydrogenase and malate dehydrogenase was 2.07±0.39 and 1.16±0.07 in the liver was noted.

Table 10: Effect of Metformin loaded microsphere on enzymes of glucose metabolism in Streptozotocin induced diabetic

rats.

	Normal control	Diabetic control	Metformin free drug	Metformin loaded microsphere	Metformin loaded microsphere & thiamine
Glucose-6- phosphate dehydrogenase liver (nmol NADP+ reduced /min/mg protein) lactate dehydrogenase	19.33±1.31	11.02±1.09**	18.96±0.76**	19.04±0.24**	19.16±0.38**
Liver (nmol pyruvate formed/min/mg protein)	63.16±2.58	78.49±2.84**	62.80±5.93**	63.25±4.47**	63.07±4.82**
Succinate dehydrogenase Liver (umol NADH oxidized /min/mg protein)	4.16±0.40	2.07±0.39**	5.89±0.68**	4.63±0.29**	4.15±0.21**
malate dehydrogenase Liver (umol NADH oxidized /min/mg protein)	1.56±0.14	1.16±0.07**	1.45±0.21**	1.50±0.21**	1.54±0.12**
Plasma (umol NADH oxidized/min/ml)	0.88±0.13	0.52±0.10**	0.82±0.19**	0.84±0.15**	0.86±0.18**

Values expressed as mean SEM (n=6). ** *p<0.01, in comparison with normal and diabetic control*

Carbohydrate metabolic enzymes & glycogen

The results of glucose-6-phosphate dehydrogenase in the diabetic control group, metformin 80 mg/kg, metformin loaded microsphere, and metformin loaded microsphere with thiamine treated groups are viz., 11.02±1.09, 18.96±0.76, 19.04±0.24, and 19,16±0.38 IU/h/mg protein **(Table 11),** and the succinate dehydrogenase was 2.07±0.39,5.89±0.68, 4.63±0.29 and 4.15±0.2 IU/mg protein **(Table 11);** malate dehydrogenase in liver tissue are 1.16±0.07, 1.45±0.21, 1.50±0.21 and 1.54±0.12 IU/h/mg protein and liver glycogen in liver tissue 1.07±0.93, 4.52 ±1.82, 4.50±1.29 and 4.53±1.30 Rodriguez et al found the relationship between nonalcoholic liver disease and blood glucose, serum insulin. It was found in stz induced diabetic rats[38]. With the application of formulated drug in the diabetic complication was reduced.

Table 11: Effect of Metformin loaded microsphere of liver glycogen and glycogen synthase in Streptozotocin induced diabetic albino rats.

Drug	(mg/kg)	Liver glycogen (mg/g wet tissue)	Glycogen synthase (mol UDP formed/min/mg protein)

Normal control	-	4.95±0.87	3.01±0.19
Diabetic control	150	1.07±0.93***	0.77±0.18***
Metformin free drug	80	4.52±1.82***	2.81±0.11***
Metformin loaded microsphere	150	4.50±1.29***	2.75±0.16***
Metformin loaded microsphere & thiamine	150	4.58±1.30***	2.81±0.17***

Values expressed as mean SEM (n=6). *** *p< 0.001*

CONCLUSION

The results of the whole study indicate that microcapsules prepared with guar gum as matrix material could be a suitable way to target specific ways. At the same time it may provide prolonged release in the intestine to achieve better drug therapy when the formulated drug was applied in suiss albino rats, when the formulated drug was applied in swiss albino rats, we found good results to control dibetes, lipid profile and beta cell regeration. This was due to thiamine and its antioxident activity thiamine antioxidant activity[352-354] Metformin nanoparticles also showed better antidiabetic effect[355] . But metformin in a microsphere form has not been evaluated in details so far. In this study, metformin in a microsphere form shows controlled release and sustained delivery and also better efficacy at lower dose when compared to metformin in a free form. Variation in the histopathology of liver, kidney and pancreas of the treated animals resulted from the control released design having a slow-release rate, giving less trace of liver and kidney function. Hematological and biochemical analysis revealed that ALT and AST, creatinine and urea level of metformin loaded microspheres was better tolerated when compared to the diabetic control. Also, the level of liver glycogen, Glucose-6-phosphate dehydrogenase, succinate dehydrogenase, and malate dehydrogenase content were found to be modulated significantly with the metformin loaded microsphere treated group. The significant control in blood glucose level and inclined body weight, food, and water intake were observed after 21 days of the drug induction. A similar range of VLDL and the other lipid profile, especially HDL level, offers the combined metformin-loaded microsphere reversal effect on cholesterol and cardiovascular risk. Hence, further studies have been needed to explore the molecular release behavior, enhancement mechanism, and the individual effect of this novel microsphere activity against diabetes and consequent metabolic disorders.

Chapter - 6

Anti tumor activity of prepared microsphere

Contents

Antitumor activity of prepared microsphere

1. Introduction:

Cancer is a deadly disease. Researchers are always involved to protect cancer and to develop better remedies in cancer chemotherapy. Traditional anticancer drugs develop apoptosis in various mechanism.

Metformin is a first line antidiabetic drug used for type 2 diabetes .The major role of the drug is to prevent hepatic neoglucogenesis and thus less production of glucose in liver tissues. Nowadays metformin is found to show various anticancer effects. It has already been seen to trigger the Ampk i.e. Amp related protein kinase which acts to decrease the ATP levels [355-356].

In this way cancer cells can be controlled for further progression [357-358] by activated Ampk cells.

Ampk is phosphorylated while it is activated. When it is activated the Ampk can inactivate the rapamycin (mTOR) signaling pathway which is the mammalian target. The cellular transcription and translation is ultimately influenced through its molecular effects 4E-BP and P7056 [359-360].It is a fact that can be established by animal models (7-10) EAC Elrichs ascites carcinoma and sarcoma-180 (s-180) cancer cell lines can be used for the effective antineoplastic studies in human breast cancer cell lines.

Metformin which is a major antidiabetic drug has also proven effect in anticancer chemotherapy.This is based on different studies and protocols set by different anticancer chemotherapeutic models.

Microcapsules are specific dosage forms which can be efficient releasing the drug in proper sites in the g.i. tract .Dose dependent drug release is further sustained with microspheres as there may be a big role of the spheres to provide sustained effect in drug respons at the target cells . Anticancer chemotherapy can be further sustained by the use of the metformin microcapsule in the dosage form.

Microcapsule formulations results longer duration of drug action with minimal gastric irritation. As microparticle preparations contain the drug embedded in the matrix made of guar gum and sodium alginate, resulting in a continuous slow release through a longer period [361].

Metformin decreases blood glucose levels in the patients who do not produce insulin in appreciable quantity to control hyperglycemia [368]. So, the microparticles of metformin will be very helpful to battle against hyperglycemia for a longer period [369].

Guar gum is utilised in food and pharmaceutical industries for a long time [364]. It is a natural nonionic polysaccharide obtained from the seeds of Cyamompsis tetragonolobus (Family-Leguminosae). It is hydrophilic in its nature and shows its swelling property in cold water. Its gelling property is helpful in making a delay in the drug release and so a better choice for controlled release formulations [365].

In this study, microsphere of metformin was formulated with an optimum concentration of all ingredient and guar gum was used as a matrix building material in coordination with sodium alginate to produce a controlled and prolonged release for a longer time.

Microparticles are therapeutically modified dosage form as it supplies the drug periodically in the target site for a longer duration. Drug is embedded in the matrix and released gradually with the passage of time. Guar gum was used as a matrix building material and ethyl cellulose was used as a polymer in the external phase. Multiple emulsion solvent evaporation technique was used to prepare the microsphere.

2. Formulation development:

a. Effect of process variables

Protocols	Variables				Non-variable
Guar gum	20mg	**30mg**	40mg	50mg	Stirring speed (780 rpm) Processing temperature (38ºc) Aquous phase & volume (5ml) Tween 80 (0.2%)
Span-80	0.00%	**1%**	1.7%		Guar gum (30mg), Stirring speed (780 rpm) Processing temperature (38ºc) Aquous phase & volume (5ml)
Processing temperature	28ºc	**38ºc**	44ºc		Guar gum (30mg), Stirring speed (780 rpm) Aquous phase & volume (5ml) Tween 80 (0.2%)
Stirring speed	700rpm	**780rpm**	900rpm		Guar gum (30mg), Processing temperature (38ºc) Aquous phase & volume (5ml) Tween 80 (0.2%)

Aqueous phase	**5ml**	7ml	10ml	Guar gum (30mg), Processing temperature (38ºc) Tween 80 (0.2%) Stirring speed (780 rpm)

Table 1. Effect of process variables on the properties of metformin loaded microsphere

Processing variables	Prepared Batches	Drug entrapment efficiency (DDE %)	Average particle size (µm)	Drug release (%)
Guar gum				
20	F1	30±1.45	327.08±2.45	85.25±1.58
30	**F2**	**53.1±1.52**	**392.08±1.96**	**79.65±2.56**
40	F3	59.5±1.07	397.78±3.61	67.38±1.78
50	F4	66.78±2.1	540.42±2.55	45.73±2.18
Span-80 (% v/v)				
0.00	F8	33.72 ±2.10	521.02±2.58	48.28±2.31
1%	**F9**	**51.8±2.52**	**391.18±1.52**	**72.76±1.81**
1.7 %	F10	36.82±1.95	438.01±1.46	58.45±2.95
Processing Temperature				
28	F11	41.29±2.25	515.05±1.29	61.48±3.15
38	F12	50.58±1.5	390.18±1.41	73.45±3.12
44	F13	44.60±1.8	523.25±1.81	58.42±3.45
Stirring speed				
700	F14	41.28±1.45	425.45±3.18	71.28±1.15
780	F15	52.93±1.15	395.78±2.89	71.38±1.95
900	F16	45.72±1.48	412.85±2.41	56.58±1.05
Aqueous phase Volume				
5	**F17**	**1.82±1.8**	**348.42±1.85**	**74.48±2.05**
7	F18	39.38±2.28	394.15±2.85	61.41±2.55
10	F19	33.72±2.18	525.85±1.82	53.42±2.85

Values are expressed as mean ± standard deviation (n= 6); Bold values are the selected processing variables for final formulation.**(Reference.)**

b. Preparation of Microsphere

A small quantity of sodium alginate was introduced in 5 ml of DW (distilled water) in a small beaker kept on a magnet stirrer. Guar gum was dissolved in to it with continuous stirring. Metformin weighting of 80 mg was added to it and stirring was continued for almost half an hour by a magnetic stirrer. Then the mixture was kept in a 20-gauge needle. An organic solution was made with 1 gm of ethyl cellulose and 30 ml of dichloromethane (DCM). Then 300 µl of span 80 was introduced in to this organic solution.. A primary emulsion of W/O type was prepared with the help of homogenizer. A water phase was madeprepared with 100 ml of DW and 200 µl of tween 80. The mixture was kept under a mechanical stirrer

revolving at 780-800 RPM. The primary emulsion was then dipped into the water phase drop wise by a 16-gauge needle and revolved for 3.5 h through mechanical stirrer. Small spherical microspheres were prepared which were filtered, air dried, weight measured and kept in a desiccator for future analysis [366].

c. Morphological analysis using scanning electron microscopy (SEM)

The (fig. 5) SEM studies showed the spherical surface of the microparticles. The outer part of the wall of the microparticles was rough and showing small holes due to the vaporisation of the solvent. The surface of drug-loaded microparticles was found to be smooth with small pores whereas the surface of blank microspheres was rough pores. In this study, the microsphere preparation with guar gum having smooth surface showed a slow and pro longed drug release profile while comparing with microspheres prepared without guar gum.[367]

d.X-ray diffraction (XRD) study

The powdered x-ray diffraction of metformin, drug-loaded microsphere and guar gum was shown in the fig. 7. The diffraction pattern of pure metformin and metformin loaded microsphere presented a crystalline peak, but guar gum alone produced an amorphous peak. The result showed that the crystalline peak of metformin and metformin loaded microsphere preparation were almost similar, but peak intensity decreases in the formulation diffractogram for the dilution of the drug with gum and polymer. So, it is evident that the conducted XRD experiment also resulted that metformin was adjustable with gum and other excipients.[368]

e.Fourier transforms infrared (FTIR) spectroscopy:

FTIR was performed to detect the chemical interactions between the drug metformin and all the other excipients incorporated in the microparticle preparations. The entire observed spectrum is depicted in fig. 6 when the corresponding peaks of drug-loaded microparticles

were compared with the standard spectrum of metformin. Apart from the FTIR studies, it was seen that no chemical interaction took place between the drug metformin and guar gum and polymer ethyl cellulose. The absorption peak of the drug seen at 3172 cm-1 due to N-H stretching of the primary amine group (-NH2). At 1065 cm-1 due to C-N stretching a peak of metformin was seen. At 1590 cm-1 the peak due to N-H bending of the primary amine group. The similar type of peaks found (N-H stretching, C-N stretching and N-H bending vibrations) in the spectra of guar gum having microparticles of metformin. Although, similar types of peaks were not seen in the blank microsphere preparation.[369]

f. Estimation of drug entrapment efficiency (EE)

The percentage Entrapment Efficiency (%EE) within the microspheres was found spectrophotometrically. Drug-loaded microparticles (10 mg) were smashed, powdered and dissolved in 5 mL of DCM (dichloromethane) and the solution was stirred for 10min utilising a magnetic stirrer for the total dissolution of the polymer in DCM. An excess of, 10 ml of methanol was introduced to the resultant solution, using by magnetic stirrier for 2 min at 40-45ºC and filtered. The absorbance of the final solution was detected at 270 nm utilising double beam UV-Visible spectrophotometer (UV1, Thermo Spectronic, Great Britain) using methanol as blank.

The % Entrapment Efficiency was calculated by applying the following equations:

$$= \qquad = \times 100$$

Where, Wt is the total initial amount of drug incorporated during formulation development, and Wf is the amount of free drug in the solution after formulating the drug entrapped microspheres [370].

The percentage yield and encapsulation efficiency of metformin was varing in all microparticlespreparations. The percentage yield of microspheres varing from 42.20±0.83 % to 94.84±1.88%. It was also seen that with the higher amount of guar gum in the internal

phase the percentage yield was also higher. Due to different of drug and guar gum ratio, formulations were influenced in table 2. The entrapment was increased up to an optimum ratio of drug and guar gum after which an increase in the ratio this decreases the drug loading.

a. particle size

The particle size of the microparticlesseen to be higher after a certain size range of 327.08 µm. After this optimum concentration (table 3), the particle size was larger with the incriment in the amount of the guar gum. Higher amount of guar gum in solution produced in an increase in the viscosity of the preparation which resulted bigger particle formation..[371]

3. Materials and Methods

a. Chemicals and Reagents of anti-cancer evaluation study of metformin loaded microsphere:

All cancer cells were obtained from Kolkata

and stored at 4^0C until use. The other chemicals used were of analytical grade. All solutions were passed through a 0.22 µm fitter (GVMP 01230, Millipore) and stored at 4^0C until use.

b. Cell lines and cell culture:

All cancer cell lines got from CNCRI, (Kol), India. All the

cells were incubated separately with 5% fetal bovine serum (FBS) containing penicillin and streptomycin at a concentration of 100 µg/mL each 37°C in a humid atmosphere (5% CO_2; 95% air). Cells were harvested by short incubation in 0.02% (w/v) EDTA in PBS. The cells were maintained routinely in subcultures in tissue culture flasks.

c. Preparation of the drug solution:

The solution of free metformin and metformin loaded microsphere was prepared by dissolving the test compounds in the sterile phosphate-buffered saline (PBS, pH: 6.8).

d. In-vitro Cell cytotoxicity assay of metformin loaded microsphere:

e. In-vitro detection of intracellular ROS:

f. In-vitro Flow cytometric analysis of mitochondrial membrane potential (Ψm):

g. In-vitro cleaved Caspase 3 flow-cytometry apoptosis analysis:

b. In-vitro cleaved PARP flow-cytometry apoptosis analysis:

c. In-vitro p53 flow-cytometry apoptosis analysis:

d. In-vivo toxicity study of metformin loaded microsphere evaluation of acute toxicity:

In accordance with OECD guidelines 425(Up and down procedure) the experiment was performed with swiss albino wilstar rats about 200-250 gm taken from the animal house of the Jadavpur University Kolkata, and they were maintained on a natural day–night cycle (12hr dark: 12hr light) at room temperature of about 22-25ºC in standard laboratory conditions with free access to standard food and standard pellets to diet and inclusion of water ad libitum. To female sex of rats in cages and metformin loaded microsphere was given orally at the single dose of 2000 mg/kg body weight, while for control animal, distilled water was given for the study of acute toxicity. The acute toxicity study was completed with the extract treated rats was seen closely for first 30 minutes, then up to 4 hours. The behaviour of the animal was recorded with care after the administered and occasionally rest of the days. The record was made twice a day for up to14 days. The clinical observations were made daily for the of physical condition, food, water intake, skin, fur, eyes, mucous membrane, salivation, sleep, coma, diarrhoea and convulsion in both group of rats. At the end findings of acute toxicity, the rats were weighed, and blood samples were collected by puncture of heart then histopathological examination of in the organs of liver and kidney under anaesthesia conditions in both control and treated extract group.

e. Determination of median lethal dose (LD_{50})

The oral toxic dose, where 50% of animal dies (LD_{50}-median lethal oral dose) was done accordance to the OECD guideline 423. The substance is introduced orally to the group of 3 animals at one of the defined doses: **250, 500, 1000 and 2000 mg/kg.**

f. Evaluation of toxic effect and behavioural study

Acute toxic and behavioural effects were conducted according to Lorke et al., 1983 [38]. 20 Swiss albino mice weighing 18-28 gm of each sex was divided randomly in four groups each containing five and marked according to the dose administered. Group I considered as a negative control, received PBSsoln (vehicle) and Group II, III, IV, V respectively received metformin loaded microsphere in a dose of **250, 500, 1000 and 2000 mg/kg** body weight orally. The animals observed continuously for the first 2 hours and frequently after 2 hours interval on the day of treatment. The nature and time of any adverse effect were noted, and it was carried out for 14 days. Body weights and mortality was observed.[372]

g. Histopathology study of liver and kidney tissue:

After 14 days of metformin loaded microsphere treated animals' **liver** and **kidney** was fixed in 10% buffered formalin bottles, then processed for histopathological examination. The tissue of liver and kidney was introduced in paraffin wax, serial sections (5-µm thick) obtained by cutting the embedded tissue with microtome, were mounted on 3- aminopropyl triethsilane – coated slides and dried for 24 h at 37 °C and stained with haematoxylin and eosin (H&E).

h. Sub-acute toxicity study

According to OECD guidelines 425, the rats were classified in 5 groups (each group 10 animals, 5 male and 5 female) treated by gavages with safely. Control group received distilled water while, the metformin loaded microsphere treated groups got the extract once daily for 28 consecutive days at the doses of **250, 500, 1000 and 2000 mg/kg.** Animals from each group were sacrificed. At the end of study, weight of rats was monitored in every groups after all the rats were anesthetized using ketamine (50mg/kg) and xylazine (5mg/kg) respectively and blood sample were collected with a cardiac puncture for the study of biochemical analysis and hematological parameters. All the rats were sacrificed by cervical dislocation. Important organs weight analysed in the rats like lungs, heart, liver, pancreas, and kidney.

i. Body weight, food, and water consumption

Body weight of the rats in each group was noted down before use of doses, further body weight was noted down during the experiment and finally on the day of sacrifice. The amount of food and water intake was monitored daily. The utilised amount of food and water was detected before they administered in each group, their remnants were calculated next day to get the differences, which were recorded as daily food (g./rat/day) and water consumption (ml/rat/day).

j. Blood analysis

Blood (2ml) was taken from the cardiac part of the rats for the measurement of haematological (EDTA- coated tubes) and biochemical (dry tubes) parameters after 14 days.

k. Haematological analysis

The blood sample was taken in heparinized tubes and utilised for haematological study. The parameters like haemoglobin (Hb), red blood cells(RBC), white blood cells(WBC), neutrophils(NP), lymphocytes(LC), monocytes(MC) were studied by automated analyser (Sysmex KX-21 Hematology-analyzer).

l. Biochemical analysis

Blood taken in clean dry tubes and centrifuged at the speed of 3000 rpm at 25ºC for 15 min to produce blood serum and then it was preserved at -20ºC until the study of biochemical parameters (Erbachem 5 semi-auto analyzer) like serum glutamic oxaloacetic transaminase (SGOT), (Tulip Group, Coral Clinical System, India), serum glutamic pyruvic transaminase (SGPT), Alkaline phosphatase (ALP), (Yenopoya health care Pvt ltd.), creatinine and urea (Tulip Group, Coral Clinical System, India) was done.

m. Induction of EAC solid tumor

In the EAC introduced tumor model, 24 animals were divided to six groups (eight animals per group), e.g., groups I, II, III, IV, V, VI respectively. Ascites fluid was picked from EACtumor-bearing swiss albino mouse at the log phase (day 7-8 of tumor-bearing) of the

anticancer activities was found with metformin loaded microsphere with 250 mg/kg body wt and 500 mg/kg body wt in swiss albino rats [373]

1. In-vivo solid tumor evaluation study:

The swiss albino mice was divided in I, II, III, IV groups. They were injected the cancer cells collected from the ascited cartilage swiss albino mice formulation was given to the swiss alb mice after 10 days of injecting cancer cells, the results was found after 21 days cervice dislocation. [374]

a. Tumor volume

The ratio of the developed tumors was calculated using Vernier calipers at 2-day intervals for 21 days and the tumor volume was measured using the formula:

$$V = \pi / 6 \times (D_1) \times (D_2)^2$$

Where D_1 is the longer diameter and D_2 is the shorter diameter.

b. Tumor weight

After of 21 days, all tumors (2 discs/animals) was punched out, weighed immediately, and the average weights were mentioned. All tumors were excised and divided into two portions. The first portion was used for checking of protein expression levels, and the second was used for histological and immune-histochemical measurements. The percentage inhibition was calculated by the formula:

$$\%\text{Inhibition} = 1\text{-}B/A \times 100.$$

Where A is the average weight of the control group and B is the average tumor weight of the treated group.

c. In-vivo determination of mean survival time (MST) & percentage increase in life span (%ILS):

375

d. Histopathology study of liver and kidney tissue:

e. Estimation of blood haematological and serum biochemical parameters:

f. H&E staining of EAC solid tumor section:

On day 22, swiss albino mice from each group selected randomly and sacrificed them properly by cervical dislocation, after then tumors that arised at the site of installation was excised and fixed in 10% formaldehyde and introduced in paraffin and 5µm sections were stained with hematoxylin and eosin (H&E). The slides were examined for histological changes such as a necrosis, mitotic figures, and inflammatory reactions with the help of light-microscopy. Three animals per group were used for histopathological and immune-histochemical analysis.

g. In-vitro Detection of intracellular ROS:

h. In-vivo Flow cytometric analysis of mitochondrial membrane potential (Ψm):

i. In-vivo cleaved Caspase 3 flow-cytometry apoptosis analysis:

j. In-vivo cleaved PARP flow-cytometry apoptosis analysis:

k. In-vivo p53 flow-cytometry apoptosis analysis:.

l. The effect of metformin loaded microsphere on lymphocyte proliferation and cytokine secretion:

Single cell suspension from tumors were centrifuged and saturated with 200 µL of PBS containing 2% mouse serum for 15 min at 4°C. After centrifugation, cells were stained for 20 min at 4°C with the various antibodies. T subset cells were studied flow cytometry. The monoclonal antibodies used were CD4, CD8, CD40, CD86 (BD Biosciences, Franklin Lakes, NJ, USA). For activation, single-cell suspensions of tumor cells were resuspended in RPMI containing100 ng/ml PMA (Sigma-Aldrich), 1 µg/ml ionomycin(Sigma-Aldrich), and brefeldin A (Sigma-Aldrich) for4 hours at 37°C. Intracellular staining was performedwithFITC-conjugated anti-IL10,anti-IL-17, anti-TNF-α and anti-IFN-γ (Biolegend). Annexin-V/7AAD (BioLegend, San Diego, CA, USA) staining wasperformed after EAC treatment with metformin free drug and metformin loaded microsphere. The fluorescence intensity of fluorochrome-labelledcells was checked by flow cytometry (FACSCanto II, BDBiosciences). FACSDiva software was used for measuring cell numbers, and data measurement was performed by FlowJo(FlowJo LLC, Ashland, OR, USA).

2. Result:

a. Formulation Development

This is an absolute preparation amongst the microsphere process development method showing the drug release reflects multiple emulsion solvent evaporation technique. The release of drug is an important parameter in the preparation of microspheres. Continuous release of the drug from the matrix of the microspere is the basic principle for the preparation of the microsphere to achieve the targets to manufacture a formulation which follows controlled release drug delivery. A multiple emulsion solvent evaporation (W1/O/W2) technique renders to formulate a matrix which includes the gum and the solvent to entrap the drug within the microparticle . This is the primary factor to encapsulate the drug inside the matrix. The matrix releases the drug gradually at a slow rate to fulfil the necessities of sustained action dosage form. A primary emulsion is needed to prepare in order to formulate the W1/O/W2 emulsion so that the primary emulsion should satisfy the requirements to prepare a W1/O primary emulsion type as it would maintain the water phase (W1) which is the matrix part of the formulation and the external oil phase (o) is nothing but the organic phase which helds the dichloro methane (dcm) as the organic solvent . This particular solvent system is a requirement for the preparation of the microsphere . The polymer ethyle cellulose was introduced in the W1/O primary emulsion and it was in the organic phase to maintain the formulation with span-80 in the organic phase of the W1/O emulsion. This primary emulsion is the fundamental of the W1/O/W2 multiple emulsion as it will be primarily required to hold the matrix in the microparticle. The solvent was vaporised from the water phase which was made stable by varying concentration of tween80. Variation of these parameters can show the presentation of the microsphere in a manner so that the reflection in the scanning electron microscopy (SEM) is changed dramatically. Different pictures of the microparticles in the SEM studies are quite important with the change in the variables to reflect the visual changes

in the microparticles **(Figure. 1C)**. FTIR and XRD studies also reflects the other qualities of the microparticle that there was no chemical interaction amongst the ingredients of the microparticle or the presence of the polymorphic forms.

In the manufacture of microparticles concentration of gum is an important criterion. In the present research we have utilised guar gum to build up the matrix of the microparticles. We have seen the variation and texture of the microparticles with the increasing concentration of guar gum. As guar gum is a big bulk molecule presents a big viscous preparation while stirring in water. This presented a variation in the drug entrapment. It has been found that with the increasing concentration of guar gum drug entrapment is also increased to 66.78 ± 2.1 percent which satisfied the fact that with the increase in guar gum in the internal phase the drug entrapment was also increased on the other hand particle size also increases simultaneously with the increase of guar gum concentration and it goes up to 540.42 ± 2.55 µm where the mean particle size can get for the optimized formulation f2 was 392.08±1.96 µm the surface of the microparticles was smooth and clean with a fantastic round shape of the sphere. The release of the drug changes probably for the fact that although initially it shows higher release with the increasing concentration of the gum builds the viscosity in the internal aquous phase and leaching of the medicament from the internal phase to the external phase. The mean release of the drug become 79.65±2.16% but ultimately the big viscous molecule in the internal aquous phase prevents release of the drug with increasing concentration of guar gum showing peculiar character of the guar gum.

Nextly tween 80 we used in the aquous phase to stabilize the formulation. It was found that tween 80 at 0.2 % showed maximum drug entrapment 51.18±2.52% . The solvent vaporised from the external phase helped the microsphere to become consolidated shape and the optimum size become to 391.18±1.52 (µm) . The release of the drug also showed a protocol

by releasing a mean 72.76±1.81 % as the vaporization of the solvent provided a stabilized formation.

The processing temperature is an important factor as it makes the formulation to be stabilized with the increase in temperature showing a mean temperature to solidify from the external aquous phase with stirring . Here we looked an optimum temperature of 38ºc to produce drug entrapment of 50.58±1.5% and a mean particle size of 390.18±1.41 µm showing a release of 73.45±3.12%. It was seen that with simultaneous increase from this temperature the rate of solidification may be higher but the particle size was also higher 523.25± 1.8 µm . The surface of the microparticle become smooth and round with a fine structure in the average temperature of 38ºC

The primary emulsion was prepared in the homogenizer with varying speeds. Primary emulsion (W1/O) is the basic to prepare the (W1/O/W2) emulsion microsphere system as it make stable the emulsion. If the primary emulsion (W1/O) is not fine then the final micro emulsion (W1/O/W2) will not be perfect .So there is an optimum speed of rotation (4500 rpm/) of the homogenizer which presents the emulsion system (w1/o/w2) properly stable to prepare finer microparticles with optimum size range of 395.78±2.89 µm with a finer surface. The drug entrapment 52.93±1.15 (%) and drug release 71.38±1.95(%) also presenting an optimum drug entrapment and release confirming the stability of the final micro emulsion (W1/O/W2) as the primary emulsion(W1/O) was made stableat 4500rpm.[376]

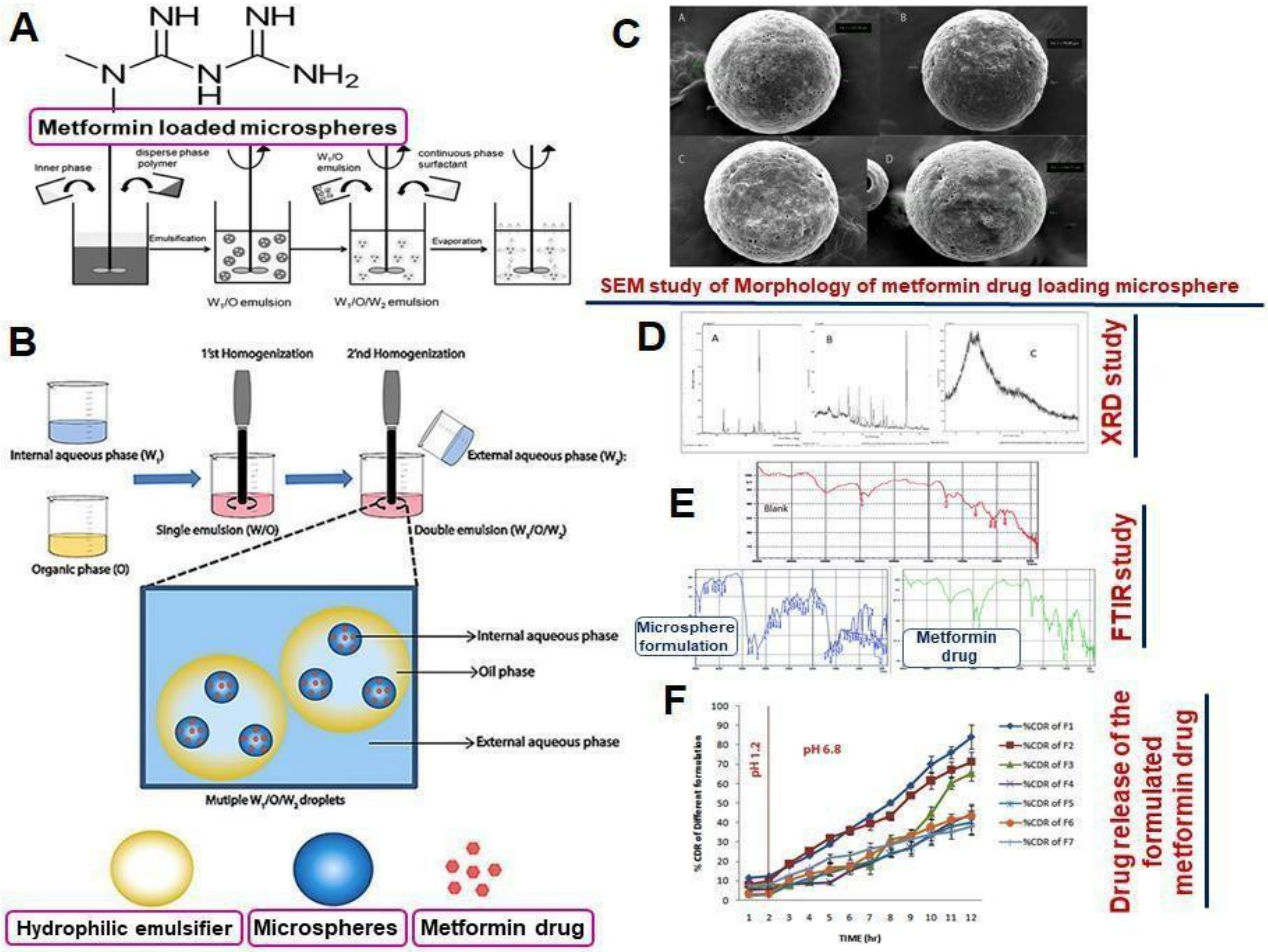
The aquous phase volume is the primary indicator of the suspension provides in the matrix as it contains (d.w) water as the vehicle and gum (guar gum) used as matrix building material in the formulation. The concentration of the vehicle is an optimum on where it will reflect (5ml) the maximum stability to the microparticle.

The particle size is also optimum 348.42±1.85 (µm)and the drug entrapment is higher 51.82±1.8% as the matrix get its maximum grip into the formulation with a good suspension

of A.p.l. in the vehicle (d.w) .So the release of the drug is also higher 74.48±2.08% as the stability of the microsphere was maintained with the optimum concentration in the internal aquous phase.

The FTIR study reports were made to check if there was any interaction between the ingredients or not. The test shows peak at 3172cm-1

Other peaks were found at 1065cm-1 and 1590cm-1 for C-N and N-H bonding in the XRD study also crystalline peaks were seen with the pure drug whereas amorphous peak was found with the gum, the microsphere was found to present polymorphic forms.



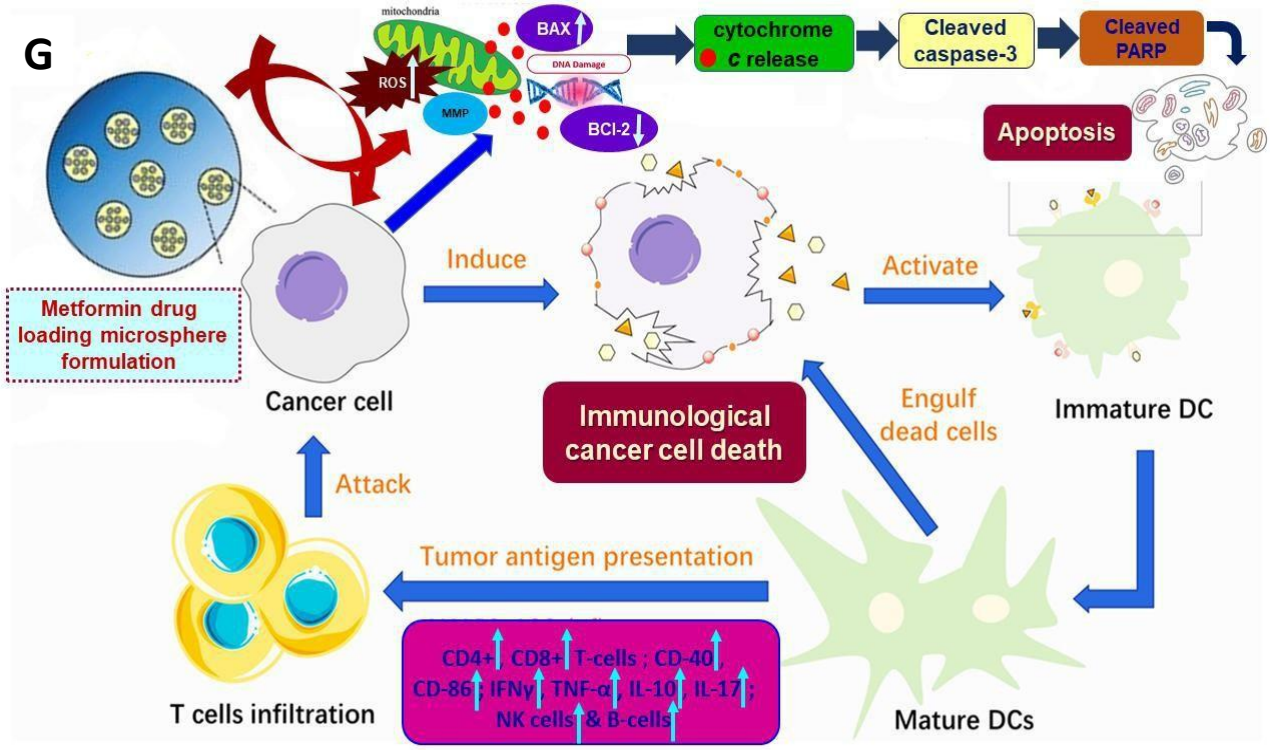


Fig. 1: Physicochemical characterization of metformin loaded microsphere. (A) (B) W1/O/W2 emulsion technique of metformin loaded microsphere preparation, (C) SEM study of morphology of metformin drug loaded microsphere, (D) XRD study of metformin loaded microsphere, (E) FTIR spectrum of drug loaded microsphere preparation, (F) In-vitro release profile of the optimized formulated metformin loaded microsphere. (G) Schematic illustration of metformin loaded microsphere nanoparticle-mediated apoptotic tumor cell death and cancer immunomoduletion.

3. In-vitro study

a. In-vitro cell cytotoxicity assay of Metformin loaded microsphere on various cancer cell-lines

To find the effect of metformin on the maturation of MDA-MB231 and EAC cells were treated with different concentrations of free metformin, metformin loaded microsphere, and blank microsphere**(0, 1.0, 5.0, 10.0, 20.0, and 40.0 mM)** for 48h and their proliferation was detected. After 48h of exposure, 0.5 µg/ml metformin loaded microparticles less impair the effective proliferation inhibition, however, a concentration of 1.0 – 40.0 µg/ml it potently diminished the cell proliferation 10.2%-84.7% respectively, as compared to free metformin,

and blank microsphere (Fig. 2). The IC_{50} value was determined and found to be as 11 µg/ml and 10 µg/ml for MDA-MB-231 and EAC respectively. Proliferation of MDA-MB231 and EAC cells was significantly inhibited by metformin loaded microsphere in a concentration-dependent manner (***P<0.001). However, when metformin loaded microsphere was used against PBMC, resulted no cytotoxicity and anti-proliferative effect indicating its target specificity **(Figure. 2).**In vitro MTT results reflected that the blank microparticles showed no major cytotoxicity after 48 h, showing guar gum microparticles as a carrier of the drug without producing any toxic manifestation. It was seen in the reports that guar gum is a natural carrier with no toxicity and biodegradable (as described earlier published research paper). Free drug and drug-loaded microsphere **(from 0.5 to 20 µg/ml)** killed viable tumor cells, but there was a natural difference between free metformin and metformin drug-loaded microsphere. [377-378]

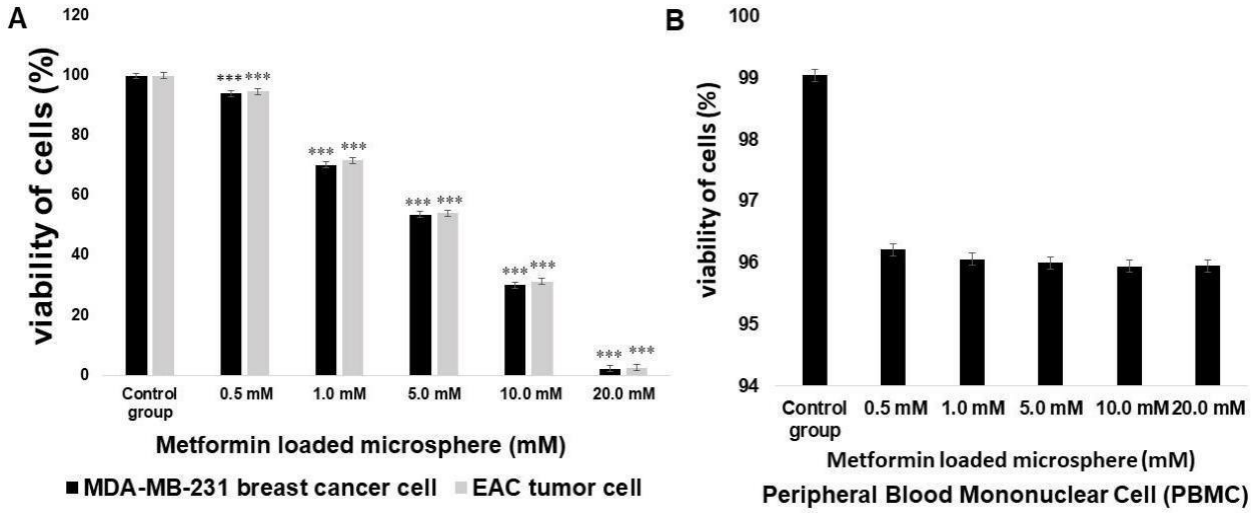


Fig. 2: MDA-MB-231breast cancer cell, EAC tumor cell (A) and PBMC (B) cells were exposed to various concentrations of metformin loaded microsphere at various dosed and cell proliferation assays were performed. Data are exposed as mean ± SEM, n= 3. MDA-MB-231: epithelial human breast cancer cell; EAC: Ehrlich Ascites Carcinoma; PBMC: Peripheral Blood Mononuclear Cell.***P<0.001

a. **Detection of intracellular ROS in-vitro MDA-MB-231 breast cancer cells:**

b. **Flow cytometric analysis of mitochondrial membrane potential (ΔΨm)on the in-vitro MDA-MB-231 breast cancer cells:**

c. **Determination of cleaved Caspase 3 flow-cytometry apoptosis analysis inthein-vitro MDA-MB-231 breast cancer cells**

d. **Determination of cleaved PARP flow-cytometry apoptosis analysis in the in-vitro MDA-MB-231 breast cancer cells**

In order to classify the type of cell death caused by metformin in MDA-MB-231 cells, flow cytometry was done to detect cleaved PARP. The executioner caspase 3 has previously been found to be activated downstream of mitochondria-mediated apoptosis in this cell line. PARP is a substrate for both caspases 3 and 7 and cleaved PARP is a hallmark of caspase-dependent apoptosis. Cleaved caspase 3, the active form of the enzyme, was detected after Metformin loaded microsphere treatment and paralleled an increase in PARP cleavage **(Figure. 2A)**. Expending flow cytometry analysis, we experimental important inhibitory effect with metformin drug-loaded microparticles in comparison with metformin free drug, and blank microspheres treated MDA-MB-231 cells **(Figure. 3A).** Also, these consequences exhibit that metformin drug-loaded microparticles accelerated cleaved PARP in a dose-dependent manner. This results that an apoptotic cell death pathway is stimulated by metformin loaded microsphere.

e. **Determination of p53 flow-cytometry apoptosis analysis in the in-vitro MDA-MB-231 breast cancer cells**

The p53 protein is a transcription factor which is active in cell cycle arrest, DNA repair and apoptosis. In MDA-MB-231 cells, this protein is down regulated by an oncogenic E6 HPV protein. So, changed expression of the p53 protein in MDA-MB-231 cells was evaluated after treatment with metformin loaded microsphere **(Figure. 3D)**. MDA-MB-231 cells expressing p53 protein became up-regulated after treatment with metformin loaded microsphere (Figure

2b-d). While treated with metformin loaded microsphere, p53 protein expression in MDA-MB-231 cells sharply got higher compared with free metformin, metformin loaded microsphere, and blank microsphere and it reached effective difference ($p < 0.001$). This result indicate that metformin loaded microsphere protects breast cancer cell growth and survival in p53-dependent ways.

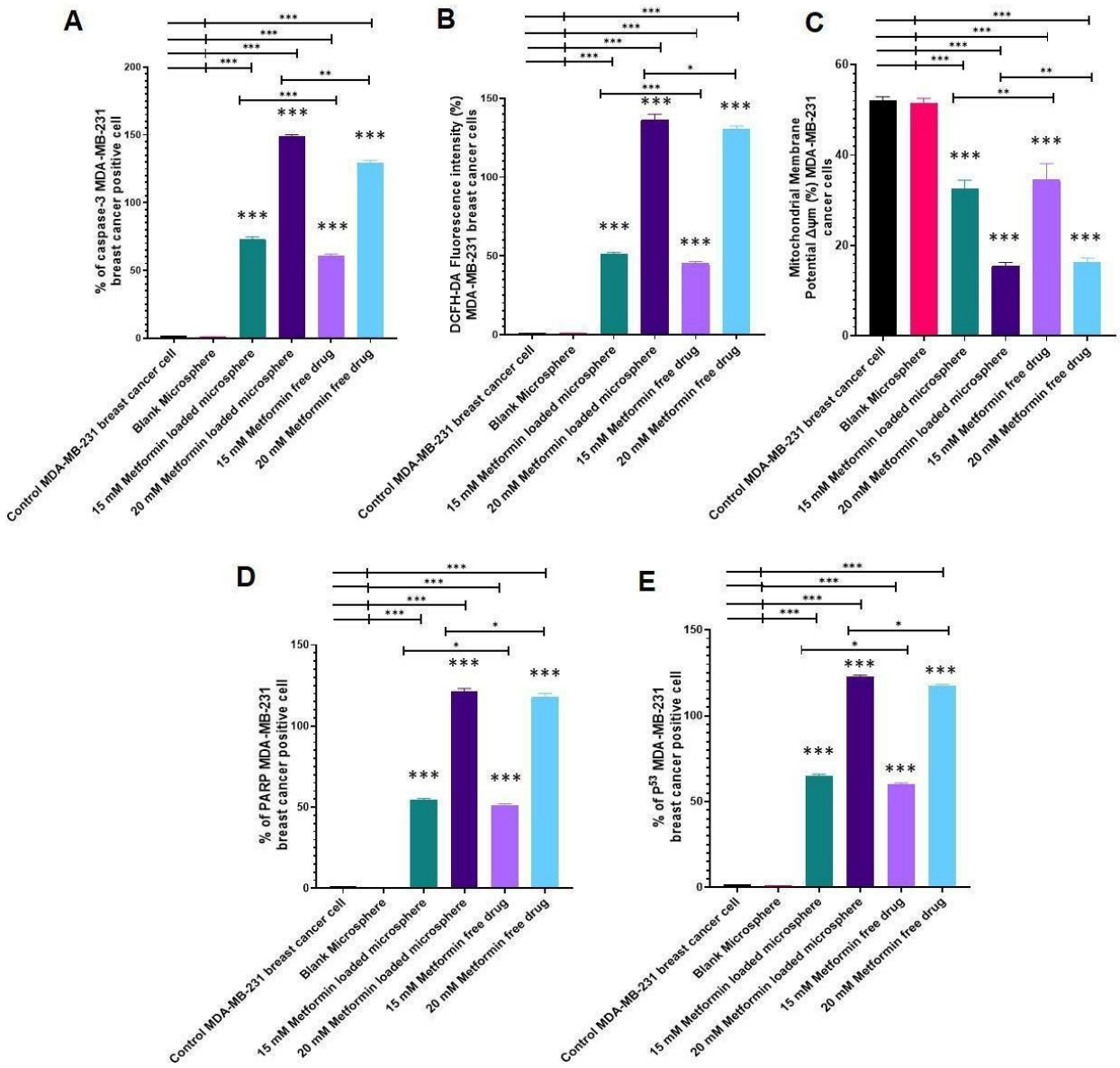


Fig. 3: This picture exhibits the oxidative-stress dependent apoptotic effect by the Metformin loaded microsphere.

4. In-vivo toxicity study of metformin loaded microsphere a.

Determination of median lethal dose (LD_{50})

Determination of minimum toxic oral dose and behavioral study

As per OECD guideline 423, **three predefined doses 250, 500 and 850 mg/kg body weight** was used in three steps taking three animals in each step. No mice found dead up to the dose of 850 mg/kg b.w. Thus, this methanolic extract was safe and non-toxic up to the maximum limit dose of**850 mg/kg body weight.**

Again 14 days experiment shown that no significant alternation in the behavior of a **daily basis 250, 500 and 850 mg/ kg. b.w.(Table 2).** Here we also saw that food intake was reduced initially but regained during the later period of study. No significant change in body weight was found concerning control, and no mortality was seen during the experiment **(Table 3).** Again, it proved that the extract has acceptability as an oral phytomedicine.

Table 2: Changes in behaviour and different parameters observed during the period of toxicity study.

Parameters	Cage-side observations
Condition of fur	Normal
Eye dullness and opacities, lacrimation	Nil
Ptosis (paralytic drooping of the eyelid or prolapsed of an organ)	Nil
Colour of faces and skin (blanching, cyanosis, vasodilatation)	Normal
Tremor, convulsion, salivation, diarrhea, lethargy, sleep, coma	Nil
Self-mutilation, walking abnormality.	Nil
Gait (style or manner of walking), posture	Normal
Sensory stimuli, grip strength, ataxia	Normal
Breathing abnormality	Nil
Circling, excitability, depression	Nil

Table 3: Cage-side observations during the period of acute toxicity and behavioural study.

Groups	No. of	Treatment	Mean body weight ± Standard deviation

	animals	mg/kg b. w.	Day 0	Day 3	Day 7	Day 14
Control	5	Distilled water 1ml	19.4±2.44	18.92±1.98	19.5±1.56	19.90±2.06
I	5	250 mg/kg b.w Metformin loaded microsphere	24.65±4.2	23.72±3.36	23.95±4.65	24.86±5.22
II	5	500 mg/kg b.w Metformin loaded microsphere	27.30±1.75	26.61±1.38	26.65±1.52	27.61±1.34
II	5	850 mg/kg b.w Metformin loaded microsphere	26.14±0.25	26.03±0.24	25.14±0.23	26.16.21

Above table represents body weights of mice throughout two weeks of acute behavioural and toxicity study.

b. Sub-acute toxicity Study:

Histopathology study of liver and kidney tissue

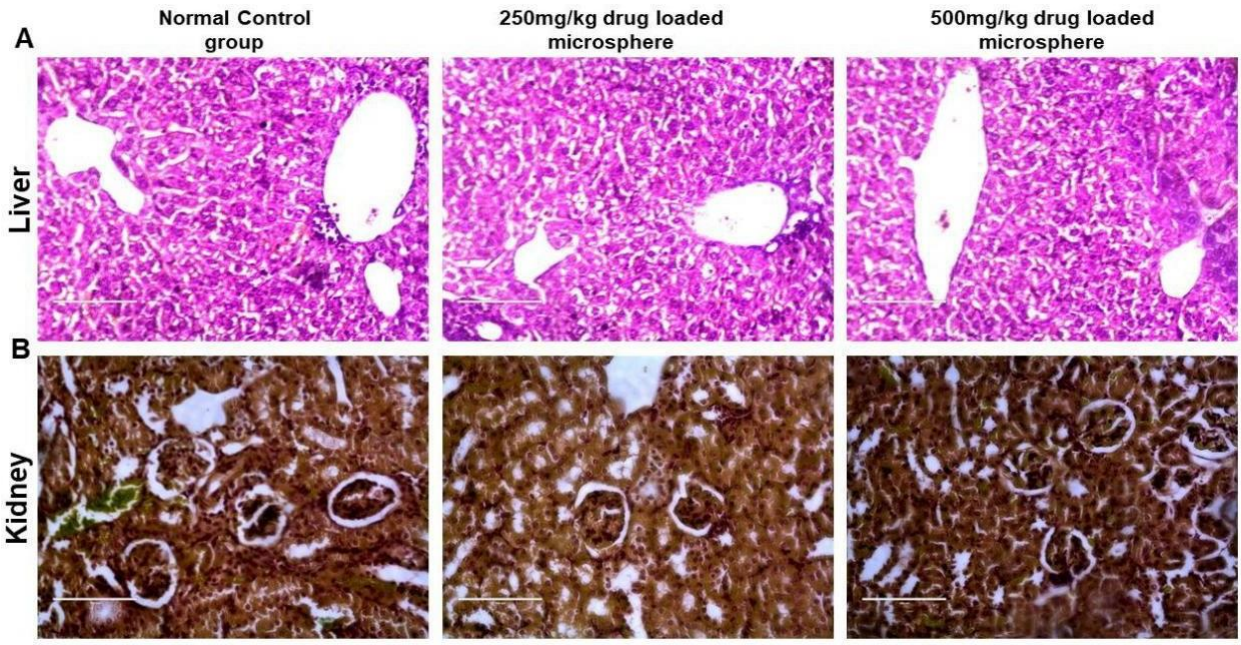


Fig. 4: H&E stained section of the liver of mice showing cell morphology of groups (A) Untreated control group, Metformin loaded microsphere 250 mg/kg b.w and 500 mg/kg b.w(B)H&E stained section of kidney of mice showing cell morphology of Untreated control group, Metformin loaded microsphere 250 mg/kg b.w and 500 mg/kg b.w.

c. Effect of metformin loaded microsphere on body weight and Organ weight

No significant change was seen in the animal's body weight and organ weights during the study. Up to 35 days of oral administration of **metformin loaded microsphere formuletion**, the food intake and water consumption were also not affected.

Table 4: Changes in body weight of rats during the treatment with different doses of metformin loaded microsphere.

Treatment group	Body weight (Gram)					
	Sex	Day 7	Day 14	Day 21	Day 28	Day 35
Control	Female	169.90±1.58	170.53±1.27	168.50±2.41	171.42±2.90	171.42±2.86
250mg/kg/b.wmetformin loaded microsphere	Female	168.47±1.29	170.53±1.14	171.43±2.27	173.42±1.30	173.42±1.40
500mg/kg/b.wmetformin loaded microsphere	Female	175.86±0.40	177.41±0.80	176.45±0.77	176.43±1.74	176.48±1.64

Above table represents body weights of rat throughout five weeks of sub-acute behavioral and toxicity study.

Table 5: Weight of the organs in gram of the female rats in the sub-acute toxicity of the metformin loaded microsphere studied and results was expressed as the mean S.E.M. of 5 rats.

Treatment group	Organ weight (Gram)					
	Sex	Lungs	Heart	Liver	Pancreas	Kidneys
Control	Female	1.29±0.04	0.54±0.05	5.29±0.02	0.49±0.03	0.51±0.01
250mg/kg/b.wmetformin loaded microsphere	Female	1.29±0.04	0.54±0.05	5.29±0.04	0.50±0.03	0.50±0.02
500mg/kg/b.wmetformin loaded microsphere	Female	1.28±0.04	0.55±0.05	5.28±0.02	0.49±0.03	0.51±0.01

Above table represents body weights of rat throughout five weeks of sub-acute behavioural and toxicity study.

d. Toxicity study of the effect of metformin loaded microsphere at different doses on blood haematological and biochemical parameter:

Here we followed all guidelines mentioned by the animal ethics committee such as OECD.

Table 6: Effect with metformin loaded microsphere at different doses on blood haematological and biochemical parameter

	with metformin loaded microsphere result (mg/kg /body weight)		
	Control	**250 mg/kg**	**500 mg/kg**
Haemoglobin (gm%)	15.31±0.36	15.34± 0.46	15.32±0.17
RBC (10^6/µl)	8.78±0.037	8.72±0.058	8.77±0.042
WBC (10^3/µl)	13.65±0.054	13.59±0.047	13.59±0.049
Neutrophil (10^3/µl)	20.73±0.648	20.61±0.649	20.72±0.591
Monocyte (10^3/µl)	2.50±0.346	2.52±0.366	2.54±0.325
Lymphocyte (10^3/µl)	71.86±0.343	71.89±0.547	71.85±0.642
Eosinophil (10^3/µl)	2.39±0.435	2.39±0.456	2.44±0.432
Platelets (10^3/µl)	1,226.10±1.638	1,237.11±1.821	1,238.12±0.601
SGOT (U/L)	90.29±0.335	92.20±0.332	93.30±0.421
SGPT (U/L)	34.60±0.214	34.62±0.242	34.68±0.218
Bilirubin Total (mg/dl)	0.45± 0.02	0.48± 0.01	0.46± 0.01
Serum Protein (g/dl)	8.19± 0.02	8.21± 0.01	8.18± 0.02
Creatinine (mg/dl)	0.38± 0.01	0.36± 0.03	0.38± 0.02
Urea (mg/dl)	59.18±4.89	60.20±3.89	60.11±4.97

Each point represents the mean ±SEM. (n=6 mice per group), P value statistically non-significant differences has when compared with control group.

5. In-vivo study

a. In-vivo solid tumor evaluation study

The average tumor volume in EAC control mice progressively enlarged with time up to **75.15±2.30** mm^3 for 21 days post-tumor implantation study**(Figure. 4C)**. In the metformin loaded microsphere-treated groups, the tumor volume was seen **33.17 ± 0.51** for **10 mg/kg b.w** metformin loaded microsphere injection through i.p and **31.27 ± 0.23** for **10 mg/kg b.w.** metformin loaded microsphere loaded microsphere respectively **(table 3).(Figure. 4A)**showed the photographic view of the tumor portion.

The body weight changes were very lower in metformin loaded microsphere treated groups in comparison to control and free drug treated groups; indicating the effect of metformin loaded microsphere protecting the tumor growth **(Table3).** Tumor weight and size was also very low in the metformin loaded microparticle treated group compared to control **(Figure. 4B),**

The rate of survival of EAC containing mice in the metformin loaded microsphere and was significantly increased as compared to free drug-treated groups and the EAC bearing control group mice. The %ILS in the metformin loaded microsphere induced were found **148.40±9.23** and **164.83±1.73** respectively (**Table 3**). That showed that microsphere of metformin had reflected significant anticancer activity in the tumor-bearing mice.

Table 7: Effect of metformin loaded microsphere on Tumor weight, Mean Survival Time (MST), Increased Life Span (ILS) and Body weight

	EAC Control ($2x10^6$ cells/mouse)	**Blank microsphere**	**250 mg/kg b.w Metformin loaded microsphere**	**250 mg/kg b.w Metformin free drug**	**500 mg/kg b.w Metformin loaded microsphere**	**500 mg/kg b.w Metformin free drug**
Tumor Weight (gm)	24.29±0.15	24.52±0.26	11.02±0.01***	11.48±0.02***	4.28±0.05***	4.02±0.07***
% of Tumor Volume	0±0	0±0	81.20±0.07***	80.40±0.02***	65.30±0.06***	65.01±0.03***

Tumor growth Inhibition (%)	0±0	0±0	24.36±0.05***	23.25±0.01***	32.57±0.06***	31.90±0.04***
MST (days)	22.35±0.20	22.61±0.38	106.16±0.15***	105.41±0.11***	130.08±0.28***	129.02±0.19***
% ILS	0±0	0±0	66.58±0.08***	67.82±0.05***	95.76±0.82***	96.01±0.56***
Body weight (gm)	39.02±0.56	38.25±0.47	23.61±0.52***	22.85±0.17***	18.70±0.61***	18.01±0.40***

Each point represents the mean ±SEM. (n=6 mice per group), ***P<0.001 statistically significant when compared with EAC control group.

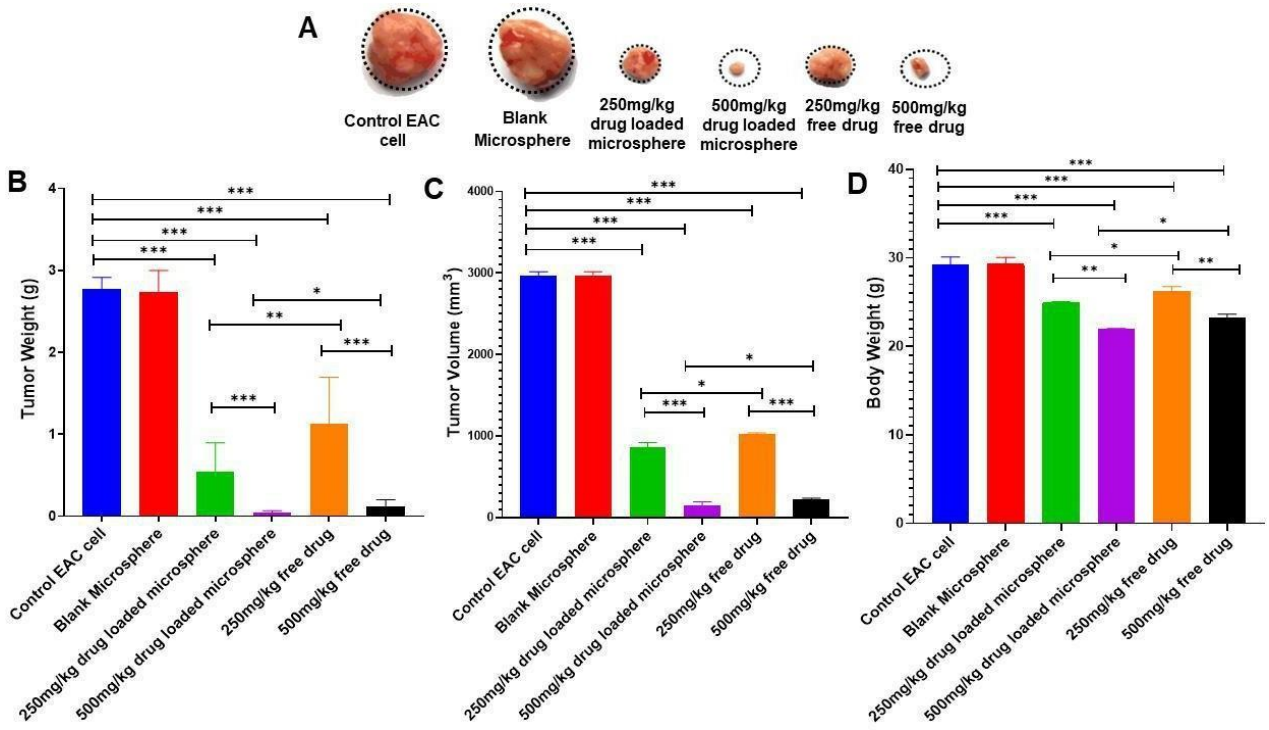


Fig. 5: Effect of metformin loaded microsphere on different groups.

b. In-vivo determination of mean survival time (MST) & percentage increase in life span (%ILS)

The survival of metformin drug loaded microsphere treated EAC bearing mice significantly higher compared to EAC bearing control group and free drug-treated groups. The %ILS increases in metformin drug loaded microsphere at **250 and 500 mg/kg b.w.** was found to be in a dose-dependent manner **(Table 1)** and **(Figure. 6).**

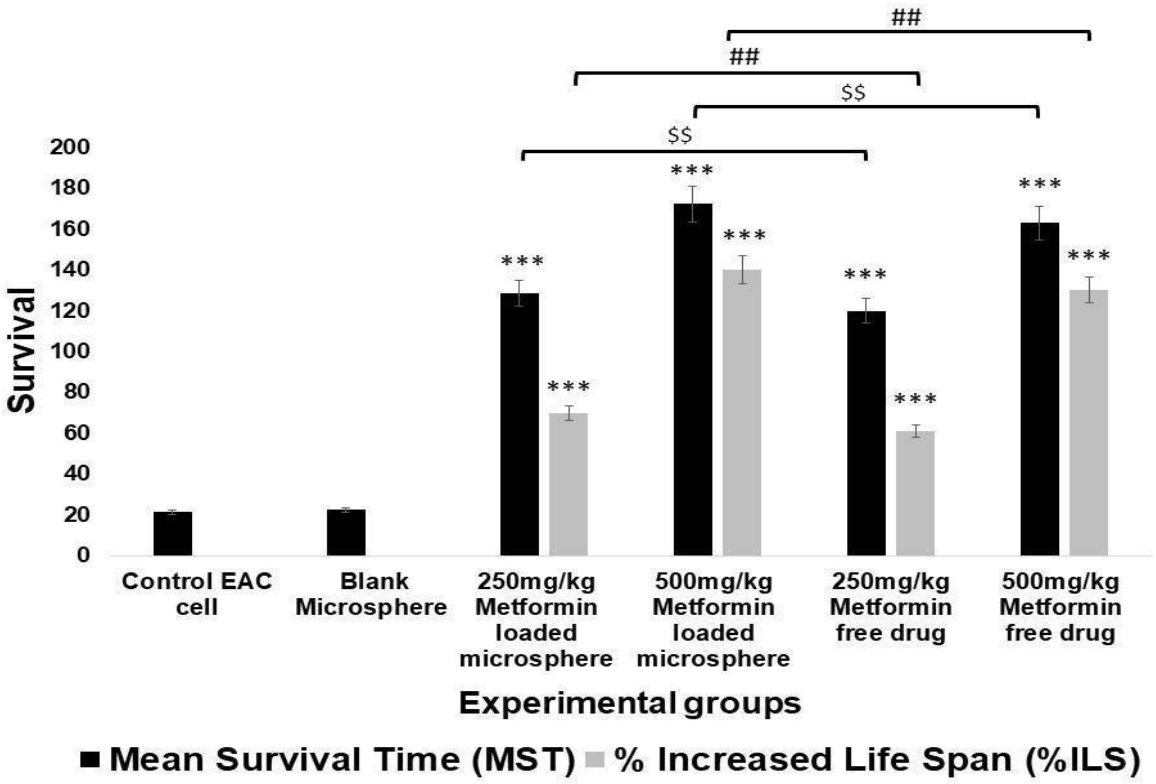


Fig. 6: Effect of metformin drug loaded microsphere treatment on solid EAC tumor. Effect of metformin drug loaded microsphere on mean survival time (MST) and percentage increase in life span (%ILS).Survival study of synthesized metformin drug loaded microspherecompared with free drug and the EAC control tumor group . Where n=6 for control and metformin drug loaded microsphere treated group.$^{***}P<0.001$, $^{\$\$}P<0.01$, $^{\#\#}P<0.01$

c. Histopathology study of liver and kidney tissue

To find the effective role of metformin drug loaded microparticle in tissue-specific metastasis in EAC carcinoma tumor models, the tumors as shown in (**Figure. 7),** were ventrally

dissected and metastatic lesions such as liver and kidney were separated and analysed by histologically (**Figure. 7).** The data showed that tumors influenced by control cell metastasis in liver and kidney whereas metformin loaded microsphere dramatically reduced this metastasis (**Figure. 7**) demonstrating that metformin loaded microsphere prevents tumor growth, and metastasis in swiss albino mice model. It showed various significant melanoma metastasis effects on EAC injected animals **(Figure. 7**). Moreover, we saw several metastatic foci in the tissue sections of liver and kidney of control EAC installed mice by histopathology using H&E staining. Overall, the data clearly produced evidence that overexpression of metformin loaded microsphere significantly reduces cancer growth.

metformin loaded microsphere **40mg/kg b.w.** As shown in (**Figure. 7B**) histopathological examination of kidney showed normal glomeruli and renal tubules in all mice groups except EAC control group. (**Figure.7B).** In kidney histopathology of the metformin drug loaded microsphere -treated mice showed an almost normal structure as found when compared with the EAC control group.

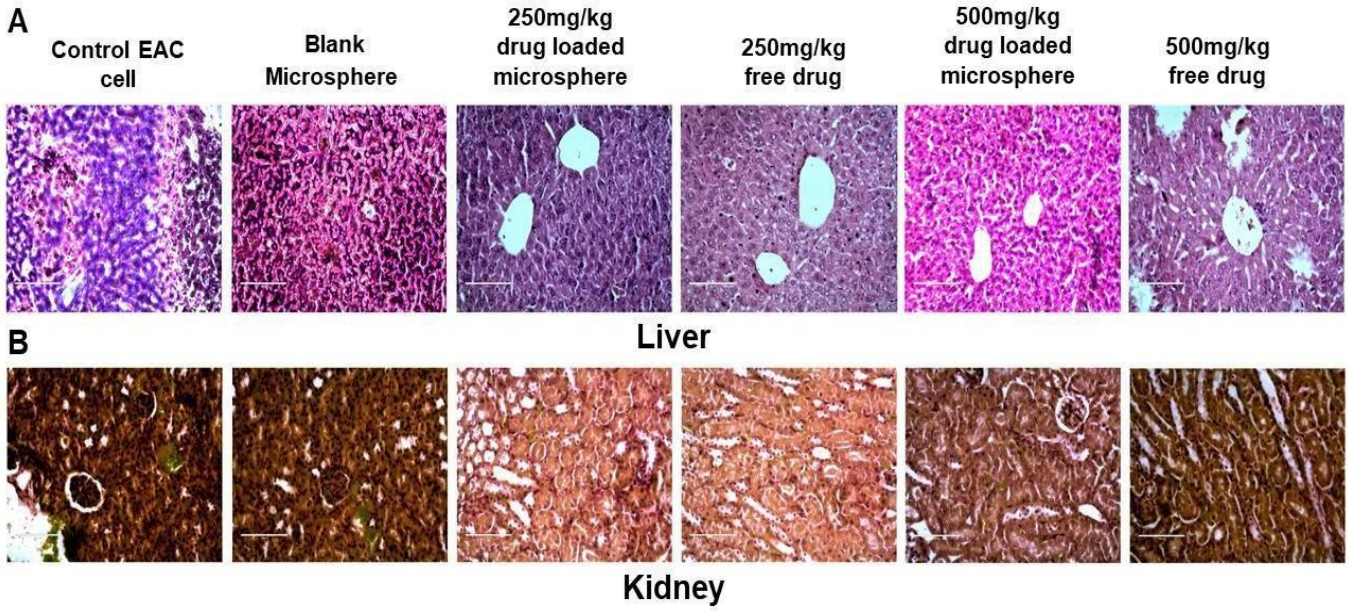


Fig. 7:H & E section of liver (A) and kidney (B) from mice showing hepatocellular profile of metformin loaded microsphere, free drug, blank microsphere compares with control untreated group, showed cellular infiltration, congestion and mild central vein dilation.

d. Effect of metformin loaded microsphere on haematological and biochemical parameters

Table 8: Effect of Metformin loaded microsphere on haematological parameters in EAC bearing swiss albino mice.

	Normal Swiss albino mice	EAC Control ($2x10^6$ cells/mouse)	Blank microsphere	250 mg/kg b.w Metformin loaded microsphere	250 mg/kg b.w Metformin free drug	500 mg/kg b.w Metformin loaded microsphere	500 mg/kg b.w Metformin free drug
Hemoglobin (gm %)	12.6 ± 0.14	5.20 ± 0.15**	5.30 ± 0.19**	8.31 ± 0.01**	8.71 ± 0.06**	12.02 ± 0.01**	11.32 ± 0.03**

Erythrocyte (RBC) (cells x $10^6/mm^3$)	9.59 ± 0.02	3.02 ± 0.01**	3.07 ± 0.03**	6.82 ± 0.02**	6.29 ± 0.04**	8.40 ± 0.01**	8.14 ± 0.02**
Leucocytes (WBC) (cells x $10^6/mm^3$)	13.4 ± 0.01	24.17 ± 0.03**	24.38 ± 0.05**	8.21 ± 0.03**	8.37 ± 0.08**	13.08 ± 0.02**	12.47 ± 0.04**
Neutrophil (%)	30.08 ± 0.06	74.11 ± 0.03**	75.29 ± 0.07**	31.15 ± 21.90*	32.47 ± 22.50*	31.10 ± 0.03**	30.28 ± 0.01**
Lymphocyte (%)	68.32 ± 0.25	27.06 ± 0.05**	26.38 ± 0.08**	46.70 ± 0.01**	46.15 ± 0.04**	60.30 ± 0.02*	61.12 ± 0.02*
Monocyte (%)	2.15 ± 0.03	1.10 ± 0.01*	1.18 ± 0.04*	1.75 ± 0.02*	1.72 ± 0.05*	2.39 ± 0.03*	2.21 ± 0.02*

Each point represents the mean ±SEM. (*n*=6 mice per group), $^{**}P<0.01$ and $^{*}P<0.05$ statistically significant when compared with the normal saline group and B16F10 control group.

Table 9: Effect of Metformin loaded microsphere on haematological parameters in EAC bearing swiss albino mice.

	Normal Swiss albino mice	**EAC Control ($2x10^6$ cells/mouse)**	**Blank microsphere**	**250 mg/kg b.w Metformin loaded microsphere**	**250 mg/kg b.w Metformin free drug**	**500 mg/kg b.w Metformin loaded microsphere**	**500 mg/kg b.w Metformin free drug**
Bilirubin Total & Direct: (mg/dl)	0.42 ± 0.02	0.37 ± 0.01**	0.38 ± 0.01**	0.31 ± 0.01**	0.31 ± 0.05**	0.26 ± 0.02**	0.26 ± 0.03**
Serum Protein (Total) (mg/dl)	6.82 ± 0.08	2.34 ± 0.11**	2.32 ± 0.17**	6.28 ± 0.08**	6.17 ± 0.04**	6.39 ± 0.08**	6.27 ± 0.02*
AST(SGOT) (IU/L)	38.19 ± 0.03	79.04 ± 0.05**	78.01 ± 0.04**	56.24 ± 0.01**	55.08 ± 0.01**	36.2 ± 0.01**	37.1 ± 0.02**
ALT (SGPT) (IU/L)	28.35 ± 0.03	66.28 ± 0.03**	64.20 ± 0.03**	42.10 ± 0.02**	43.17 ± 0.01**	29.08 ± 0.05**	30.09 ± 0.02**
Serum Alkaline Phosphates (IU/L)	77.28 ± 0.01	124.25 ± 0.03**	123.15 ± 0.01**	90.02 ± 0.03**	89.08 ± 0.01**	78.13 ± 0.02**	77.18 ± 0.02**
Creatinine (mg/dl)	0.82 ± 0.02	0.62 ± 0.02*	0.63 ± 0.02*	0.76 ± 0.01*	0.75 ± 0.01*	0.8 ± 0.02*	0.8 ± 0.01*

Each point represents the mean ±SEM. (*n*=6 mice per group), $^{**}P<0.01$ and $^{*}P<0.05$ statistically significant when compared with the normal saline group and EAC control group.

6. Effect of metformin loaded microsphere on histopathological changes of solid EAC tumor section

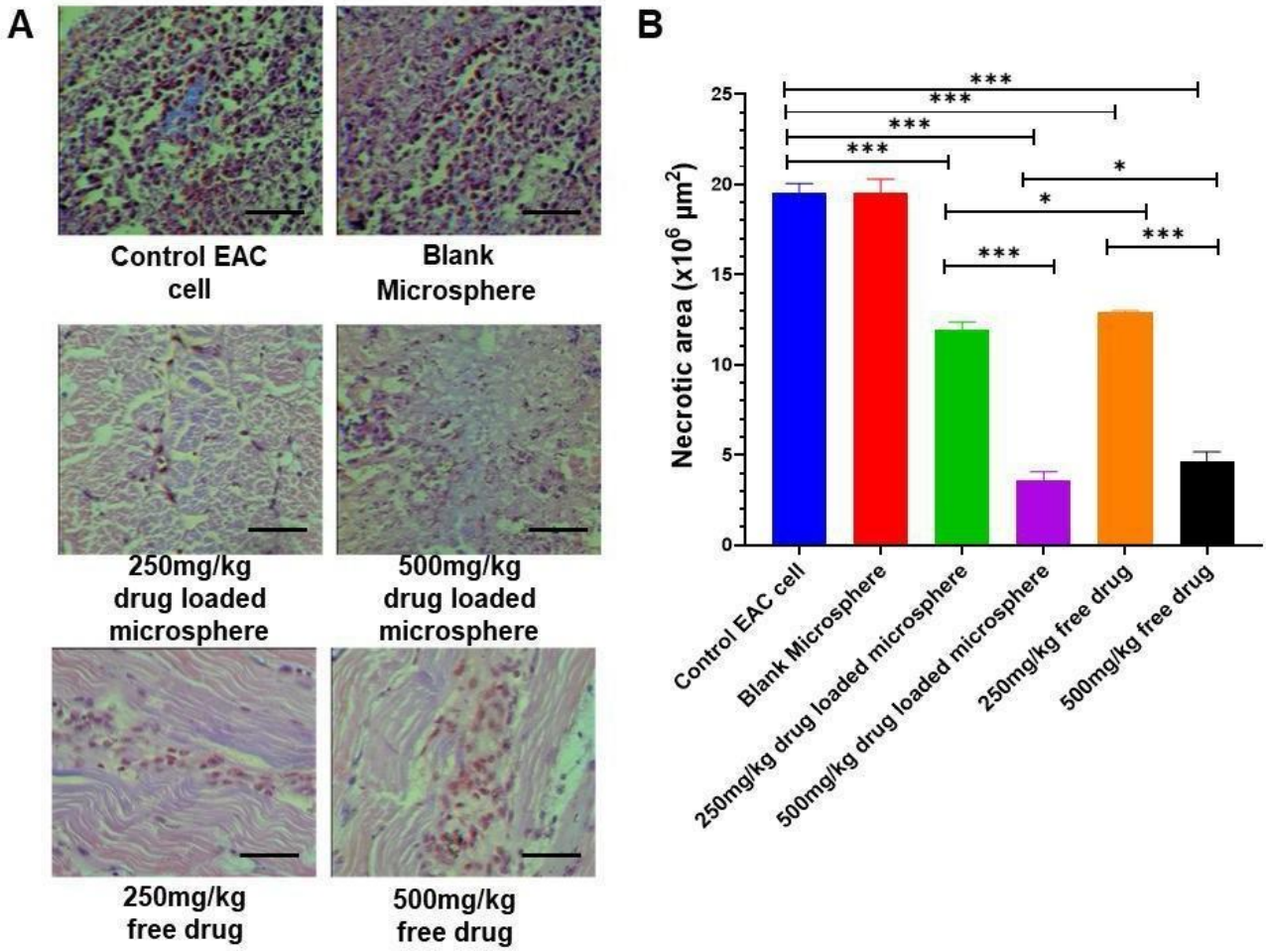


Fig. 8:*In-vivo* histopathological analyses of EAC tumor. (A) Histopathological examination of EAC solid tumor. We have shown untreated EAC-bearing mice tumor section infiltration of subcutaneous tissue with tumor cells, newly formed blood capillaries; and leukocyte infiltration, EAC tumor-bearing mice treated with metformin loaded microsphere 250 mg/kg have shown necrosis; fibrosis; skeletal muscles; destructed blood vessels, haemorrhage; extensive infiltration of the subcutaneous tissue with tumor cells compare to 250 mg/kg/b.w free drug; tumor cells with anisocytosis, EACtumor-bearing mice treated with metformin drug loaded microsphere500 mg/kg has shown moderate infiltration of the tumor cells; numerous leukocyte infiltration; large numbers of tumor cells introduced in the skeletal muscles; less infiltration with tumor cells; extensive necrosis and fibrosis; islets of viable tumor cells; extensive necrosis compare to free drug 500mg/kg/b.w. Tissue sections was stained with H&E dyes (scale bar = 100 µM). (B) The quote of the percentage of necrotic area in sections of tumors harvested on d 22 post-treatment. In each section per tumor four

random areas were counted and three tumors were found in each treatment group. The quota of the percentage of necrosis was found using ImageJ software. Mean ± SEM, n=3, $^{***}P<0.001$

Table 10: Apoptotic effect of metformin loaded microsphere on the histopathological changes in solid tumor (EAC) bearing Mice *(n*=3)

Group	Tumor Infiltration at Margin	Inflammatory cells Infiltration at Edge	Mitotic Index (no of MFs in 12 HPFs)	Apoptotic index (no of Abs in HPFs)
EAC Control	+++	+++	26	3
Blank microsphere	+++	+++	27	3
250 mg/kg b.w Metformin loaded microsphere	++	++	5	11
250 mg/kg b.w Metformin free drug	++	++	5	10
500 mg/kg b.w Metformin loaded microsphere	+	+	3	20
500 mg/kg b.w Metformin free drug	+	+	3	21

Tumor sections from three different tumors in each group, and four randomly selected areas from each tumor were analysed (12 HPFs); MF= Mitotic figure; HPF= High power field (X400); Abs= Apoptotic body; (+), mild; (++), moderate; (+++), sever.

7. Detection of intracellular ROS in in-vivo EAC tumor cells

8. Flow cytometric analysis of mitochondrial membrane potential (ΔΨm)on the in-vivo EAC tumor cells

9. Determination of cleaved Caspase 3 flow-cytometry apoptosis analysis in thein-vivo EAC tumor

10. Determination of cleaved PARP flow-cytometry apoptosis analysis in the in-vivo EAC tumor

In order to classify the type of cell death caused by metformin in EAC cells, flow cytometry was done to detect cleaved PARP. The executioner caspase 3 has previously been detected to be activated downstream of mitochondria-mediated apoptosis in this cell line (28). PARP is a substrate for both caspases 3 and 7 and cleaved PARP is a hallmark of caspase-dependent apoptosis. Cleaved caspase 3, the active form of the enzyme, was found after metformin loaded microsphere treatment and paralleled an increase in PARP cleavage **(Figure. 2A)**. Expending flow cytometry analysis, we experimental important inhibitory effect with metformin drug-loaded microspheres compared to metformin free drug, and blank microspheres treated EAC cells **(Figure. 9D)**. Also, these consequences exhibit that metformin drug-loaded microspheres triggered cleaved PARP in a dose-dependent manner. This suggests that an apoptotic cell death pathway is encouraged by metformin loaded microsphere.

11. Determination of p53 flow-cytometry apoptosis analysis in the in-vivo EAC tumor

The p53 protein is a transcription factor which takes part in cell cycle arrest, DNA repair and apoptosis. In EAC cells, this protein is down regulated by an oncogenic E6 HPV protein. So, changed expression of the p53 protein in EAC cells was evaluated after treatment with metformin loaded microsphere **(Figure. 9E).** EAC tumor cells expressing p53 protein became up-regulated after treatment with metformin loaded microsphere **(Figure. 9E).**After treatment with metformin loaded microsphere, p53 protein expression in EAC cells highly

increased compared with free metformin, metformin loaded microsphere, and blank microsphere and it reached significant difference (***P<0.001). This result indicate that metformin loaded microsphere inhibits breast cancer cell growth and survival in p53-dependent ways.

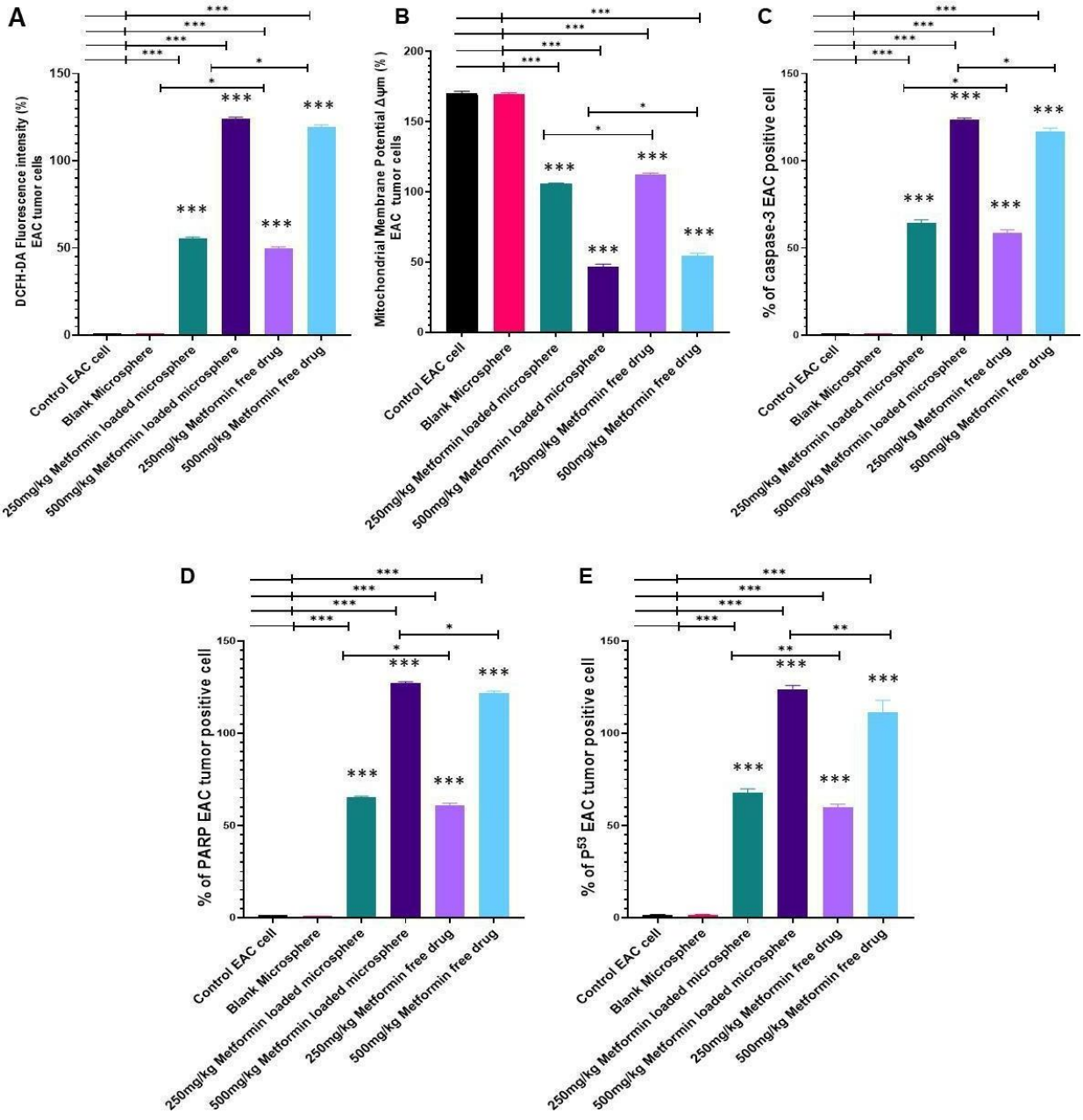


Fig. 9: This picture exhibits the oxidative-stress dependent apoptotic effect by the metformin loaded microsphere on in-vivo EAC induced swiss albino mice model. (A) This picture exhibits the Metformin loaded microsphere-induced ROS generation. In-vitro DCFH-DA

ROS fluorescence intensity of EAC solid tumor cells on different concentration drug treatment. (B) This metformin loaded microsphere-induced apoptosis through the loss of MMP potential Δψm. In-vitro mitochondrial membrane potential Δψm assay of EAC solid tumor cells on different concentration drug treatment. (C) This metformin loaded microsphere-induced apoptosis. In-vivo caspase-3 assay of EAC solid tumor on different concentration drug loaded microsphere treatment. (D) metformin loaded microsphere-induced apoptosis through p53 up-regulation. In-vitro p53 assay of EAC solid tumor cells on different concentration drug loaded microsphere treatment. (E) metformin loaded microsphere-induced apoptosis. In-vitro PARP assay of EAC solid tumor cells on different concentration drug loaded microsphere treatment. Values are mean ± SD with $n = 3$.
$^{***}P<0.001$, $^{**}P<0.01$, $^{*}P<0.05$

12. Metformin loaded microsphere treatment modulates local and systemic immune response,cytokine secretion and increases T-cells infiltration into tumor:

we found the T cell subsets present in the tumor of metformin loaded microsphere treated EAC mice by flow cytometry, 21 days after treatment. The number of **CD4+** and **CD8+** T cells increased in the metformin loaded microsphere -treated group compared with the control and free drug treated groups (**Figure 10A, 10B).** Metformin drug loaded microsphere also increased the numbers of **IFNγ**,**TNF-α, IL-10** and **IL-17**concentrations **(Figure 10D, 10E 10H, 10I).**These results suggest that T cells lung infiltration is connected to the anti-metastatic response induced by the drug metformin.

In addition to a local immune response, metformin treatment of EAC-challenged mice promoted a systemic antitumor response. metformin -treated mice exhibited significantly increased **IL-10** and **IL-17** concentrations in tumor homogenates compared with concentrations in the control group **(Figure 10H, 10I).**These results show that metformin modulates cytokine profiles locally and systemically in response to EAC challenge.

It is seen that maturation of DCs is associated with increased expression of MHC class II and costimulatory molecules, such as CD40, CD80, and CD86 on the cell surface. Our results

showed that metformin loaded microparticle treatment greatly increased the levels of costimulatory signals (**CD-40** and **CD-86)**, suggesting that these DCs are matured and activated to promote the antitumor T-cell response and induce cytokine secretion**(Figure 10C, 10G).**We also seen by flow cytometric results that the lymphoid cell population for **natural killer (NK) cells** was significantly and positively affected by metformin loaded microsphere (Figure. 10F). After initial response of systemic immune response, we profiled infiltrating leukocytes in the distant tumors. Specifically, along with the CD8+ and CD4+ T cells, the percentages of NK cells significantly increased in the metformin loaded microsphere treated group compared to the control group and the free metformin drug **(Figure 7C–E),**and the percentage of **B-cells** significantly increased in metformin loaded microparticle treated group compared to the control group and the free drug treatment **(Figure. 7F).**These results suggest that metformin loaded microsphere plays an important role in promoting the dramatically increased NK cell infiltration and accumulation in the distant tumor sites and activating tumor-specific T-cells responses to control the distant tumors, and evokes B-cells infiltration to the distant tumors, which can potentially induce antitumor humoral immune responses**(Figure. 10J).**

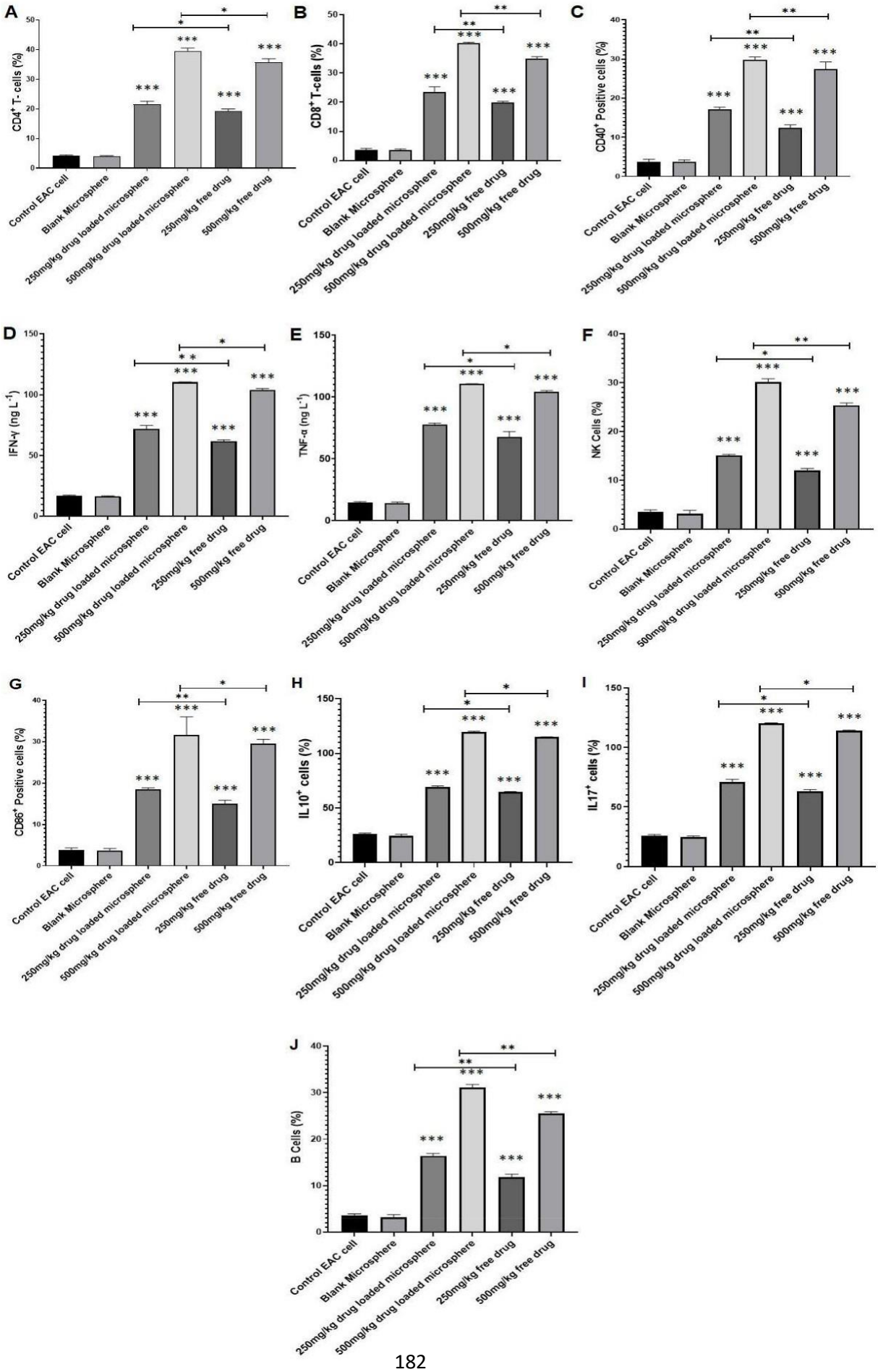

A
CD4+ T- cells (%)
B
CD8+ T-cells (%)
C
CD40+ Positive cells (%)
D
IFN-γ (ng L-1)
E
TNF-α (ng L-1)
F
NK Cells (%)
G
CD86+ Positive cells (%)
H
IL10+ cells (%)
I
IL17+ cells (%)
J
B Cells (%)
Control EAC cell
Blank Microsphere
250mg/kg drug loaded microsphere
500mg/kg drug loaded microsphere
250mg/kg free drug
500mg/kg free drug

Fig. 10: Metformin loaded microsphere nanoparticle drug therapy modulate immune checkpoint and activates the antitumor immune response. Immune microenvironment remodulation within EAC solid tumor after formulated drug therapy compare with free drug and EAC control group. CD4+ T-cells;(B) CD8+T-cells;(C) CD40+ cells; proinflammatory cytokines (D) IFNγ, (E) TNF-α, (H) IL-10 and (I) IL-17; (G) CD86+ cells;(F) NK cells;(J) B-cells.Significant statistical differences analysiswas assessed using *t*-test to determine differences within groups and between groups, respectively. Results are presented as mean (SD).

13. Discussion:

Deassociation of cancer cells from extracellular matrix is an early step in the metastatic cascade. Here we show that Metformin loaded microspheres formulation treatment completely prevents proliferation and immunological modulation of in-vitro MDA-MB-231 breast cancer cells and in-vivo EAC mice model.

The anticancer activity of the administered of drug-loaded microparticles in swiss albino mice with EAC carcinoma was resulted that the tumor volume and tumor weight from the metformin loaded microspheres treated group was significantly lower than those of the other free drug and other groups. At the same time, the size of the tumor nodes was very smaller than those of the other groups. The in-vivo survival rates of each group was also examined. After administration of saline and blank microspheres, all mice died within **23 days** due to the rapid growth of the tumors. The median survival time in the metformin loaded microparticles group was significantly longer than that in the free metformin, blank nanofibrous microspheres groups. The combined nanofibrous microspheres treatment resulted in dramatic inhibition of angiogenesis in the tumors. The microvessel density (MVD) in the metformin loaded microparticles group dramatically lower than that in the free metformin drug group. The results implied that anti-angiogenesis may be another mechanism of inhibiting tumor progression by the metformin microspheres in-vivo mice model.

The in-vitro data for metformin loaded microparticlesindicate that they exert similar cytotoxicity profiles as Metformin on cancer cells. Based on these promising in-vitro data, in-vivo efficacy studies were conducted in in-vivo mice model. Experiments were performed to assess the efficacy of metformin microspheres as a treatment for EAC carninoma and to examine metformin loaded microspheres as a delivery system targeted to the tumor site. Carcinoma tumors were induced using EAC cells. Our results summarize the consequences

of the in-vivo efficacy studies using Metformin -loaded microparticles. Mice bearing tumors were treated with microspheres containing metformin. metformin -loaded microspheres inhibited tumor growth more effectively than did the metformin solution at weeks 2 and 3 after treatment. In addition, the metformin microparticles inhibited approximately 20% more tumor growth as compared with the metformin solution at week 4 following treatment.

To analyse the antiproliferative mechanism, cell apoptosis analysis was conducted. Significant cell apoptosis was seem after treatment with metformin loaded microspheres, and the total apoptotic ratio of the metformin loaded microspheres increased respectively comparable with the free drug. Similarly, the total apoptotic percentage of the Metformin loaded microparticles group was much higher than that of the free drug treatment dose dependently. This phenomenon was consistent with the antiproliferative activity, and both were attributed to the improved release behaviour of metformin loaded microspheres. The morphological study showed the presence of compaction, condensation and segregation of the nuclear chromatin after treatment with the microsphere release supernatant, exhibiting typical apoptosis morphological features.

Diabetics using metformin have a lower risk of cancer and metformin has been found to enhance neoadjuvant therapy for diabetic breast cancer patients. Metformin has previously been shown to inhibit proliferation of various types of cancer cells. In addition, metformin has been shown to promote cell death of some cancer cells through activation of apoptotic pathways. The data presented here confirm metformin loaded microparticles -induced killing of aggressive breast cancer cells through apoptosis in breast cancer cells in a p53-dependent manner. This mode of cell death is dependent on PARP and based of caspase activity. PARP-mediated cell death has been found under a number of different circumstances, including treatment with DNA alkylating agents, following oxidative DNA damage, treatment with neurotoxic compounds, and ischemia. PARP-dependent cell death appears to be a unique form of programmed cell death that is distinct from apoptosis (29). Here we show that metformin treatment of MDA-MB-231 breast cancer cells enhances both PAR synthesis and AIF uptake into the nuclear compartment. PARP-dependent cell death of metformin treated cells appears to be delayed relative to apoptotic cell death and is associated with changes in mitochondrial morphology. In metformin loaded microspheres treated breast cancer cell lines

examined, mitochondria become enlarged and this is blocked by PARP and caspase activation.

To further study the apoptosis mechanism, the related proteins were studied by flowcytometry. The relevant pro-apoptosis proteins, including caspase-3 and PARP, upregulated after treatment with the release supernatants of metformin loaded microspheres The in-vivo antitumor activities of different drug formulations was investigated on EAC tumor-bearing swiss albino mice. To further estimate the antitumor activity, histological (hematoxylin/eosin (H&E) analyses were found to tumor tissues from different group.

In this study, we have seen a nano-immunotherapy, which metformin loaded microspheres with a metformin loaded microparticles checkpoint inhibition for treating breast cancer cells. We tested the effect of our nanoimmunotherapy in a synchronous mode of neuroblastoma.

After initial sign of systemic immune response, we further profiled infiltrating leukocytes in the distant tumors. The percentage of CD40+ and CD86+ leukocytes in the total tumor cells significantly increased in the metformin loaded microparticles treatment group, compared to the PBS control group and the free drug. Specifically, the percentages of NK cells, CD8+ T cells and CD4+ T cells all significantly increased in the metformin loaded microspheres treated group compared to the control group and metformin free drug treated group, while the percentage of B cells significantly increased in metformin loaded microspheres treated group compared to the control group and free drug treated group. These results suggest that metformin loaded microspheres checkpoint blockade plays an important role in promotingthe dramatically increased NK cell infiltration and accumulation in the distant tumor sites and activating tumor-specific T cells responses to control the distant tumors, while metformin loaded microspheres evokes B cells infiltration to the distant tumors, which can potentially induce antitumor humoral immune responses. There was also though statistically significant, increase in the percentage of regulatory T cells in tumors treated with metformin loaded microspheres, which may have contributed to the increased CD8+ T cells activity. A combination of these immune response was likely required for the eradication of the primary tumor and inhibition of distant tumor, supporting the increased efficacy of metformin loaded microspheres combined with immune checkpoint blockade therapy.

14. Conclusion :

Interestingly, in-vitro and in-vivo studies in which we observed the highest efficiency for particles composed of metformin loaded microsphere which can deliver metformin in the fastest manner, correlates with their apoptotic and immunological cell death activity. Moreover, the size also determines their uptake by breast cancer cells. These advantageous features indicate that metformin loaded microspheres can enhance the efficiency of chemotherapy against breast cancer as these metformin -loaded microcarriers could be potentially administrated as oral dose. Based on their high antigen binding efficiencies, metformin loaded microsphere conjugates could be useful for the delivery of various anticancer drugs for enhanced therapies for cancer.

In summary, we have shown that the Metformin loaded microsphere can lead to tumor regression even in established aggressive breast cancer metastases and achieve long-term protection against tumor recurrence. Although this treatment regimen will be challenging for direct clinical translation due to the difficulty to identify optimal dosing levels and schedules for each component, our protocol provides a framework of potentially curative strategy for breast cancer metastases via the induction of memory T-cell response.

Chapter - 7

Summary and future works

Contents

SUMMARY AND FUTURE WORKS

1. Introduction

The aim of the research is to produce an effective therapeutic concentration in the target area. This is not very easy to prepare target oriented dosage form. for this reason we prepared one matrix which contains the active ingredient. The drug is embedded in the matrix by the use of two gum. Here we selected sodium alginate and guar gum to prepare the gummy matrix and its release also continuous. Overall drug release is maintained by continuous and steady release of the medicament. Thus the optimum level of drug release can be obtained in 24 hours. In vivo reports also suggests an effective and controlled care of diabetes mellitus type-2. It is evident due to the slow continuous release of the drug through a constant level. Even its antidiabetic approach becomes more applicable with its use with an antioxidant thiamine. The anti tumor effect of metformin microsphere is also seen. Various anticancer studies have been performed with human breast cancer cell lines and MDA MB 231 solid tumor cell lines. The apoptotic pathway is also studied in this research work. So we here done the research work to establish metformin microsphere as a suitable and effective dosage form in the controlled release.

2. Formulation development

Microspheres are prepared with the desired release to be continuous and moderate. To achieve this goal various methods are optimized. Double emulsion solvent evaporation technique[379] is most convenient. Here we prepare one gummy matrix to pack the active ingredient into it. Here we have used guar gum to bind with sodium alginate in the gummy matrix [380]. One non aqueous phase is required also. We have got dichloromethane (DCM) for this purpose as a solvent. Ethyl cellulose is the polymer used for this purpose and to stabilize this liquid phase span-80 is added. Tween-80is added in the water phase. Microsphere is an effective tool of drug development. The sustained release dosage form or controlled release drug delivery is achieved by microsphere [381]. This is again one important aspect for modern day drug delivery research. Delivery of the active ingredient in the proper site with an effective therapeutic concentration is very important is modern day drug delivery research [382].

With this aim to provide adequate amount of drug in the target site. Novel drug delivery is introduced. Microsphere is a very important part of that. In this research work we have used guar gum as a viscosity builder in the formulation. The study shows that with the increase in the concentration of guar gum the

particle size is increased [383]. When the drug : guar gum is 1:0.37 the average particle size increased to 192.08 µm whereas when its (1:1) the particle size is 556.48 µm. This particular fact is observed only due to the viscosity building capacity of guar gum. With the increase in guar gum in the w1 phase increases the volume in the W1 phase resulting an increase in the particle size [384].

The drug entrapment is also studied. This is nothing but the capacity to entrap the drug in the formulation. The amount of drug entrapped in the gum is shown by drug entrapment efficiency or DEE. In this formulation it is seen that guar gum has increased in the DEE to a certain extent i.e. the first four batches (f1 – f4) but after that it decreases. As at first with the increase in the guar gum concentration the leaching of the drug increases from W1 phase to the W2 so DEE also increases. But after a certain time with the increase in gum concentration the DEE is decreasing. Guar gum is a bulk material which forms some undiagnosed properties in the formulation. There is a formation of in homogeneous emulsion in the W1 phase. This may push the dispersed phase droplets from the inner phase to the external phase . Viscosity is already raised due to the increase in the concentration of gum in the W1 phase. And the mass transfer occurs from the internal to the external phase [385].

It has been observed that with the increase in the concentration of san-80 the average particle size is increased. But with 1 % concentration of span-80 it produces good DEE and average particle size is also moderate with a higher drug release [386].

Different processing temperature are found to follow the various formulation. It has been found from the results that 38 ºc is optimum for the processing. As it has seen to provide maximum drug entrapment with proper particle size and higher release.

Various stirring speed being followed for study. At 780 rpm the microspheres show good release. The drug entrapment and the particle size is also higher.

In the experiment we have seen 5ml is the proper aquous phase volume. The drug entrapment, average particle size and drug release is higher with this aquous phase volume.

SEM pictures of the prepared matrix microsphere found to be circular particles in the picture. The roughness in the outer surface of the matrix microsphere is due to the rapid evaporation of the solvent [387-388].

The FTIR studies are perd. there is no interaction between the metformin and the formulation. So FTIR pictures do not show any chemical interaction.

The XRD studies are performed. In that we find that pure drug metformin is showing sharp peak. The microsphere for the drug and polymorphic forms for the gum. The guar gum shows amorphous peak in its WRD figures. This can be explained by the X-Ray crystallography theory.

This says that the crystalline substances will show sharp peaks. It is due to their chemical structure.

3. Antidiabetic study

Antidiabetic study is performed for the in vivo effect of the prepared formulation. There are several parameters which are studied in this part.

a. Effect in blood glucose level (fasting)

In the fasting blood glucose level we have seen severe decrease in high blood sugar level. This was observed in streptozotocin induced induced diabetic rat model. Control of blood sugar is observed in microsphere treated groups as well as microsphere treated groups as well as microsphere with thiamine treated groups. This was a 21 day experiment [389-407].

b. Effect in post pardinal (PP) blood sugar

With the 21 days of anti diabetic experiment the PP-Blood glucose level also controlled. This is too the fact of its anti diabetic effect. The streptozotocin induced diabetic rats showed control in type-II diabetic melitus with metformin microsphere and metformin microsphere with thiamine.

c. Effect of total cholesterol and triglyceride

It has been proved that with the use of metformin microsphere total cholesterol and triglyceride will be controlled. Metformin microshere with thiamin also controls it in a satisfactory way.

d. Control over HDL and LDL

HDL is high density lipo protein and LDL is low density Lipoprotrein which should be in a specified amount in the body. Metformin microsphere and metformin microsphere with thiamin help to control that [403-408]

e. Histopathology

Histopathologic studies were done of the liver, kidney and pancreas. Histology of normal control groups exhibits no change in its structure. The diabetic

control group liver cells show damage in its overall shape. Those treated with metformin treated microsphere their portal vein is found to recover the deformities. Histopathology was done by 10x of the light microscope.

We performed the histology of the pancreatic cells also. This was done under the 10x of the light microscope. In the diabetic control group lot of deformities was seen. We see some developed lobular arrangement in metformin microsphere treated group and metformin microsphere thiamin treated groups. The cell proliferation in the islets of langerhance tissues was also seen in the drug treated and microsphere treated groups.

The kidney tissue were subjected for histopathological tests also. It was done in 10x light microscope. In the diabetic control group the glomerular vessels were destructed. The shape of the bowman's capsule was also destructed. The endocytic vacuole was also not found in the control group.

But there was development found in the 80mg/Kg body wt. drug treated group after 21 days. It is also seen that healing in glumerular and bowmans capsule structure with microsphere with thiamine treated groups. IMPROVEMNETS IN THE VACUOLe structure and also the overall irregularities found with the microsphere treated and microsphere with thiamin treated group.

4. Anticancer study

Cell lines and cell culture for the experiment –

for this anticancer research work we used two types of cell lines. We used MDA –MB 231 (aggressive breast cancer cell line) and Ehrlich ascites carcinoma (EAC) cells. The cells were arranged from chittaranjan national cancer research institute, Kolkata, India. The incubation of cells was done. It was done by 5 % foetal bovine serum albumin (FBV) with penicillin and streptomycin (100 mg/ml) at 37 c in a hid atmosphere 5% CO_2, 95% AIR)> CELLS Were harvested after that short incubation in 0.02% (W/V) EDTA in PBS was done.

A. IN Vivo study

Measurement of different parameters like tumors weight , tumor volume , mean survival time, body wt etc are done. This is done for all the animal group with formulated drug in different doses,

It has been seen that with 250mg/k body wt and 500 mg/kg body wt the results are good. so we can say that apoptotic activities is good in the

formulated drug. Determination of mean survival time and percentage of in invase in the life span is found with the formulated metformin.

Again it has been found with different groups containing control , block, microsphere and the formulated metformin with two divide dose i.e. 250mg/ kg body wt and 500 mg/kg body wt. (35-45)

a. Histopathology

Histopathology of liver and kidney tissue was done . hematotoxin and eosin is used for staining , liver cells were finely visible, billiary canals present in the centre . As the controlled release formulation having slow rate of release so effect on liver is less. Here the formulation effectively controls the matstasis . as we have noticed several signs of metastasis . so we can say it block the metastasis of cancer cells.

b. Formulation effect on hematological and biochemical parameters

Metformin loaded microsphere used in the dose of 10mg/kg B.w . it is seen that RBC Count is controlled in comparison to control mice. Generally it is found to control WBC, RBC and haemoglobin.

ALT and AST are controlled with metformin microsphere treated groups and metformin microsphere with thiamin treated groups. These results are compared with the control. This shows that anticancer effect of formulated metformin microsphere is higher than that of powder metformin.

The creatinine level of the control is higher. The metformin powder drug treated group is providing some cure. But with the formulated metformin the creatinine level improvement is seen.

c. Histopathology study of solid EAC tumor section

The control less (EAC) are not treated with formulated drug. So we find signs of new blood vessels, capillaries and leukocytes infiltration.

But while we see the tissue sections of metformin loaded microsphere 250mg/kg bw. There is necrosis, fibrosis, destruction of blood vessels etc. the necrosis of cells and tissues is severe in comparison to metformin powder drug.

d. Detection of intracellular ROs

In EAC tumor cells we see intracellular ROs generation. This intracellular ROs generation is found in response to metformin formulation treated animals. So it implied that the formulation is working against cancer cells.

e. Flow cytometric analysis

It is for the in Vivo eac tumor cells. The mitochondrial membrane is studied with jc1 staining. The eac cells show response with free metformin powder formulated metformin with jc1 staining. Mitochondrial membrane potential is found with jc1 staining. The intensity is higher with formulated drug.

f. Cleaved caspase 3 detection in flow cytometry

They apoptotic analysis is with the cleft caspase 3 is found. Caspase 3 is an Important parameter and are effective in decreasing vital cellular components.

g. Determination of cleaved PARP

it is done for the effect of drug and the formulation in the cancer cell. PARP works as a substrate for both Casper three and seven. Slave PARP is an identification of caspase development apoptosis. From the flow cytometry analysis we find the inhibitory effect the metformin free drug and metformin microsphere. So the apoptosis is seen.

h. Study of p53 flow cytometry

The p53 Jain is important to protect cancer. It helps in the cell cycle arrest DNA repair and apoptosis. M e a c cells the cells become in effective. It is seen with the formulated drug some better response of p53 bearing cells to prevent cancer. The p53 protein expression is higher with treated drug in breast cancer cell.

In vitro studies

a. Cell cytotoxicity assay for formulated drug

The effect of the free drug and formulated drug is studied in MDA MB 231 breast cancer cell line and EAC bearing mice. It is seen after mtt essay that guar gum containing microsphere showing no toxic effects.

b. Detection of intracellular ROs

ROs is reactive oxygen species and this is produced in response to chemotherapeutic drug. And this is cytotoxic too. Intracellular ROs production

is studied in MDA MB 231 breast cancer cell lines. This is done by DCFH DA staining. This die generates fluorescence while reacts with ROs. After treatment with metformin free drug and metformin microsphere we see r o s generation. At the same time blank microsphere do not produce ROs.

c. Flow cytometry to study the mitochondrial membrane potential

Mitochondrial membrane potential is studied. This is done by jc1 staining. The optic of jc1 staining is higher in metformin loaded microsphere. So mitochondrial membrane potential is higher. This indicate higher apoptic activity.

d. Detection of cleaved PARP

This is done by flow cytometry. Cleaved p a r p is an indicator of apoptosis. P a r p is a substrate for both caspase 3 and 7. With metformin loaded microsphere cleaved p a r p formation is higher. This is a sign of higher apoptotic activity

e.Apoptotic activity in p53 cells

The p53 protein is very effective in DNA repair and cell cycle arrest. This cell is very effective in producing apoptosis. Both in MDA MB 231 breast cancer cell lines this p53 cells found to be down regulated. But after the treatment with metformin drug loaded microsphere the p53 cells become up regulated. This is an indication of apoptotic activity.

f. Future work

Metformin microsphere prepared by double emulsion solvent evaporation technique. Here we have used guar Gam as a matrix building material. The drug is embedded in the matrix. In the external organic phase we have used dichloromethane (DCM) as a solvent. Here in this formulation we have used ethyl cellulose as a polymer. For the preparation of the microsphere here it took three hours. But if we mix hydroxy propyl methyl cellulose (HPMC) in a certain ratio with ethyl cellulose that time may become reduced. It seems that rotating time in the water phase will be reduced.

Conclusion

This work confirms that drug is embedded in the matrix. The matrix is build up by sodium alginate and guar gum. The polymer used in the organic phase is ethyl cellulose. Dichloromethane or DCM is the vehicle used in the organic phase . microsphere are prepared.

Targeted drug delivery is a better therapy for the drug delivery research . in this work metformin formulated microsphere are given orally to the streptozotocin induced diabetic rat model. In this context we have seen that the formulated microsphere controls the diabetic management very well. This is found in both fasting and p.p. blood sugar .

At the same time the formulated microsphere also controls the drug delivery and lipid profile, the serum HDL and LDL and cholesterol level is under control.

The formulated rug also controls the ALT , AST and serum creatinine level. Thus it controls the blood sugar, lipid profile, urea, cretinine and other biochemical factors like, SGOT, SGPT, ALT etc.

The formulated microsphere also found to control the anticancer research.

Various flow cytometric studies were performed to detect the intracellular ROS generation . in vitro detection of MDA-MB 231 breast cancer cell lines. The in vitro flow cytometry was done to detect the mitochondrial membrane potential.

Cleaved caspase- 3 flow cyto metric analysis was done to detect the cancer cells. Flow cytometry was done to detect the apoptotic analysis in MDA MB 231 cell lines. The flow cytometry was also done to detect the in vitro apoptotic analysis of P53 genes.

Evaluation of acute toxicity was measured with animal study. LD 50 was measured with the OECD guidelines.

Histopathological study of liver and kidney tissue were done to find out the apoptotic pathway with the formulated drug.

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