Protective role of *Swietenia mahagoni* against high fat diet and low dose of streptozotocin induced diabetes

Thesis submitted in partial fulfillment for the requirements of the degree of
MASTER OF PHARMACY
(2016)

Submitted by

MS. SHILPA DAS
B.PHARM.
Examination Roll. No. M4PHA1622
Registration No. 129117 of 2014-2015

Under the guidance of

DR. SAIKAT DEWANJEE
Assistant Professor
Department of Pharmaceutical Technology,
Faculty of Engineering & Technology
Jadavpur University Kolkata- 700032, India.

Protective role of *Swietenia mahagoni* against high fat diet and low dose of streptozotocin induced diabetes

Thesis submitted in partial fulfillment for the requirements of the degree of

MASTER OF PHARMACY

(2016)

Submitted by

MS. SHILPA DAS

B.PHARM.

Examination Roll. No. M4PHA1622

Registration No. 129117 of 2014-2015

Under the guidance of

DR. SAIKAT DEWANJEE

Assistant Professor

Advanced Pharmacognosy Research Laboratory

Department of Pharmaceutical Technology

Faculty of Engineering & Technology

Jadavpur University

Kolkata- 700032, India.

CERTIFICATE OF APPROVAL

This is to certify that the thesis entitled "Protective role of Swietenia mahagoni against high fat diet and low dose of streptozotocin induced diabetes" has been carried out by Ms. Shilpa Das under my supervision at the department of Pharmaceutical Technology, Jadavpur University, Kolkata- 700032. She has incorporated her findings into this thesis of the same title, being submitted by her, in partial fulfillment of the requirements for the degree of "Master of Pharmacy" of this university. She has pursued this research work, independently and with proper care and attention to my entire satisfaction.

Dr. Saikat Dewanjee

Project Supervisor

Department of Pharmaceutical Technology,

Jadavpur University

Kolkata 700032, India

Prof. (Dr.) Biswajit Mukherjee

Head of the department,

Department of Pharmaceutical Technology,

Jadavpur University

Kolkata 700032, India.

Dean, faculty of Engg. & Tech.

Jadavpur University

Kolkata- 700032, India.

DECLARATION OF ORIGINAL AND COMPLIANCE OF ACADEMIC ETHICS

I hereby declare that this thesis contains literature survey and original research work pursued by me. As a part of my thesis work entitled "Protective role of *Swietenia mahagoni* against high fat diet and low dose of STZ induced diabetes".

All the information in this document have obtained and presented in accordance with academic rules and ethical conduct.

I also declared that as required by thesis rules and conduct, I have fully cited and referenced all the materials and results that are not original to this work.

Name: Shilpa Das

Examination Roll No. M4PHA1622

Registration No. 129117 of 2014-2015

Thesis title: "**Protective role of *Swietenia mahagoni* against high fat diet and low dose of streptozotocin induced diabetes".**

Date:

Place:

Acknowledgements

I convey sincerest regard and deepest gratitude to my guide Dr. Saikat Dewanjee, Assistant Professor "Department of Pharmaceutical Technology", Division of Pharmacognosy of Jadavpur University for his guidance, support and inspiration, without which this project would never have been possible.

I also grateful to Prof. (Dr.) Biswajit Mukherjee, Head of the Department of Pharmaceutical Technology for providing me the facility to do this job and all the faculty member of this department for their invaluable suggestion and kind co-operation.

I express my sincere gratitude and special thanks to Mr. Tarun Kumar Dua, Mr. Niloy Bhattacharya, Mrs. Ritu Khanra, Ms. Sujata Barma, Ms. Swarnalata Joarder, Mr. Subhodeep Das, Mr. Sonjit Das for giving me the strength to carry out this work and inspiring me to scale new heights.

I prefer to say thanks to all of my friends and relatives who helped me directly or indirectly during my research work.

I am thankful to the authorities of Jadavpur University for providing me facilities to complete my work successfully.

I am sincerely thankful to the staff of this department. I will always remember them.

Finally I exceedingly thankful for my parents for giving me the strength and unlimited blessing that enable me to reach to this stage of life.

Date: ----------

Place: *(Shilpa Das)*

CONTENTS

List of figures

mg/kg) were compared with standard drug glibenclamide (1 mg/kg) and it was found that the hypoglycemic and hypolipidemic effect of AESM (200 mg/kg) were comparable to that of glibenclamide (1 mg/kg).

Figure 5.4. Group II exhibited thickening of glomerular basement membrane (red arrows) which indicates glomerular damage, while, Group I exhibited normal glomerular arrangement in kidney sections. Group III, IV and V indicated significant improvement in podocyte structure comparable to that of Group I. Group I: Normal control; Group II: T2D control, Group III: T2D rats treated with AESM (100 mg/kg, orally); Group IV: T2D rats treated with AESM (200 mg/kg, orally); Group V: T2D rats treated with glibenclamide (1 mg/kg, orally). The observed effects of AESM (100 and 200 mg/kg) were compared with standard drug glibenclamide (1 mg/kg) and it was found that the hypoglycemic and hypolipidemic effect of AESM (200 mg/kg) were comparable to that of glibenclamide (1 mg/kg).

Figure 5.5. Group II exhibited abnormal architecture of liver tissue and distranded central vein (red arrows), while, Group I showing normal architecture. Group III, IV and V indicated significant improvement in tissue architecture comparable to that of Group I. Group I: Normal control; Group II: T2D control, Group III: T2D rats treated with AESM (100 mg/kg, orally); Group IV: T2D rats treated with AESM (200 mg/kg, orally); Group V: T2D rats treated with glibenclamide (1 mg/kg, orally). The observed effects of AESM (100 and 200 mg/kg) were compared with standard drug glibenclamide (1 mg/kg) and it was found that the hypoglycemic and hypolipidemic effect of AESM (100 mg/kg) were comparable to that of glibenclamide (1 mg/kg).

List of tables

Chapter 1

INTRODUCTION

1. INTRODUCTION

1.1. Traditional medicine and drug discovery

Plants have been used as medicine by a majority of cultures around the world. Currently these herbal medicines are being investigated as possible targets for drug development. During recent years, herbal medicine has began to develop into a scientifically based system of healing. Due to demands from both the public and medical practitioners, studies leading to the scientific explanation of plant therapeutic capabilities are allowing this practice to gain increasing credibility and acceptance within medical community (Naveen and Urooj, 2014).

By definition 'traditional' use of herbal medicines implies substantial historical use, and this is certainly true for many products that are available as 'traditional herbal medicines'. In many developing countries, a large portion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet health care needs. Although modern medicine may exist side by side with such traditional practice, herbal medicines have often maintained their popularity for historical and cultural reasons. Such products have become more widely available commercially, especially in developed countries. In this modern setting, ingredients are sometimes marketed for uses that were never contemplated in traditional healing systems from which they emerged. An example is the use of ephedrine for weight loss or athletic performance enhancement (Naveen and Urooj, 2014). While in some countries, herbal medicines are subject to rigorous manufacturing standards, this is not so everywhere. In Germany, for example, where herbal products in the marketplace are marketed and regulated as dietary supplements, a product category that does not require preapproval of products on the basis of any of these criteria.

The pharmacological treatments of the diseases began long ago with the use of herbs. Methods of folk healing throughout the world commonly used herbs as part of their tradition. Some of these traditions are briefly described below, providing some examples of the array of important healing practices around the world that used herbs for this purpose. Traditional Chinese medicine has been used by Chinese people from ancient times. More than 12000 items of traditional medicines are used by traditional healers. Ayurveda is a medical system primarily practiced in India that has been known for nearly 5000 years. It includes diet and herbal remedies, while emphasizing the body, mind and spirit in disease prevention and treatment.

One of the most difficult issues to contend with in translating traditional herbal practices into conventional 'Western' medicine is the individualization of prescriptions containing multiple herbal and other ingredients. There is little incentive for standardization of products for a mass market, when the intention has been to provide an individual prescription. To the small grower or the traditionally trained herbalist, standardization means understanding the growth conditions, the time of harvesting, the manner of extraction or other preparation of material so that a reliable active ingredient can be offered to people.

In USA, there is both small scale and large scale production of herbal products and there can be wide variation in their content and quality in the marketplace. Regulation in the USA does not yet require that dietary supplement manufacturer adhere to standard manufacturing practices, and so quality is not guaranteed.

For herbal products in common use, evidence of efficacy may be based upon traditional uses, testimonials, clinical studies, both controlled and uncontrolled, and randomized, double blind, and placebo-controlled trials.

Safety of some herbal ingredients has been recently called into question, in part because of the identification of adverse events associated with their use and increasingly because of the demonstration of clinically relevant interactions between herbs and prescription drugs.

Findings new habitats of plants and identify new biologically active natural products in plants and plants material used in traditional medicine has been a continuous challenge. The use of medicinal plants in the form of crude extracts presents several difficulties. The amount of the bioactive compounds from plants may vary with both locality and season in which they are collected. Also bioactive molecules of many plants are powerful poisons when taken in excess, and if the plant extract contains a lower content of bioactive compounds than usual, sub- optimal dosage may not be effective. Medicinal properties of many plants are also rapidly lost on storage, for example bioactive molecules of foxglove leaves decompose on long storage, unless dried quickly after collection. Furthermore, crude extract from many medicinal plants may contain, in addition to the bioactive molecules, other constituents which have harmful effects. For example aristolochic acid presents in a Chinese plant, *Aristolochia fangch* are nephrotoxic and carcinogenic compounds closely associated with renal failure. It is therefore important to isolate and identify the bioactive molecules from plant extracts. The advantage of using pure drugs instead of crude extracts may allow an improvement in the efficacy and moderation of side effects. Pure bioactive molecules can frequently be synthesized economically, thus preventing dependence on plant as a source. Therefore, the phytochemical research based on ethno pharmacology is considered an effective approach in drug discovery, however in this case no information are available on the nature of secondary metabolite; thus all the extraction /purification/separation process are performed in order to “find and follow” the supposed pharmacological activity with the final aim to isolate and identify the bioactive

compounds with high sensitivity and selectivity (Gray *et al.*, 2010). The history of the extraction of natural products dates back to Mesopotamian and Egyptian times, where production of perfumes or pharmaceutically active oils and waxes was a major business. After the short historical review we should consider the language used. In German, "Natural plant extraction" is equivalent to "Phytoextraction", which in English terms means extraction achieved by plants (Cannell, 1990). A natural product is a chemical compound or substance produced by a living organism. They may be extracted from tissue of terrestrial plants, marine organism or microorganism fermentation. In that respect any biological molecule is a natural product, but in general the term is reserved for secondary metabolites (carotenoids, phytosterines, saponins, phenolic compounds, alkaloids, glycosinates, terpenes etc.) produced by an organism. The feed material for the extraction of natural products from plants can be leaves, flowers, branches, bark, rhizomes, roots, seed, fruits and active pharmaceutical ingredients content is usually ranging between 0.3-3% with seasonal fluctuations in period and production area .

Extraction of plant materials can be done by various extraction procedures such as maceration, infusion, digestion, decoction, percolation, hot continuous extraction (soxhlation), counter current extraction, ultrasound extraction, supercritical fluid extraction etc. It is very essential to choose an appropriate process of extraction, to conduct further separation, identification and characterization of bioactive compound. Extraction process or techniques have some common objectives including extract of different bioactive compounds from complex plant sample, to increasing the selectivity of analytical methods, increasing the sensitivity of bioassay by increasing the concentration of targeted compounds, converting the bioactive compounds into a more suitable form for detection and separation and providing a

strong and reproducible method that is independent of variations in the sample matrix (Smith, 2003). After extraction it is necessary to isolate and evaluate the active compound which has desired action and followed by the toxicity studies. To obtain isolated active compounds, the plant extracts are first qualitatively analyzed by chromatographic methods.. For purification and isolation, the active plant extracts are sequentially fractionated. Usually, plant extract contains thousands of chemical constituents but only a few components are bioactive (Li *et al.*, 2011). Some chemical constituents have therapeutic value so used in drug formulation but some are very potent and produce undesirable effect. Therefore it is essential to separate out those compounds which are responsible for therapeutic effect is called as active constituents. The used of isolated active constituents is obvious, since these compounds are having a fixed and definite physiological effect. So the isolating and using the compounds in formulations will potentiate the activity.

Isolation is a part of natural product chemistry, through which it is possible to separate different components and biologically active ones which can be incorporated as the ingredients in the modern system of medicine. Column chromatography technique is widely used for the separation, isolation, and purification of chemical constituents from natural drugs. Further, it is essential to identify and measure all of the bioactive constituents of medicinal plants to ensure the reliability and repeatability of clinical research and enhance quality control from pharmacologically beneficial and hazardous perspectives. However, the inherent complexity and variability of botanical extracts has presented significant challenges for separation and detection methods enabling rapid analysis of the chemical composition of medicinal plants (Marston *et al.*, 2009). After verifying the purity of an isolated active compound, the structure is determined by spectroscopic methods (UV, IR, MS and NMR).The development of advanced

analytical techniques it helps to identification of isolated compound, characterization of their chemical structure and bioactivity, quantification in the natural source, product development, quality control in their dosage forms etc. Due to the complexity of these natural matrices, the use of advanced analytical techniques (such as Mass Spectrometry (MS), Nuclear Magnetic Resonance (NMR), High performance Liquid Chromatography (HPLC), Capillary Electrophoresis (CE), HPLC-NMR, HPLC-MS, GC-MS and CE-MS) is mandatory in order to carry out studies. Once the chemical structure is defined, total or partial synthesis and preparation of derivatives or analogues can be considered, and modulation of the biological activity and definition of the structure activity relationship can be carried out. After completing all these steps, large scale isolation (it may necessary to collect the plant again) or partial or total synthesis is required for pharmacological evaluation in preclinical, clinical and toxicological trials aimed at future therapeutic use. Furthermore, in pharmacokinetic and pharmacodynamic studies, determination and quantification of trace metabolites of natural products in complex biological matrices also requires sophisticated analytical methods.

1.2. Diabetes- caused by high fat diet

Diabetes mellitus is a group of metabolic diseases characterized by elevated blood glucose levels (hyperglycemia) resulting from defects in insulin secretion, insulin action or both. Insulin is a hormone manufactured by the beta cells of the pancreas, which is required to utilize glucose from digested food as an energy source. Chronic hyperglycemia is associated with micro vascular and macro vascular complications that can lead to visual impairment, blindness, kidney disease, nerve damage, amputation, heart disease and stroke. In 1997, an estimated 4.5% of the US population had diabetes. Direct and indirect health care expenses were estimated at$98 billion (Loghmani, 2005).

Obesity is associated with several conditions, the most devastating of which may be type 2 diabetes. At the turn of this century 171 million individuals were estimated to have diabetes and this is expected to increase to 366 million by 2030 (Wild *et al.*, 2004). Both obesity and type 2 diabetes are associated with insulin resistance. But most obese, insulin resistant individuals do not develop hyperglycemia. Under normal conditions, the pancreatic islet β-cells increase insulin release sufficiently to overcome the reduced efficiency of insulin action, thereby maintaining normal glucose tolerance (Parley and Kipnis, 1966). For obesity and insulin resistance to be associated with type-2 diabetes, β-cell must be unable to compensate fully for decreased insulin sensitivity. β-cell dysfunction exists in individuals who are at high risk of developing the disease even when their glucose levels are still normal (Kahn, 2001). Non-esterified fatty acids induced insulin resistance and impair β-cell function, making them a likely culprit. The most critical factor in the emergence of metabolic diseases is obesity. Adipose tissue modulates metabolism by releasing (Non essential fatty acids) NEFAs and glycerol, hormones-including leptin and adiponectin-and proinflammatory cytokines (Wellen and Hotamisligil, 2005). In obesity, the production of many of these products is increased. Retinol-binding-protein-4 induces insulin resistance through reduced phosphatidylinositol-3-OH kinase [PI(3)K] signaling in muscle and enhanced expression of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase in the liver through a retinol dependent mechanism (Yang *et al.*, 2005). By contrast, adiponectin acts as an insulin sensitizer, stimulating fatty acid oxidation in an AMP activated protein kinase (AMPK) and peroxisome proliferators activated receptor α (PPAR-α)-dependent manner (Scherer, 2006; Kadowaki, 2006). In addition to adipocyte derived factors, increased release tumor necrosis factor-α (TNF-α), interleukin-6(IL-6), monocyte chemo attractant protein 1(MCP1) and additional products of macrophages and

other cells that populate adipose tissue might also have a role in the development of insulin resistance. TNF-α and IL-6 act through classical receptor mediated processes to stimulate both the c-Jun amino-terminal kinase (JNK) and the IκB kinase-β (IKK-β) nuclear factor–κβ (NF-κβ) pathways, resulting in up regulation of potential mediators of inflammation that can lead to insulin resistance.

Pathways involving the induction of suppression of cytokine signaling (SOCS) proteins and inducible nitric oxide synthase may be involved in mediating cytokine-induced insulin resistance. Secretion of these proinflammatory proteins, particularly MCP-1 by adipocytes, endothelial cells and monocytes, increases macrophage recruitment and thereby contributes to a feed forward process (Weisberg , 2003).

The release of NEFAs may be single most critical factor in modulating insulin sensitivity. Increased NEFA levels are observed in obesity and type 2 diabetes, and are associated with the insulin resistance observed in both (Reaven *et al*., 1988; Boden, 1997). Insulin resistance develops within hours of an acute increase in plasma NEFA levels in humans. Conversely, insulin-mediated glucose uptake and glucose tolerance improve with an acute decrease in NEFA levels after treatment with the antilipolytic agent acipimox. Increased intracellular NEFAs might result in competition with glucose for substrate oxidation leading to the serial inhibition of pyruvate dehydrogenase, phosphofructokinase and hexokinase II activity (Randle *et al.*, 1963). It has also been proposed that increased NEFA delivery or decreased intracellular metabolism of fatty acids results in an increase in the intracellular content of fatty acid metabolites such as diacylglycerol (DAG), fatty acyl-coenzyme A (fatty acyl-CoA), and ceramides, which in turn activate a serine / threonine kinase cascade leading to serine / threonine phosphorylation of insulin receptor substrate-1 (IRS1) and insulin receptor substrate-

2 (IRS-2), and a reduced ability of these molecules to activate PI(3)K. Subsequently, events downstream of insulin-receptor signaling are diminished.

1.3. Aim and Objective of the project work

Current available therapy for diabetes includes insulin and various oral hypoglycemic agents such as sulphonylureas, metformin, glucosidase inhibitors, troglitazone, etc. But these are reported to produce serious side effects such as liver problems, lactic acidosis and diarrhea. Various plant species have been reported to possess anti diabetic properties. Biological action of the plants are related to chemical composition that are rich in phenolics, alkaloids, flavonoids, terpenoids, coumarins, and glycosides usually shows positive effects. From the literature review study, we came to know that *Swietenia mahagoni* is rich in flavonoids, terpenoids, coumarins, tannins.

So in this project work I have decided to use the extract of the fruit exocarp of *Swietenia mahagoni* for treatment of high fat diet and low dose STZ induced diabetes. By evaluating the antioxidant potential of this important natural fruit's exocarp *of Swietenia mahagoni* , the primary aim is to establish the extract of the plant used in the treatment of type 2 diabetes. So the aim is to evaluate the antioxidant potential of the selected species against type-2 diabetes, where the antioxidant enzymes SOD, CAT, GSH etc are damaged. The extract of the fruit exocarp acts as protective effect against type 2 diabetes.

1.4. Basis of Plant Selection

Swietenia mahagoni seeds have been applied as a folk medicine for the treatment of hypertension, diabetes, and malaria. The seeds have also been reported to have therapeutic value for treatment of cancer, amoebiasis, coughs, chest pains and intestinal parasitism. The biologically active ingredients, tetranortriterpenoids and fatty acids are considered to be

responsible for these therapeutic effects. After the literature review we can see that the extract of seeds, bark, leaf of the *Swietenia mahagoni* had been used in the treatment of inflammation, diabetes, diarrhea, HIV etc. The parts of the plant have been used locally to treat many human ailments such as malaria, diabetes, diarrhea and hypertension. The fruit of the plant is used as a powerful anti-hyperglycemic drug (Naveen and Urooj, 2014). This plant has the positive role for the correction of oxidative stress and hyperlipidaemia. It has also activity against inflammation, mutagenecity and tumor. This plant has also medicinal values like antimalarial and antidiarrheal effects. The plant extracts have been accounted to possess antibacterial and antifungal activities. The seed of *Swietenia mahagoni* is a natural agonist of Peroxisome Proliferators Activated Receptor (De *et al*., 2011).Various parts of the *Swietenia mahagoni* have been used as folk medicine for the treatment of hypertension, malaria, cancer, amoebiasis, chest pain, fever, anemia, diarrhea, dysentery, depurative and intestinal parasitism (Radahe *et al*., 2013). In some African countries the seed oil is used as an alternative body ointment therapy for a range of skin cuts, itches and wound to ameliorate the healing process (Naveen *et al*., 2014). In Indonesia, *Swietenia mahagoni* seed is used as folk medicine to cure diabetes. The focus of the study is to search for natural source which can lower the blood glucose level of fat induced type 2 diabetic rats. From the literature review study we came to know that the seeds are mainly used in diabetes treatment. Seed extract acted as an antagonist to the peroxisome proliferators activated receptor γ in both the yeast two-hybrid system and diabetic mice. The activity was comparable to that of standard drug rosiglitazone (Li *et al*., 2005). Here, we used the aqueous extract of the exocarp of *Swietenia mahagoni* fruit for the treatment of diabetes induced rats.

Chapter 2

LITERATURE REVIEW

2. LITERATURE REVIEW

2.1. Description of investigated plant

Swietenia mahagoni (Linn.) Jacq. commonly known as Sky Fruit plant, is a small, leafy, medium sized tree, consists of pinnate shaped leaf, mostly grown at moist well drained soil. It is generally found in India and some African countries, but native to West Indies.

2.2. Ecological niche of *Swietenia mahagoni*

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Sapindales

Family: Meliaceae

Genus: *Swietenia*

Species: *mahagoni* Linn.

Fig. 2.1. Fruit of *Swietenia mahagoni*

2.3. Vernacular names

English: Big leaf mahogany

Hindi: Mahogany vriksha

German: Echtes mahagony

Bengali: Mahogani

Thailand: Mahokkani-baiyani

French: Acajoudu Honduras, acajou du venezuela

Spanish: Caoba, mara, domingo

Malayalam: Mahagoni

Tamil: Peddakulamaghani

2.4. Synonyms

Cedrela mahagoni L.

Swietenia acutifolia Stokes

Swietenia fabrilis Salisb.

Swietenia mahagoni var.praecociflora Hemsi

2.5. Distribution

Swietenia mahagoni is a large, deciduous and economically important timber tree native to West Indies. This timber tree is mainly cultivated at tropical zone such as India, Malaysia and Southern China. *Swietenia mahagoni* is a valuable timber tree closely related to the African genus *Khaya* and one of the popular medicine in Africa (Sahgal *et al*., 2009). Around the world, the plant is commonly known as West Indies mahogany, caoba, caoba dominicana or acajou. It is one of the species of genus Swietenia which belongs to chinaberry family, meliaceae (Naveen and Urooj, 2015). The tree is native in parts of Dade and Monroe countries as well as Bahamas,

and in the Western Caribbean from Hispaniola and including Cuba and Jamaica. It was introduced into Puerto Rico and the virgin islands more than 250 years ago. In South Florida, it occurs in high coastal hardwood hammocks, often in dry, rocky sites, in full sun or nearly full sun.

2.6. Morphological Description

The plant attains a height of about 1290cm, with a crown diameter between 915 and 1524cm. Trunk can be 92-94 cm in diameter. The canopy is symmetrical with a regular outline and individuals all have a more or less identical crown form. The plant bears dark green-colored leaves that are alternate, and pinnately compound. The leaflet shape is lanceolate or ovate. The plant produces inconspicuous green flowers peri-annually. Fruits are a woody capsule, brown-colored with oval to pear shape, attaining a length of 8-16 cm, which does not attract wildlife (Naveen and Urooj, 2014). After a period of winter rest, leaflets of the existing foliage turn brown and the leaves begin to abscise sometime in March to May. This is a brief process as the tree is semi-deciduous. Old leaves are shed suddenly and are quickly replaced by fresh new foliage just before or during the onset of new growth. As the tree gets older, the trunk usually divides into multiple branches around 4 to 8 feet above ground. These upright multiple leaders significantly reduce the wind and storm tolerance of any tree including Mahogany. From spring flowers, the fruits develop slowly through the summer, fall and winter. Usually only one flower on any leafy shoot becomes a fruit. Fruits are a woody capsule, brown-colored with oval to pear shape, attaining a length of 8-16 cm, which does not attract wildlife (Fig. 2.1). The branches of the tree droop as the tree grows. They are conspicuous in fall and winter as large, oval, woody, silvery capsule from 2 to 5 inches long. The mature capsule stands more or less erect, supported

by a thick pedicel. The seeds are flat, long winged, about 2 to 2.5 inches long and 0.5 inches wide .

2.7. Medicinal uses

The parts of the plant have been used locally to treat many human ailments such as malaria, diabetes, diarrhea and hypertension. The fruit of the plant is used as a powerful anti-hyperglycemic drug (Naveen and Urooj, 2014).This plant has the positive role for the correction of oxidative stress and hyperlipidaemia. It has also activity against inflammation, mutagenecity and tumor. This plant has also medicinal values like antimalarial and antidiarrheal effects. The plant extracts have been accounted to possess antibacterial and antifungal activities. The seed of *Swietenia mahagoni* is a natural agonist of Peroxisome Proliferators Activated Receptor (De *et al*., 2011).Various parts of the *Swietenia mahagoni* have been used as folk medicine for the treatment of hypertension, malaria, cancer, amoebiasis, chest pain, fever, anemia, diarrhea, dysentery, depurative and intestinal parasitism. In some African countries the seed oil is used as an alternative body ointment therapy for a range of skin cuts, itches and wound to ameliorate the healing process (Naveen and Urooj, 2014). In Indonesia, *Swietenia mahagoni* seed is used as folk medicine to cure diabetes.

2.8. Ethnomedicinal uses

Swietenia mahagoni has been used in Asia and other countries to treat diverse ailments based on its antimicrobial, anti-inflammatory, antioxidant effects, antimutagenic, anticancer, antitumor and antidiabetic activities. Almost all parts of the plant are used in traditional medicine for the treatment of various human ailments. The fruit of *Swietenia mahagoni* has been used commercially in health care products for the improvement of blood circulation and skin condition. In Malaysia, the seeds are used traditionally to treat hypertension, diabetes and relief

pain. An Bolivian Amazonian ethnic group has used the seeds for leshmaniasis and as an abortion medicine. In Indonesia, seeds have been used as folk medicine for treatment of diabetes, hypertension and malaria (Nagalakshmi *et al*., 2001).

2.9. Scientifically explored pharmacological activities

2.9.1. Antimicrobial activity

Swietenia mahagoni has a potent antimicrobial activity on a variety of microorganisms, including pathogenic microorganisms. The various phytochemicals present in the plant are responsible for the observed antimicrobial activity. Most of the areal parts of the plant have been shown to significantly inhibit the propagation of various microorganisms. A crude methanolic extract of the seed has been shown to inhibit growth of 5 Gram-positive and 9 Gram-negative bacteria. Further, the extract of the seed at a concentration of1 mg/ml has been shown to inhibit growth of the fungus *Candida albicans* (Sahgal *et al*., 2009). The methylene chloride extract inhibited growth of seven species, while the methanol extract was active only against *Rhizoctonia solani* (Goun *et al*., 2003). The methanol extract of the seed also had an inhibitory effect on *Candida albicans* in both *in vivo* and *in vitro* assays. *In vitro* disc diffusion assays showed a minimum inhibitory concentration of the extract to be 12.5 mg/dl. The extract had a deleterious effect on cell structures of *Candida albicans*, causing morphological change and death, as evidenced by electron microscope images of extract-treated fungi. Treatment of fungus-infected mice with seed extract reduced colony forming units in the kidney and the blood when compared to positive control mice (Sahgal *et al*., 2011). The oil extracted from the seed reduced growth rates of several diseases causing by bacterial (*Shigellady senterial, Salmonella typhi* and *Staphylococcuss aureus)* and fungal (*Macrophomina phascolma, Alternaria alternate* and *Curvularia lunat*a pathogens (Shipar *et al*., 2004). The alcoholic and aqueous extracts of the leaf,

stem/bark and root have inhibitory effects on *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris* and *Bacillus subtilis.*

2.9.2. Anti-inflammatory effect

The methanolic extract of *Swietenia mahagoni* seed has an ameliorating effect on paw edema induced by carrageenan and arachidonic acid, acetic acid-induced writhing, ear inflammation induced by croton oil, cotton pellet-induced granuloma and Freund's adjuvant-induced polyarthritis in rats. The extract significantly reduced the acetic acid induced writhing in rats; the writhing reducing effect was superior to the standard ibuprofen. Carrageenan-induced paw edema was reduced by 56.8% and 68.0% in rats treated with doses of 50 and 100 mg/kg extract, respectively. Croton oil-induced ear inflammation was reduced by 7.35% at 50 mg/kg dose and 47.06% at 100 mg/kg. Polyarthritis induced by Freund's adjuvant was reduced by 53.79%, which was more than the positive control, ibuprofen. Cotton pellet-induced granuloma was reduced by 28.29% at 50 mg/kg and by 42.86% at a dose of 100 mg/kg; the effect of the extract was far more than the standard drug ibuprofen (14.29%). The extract also significantly increased the intraperitoneal count of white blood cells and macrophages (Ghosh *et al*., 2009). Although there are many studies which indicate effective anti-inflammatory activity of *Swietenia mahagoni in* animal models, the mechanism of action has yet to be explored.

2.9.3. Hepatoprotective effect

The petroleum and 80% aqueous methanol extracts of *Swietenia mahagoni* bark showed hepatoprotective effect against paracetamol-induced hepatic damage in male Wistar rats. Treatment with *Swietenia mahagoni* bark extract significantly reduced the alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and serum bilirubin levels, suggesting the hepatoprotective effect. Histopathology revealed that the liver cells in the

extract treated group were indistinguishable from liver cells of healthy rats. The extract also significantly reduced the thiobarbituric acid-reactive substances, when compared to the paracetamol-treated group. Bark extract was also reported to increase the reduced glutathione level in the liver. The above findings indicate that the hepatoprotective efficacy of the bark extract may be mediated through the modulation of lipid peroxidation and the augmentation of endogenous enzymatic and non-enzymatic antioxidant defense systems. To determine safety for human use, acute toxicity tests were carried out on Swiss albino mice; the dose at which 50% of recipients died (LD50), for orally administered bark extract, was 200 mg/kg (Halder *et al*., 2011).

2.9.4. Anti-diarrheal activity

The ethanolic, methanolic and aqueous extracts of *Swietenia mahagoni* seed show antidiarrheal activity in castor oil-induced diarrhea as well as in charcoal-induced gastrointestinal motility in Wistar albino rats. The ethanolic, methanolic and aqueous extracts of seeds at various concentrations (50, 100, 200 and 300 mg/kg) were used in this study. Among the three solvent extracts, the ethanolic extract showed the most potent antidiarrheal activity, as evidenced by reduction in the rate of defecation and improved consistency of faeces. Treatment with the extract produced a profound decrease in intestinal transit and significantly inhibited castor oil-induced enter pooling compared to standard drugs diphenoxylate (50 mg/kg) and atropine sulfate (2.5 mg/kg). The delayed onset of diarrhea, inhibition of castor oil-induced enter pooling and the suppressed propulsive movement all support the traditional claim that *Swietenia mahagoni* functions as an antidiarrheal drug in the Indian system of medicine without any side effects (Hajra *et al*., 2012).

2.9.5. Antioxidant effect

Studies have reported that the seed of *Swietenia mahagoni* possesses antioxidant activity. The methanol extract of the seed was shown to be a potent antioxidant in various *in vitro* assays (i.e., xanthine oxidase assay, hydrogen peroxide scavenging activity, ferric-reducing antioxidant power (FRAP) assay and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay). The methanol extract inhibited superoxide formation with a value of 47.2%, which was less than standard drug allopurinol (87.51%). Hydrogen peroxide scavenging activity of the extract was found to be 49.5, which was comparable to standard drug ascorbic acid (51.1). The seed extract had FRAP activity of 0.728 mmol Fe^{++}/g, which was higher than the FRAP activity of ascorbic acid (0.405). The extract showed a DPPH-scavenging activity of 23.29% at 1 mg/ml concentration, this value was less than that of ascorbic acid (Sahgal *et al*., 2009).

2.9.6. Gastro protective effect

The ethanol extract from the seed of the *Swietenia mahagoni* has shown gastro protective activity against ethanol-induced gastric mucosal injury in rats. Six groups of rats were orally fed with carboxymethyl cellulose, omeprazole (standard drug, 20 mg/kg) or seed extract (50, 100, 200 and 400 mg/kg) one hour before oral administration of absolute ethanol (to generate gastric mucosal injury). The carboxymethyl cellulose group exhibited severe mucosal injury, whereas pre-treatment with plant extract provided significant protection of gastric mucosa in rats. The carboxymethyl cellulose group showed severe damage to gastric mucosa (edema and leucocyte infiltration of sub-mucosa) compared to the plant extract-treated group, which showed gastric protection as evidenced by histological observations. The authors also carried out similar studies using ethanol extract of mahagoni leaf. The leaf extract significantly reduced the mucosal injury and increased the mucus secretion when compared to the control group; the effect was similar to

standard drug omeprazole. The extract, in addition, reduced the edema and leucocyte infiltration into the sub-mucosal layer (Alrdahe *et al*., 2010).

2.9.7. Anti-diabetic effect

Aqueous-methanol extract of *Swietenia mahagoni* seed has been reported to exhibit hypoglycemic and antihyperlipidemic potency in streptozotocin-induced diabetic rats. Oral feeding of the extract to diabetic rats for 21 d lowered the blood glucose level and improved liver glycogen content. Furthermore, treatment with the seed extract normalized the levels of serum urea, uric acid, creatinine, cholesterol, triglyceride and lipoproteins. In addition, the extract increased the activity of antioxidant enzymes and reduced the oxidative stress in liver, kidney and skeletal muscles. The ethanolic extract of *Swietenia mahagoni* seed inhibited α-amylase to an extent of 70.33% at a concentration of 200 μg/mL. Seed extract acted as an antagonist to the peroxisome proliferator activated receptor γ in both the yeast two-hybrid system and diabetic mice. The activity was comparable to that of standard drug rosiglitazone (Li *et al*., 2005).

2.9.8. Anti-HIV effect

Methanol extract of *Swietenia mahagoni* bark is reported to exhibit anti-HIV-1 activity by inhibiting a key enzyme, HIV protease, which is required by the virus to replicate in host cells. Bark extract also suppressed the formation of syncytia in co-cultures of human acute lymphoblastic leukemia cell line (MOLT-4) and MOLT-4/HIV-1 cells (Otake *et al*., 1995).

2.9.9. Immunomodulatory effect

The methanolic extract of seeds enhanced the immune efficiency, as assessed by neutrophil adhesion, phagocytic index by carbon clearance, hem agglutinating antibody titer and delayed type hypersensitivity responses in rat models. Oral administration of the extract significantly increased the neutrophil adhesion to nylon fiber, when compared to control group. In addition, it

showed a significant increase in circulating antibody titer and phagocytic index in carbon clearance in a concentration-dependent manner. The effect may be mediated by significantly increasing circulating antibody titer (Hajra *et al*., 2012).

2.9.10. Insect repellent and larvicidal effect

The petroleum ether and methanol extracts of *Swietenia mahagoni* leaves both showed insect repellent and larvicidal activity against mosquito *Culex quinquefasciatus* in a laboratory bioassay. The extracts were applied to mosquito larvae of all stages at various concentrations. The extract lad to cent percent mortality in the 2nd instar larvae at a concentration of 50 ppm. Exposure to the extract also caused significant mortality in the 1st, 3rd and 4th instar larvae. The extracts showed 100% repellent power for up to 135 min. No mosquito bites were observed during the repellent period. The safety of the extracts was assayed using larvae of *Gambusia affinis*, tadpole of *Bufo* and *Chironomus*. The extracts did not cause mortality in these larvae at any of the tested concentrations (Adhikari *et al*., 2012). Along with the larvicidal and insect repellent activity, the acetone, methanol and water extracts of the leaf showed anti-feedant property against the red flour beetle, *Tribolium castaneum* Herbst. The toxic effect of the extract on the beetle was significant (Mostafa *et al*., 2012).

Chapter 3

PHYTOCHEMISTRY

3. PHYTOCHEMISTRY

3.1. Introduction

Phytochemicals can be defined, as chemicals produced by plants. These are natural occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. It contains complex mixture of many plant metabolites. The isolation and the use of natural products such as digoxin, morphine and quinine has resulted in replacing the plant extracts used with single chemical entities. There is a basic supposition that any plant possessing clinical effectiveness must contain an active principle which can completely replace the plant extract.

Different extracts of *Swietenia mahagoni* have been tested for the presence or absence of primary and secondary bioactive compounds like limonoids, terpenoids, saponins, flavonoids, oil etc. It has been found to be a rich source of limonoids. Swietenine acetate, swietenolide diacetate, swietenolide tiglate were found in the extraction of seed, that was reported (Taylor and Taylor, 1983). The bark of this plant contains tannin but no alkaloid is present. Cyclomahogenol, a new tetracyclic triterpene, has been identified in the leaves of the plant. The methanolic extract of seeds contains alkaloids, terpenoids, anthraquinones, cardiac glycosides, saponins and volatile oils. Two potent antimicrobial limonoids (swietenolide and 2-hydroxy-3-O-tigloylswietenolide) have been isolated from the methanolic extracts of the seed. Some mexicanolide type limonoids, swietmanins, 2-hydroxy-3-O-isobutyryl-proceranolide, 2-hydroxy- 3- O- benzoylproceranolide, a new andirobin type limonoid, swietmanin J and some known compounds have been isolated from the fruits. Two common as well as novel limonoids, swiemahogins A and B have been isolated from the twigs and leaves (Naveen and Urooj, 2014).

3.2. Collection and identification of selected plants

Fruits of *Swietenia mahagoni* were collected in the month of September from Kalyani, Nadia, West Bengal, India. The plants were authenticated by the Taxonomists, Botanical Survey of India, Howrah, India [Ref.CNH/1-1(64)/06]. The voucher specimens have been deposited at Advanced Pharmacognosy Research Laboratory, Department of pharmaceutical Technology, Jadavpur University for future references.

3.3. Extraction

Exocarps of fruits of the *Swietenia mahagoni* were collected by cutting the fruits. The exocarp of the fruits were cut into small pieces and dried in the sunlight for 2 weeks. Then those were pulverized in an electrical grinder into fine powder. The powdered plant materials were macerated with methanol with occasional shaking and stirring. The methanolic extract of *Swietenia mahagoni* (MESM) were filtered to remove particulate matters and dried in vacuum in a rotary evaporator at 40°C to powdered extract for further experiments. The extract was stored in desiccators for use in subsequent experiments.

3.4. Qualitative Phytochemical Analysis

MESM was used for preliminary qualitative screening of phytochemicals such as alkaloids (Dragendorff test), flavonoids (Shinoda test), Carbohydrate (Iodine test), phenols (phenol test), saponins (Foam test), sterols and terpenes (Liberman-Burchard) and tannins.

3.4.1. Test for alkaloids

Extract was dissolved individually in dilute Hydrochloric acid and the solutions were filtered.

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate marked the presence of alkaloids.

Hager's Test: Filtered solutions were taken in a test tube and Hager's reagent (saturated picric acid solution) was added with it. Presence of alkaloids was confirmed by the formation of yellow colored precipitate.

Dragendroff test: A small quantity of test sample was treated with few drop of dilute hydrochloric acid and filtered. The filtrate was then treated with Dragendroff's reagent. Presence of orange brown precipitate indicated the presence of alkaloids.

Wagner test: A small quantity of test sample was treated with few drop of dilute hydrochloric acid and filtered. The filtrate was treated with Wagner's reagent. Presence of reddish brown precipitate suggested the presence of alkaloids.

3.4.2. Test for flavonoids

Shinoda test: To 1 ml. of the extract, magnesium turning and 1-2 drops of concentrated hydrochloric acid were added. Formation of pink colour indicates the presence of flavonoids.

Lead acetate Test: 4-5 drops of lead acetate solution was added to the fraction's solutions. Formation of yellow color precipitate marked the presence of flavonoids.

3.4.3. Test for steroids

Salkowski's test: The extract was dissolved in 2 ml. of chloroform and equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer turns red and lower layer turns yellow with green fluorescence, indicating the presence of steroids in the extract.

3.4.4. Test for carbohydrates

Iodine test: Two ml. of dilute iodine solution was added to the extract. The appearance of blue colour indicates the presence of starch.

3.4.5. Test for saponins

Foam test: About 1 ml. of alcoholic extract was diluted with 20 ml. of distilled water and was shaken in a graduated cylinder for 15 min. The formation of 1 cm layer of foam indicates the presence of saponins.

3.4.6. Test for tannins

Small quantity of test sample was dissolved in minimum amount of distilled water and filtered. The filtrate was treated with 10% aqueous potassium dichromate solution. Development of yellowish brown precipitate demonstrates the presence of tannins.

3.4.7. Test for volatile oil

To a small quantity of test sample add few drops of tincture alkana, red colour indicates presence of volatile oil.

3.5. Isolation of compounds from *Swietenia mahagoni*

Methanol extract was subjected to silica gel (100-120 mesh) column chromatography using mixtures of n-hexane-ethyl acetate-methanol of increasing polarity. Eluents have been collected from where we get four fractions (1-4) through Thin Layer Chromatography (TLC) screening and we also get three compounds. Compounds (S1, S2, S3) were purified by direct crystallization method with the help of methanol. The resulting solutions were allowed to stand at room temperature which resulted white crystals (S1), white crystals (S2), white amorphous powder (S3). The crystals were separated and washed with excess cold hexane (3times) and cold methanol (minimum quantity). The crystals were dried under vacuum. S1 gave single spot on TLC plate after chromatograph with hexane.S2 gave single spot on TLC plate after chromatograph with ethyl acetate. S3 gave single spot on TLC plate after chromatograph with ethyl acetate. The dried compounds were subjected to NMR analysis for structure interpretation.

3.6. Results

Qualitative analysis of *Swietenia mahagoni* suggests the presence of various phytochemical constituents such as such as alkaloids, carbohydrates, steroids, flavonoids, tannins, saponins and volatile oils in individual fractions have been shown in table 3.1.

Table 3.1. Results of phytochemical analysis

Phytochemicals	Observation	Inference
Alkaloids	Colour changes into Yellow – orange colour	+
Flavonoids	Colour change to pink	+
Steroids	Colour change to green colour	+
Carbohydrate	Colour changes into green-blue colour	+
Saponins	Formation of frothing	+
Volatile oils	Formation of red colour	+
Tannins	Yellowish brown ppt.	+

("+" indicates positive result of the test)

Compounds S1, S2 and S3 have been isolated from the methanolic extract of *Swietenia mahagoni*. The chemical structures of isolated compounds would be identified by the combination of data of NMR (^{1}H, ^{13}C), Mass, UV and IR spectroscopy. ^{1}H spectrum of compound S1 ready to analysis but ^{13}C NMR, MASS, UV and IR spectrum are not ready to analysis. And ^{1}H NMR, ^{13}C NMR, MASS, UV and IR spectrum of compound S2 and S3 are not ready to analysis. The ^{1}H NMR spectra of compounds S1 have been shown here under (Figure 3.1.). On the basis of ^{1}H NMR signals, it would be perceived that the isolated materials are non-polar organic compound.

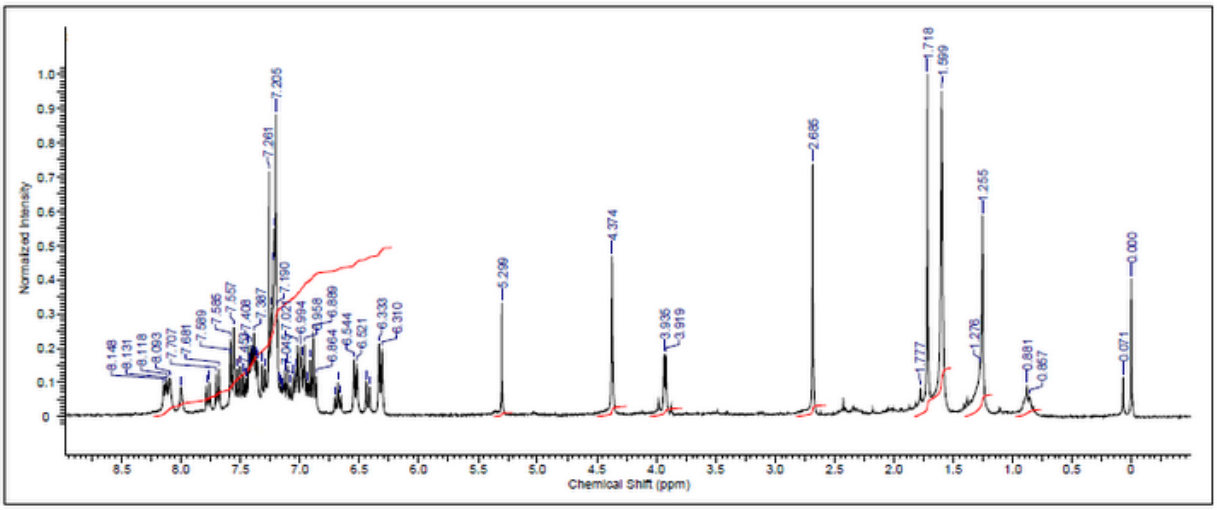

Fig. 3.1. ^{1}H spectrum ($CDCl_3$) of compound S1

Chapter 4

IN VITRO ANTIOXIDANT ACTIVITY

4. *In vitro* antioxidant activity

4.1. Introduction

The role of free radical reactions in disease pathology is well established, suggesting that these reactions are necessary for normal metabolism but can be detrimental to health as well. The reactive oxygen species (ROS), super oxide ion($\cdot O_2^-$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) produced during atmospheric oxygen metabolism are involved in various human diseases such as ischemic heart disease, Alzheimer's disease, aging, cancer, inflammation, diabetes, rheumatoid arthritis and atherosclerosis. ROS participate in the direct oxidative damages of the structural and functional macromolecules within the cells and induces apotototic cell death. The antioxidant properties of phytoconstituents have a correlation with oxidative stress defense. Antioxidant agents from natural origin have attracted special interest because they can protect human body from free radicals. Numerous medicinal plants and their formulations are used as antioxidant in ethno medical practices as well as in traditional Indian medicines. Human body cells utilize oxygen to break down biomolecules for the generation of energy. Free radicals are produced in this process in the form of byproducts. These free radicals are highly active because they possess unpaired electron. Normally free radicals are beneficial for oxidative energy production and killing the invaded bacteria in to the cells. Almost all cells posses antioxidant defense and repair system to protect them against oxidative damages but unfortunately not sufficient entirely all the time. The oxygen centered free radicals are called reactive oxygen species (ROS) which includes superoxide anion radical (O_2^-), hydroxyl radical (OH^-), nitric oxide (NO^-) and hydrogen peroxide (H_2O_2) and other molecules. In healthy individuals, the production of free radicals is balanced with the anti-oxidative defense system. When this equilibrium gets unbalanced, it results the generation of free radicals beyond the limit

and ultimately depletion of antioxidant levels. Exogenous free radical when coupled with endogenous free radicals results the elevation of ROS which consequences oxidation of cellular lipids, nucleic acids, proteins, carbohydrates and other biomolecules and leads to a number of diseases like cancer, atherosclerosis, diabetes mellitus, coronary heart diseases and various other degenerative diseases antioxidants play important role against oxidative damage of ROS. Antioxidants are important in the prevention of human diseases by functioning either as free radical scavengers, complexes of pro-oxidant metals, reducing agents or quenchers of singlet oxygen formation. The requirement of antioxidants of human being is solely filled by dietary vegetables, which play an important role in preventing oxidative damages. Plants also need to protect themselves from free radical damages with the help of their own metabolites, so they develop a number of different classes of antioxidants. The pigments, flavonoids, coumarines, phytosterols, proanthrocyanidines, tannins, essential oils, resins and gums are responsible phytochemicals for antioxidant activity. Thus the present investigation was directed to investigate the antioxidant activity with the help of some selective assays.

4.2. *In vitro* antioxidant assay

4.2.1. Determination of DPPH radical scavenging activity

The DPPH assay measured hydrogen atom (or one electron) donating activity and hence provided an evaluation of antioxidant activity due to free radical scavenging. Radical scavenging activity of plant extracts against stable DPPH (1,1-diphenyl-2-picrylhydrazyl hydrate, Sigma-Aldrich, Steinheim, German) was determined spectrophotometrically. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep—violet to light—yellow) were measured at 515 nm on a UV/visible light spectrophotometer. The free radical scavenging activity of methanol extract of *Swietenia*

mahagoni was measured by DPPH (1, 1-diphenyl-2-picryl-hydrazyl) employing the method described by Blois, (1958). The antioxidant activity of the extract was expressed as IC50. The IC50 value was defined as the concentration (in μg/ml) of extracts that inhibits the formation of DPPH radicals by 50%.

4.2.2. Determination of total phenolic compounds

The total concentration of phenolics in *Swietenia mahagoni* was determined according to the method Singleton *et al.* (1999). Briefly, 0.1 ml of each extract solution (contains 500 μg of extract) was transferred to a 100 ml Erlenmeyer flask, and then the final volume was adjusted to 46 ml by the addition of distilled water. Afterward, 1 ml of Folin-Ciocalteu reactive was added into this mixture and after 3 min 3 ml of sodium carbonate (2%) was added. Subsequently, the mixture was shaken on a shaker for 2 hour at room temperature. The total concentration of phenolics in mahagoni extract was determined according to the method. Briefly, 0.1 ml of each extract solution (contains 500 μg of extract) was transferred to a 100 ml Erlenmeyer flask, and then the final volume was adjusted to 46 ml by the addition of distilled water. Afterward, 1 ml of Folin-Ciocalteu reactive was added into this mixture and after 3 min 3 ml of sodium carbonate (2%) was added. Subsequently, the mixture was shaken on a shaker for 2 hour at room temperature, and then absorbance was measured at 760 nm. Pyrocatechol (Sigma) was used as the standard for the calibration curve. The estimation of phenolics in the fractions was carried out in triplicate, and the results were averaged. The phenolic compound content was determined as pyrocatechol equivalents using the following linear equation based on the calibration curve: $A = 0.0034C - 0.058$, $R2 = 0.9996$. A is the absorbance, and C is pyrocatechol equivalents (μg).

4.2.3. Determination of total flavonoids

Total soluble flavonoids content in *Swietenia mahagoni* extract were determined with aluminium nitrate and potassium acetate using quercetin as the standard Hsu (2006). The flavonoid contents of the extracts in terms of quercetin equivalent using the following linear equation based on the calibration curve: $A = 0.0067C + 0.0132$, R2 = 0.999. *A* is the absorbance, and *C* is quercetin equivalents (μg).

4.3. Results

4.3.1. DPPH radical scavenging activity

The DPPH radical scavenging capacity of was measured at 517 nm. Figure 4.1. illustrates a steady increase of percentage scavenging activity with concentration of *Swietenia mahagoni*. The IC50 value was 30 μg/ml for extract.

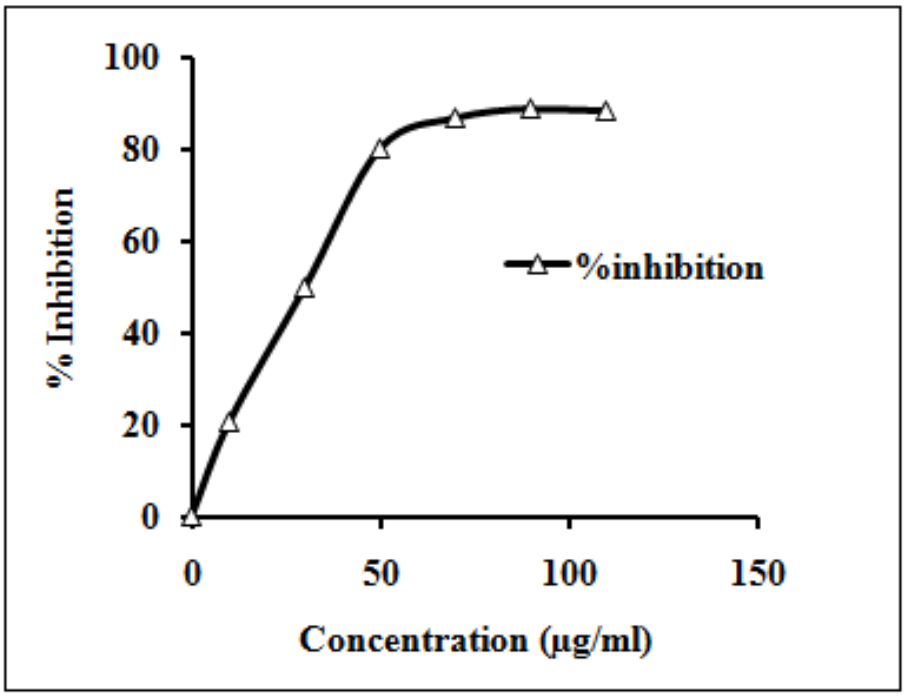

Fig. 4.1. DPPH scavenging activity

4.3.2. Quantification of total phenolics and flavonoids

Total phenolic contents compounds of *Swietenia mahagoni* were expressed as mg of pyrocatechol equivalent per gram of dry weight of extract. The level of total polyphenolic compounds was ~19.71 mg/g^{DW} of pyrocatechol equivalent per gram of extract. Total flavonoids content of the extract was found to be ~43.75 mg/g^{DW} of quercetin equivalent per gram of dry weight.

Chapter 5

Effect of the extract of *Swietenia mahagoni* on high fat diet and STZ induced type-2 diabetes model

5. Effect of the extract of *Swietenia mahagoni* on high fat diet and streptozotocin induced type-2 diabetes model

5.1. Introduction

Type-2 diabetes is characterized by excessive hepatic glucose production, decrease insulin secretion, and insulin resistance (Grodsky, 2000). There is considerable evidence that hyperglycemia results in the generation of reactive oxygen species (ROS), ultimately leading to increased oxidative stress in a variety of tissues. In the absence of an appropriate compensatory response from the endogenous antioxidant network, the system becomes overwhelmed, leading to the activation of stress sensitive intracellular signaling pathways. One major consequence is the production of gene products that cause cellular damage and are ultimately responsible for the late complications of diabetes. The ability of antioxidants to protect against the effects of hyperglycemia and free fatty acids *in vitro*, along with the clinical benefits often reported following antioxidant therapy, supports a causative role of oxidative stress in mediating these abnormalities (Evans *et al*., 2001).

Concurrent with the obesity epidemic, the incidence of type-2 diabetes is increasing in an alarming rate. Type-2 diabetes arises when the endocrine pancreas fails to secrete sufficient insulin to cope with the metabolic demand because of acquired β-cell secretory dysfunction or decreased β-cell mass (Linghor *et al*., 2002). Oxidative stress resulting from increased production of ROS plays a key role in the pathogenesis of late diabetic complications. In uncontrolled diabetes, the level of superoxide dismutase, the enzyme responsible for inactivating the superoxide radical, along with the levels of antioxidants vitamin E and α-lipoic acid are decreased. There is also some evidence that a deficiency in erythrocyte catalase, an enzyme responsible for the removal of H_2O_2 is associated with increased frequency of diabetes.

In vivo studies reveal that oxidative stress due to hyperglycemia occurs before late complications (King & Brownlee, 1996). One area of intense study has been the regulation of stress activated signaling pathways including nuclear factor-κβ, p38 MAPK, NH_2-terminal Jun kinases/stress activated protein kinases (JNK/SAPK), advanced glycosylation end products(AGE)/ receptor for AGE (RAGE) and protein kinase C (PKC). In the process of mitochondrial respiration, molecular oxygen is essential for the complete metabolism of glucose another substrates during the production of ATP. During the course of normal oxidative phosphorylation, however between 0.4 and 4% of all oxygen consumed is converted into the free radical superoxide ($\cdot O_2^-$). Subsequently, $\cdot O_2^-$ can be converted into other ROS and reactive nitrogen species (RNS). This $\cdot O_2^-$ is normally eliminated by antioxidant defenses. $\cdot O_2^-$ molecules within the mitochondria are quickly converted to H_2O_2 by the key mitochondrial enzyme, SOD_2. H_2O_2 is then either detoxified to H_2O and O_2 by glutathione peroxidase (in the mitochondria), or diffuses into the cytosol and is detoxified by catalase in peroxisomes. However, in the presence of reduced transition metals such as Cu or Fe, H_2O_2 can be converted to the highly reactive $\cdot OH$ radical (Fenton reaction). Excessive levels of ROS lead to the damage of proteins, lipids, and DNA. Thus, the endogenous antioxidant systems exist within cells to neutralize ROS, and these systems are critical to maintaining proper cellular function. A major cellular antioxidant is reduced glutathione (GSH), which is regenerated most efficiently by glutathione reductase and reduced nicotinamide adeninedinucleotide phosphate. It can also be regenerated by lipoic acid (LA) in concert with other antioxidants. When the endogenous antioxidant network fails to provide a sufficient compensatory response to restore cellular redox balance, GSH levels fall and oxidative stress ensues. In addition to their ability to directly inflict damage upon cellular macromolecules, ROS play a significant role in activating stress sensitive signaling pathways

that regulate gene expression resulting in cellular damage antioxidant systems exist within cells to neutralize ROS, and these systems are critical to maintaining proper cellular function. A major cellular antioxidant is reduced glutathione (GSH), which is regenerated most efficiently by glutathione reductase and reduced nicotinamide adenine di nucleotide phosphate. It can also regenerated by LA in concert with other antioxidants. When the endogenous antioxidant network fails to provide a sufficient compensatory response to restore cellular redox balance, GSH levels fall and oxidative stress ensues. In addition to their ability to directly inflict damage upon cellular macromolecules, ROS play a significant role in activating stress sensitive signaling pathways that regulate gene expression resulting in cellular damage.

Several explanations have been offered to account for how elevated free fatty acid (FFA) could result in insulin resistance. The glucose fatty acid cycle (Randle hypothesis) was the first to be widely accepted (Steppan *et al*., 2001). Randle reasoned that the increased availability of FFA would cause an increase in the ratio of mitochondrial acetyl-CoA **:** CoA and reduced nicotinamide adenine dinucleotide : nicotinamide adenine dinucleotide, resulting in: 1) inactivation of the pyruvate dehydrogenase complex, 2) reduced glucose oxidation and increased intracellular citrate, 3) inhibition of phosphofructokinase, 4) accumulation of glucose-6-phosphate, and ultimately, 5) inhibition of hexokinase II activity. The net result would be an accumulation of intracellular glucose and the concomitant decrease in muscle glucose uptake. However, in contrast to the Randle hypothesis, which predicts that increased FFA availability would lead to an increase in i.m. glucose-6-phosphate, recent studies have indicated that the decrease in muscle glycogen synthesis in healthy subjects caused by fat infusion was preceded by a reduction in i.m glucose-6-phosphate levels (Roden *et al*., 1996). FFA leads to a decrease in the intracellular concentration of glucose. These results provide the basis for implicating the

glucose transport system (as opposed to hexokinase II or other intracellular sites) as the rate-controlling step for fatty acid induced insulin resistance (Shulman, 2000).

Insulin resistance in obesity is evident before the development of chronic hyperglycemia. Therefore, it is unlikely that insulin resistance, at the prediabetic stage, results from oxidative stress triggered by hyperglycemia. However, the strong association of obesity and insulin resistance (Sims *et al*., 1973) suggests that a major mediator of oxidative stress-induced insulin resistance at the prediabetic stage might be a circulating factor secreted by adipocytes. In this regard, several possible candidate molecules have been suggested including TNF-α (Hotamisligil & Spiegelman, 1994), leptin (Cohen *et al*., 1996), FFA (Randle *et al*., 1988). However, the evidence is strongest that FFA are the most likely link between obesity and insulin resistance (Boden, 1997). Plasma FFA content is increased in many states of insulin resistance including obesity and type 2 diabetes (Gordon, 1960 & Frayn, 1993). There is an inverse relationship between fasting plasma FFA concentrations and insulin sensitivity (Perseghin *et al*., 1997). There is an even stronger relationship between the accumulation of intramyocellular triglyceride and insulin resistance.

Although there exists a surplus of animal models (spontaneous as well as induced) available for the study of type 2 diabetes, the pattern of disease initiation and development in most of them do not appear to be closely analogous to the clinical situation in humans. However, there are certain genetic models namely Zucker diabetic fatty (ZDF) rat and db/db mouse which develop diabetes spontaneously resembling human type 2 diabetes, the development of diabetes in them is predominantly genetically determined unlike in humans (Luo *et al*., 1998). Moreover, the observations derived from these highly inbred genetic strains may not always be satisfactorily extended to the human population as a whole because of the large heterogeneity in

the latter. In addition, these animals are expensive and are not easily available for the investigative purposes as well as regular screening experiments. Further, in induced diabetic models, most of the animals (adult or neonates) requires relatively high dose of STZ (STZ>50 mg kg−1) (Shafrir, 2003). The development of hyperglycemia in these rats following STZ injection is primarily due to the direct pancreatic beta cell destruction, and resulting insulin deficiency rather than the consequence of insulin resistance (Rerup, 1970). Thus, they depict symptoms and characteristics typical more of human type 1 than type 2 diabetes and further are notvery responsive to the effects of drugs like insulinotropic (e.g. glipizide, tolbutamide) and insulin-sensitizing compounds (e.g. pioglitazone, rosiglitazone) (Wier *et al*., 1981 & Portha *et al*., 1994). In contrast, the rats fed with high-fat diet (HFD) develop obesity, hyperinsulinemia, and insulin resistance and not frank hyperglycemia or diabetes, thus limiting the screening of agents on controlling the blood glucose level (Srinivasan *et al*., 2004). Hence, there exists a continued quest among the investigators for establishing the ideal animal model for type 2 diabetes either by way of modification of the existing methods or by developing new methodologies or a combination of both (Reed *et al*., 2000). Thus, we initiated this study with the objective of developing a suitable type 2 diabetic rat model that would closely mimic the natural history of the disease events (from insulin resistance to beta cell dysfunction) as well as metabolic features of human type 2 diabetes and on the other hand would be cheaper, easily available and useful for the investigation as well as preclinical testing of various compounds viz. insulin sensitizers and insulinotropics for the treatment of type 2 diabetes. The materialization of the disease pattern was achieved by combining the feeding of high fat diet which produced insulin resistance and low dose of STZ treatment that caused the initial beta cell dysfunction and subsequently the frank hyperglycemia in non-genetic, male wistar rat. Though attempts have

been made previously by the other investigators for developing suitable animal models for type 2 diabetes by injecting STZ into genetically insulin-resistant animals (spontaneous hypertensive rats) or by a combination of high fat diet and low dose sreptozotocin treatment in normal rats and mice (Zang *et al*., 2003), the rat model described in this paper is unique in so far as the approach adopted towards the development of the model as well as its suitability for pharmacological screening is concerned.

Medicinal plants are blessed with a wide array of phytochemicals with various physiological actions; most of them are beneficial to human health and well being. *Swietenia mahagoni* Jacq. is a small leafy, medium sized tree native to the West Indies. Around the world, the plant is commonly called as West Indies mahogany, caoba, caoba dominicana or acajou. It is one of the species of genus Swietenia which belongs to chinaberry family, meliacea (Elbart, 1978). The parts of the plant have been used to treat many human ailments such as malaria, diabetes, diarrhea, astringent, hypertension etc. locally. The fruit of the plant is used as the powerful anti-hyperglycemic drug. The seed oil is being used as an alternative body ointment therapy for a range of skin cuts, itches and wounds to ameliorate the healing process in African countries. Decoction of bark is used to increase appetite, as an energizer in case of tuberculosis, to treat anemia, diarrhea, dysentery, fever and toothache. The decoction of leaves is used to treat nerve disorders, the infusion of seed to relief from chest pain (Bacsal *et al.*, 1997).There are no data regarding the toxic effects, dose and long term side effects of treatment in the animal system. The present study evaluates the effect of extract of exocarp of the fruits on high fat diet and STZ induced diabetic rat model.

5.2. Materials and methods

5.2.1. Preparation of extract

Swietenia mahagoni (Meliaceae) fruits were collected in the month of September from Kalyani, Nadia, West Bengal. Plant was authenticated by the Taxonomists of the Botanical Survey of India, Howrah, India. The voucher specimens [Ref.CNH/1-1(64)/06] have been deposited at Advanced Pharmacognosy Research Laboratory, Department of Pharmaceutical Technology, Jadavpur University, Kolkata. The exocarpus of the fruits of *Swietenia mahagoni* were collected. The exocarp of the fruits were cut into small pieces and dried in the sunlight for two weeks. Then those were pulverized in an electrical grinder into fine powder. The powdered plant materials were macerated with water with occasional shaking and stirring. The aqueous extract of *Swietenia mahagoni* (AESM) were filtered to remove particulate matters and dried in vacuum in a rotary evaporator at 40°C to powdered extract for animal study. The testing sample was prepared by dissolving lyophilized powder in distilled water containg 1% tween 80 prior to *in vivo* experiment.

5.2.2. Animals

Healthy male Wister rats (140±20g) were lodged in cages (polypropylene) under standard lab condition of light: dark cycle (12 h: 12 h), relative humidity (55 ±5%), temperature (25±2°C), standard diet and water *ad libitum*. The animal experiments were performed at the Department of Pharmaceutical Technology, Jadavpur University, India. All the rats were provided with commercially available rat normal pellet diet (NPD) (Amrut Diet, New Delhi) and water *ad libitum*, prior to the dietary manipulation. The principles of Laboratory Animals care (PHS, 1986) and the instructions given by our institutional animal ethical committee (Registration No: 0367/01/C/CPCSEA) were followed throughout the experiment.

5.2.3. Chemical used

Streptozotocin (STZ) was purchased from Sisco Research Laboratories Pvt.Ltd. (India). All other chemical used in the study were of analytical reagent and obtained from Sisco Research Laboratories (India), Qualigens (India/Germany), SD fine chemicals (India). The feed ingredients such as casein and cholesterol both from HIMEDIA (India), vitamin and mineral mix from Sarabhai chemicals, Boroda, India. Kits for measurement of serum biochemical parameters were purchased from Span diagnostic Ltd., India.1-Chloro-2, 4-dinitrobenzene, ammoniumsulphate, 2,4- dinitrophenylhydrazine, ethylene diaminetetraacetic acid, 5,5-dithiobis(2-nitrobenzoic acid), N-ethylmaleimide, nitro blue tetrazolium, reduced nicotinamide adenine dinucleotide, potassium dihydrogen phosphate, phenazine methosulphate, sodium pyrophosphate, reduced glutathione, sodium azide, thiobarbituric acid, 5-thio-2-nitrobenzoic acid and trichloro acetic acid were purchased for sisco Research Laboratory, Mumbai, India. Glibenclamide (D Con 2.5 mg) was purchased from Grandix Pharmaceutical.

5.2.4. Development of high fat diet -fed and low dose STZ induced type 2 diabetic rats

The 30 rats were allocated into a dietary regimen by feeding High Fat Diet (HFD) (58% fat, 25% protein and 17% carbohydrate, as a percentage of total kcal) ad libitum, for the initial period of 2 weeks (Reed *et al*., 2000). The composition (Table 5.1) and preparation of HFD as were described elsewhere (Srinivasan *et al*., 2004).

After two weeks of high fat diet treatment, twenty four rats (without control group) were injected intraperitoneally (i.p) with low single dose of STZ (35 mg/kg b.w). The fat-fed/STZ diabetic rats were randomly divided into four groups consisting each of six rats after 3 weeks of dietary manipulation (i.e., after 1 week of STZ injection), while control groups were given vehicle. One week after STZ injection, the fasting blood

Table 5.1. Composition of HFD

Ingredients	Diet(g/kg)
Powdered NPD	365
Lard	310
Casein	250
Cholesterol	10
Vitamin and mineral mix	60
Yeast powder	01
Sodium chloride	01

glucose levels were appraised and the animal exhibiting fasting blood glucose levels 170±30 mg/dl were considered to be type 2 diabetic (T2D) rats and included for the further experiment. The rats were continued with high fat diet throughout the course of the study.

5.2.5. *In vivo* assay

5.2.5.1. Experimental Design

The wistar rats were divided into five groups and received the treatment as follows for 28 days.

Group I: Normal control rats were administered 1% tween 80 (2 ml/kg b.w, orally) in normal saline daily.

Group II: T2DM control rats were administered high fat diet + 1% tween 80 (2 ml/kg b.w, orally) in normal saline daily.

Group III: T2DM rats were administered high fat diet + AESM (100 mg/kg b.w., orally) daily.

Group IV: T2DM rats were administered high fat diet + AESM (200 mg/kg b.w., orally) daily.

Group V: T2DM rats were administered high fat diet + glibenclamide (1 mg/kg b.w., orally) daily.

Extract administration to the rats of group III was performed early in the morning and at fasting state by gavage. Animals of the control group (Group I) were subjected to gavage of distilled water like group II at the time of extract treatment to the animals of group III to keep all the animals under the same experimental condition and stress imposition if any due to treatment of extract and animal handling. Starting from first day of extract treatment to diabetic rats, fasting blood glucose levels (12 hours after feed delivery) in all the groups were measured by single touch glucometer (0, 1, 3, 7, 14, 21, 28 days) by collecting blood from the tail vein, and fasting glucose level was monitored by single touch glucometer. On day 29th, the treated rats were euthanasia and sacrificed by cervical dislocation. For haematological assays, blood samples were collected from retro orbital venous plexus in eppendroff tubes rinsed with anticoagulant before sacrificing. Liver, kidney, heart, pancreas were excised, cleaned and washed three times with PBS to remove adherent blood. The organs were homogenized in 0.1 M Tris-HCL-0.001 M EDTA buffer (PH 7.4) and centrifuged (12,000g, 30 min) at 4°C. The supernatant were collected and used for assaying biochemical parameters.

5.2.5.2. Serum biochemical parameters

Serum biochemical parameters viz. alanine amino transferase (ALT), aspartate amino transferase (AST), cholesterol and triglycerides were estimated by commercially available kits (Span diagnostic Limited, India).

5.2.5.3. Assessment of antioxidant markers related to organ dysfunction

The antioxidant enzymes superoxide dismutase (SOD) estimation was done by the protocol developed by Kakkar and co-workers (1984), catalase (CAT) activity was measured by the

method of Aebi (1984), glutathione-S-transferase (GST) activity was estimated by the method of Habig and co-workers (1974). Nonenzymatic antioxidant, reduced glutathione (GSH) level was assayed by the method of Hissin and Hilif (1973).

5.2.5.4. Histological studies

The rat organs from different groups were fixed in 10% formaldehyde and were processed for paraffin sectioning. Organ sections (5μm) were stained with hematoxylin and eosin to study the histology of organs (Dewanjee *et al*., 2013).

5.3. Effect of AESM against HFD and low dose streptozotocin induced type 2 diabetes

5.3.1. Effect on haematological and serum biochemical parameters

The effects of different treatments on serum biochemical parameters are shown in Table 5.2. The animals treated with HFD and STZ showed a significant ($p < 0.01$) increase of glycated haemoglobin whereas, the AESM treated groups showed significant improvement. The animals treated with HFD and STZ showed a significant ($p < 0.01$) increase in AST, ALT, triglyceride levels, whereas the levels of HDL cholesterol as found to decrease significantly ($p < 0.01$). Animals of group IV and group V received the combined treatment of HFD and extract (100 mg/kg and 200 mg/kg respectively) showed significant improvements ($p <0.05$-0.01) of haematological and biochemical parameter. The effect of different treatments on blood glucose level (0, 1, 3, 7, 14, 21, 28 days) is shown in the table 5.3. The animals treated with HFD and low dose STZ (Group II) showed a significant ($p < 0.05$) increase of blood glucose level whereas, the AESM treated groups (Group III and Group IV) showed significant decrease of blood glucose level ($p < 0.01$) that is almost similar to normal control (Group I) animals and standard drug glibenclamide treated group (Group V).

Table 5.2. Effect of AESM on serum biochemical parameters of T2D rats

Serum Parameters	Group I	Group II	Group III	Group IV	Group V
Total cholesterol (mg/dl)	87.97 ± 5.34	154.2 ±11.44$^{\#}$	115.95 ±8.67	106.97 ±6.34**	97.38 ±7.72**
HDL-cholesterol (mg/dl)	32.69 ±3.22	16.58 ±2.04$^{\#}$	25.36 ±2.76	27.44 ±2.04	31.92 ±2.86**
LDL-cholesterol (mg/dl)	27.93 ±6.74	109.2 ±15.13$^{\#}$	56.34 ±5.71	45.36 ±7.97**	37.23 ±5.09**
Triglycerides (mg/dl)	116.64 ±13.97	201.97 ±19.13$^{\#}$	155.97 ±15.54	136.45 ±13.21*	133.30 ±14.57*
ALT (IU l^{-1})	67.23 ±3.94	104.55 ±7.33$^{\#}$	84.37 ±5.74	80.72 ±6.17*	76.24 ±6.53
AST (IU l^{-1})	46.36 ±3.77	74.53 ±5.13$^{\#}$	54.32 ±5.14	57.93 ±5.04	49.79 ±6.09*
Glycated haemoglobin (mg g^{-1} haemoglobin)	0.26 ±0.43	0.71 ±0.63$^{\#}$	0.52 ±0.57	0.43 ±0.67*	0.37 ±0.33**
Urea (mg dl^{-1})	30.36 ±2.04	67.21 ±4.37$^{\#}$	47.57 ±3.26	45.14 ±4.04**	41.39 ±3.27**
Lactate dehydrogenase (U l^{-1})	204.73 ±8.74	296.96 ±14.73$^{\#}$	255.21 ±15.27	244.45 ±13.04*	237.71 ±9.86*
Creatinine kinase (IU mg^{-1} protein)	4.65 ±0.43	8.37 ±0.53$^{\#}$	6.57 ±0.47	6.24 ±0.39	5.83 ±0.50**
Insulin (µg dl^{-1})	2.76 ±0.18	1.43 ±0.13$^{\#}$	2.14 ±0.12	2.07 ±0.11*	2.07 ±0.11*

The Data were expressed as mean ± SE (n = 6). $^{\#}p < 0.01$ compared with normal control group. $^{*}p < 0.05$ compared with diabetic control group. $^{**}p < 0.01$ compared with diabetic control group. Gr I: Normal; Gr I: Normal; Gr II: T2D control, Gr III: T2D + 100mg/kg AESM ; Gr IV: T2D +200mg/kg AESM; Gr V: T2D + glibenclamide (1 mg/kg, p.o.).

5.3.2. Effect of antioxidant enzymes

The antioxidant enzymes protect biological macromolecules from oxidative damage. Therefore, the activities of antioxidant enzymes (CAT, SOD, GST, G6PD) and other cellular metabolites (GSH, GR) have been done in this study (Figure 5.1 and 5.2). CAT levels were significantly decreased in liver, kidney, heart ($p < 0.01$) of T2D control rats (Group II) as compared with normal (Group I) rats. Concurrent treatment of AESM (100 mg/kg and 200 mg/kg respectively) along with HFD could significantly ($p < 0.05$) increase CAT activities in aforementioned tissues. In the study of SOD activities, a significant ($p < 0.01$) depletion of SOD

Table 5.3. Effect of AESM on fasting blood glucose level of T2D rats

Groups	Fasting blood glucose level (mg/dl)in days						
	0	**1**	**3**	**7**	**14**	**21**	**28**
Group I	75.02 ±4.76	76.39 ±5.33	74.73 ±7.14	77.01 ±6.74	75.21 ±3.76	76.23 ±4.71	75.37 ±5.32
Group II	171.76 ±14.76$^{\#}$	172.97 ±12.01$^{\#}$	178.27 ±14.43$^{\#}$	181.21 ±16.74$^{\#}$	188.77 ±18.43$^{\#}$	196.21 ±18.43$^{\#}$	193.22 ±17.44$^{\#}$
Group III	172.21 ±16.14$^{\#}$	167.45 ±13.41$^{\#}$	147.24 ±9.04*	136.46 ±17.31**	130.22 ±14.21**	127.98 ±16.33**	123.26 ±12.43**
Group IV	173.24 ±15.09$^{\#}$	158.76 ±14.33$^{\#}$	143.27 ±11.47*	129.29 ±14.13**	120.12 ±15.34**	114.76 ±12.61**	107.37 ±13.45**
Group V	172.22 ±13.43$^{\#}$	166.45 ±11.46$^{\#}$	137.29 ±14.37**	126.65 ±15.12**	109.72 ±8.79**	98.33 ±10.03**	90.12 ±8.33**

Data were expressed as mean ± SD (n=6). $^{\#}p< 0.01$ compared with Group I; $^{*}p< 0.05$ compared with Group II; $^{**}p< 0.01$ compared with Group II. Group I: Normal control; Group II: T2D control, Group III: T2D rats treated with AESM (100 mg/kg, p.o.); Group IV: T2D rats treated with AESM (200 mg/kg, p.o.); Group V: T2D rats treated with glibenclamide (1 mg/kg, p.o.).

level was observed in the selected tissues of T2D control (Group II) rats. Treatment with AESM (100 mg/kg and 200 mg/kg) along with HFD could significantly ($p < 0.05$) increase SOD activities in tissues. In the study of GST activities, a significant ($p < 0.01$) depletion of GST level was observed in the selected tissues of T2D control (Group II) rats. Treatment with AESM ((100 mg/kg and 200 mg/kg) along with HFD could significantly ($p < 0.05$) increase GST activities in tissues. T2D control rats could significantly ($p < 0.01$) reduce GSH levels in selected tissues of experimental rats (Group II). However, simultaneously administration of AESM (100 mg/kg and 200 mg/kg respectively) along with HFD could significantly improve GSH level of experimental rats (Group III and Group IV) when compared with toxic control animals (Group II). G6PD levels were significantly decreased in liver, kidney, heart ($p < 0.01$) of T2D control rats (Group II) as compared with normal (Group I) rats. Concurrent treatment of AESM (100 mg/kg and 200 mg/kg respectively) along with HFD could significantly ($p < 0.05$) increase G6PD activities in tissues. GR levels were significantly decreased in liver, kidney, heart ($p < 0.01$) of T2D control rats (Group II) as compared with normal (Group I) rats. Concurrent treatment of AESM (100

mg/kg and 200 mg/kg respectively) along with HFD could significantly ($p < 0.05$) increase GR activities in tissues. GPx levels were significantly decreased in liver, kidney, heart ($p < 0.01$) of T2D control rats (Group II) as compared with normal (Group I) rats. Concurrent treatment of AESM (100 mg/kg and 200 mg/kg respectively) along with HFD could significantly ($p < 0.05$) increase GPx activities in aforementioned tissues. For standard drug (Glibenclamide) treated animals (Group V) SOD, CAT, GSH, GST, G6PD, GPx, GR levels were significantly increased.

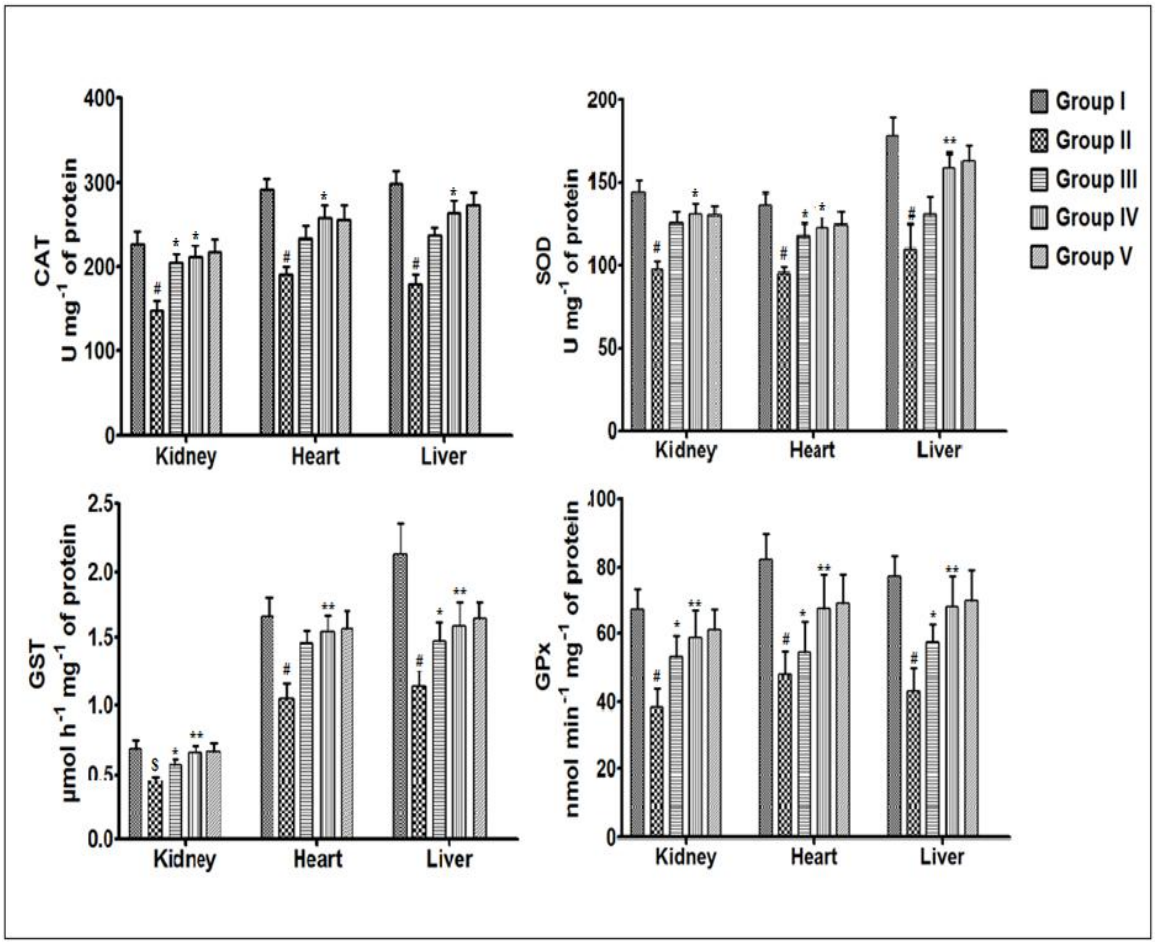

Fig 5.1. Effect of AESM on endogenous antioxidant enzymes (SOD, CAT, GPx, GST) levels in the myocardial tissues of T2D rats. Data were expressed as mean ± SD (n = 6). $^{\$}p < 0.05$ compared with Group I; $^{\#}p < 0.01$ compared with group I; $^{*}p < 0.05$ compared with Group II; $^{**}p < 0.01$ compared with Group II.Group I: Normal control; Group II: T2D control, Group III: T2D rats treated with AESM (100 mg/kg, orally); Group IV: T2D rats treated with AESM (200 mg/kg, orally); Group V: T2D rats treated with glibenclamide (1 mg/kg, orally).

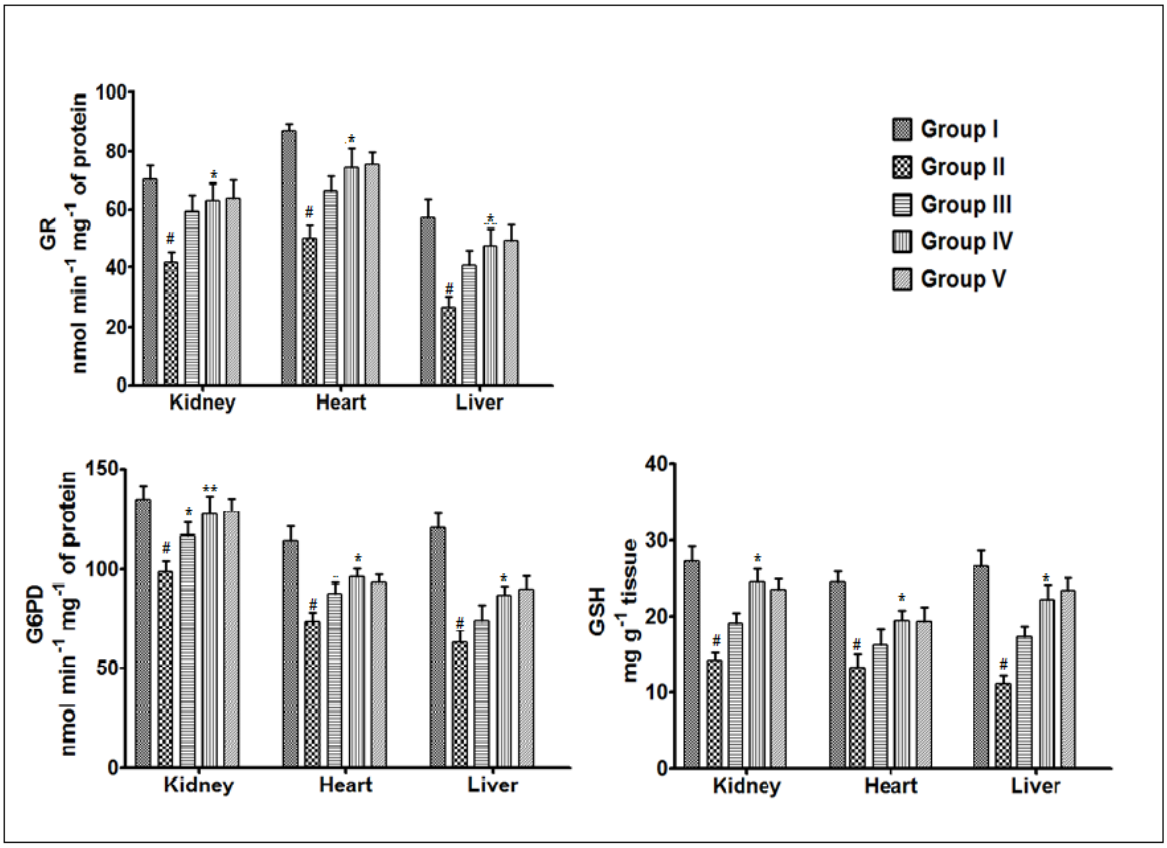

Fig 5.2. Effect of AESM on endogenous antioxidant enzymes (GR, G6PD, GSH) levels in the myocardial tissues of T2D rats. Data were expressed as mean ± SD (n = 6). $^{\#}p < 0.01$ compared with group I; $^{*}p < 0.05$ compared with Group II; $^{**}p < 0.01$ compared with Group II. Group I: Normal control; Group II: T2D control, Group III: T2D rats treated with AESM (100 mg/kg, orally); Group IV: T2D rats treated with AESM (200 mg/kg, orally); Group V: T2D rats treated with glibenclamide (1 mg/kg, orally).

5.3.3. Histological assessment

Heart, kidney and liver from the animals (normal and experimental) were immediately fixed in formalin (10% buffered) after sacrifice and were managed for paraffin sectioning. Sections (thickness ~ 5 μm) were stained (eosin & hematoxylin) to assess under light microscope.

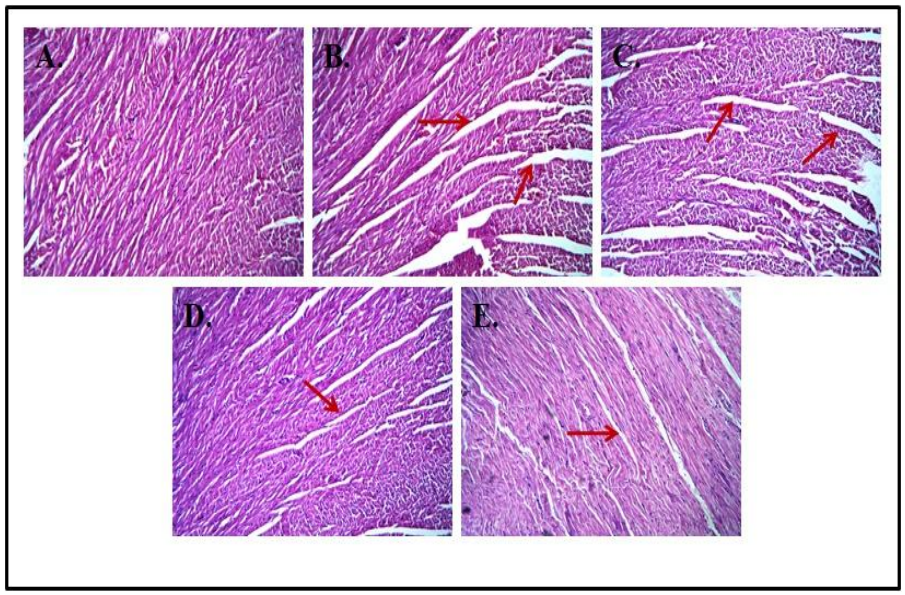

Fig 5.3. Group II (B) exhibited degeneration of interstitial tissues (red arrows), while, Group I exhibited general radiating pattern of heart section. Group III, IV and V indicated significant improvement in myofibrillar arrangement in heart tissues comparable to that of Group I. Group I: Normal control; Group II: T2D control, Group III: T2D rats treated with AESM (100 mg/kg, orally); Group IV: T2D rats treated with AESM (200 mg/kg, orally); Group V: T2D rats treated with glibenclamide (1 mg/kg, orally). The observed effects of AESM (100 and 200 mg/kg) were compared with standard drug glibenclamide (1 mg/kg) and it was found that the hypoglycemic and hypolipidemic effect of AESM (200 mg/kg) were comparable to that of glibenclamide (1 mg/kg). However,

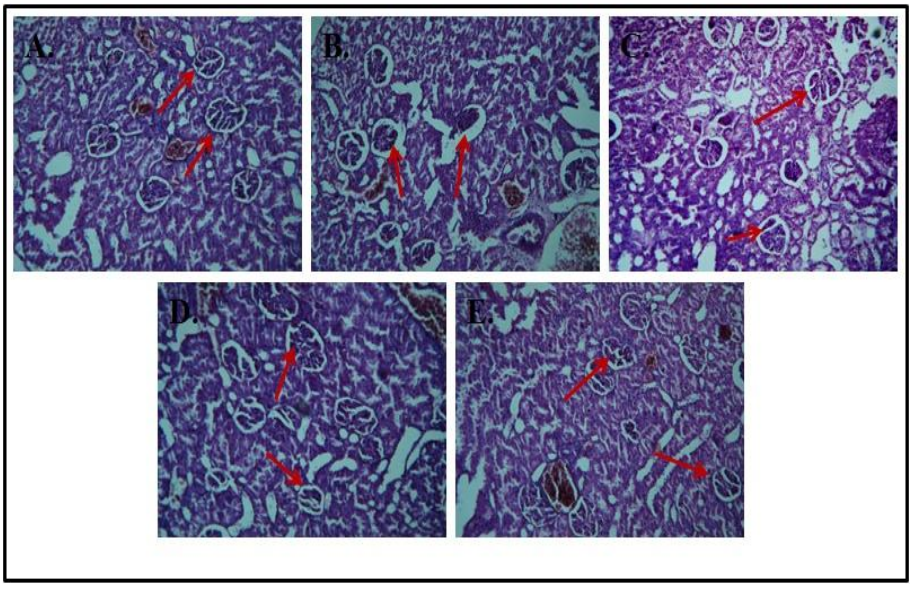

Fig 5.4. Group II exhibited thickening of glomerular basement membrane (red arrows) which indicates glomerular damage, while, Group I exhibited normal glomerular arrangement in kidney sections. Group III, IV and V indicated significant improvement in podocyte structure comparable to that of Group I. Group I: Normal control; Group II: T2D control, Group III: T2D rats treated with AESM (100 mg/kg, orally); Group IV: T2D rats treated with AESM (200 mg/kg, orally); Group V: T2D rats treated with glibenclamide (1 mg/kg, orally). The observed effects of AESM (100 and 200 mg/kg) were compared with standard drug glibenclamide (1 mg/kg) and it was found that the hypoglycemic and hypolipidemic effect of AESM (200 mg/kg) were comparable to that of glibenclamide (1 mg/kg).

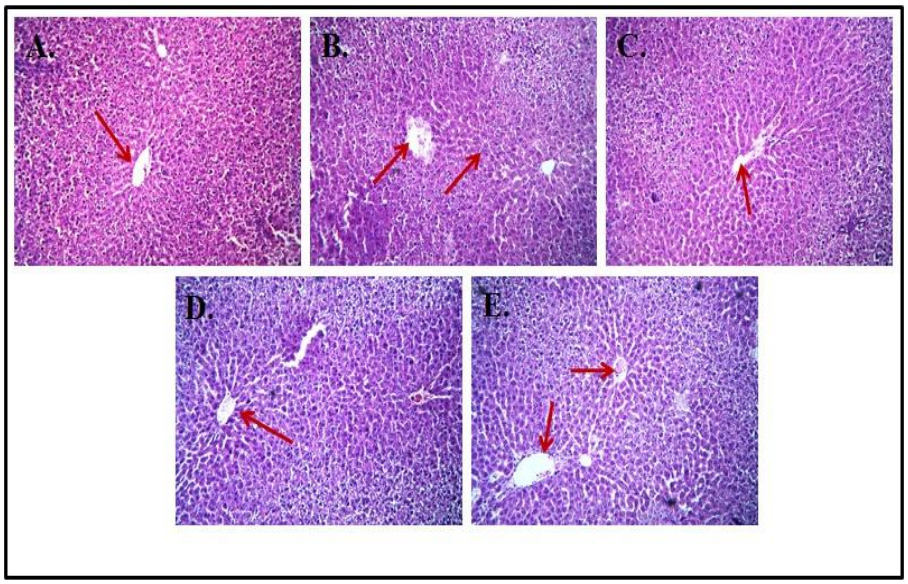

Fig5.5. Group II exhibited abnormal architecture of liver tissue and distranded central vein (red arrows), while, Group I showing normal architecture. Group III, IV and V indicated significant improvement in tissue architecture comparable to that of Group I. Group I: Normal control; Group II: T2D control, Group III: T2D rats treated with AESM (100 mg/kg, orally); Group IV: T2D rats treated with AESM (200 mg/kg, orally); Group V: T2D rats treated with glibenclamide (1 mg/kg, orally). The observed effects of AESM (100 and 200 mg/kg) were compared with standard drug glibenclamide (1 mg/kg) and it was found that the hypoglycemic and hypolipidemic effect of AESM (100 mg/kg) were comparable to that of glibenclamide (1 mg/kg).

5.4. Discussion

High fat diet is a major cause of obesity with simultaneously insulin resistance in the western countries (Zheng et al., 2012). High fat diet (ad libitum) and low single dose of STZ (35 mg/kd body weight) induced diabetes mellitus has been considered to be the best experimental model of T2D in term of insulin resistance (Bhandari et al., 2013). Therefore, high fat diet + low single dose of STZ model was employed in this study to evaluate protective effect of AESM. Reduction of the blood glucose level is the principle approach of diabetic therapy. Inclusion of low dose of STZ caused incomplete destruction of β-cell population in islet of Langerhans. In this study, significant reduction of serum insulin level was observed. Insulin is known to activate

lipoprotein lipase which catalyses the hydrolytic breakdown of lipids during normal physiological status (Khanra et al., 2015).

Therefore, lower insulin level coupled with insulin resistance during diabetic condition causes hyperlipidemia. In this study, high concentrations of serum lipids viz. cholesterol and triglycerides were observed in T2D rats. AESM treatment could significantly attenuated hyperlipidemia, which would be corroborated with the reversal of insulin resistance and/or elevation of insulin secretion. A persistently elevated blood glucose level ensures increased glycosylation of different functional proteins including haemoglobin (Khanra et al., 2015). In this study, a significant elevation of glycosylated haemoglobin was observed in T2D rats. Increased CK and LDH contents are the major indicators of cellular damage (Patel et al., 2014). These membrane bound enzymes come into the blood during cellular injury. In this study, CK and LDH levels in the sera were significantly raised in T2D rats over control, which revealed the occurrence of hyperglycemia mediated cytotoxicity during diabetes mellitus. AESM treatment significantly attenuated serum levels of CK and LDH, which indicated the cyto-protective role of test extract during diabetes mellitus.

Increase level of blood glucose facilities generation of ROS which directly participate in the pathological occurrence in DIABETES MELLITUS. organ damage is a critical reason of morbidity and mortality of the DIABETES MELLITUS patients (Manna & Sil et al., 2012). Earlier reports revealed that hyperglycemia mediated excessive ROS generation plays predominant role in diabetes related organ dysfunction. In this study, a significantly high ROS production was observed in kidney, liver T2D rats. An enhanced generation of ROS would result in the increases in lipid peroxidation, protein carbonylation with concomitant depletion of endogenous antioxidant molecules viz. antioxidant enzymes and GSH (Dua et al., 2015).

Therefore, it would be concluded that myocardial tissues experienced to redox challenge/oxidative stress during diabetes mellitus. AESM treatment could significantly attenuated intracellular ROS levels in the myocardial tissues of T2D rats. AESM could produce the effect either by direct scavenging ROS and/or indirectly by inhibiting ROS generation through its hypoglycemic effect. Decrease in the levels of ROS in the myocardial tissues in AESM treated T2D rats caused the reduction of peroxidative damage of cellular lipid and carbonylation of protein.

The present study describes the protective role of *Swietenia mahagoni* against T2D caused by HFD and low dose STZ (35mg/kg) employing suitable *in vivo* preclinical assays. Haematological and serum biochemical parameters stay earlier indicators of any pathophysiological state. In this study, a considerable increase of glycated haemoglobin was observed in T2D control rats, whereas after the treatment with AESM, a considerable reduction of glycated haemoglobin was observed that is almost similar to the marketed standard antidiabetic drug glibenclamide. Blood glucose level also significantly decreased after the treatment with AESM. As well as total cholesterol, ALT, AST, LDL, urea, lactate dehydrogenase, creatinine levels are significantly increased in T2D control rats (Group II) and HDL, insulin levels are significantly decreased in Group II diabetic control rats. Combined treatment with HFD and AESM (Group III and Group IV) total cholesterol, ALT, AST, LDL, urea, lactate dehydrogenase, creatinine levels are significantly decreased and HDL, insulin levels are significantly increased.

Antioxidant enzyme CAT, SOD, GST, G6PD is considerable to be the first line of cellular defense against oxidative stress (Das *et al*., 2010). On the other hand, second line of defense includes the nonenzymatic radical scavenger GSH, which scavenges residual free

radicals escaping decomposition by the antioxidant enzymes. In the present study Type 2 diabetes significantly reduced the activities of all antioxidant enzymes as well as GSH in liver of experimental animals. However the combined treatment of HFD and AESM could significantly prevent the alteration of antioxidant enzymes. SOD and CAT are most important radical scavenging enzymes. SOD acts by quenching the superoxide radicals leading to the formation of hydrogen peroxide and molecular oxygen, while CAT is well known to detoxify hydrogen peroxide (Dewanjee *et al*., 2013). GST protects cells against oxidants by removing free radicals. Significant reduction of these antioxidant enzymes during T2D may be due to an enhanced production of ROS and down regulation in the synthesis of antioxidant enzymes. Thiol based antioxidant system, GSH helps to protect cells from ROS (Das *et al*., 2010). The GSH level significantly decreased in the liver in diabetic control rats whereas the GSH level increased after the treatment with AESM.

The histological sections Fig 5.3(heart, x 100), Fig 5.4 (kidney, x 100) and Fig 5.5 (liver, x 100) of T2D rats proves the beneficial effect of AESM on different organs during diabetes. Fig 5.3 indicates that Group II (T2D control) exhibited degeneration of interstitial tissues, while, Group I (normal control) exhibited general radiating pattern of heart section. Group III , IV and V indicated significant improvement in myofibrillar arrangement in heart tissues comparable to that of Group I. Fig 5.4 shows that Group II exhibited thickening of glomerular basement membrane which indicates glomerular damage, while, Group I exhibited normal glomerular arrangement. AESM treatment successfully prevented glomerular matrix enlargement in diabetic kidney sections. Fig 5.5 clearly proves protective activity of AESM on diabetic liver damage Group II exhibited abnormal architecture of liver tissue and distranded central vein, while, Group

I showing normal architecture. Group III, IV and V indicated significant improvement in tissue architecture comparable to that of Group I.

After some literature review it was observed that AESM has effect against T2D (Naveen and Urooj, 2014). Medicinal plants and their preparations are being used for thousands of years in all types of traditional medicinal practices because of their ease of availability and cost They are rich sources of numerous bioactive components which can prevent, treat and also can help in the management of several diseases/disorders and till date only few plants have been explored for their potential pharmacological activities. Although their long usage history in treating the ailments, there is very less documentation in the scientific literature regarding the dose, adverse side effects, route and form of administration of the medicinal plants. The maximum allowable dose and adverse side effects can be evaluated by toxicological study using the animal models. The present study evaluates the safety of the *Swietenia mahagoni* in its maximum allowable dose prescribed by the OECD guideline. There were no sudden reactions such as hypersensitivity and allergic reactions following the administration of extract. In addition, there was no behavioral or physical change observed during the whole study period. The changes of glucose level, serum parameters, antioxidant enzyme levels are also comparable to normal group (Group I) and marketed standard drug glibenclamide treated group (Group V). Here we observed the presence of phenolics and flavonoids are potent antioxidants, which prevent apoptosis caused by oxidants. Therefore, phenolics, flavonoids, ascorbic acid and saponins within AESM would offer a cumulative effect to ensure the protection against diabetes.

Chapter 6

Conclusion

Conclusion

The phytochemical investigations, isolation and protective role of *Swietenia mahagoni* against experimentally induced diabetes has been represented here in this thesis. The thesis is furnished with the characterization and isolation of compounds and exploration of the antioxidative efficacy of the plant.

Chapter 1 is an introductory part which deals with traditional medicine and drug discovery. It also gives concept and research scheme about diabetes and objective of this work and basis of plant selection.

Chapter 2 deals with literature review, where previous research work of *Swietenia mahagoni* has been discussed. It also discussed about the ethnomedicinal documentation of this plant.

Chapter 3 deals with the methodology of extraction, preliminary phytochemical screening and isolation for the presence phytochemicals in *Swietenia mahagoni*. From phytochemical study we have observed the presence of alkaloids, flavonoids, saponins, steroids, carbohydrate, volatile oil, tannins in the extract of *Swietenia mahagoni*.

Chapter 4 is about the *in vitro* antioxidant study of the extract by DPPH assay, total phenolic content and total flavonoid content. On the basis of the result it could be concluded that *Swietenia mahagoni* had very good antioxidant activity.

Chapter 5 is about the effect of the aqueous extract of *Swietenia mahagoni* against high fat diet and low dose streptozotocin induced diabetic model. The presence of dietary antioxidants namely flavonoids, phenolics in the test extracts had contribute in overall protection against diabetes. In conclusion, augmentation of *Swietenia mahagoni* can be a novel strategy for attenuate diabetes in future.

REFERENCES

References

Adhikari, U., Singha, S., Chandra, G., 2012. *In vitro* repellent and larvicidal efficacy of *Swietenia mahagoni* against the larval forms of *Culex quinquefasciatus* Say. Asian Pacific Journal of Tropical Biomedicine 2(1), 260-264.

Aebi, H., 1984. Catalase invitro. Method Enzyme 105, 121-126.

Alrdahe, S.S, Abdulla, M.A., Razak, S.A., Kadir, F.A., Hassandarvish, P., 2010. Gastroprotective activity of *Swietenia mahagoni* seed extract on ethanol-induced gastric mucosal injury in rats. World Academy of Science, Engineering and Technology 43, 883-887.

Boden, G., 1997. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes 46, 3-10.

Cannell, R.J.P., 1990. How to approach the isolation of a natural product, in Natural Products Isolation, in methods in biotechnology. Humana Press Inc. 4, 1-52.

Cohen, B., Novick, D., Rubinstein, M., 1996. Modulation of insulin activities by leptin. Science 274, 1185-1188.

Das, A.K., Bag, A., Sahu, R., Dua, T.K., Simha, M.K., Gangopadhyaya, M., et al., 2010. Protective effect of *Corchorus olitoritus* leaves on sodium arsenic induced toxicity in experimental rats. Food and Chemical Toxicology 48, 326-335.

De, D., Chatterjee, K., Ali, K M., Bera, T K., Ghosh, D., 2011. Antidiabetic potentiality of the aqueous methanolic extract of seed of *Swietenia mahagoni* (L.) Jacq. In streptozotocin-induced diabetic male albino rat:a correlative and evidence based approach with antioxidative and antihyperlipidemic activities. Evid Based Complement Alternat Med. 89, 2807.

Dewanjee, S., Sahu, R., Karmakar, S., Gangopadhayay, M., 2013. Toxic effects of lead exposure in wistar rats : Involvement of oxidative stress and the beneficial role of edible jute (Corchorus olitorius) leaves. Food and Chemical Toxicology 55, 78-91.

Dewanjee, S., Sahu, R., Karmakar, S., Gangopadhyay, M., 2013. Toxic effect of lead exposure in Wistar rats: Involvement of oxidative stress and the beneficial role of edible jute (*Corchorus olitorius*) leaves.Food and chemical toxicology 55, 78-91.

Evans, J., Goldfine, I., Maddux, B., Grodsky, G., 2002.Oxidative stress and stress-activated signaling pathways: A unifying hypothesis of type2 diabetes. Endocrine Reviews 23, 599-622.

Frayn, K.N., 1993. Insulin resistance and lipid metabolism. Current Opinion in Lipidology4, 197-204.

Ghosh, S., Besra, S.E., Roy, K., Gupta, J.K., Vedasiromoni, J.R., 2009. Pharmacological effects of methanolic extract of *Swietenia mahagoni* Jacq (Meliaceae) seeds. International Journal of Green Pharmacy 3(3), 206-210.

Gordon, E.S., 1960. Non-esterified fatty acids in blood of obese and lean subjects. The American Journal of Clinical Nutrion8, 740-745.

Goun, E., Cunningham, G., Chu, D., Nguyen, C., Miles, D., 2003. Antibacterial and antifungal activity of Indonesian ethnomedical plants. Fitoterapia 76, 592-596.

Gray, M.J., Chang, D., Zhang, Y., Liu, J., Bensoussan, A., 2010. Development of liquid chromatography/mass spectrometry methods for the quantitative analysis of herbal medicine in biological fluids: a review. Biomedical Chromatography 24, 91-103.

Grodsky, G.M., 2000.Kinetics of insulin secretion : underlying metabolic events in diabetes meliitus. Diabetes mellitus: a fundamental clinical text, Philadelphia

Habig, W.H., Pabst, M.J., Jacoby, W.B., 1974. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry 249, 7130-7139.

Hajra, S., Mehta, A., Pandey, P., 2012. Evaluation of the antidiarrhoeal activity of *Swietenia mahagoni* seed extracts. International Journal of Pharmacy and Pharmaceutical Sciences 8(5), 416-421.

Hajra, S., Mehta, A., Pandey, P., 2012. Immunostimulating activity of methanolic extract of *Swietenia mahagoni* seeds. International Journal of Pharmacy and Pharmaceutical Science 4(1), 442-445.

Haldar, P.K., Adhikari, S., Bera, S., Bhattacharya, S., Panda, S.P., Kandar, C.C., 2011. Hepatoprotective efficacy of *Swietenia mahagoni* L. Jacq. (Meliaceae) bark against paracetamol induced hepatic damage in rats. Indian Journal of Pharmaceutical Education and Research 45(2), 108-113.

Hissin, P.J. & Hilif, R.A., 1973. A flurometric method for the determination of oxidized and reduced glutathione in tissue. Analytical Biochemistry 74, 214-226.

Hotamisligil, G.S., Spiegelman, B.M., 1994. Tumornecrosis factor α: a key component of the obesity-diabetes link. Diabetes 43, 1271-1278.

Kadowaki, T., 2006. Adiponectin and adiponectin receptors in insulin resistance, diabetes and the metabolic syndrome. Journal of Clinical Investigation 116, 1784-1792.

Kahn, S.E., 2001. The importance of β-cell failure in the development and progression of type2 diabetes. Journal of Clinical Endocrinology and Metabolism 86, 4047-4058.

Kakkar, P., Das, B., Viswanathan, P., 1984. A modified method for assay of superoxide dismutase. Indian Journal of Biochemistry and Biophysics 21, 131-132.

Khanra, R., Dewanjee, S., Dua, T.K., Sahu, R., Gangopadhyay, M., Feo De, V., Haq, M., 2015. Abroma augusta L. (Malvaceae) leaf extract attenuates diabetes induced nephropathy and cardiomyopathy via inhibition of oxidative stress and inflammatory response. Journal of translational medicine 13, 6.

King, G.L., Brownlee, M**.,** 1996. The cellular and molecular mechanisms of diabetic complications. Endocrinology Metabolism Clinics of North America 25, 255-270.

Li, D.D., Chen, J.H., Chen, Q., Li, G.W., Chen, J., Yue , J.M., *et al*., 2005. *Swietenia mahagoni* extract shows agonistic activity to PPARγ and gives ameliorative effects on diabetic *db/db* mice. Acta Pharmacological Sinica. 26(2), 220-222.

Li, S.P., Zhao, J.,Yang, B., 2011. Strategies for quality control of Chinese medicines. Journal of Pharmaceutical and Biomedical Analysis 55, 802-809.

Linghor, M., Buettner, C.J., Rhodes, C., 2005. Type2 diabetes-a matter of β-cell life and death? www.sciencemag.org 307, 380-383.

Loghmani, E., 2005. Diabetes mellitus Type1 and Type2.Gudelines for adolescent nutrition services 2005, 167-181.

Luo , J., Quan, J., Tsai, J., Hobensack, C.K., Sullivan, C., Hector, R., et al., 1998. Nongenetic mouse models of non-insulin-dependent diabetes mellitus.Metabolism 47, 663-668.

Marston, A., Hostettmann, K., 2009. Natural product analysis over the last decades. Planta Medica 75, 672-682.

Mostafa, M., Hemayet, H., Hossain, M.A., Biswas, P.K., Haque,M.Z., 2012. Insecticidal activity of plant extracts against *Tribolium castaneum* Herbst. Journal of Advanced Scientific Research 3(3), 80-84.

Nagalakshmi, M.A.H., Thangadurai, D., Muralidara, D., Pullaiah, R.T., 2001. Phytochemical and antimicrobial study of *Chukrasia tabularis* leaves. Fitoterapia 72, 62-64.

Naveen, P., Urooj, A., 2015. Preclinical Safety Evaluation of *Swietenia mahagoni* leaf in Wistar Rats. International Journal of Pharmacy and Pharmaceutical Sciences 7, 294-297.

Naveen, Y.P., Urooj,A., 2015. Preclinical safety evaluation of *Swetenia mahagoni* leaf in Wister rat.International Journal of Pharmacy and Pharmaceutical Sciences 7, 294-297.

Otake, T., Mori, H., Morimoto, M., Ueba, N., Sutardjo, S., Kusumoto, I.T., Hattori, M., Namba, T., 1995. Screening of indonesian plant extracts for anti-human immunodeficiency virus — type 1 (HIV-1) activity. Phytother Research 9(1), 6-10.

Perley, M. & Kipnis, D.M.,1966. Plasma insulin responses to glucose and tolbutamide of normal weight and obese diabetic and nondiabetic subjects. Diabetes 15, 867-874.

Perseghin, G., Ghosh, S., Gerow, K., Shulman, G.I., 1997. Metabolic defects in lean nondiabetic offspring of NIDDM parents: a cross sectional study. Diabetes 46, 1001-1009.

PHS. Public Health Service Policy on Humane Care and Use of Laboratory Animals, Washington, D.C., U.S., Department of Health and Human Services 1986. Available from Office for Protection from Research Risks, Building 31, Room 4B09, NIII, Bethesda, MD 20892, 1986

Portha, B., Giroix, M-H., Serradas, P., Morin, L., Tormo, M-A., Bailbe, D., 1994. Cellular basis for glucose refractoriness of pancreatic B-cells in non insulin dependent diabetes. Insulin secretion and pancreatic B cell research.UK: Smith-Gordon, 461-472.

Randle, P.J., Garland, P.B., Hales, C.N., & Newsholme, E.A., 1963. The glucose fatty acid cycle:it's role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet i, 785-789.

Randle, P.J., Kerbey,A.L., Espinal,J., 1988. Mechanisms decreasing glucose oxidation in diabetes and starvation: role of lipid fuels and hormones. Diabetes Metabolism Research and Reviews 623, 638.

Reaven, G.M., Hollenbeck, C., Jeng, C.Y., Wu, M.S., & Chen, Y.D., 1988. Measurement of plasma glucose, free fatty acid, lactate and insulin for 24h in patients with NIDDM.Diabetes 37, 1020-1024.

Reed, M.J., Meszaros, K., Entes, L.J., Claypool, M.D., Pinkett, J.G., Gadbois, T.M., *et al*., 2000. A new rat model of type 2 diabetes: the fat-fed, streptozotocin-treated rat. Metabolism 49, 1390-1394.

Rerup, C.C., 1970. Drugs producing diabetes through damage of the insulin secreting cells. Pharmacological Reviews 22, 485-518.

Roden, M., Price, T.B., Perseghin, G., Petersen, K,F., Rothman, D.L., Cline, G.W.,*et al*., 1996. Mechanism of free fatty acid-induced insulin resistance in humans Journal of Clinical Invest 97, 2859-2865.

Sahgal, G., Ramanathan, S., Sasidharan, S., Mordi, M.N., Ismail, S., Mansor, S.M., 2009. *In vitro* antioxidant and xanthine oxidase inhibitory activities of methanolic *Swietenia mahagoni* seed extracts. Molecules 14(11), 4476-4485.

Sahgal, G., Ramanathan, S., Sasidharan, S., Mordi, M.N., Ismail, S., Mansor, S.M., 2009. Phytochemical and antimicrobial activity of *Swietenia mahagoni* crude methanolic seed extract. Asian Pacific Journal of Tropical Biomedicine 26(3), 274-279.

Sahgal, G., Ramanathan, S., Sasidharan, S., Mordi, M.N., Ismail, S., Mansor,S.M., 2011. *In vitro* and *in vivo* anticandidal activity of *Swietenia mahogani* methanolic seed extract. Asian Pacific Journal of Tropical Biomedicine 28(1), 132-137.

Scherer, P.E., 2006. Adipose tissue: from lipid storage compartment to endocrine organ. Diabetes 55, 1537-1545.

Shafrir, E., 2003. Diabetes in animals: Contribution to the understanding of diabetes by study of its etiopathology in animal models. Diabetes mellitus, 231-255.

Shipar, M.A.H., Chowdhury, R., Majid, M.A., Uddin, M.H., Rahman,I.M.M., 2004. Physico-chemical characterization, antimicrobial activity and toxicity analysis of *Swietenia mahagoni* seed oil. International Journal of Biological Engineering 6(2), 350-354.

Shulman, G.I., 2000. Cellular mechanisms of insulin resistance. Journal of Clinical Invest 106, 171-176.

Sims, E.A., Danforth, Jr.E., Horton, E.S., Bray, G.A., Glennon, J.A., Salans, L.B., 1973. Endocrine and metabolic effects of experimental obesity in man. Recent Progress in Hormone Research 29, 457-496.

Smith, R.M., 2003. Before the injection-modern methods of sample preparation for separation techniques. Journal of Chromatography A1000 (1-2), 3-27.

Srinivasan, K., Patole, P.S., Kaul, C.L., Ramarao, P., 2004. Reversal of glucose intolerance by pioglitazone in high-fat diet fed rats. Methods Findings in Experimental and Clinical Pharmacology 26, 327-333.

Steppan, C.M., Bailey, S.T., Bhat, S., Brown, E.J., Banerjee, R.R.,Wright, C.M., *et al*.,2001. The hormone resist in links obesity to diabetes. Nature 409, 307-312.

Taylor, A.R.H., Taylor, D.A.H., 1983. Limonoids extractives from Swietenia macrophylla. Phytochemistry 22, 2870-2871.

Weir, G.C., Clore, E.T., Zmachinski, C.J., Bonner-Weir, S., 1981. Islet secretion in a new experimental model for non insulin dependent diabetes. Diabetes 30, 590-595.

Weisberg, S.P., 2003. Obesity is associated with macrophage accumulation in adipose tissue. Journal of Clinical Investigation 112, 1796-1808.

Wellen, K.E & Hotamisligil, G.S., 2005. Inflammation,stress and diabetes. Journal of clinical investigation 115, 1111-1119.

Wild, S., Roglic, G., Green, A., Sicree, R., King, H., 2004. Global prevalence of diabetes : estimates for the year 2000 and projections for 2030. Diabetes Care 27, 1047-1053.

Yang, Q *et al*., 2005. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type-2 diabetes. Nature 436, 356-362.

Zhang, F., Ye, C., Li, G., Ding, W., Zhou, W., Zhu, H., et al., 2003. The rat model of type 2 diabetes mellitus and its glycometabolism characters. Journal of Experimental Animal 52, 401-407.