Enhancing the Bioavailability of Some Therapeutically Potent Plant Secondary Metabolites through Development of Novel Delivery Systems

Synopsis Submitted By
Ranjit Kumar Harwansh, M. Pharm
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School of Natural Product Studies
Department of Pharmaceutical Technology
Faculty Council of Engineering & Technology
Jadavpur University
Kolkata, India

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JADAVPUR UNIVERSITY
KOLKATA – 700032, INDIA

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1. Title of the thesis:

Enhancing the bioavailability of some therapeutically potent plant secondary metabolites through development of novel delivery systems

2. Name of the candidate:

Ranjit Kumar Harwansh, *M. Pharm*

3. Name, designation & institution of the supervisor:

Prof. Pulok K Mukherjee, *M. Pharm, PhD, FRSC*
Director, School of Natural Product Studies
Department of Pharmaceutical Technology
Jadavpur University, 188, Raja S.C. Mullick Road,
Kolkata - 700032, India

1. Background of the study

The plant secondary metabolites (flavonoids, alkaloids, terpenoids, glycosides, etc.) have been exploited as medicine in pharmaceutical, medical and food research for their tremendous therapeutic benefits. Several phytomolecules are used as therapeutic agents, nutraceuticals and dietary supplements because of their beneficial effects. Flavonoids are a diverse group of polyphenolic compounds present in plants and natural products. Studies have indicated that the flavonoids exhibit antiproliferative activity toward coronary heart disease, lung cancers, and other types of cancers. Polyphenols are abundant micronutrients in our diet, and evidence for their role in the prevention of degenerative diseases is emerging. Flavonoids have also been used in skin rejuvenating creams to heal and moisturize aged and sun burnt skin. Recent interest has increased in the potential protective effects of phenolic compounds against oxidative stress induced diseases (cardiovascular diseases and cancers) caused by reactive oxygen species (ROS) and other free radicals.

A great deal of herbal products for therapeutic application is being acquired through the use of phytopharmaceuticals, and information is increasing quickly with greater understanding of molecular mechanisms of diseases. Nevertheless, favorable drug action alone against the disease is insufficient to satisfy the medical community. In addition, avoiding unwanted side effects at the site of action is equally important. The pharmacological activity of any administered drug relies not only on its therapeutic efficacy but also on the bioavailability at the administered site. The several phytopharmaceuticals and plant secondary metabolites are used as therapeutic agents, nutraceuticals and dietary supplements for development and promotion of human health because of their beneficial importance. Though, the phytopharmaceuticals exhibit diverse therapeutic effects like antioxidants, anti-inflammatory, hypoglycaemic, hepatoprotective, UV-protective, anticancer, and antiobesity effect etc. The efficacy of any phytopharmaceuticals depends on effective dosage form and delivery systems. Apart from its valuable pharmacological effects suffer from poor oral bioavailability due to low aqueous solubility, low permeability, gastro intestinal degradation (instability in G.I. pH) and extensive first-pass metabolism resulted into low elimination half-life ($t_{1/2}$). To overcome these problems of therapeutic efficacy and bioavailability of phytomolecules, developing novel drug delivery systems (NDDS) are introduced in the phytopharmaceuticals with better absorption profile is of prime focus.

The application of NDDS is an important approach toward solving bioavailability-related problems associated with phytomolecules. Several novel herbal drug delivery systems specifically liposomes, transfersomes, ethosomes, niosomes, phytosomes, herbosomes, dendrimers, micro/nanoparticles, micro/nanoemulsions (NEs), micelles, etc., have been successfully employed for the delivery of phytopharmaceuticals. The novel formulations have notable advantages compared to conventional formulations like enhancement of solubility and stability, membrane permeability and bioavailability, improved pharmacological activity through sustained-release profile, and reduced

toxicity. The novel systems of herbal medicine have the capability to deliver the drug at a rate directed by the needs of the body for an extended period of time, and it should channel the bioactive molecule of herbal products to the site of action. Therefore, the NDDS have a great future for enhancing the therapeutic activity and overcoming problems attributed with herbal medicine. Most of bioactive agents (e.g., nutraceuticals and pharmaceuticals) intended for oral administration are found as highly hydrophobic compounds with low water solubility and poor bioavailability. Furthermore, poor solubility also leads to lower absorption in the gastrointestinal tract (GIT) and therefore limited therapeutic activity. The application of nano-encapsulation to functional food, medical and pharmaceutical industries have received great attention from the scientific community. Forced back by the increasing consumer demand for quality and safer medicinal herbal/food products for the promotion of better health, researchers are currently concentrating their efforts in nanotechnology to address topics relevant to food-herb as medication.

Nanoemulsion drug delivery system is particularly suited for encapsulating the phytopharmaceuticals as it prevents their degradation and improves their bioavailability. Nanoemulsion as a carrier system is a particularly convenient means of loading, protecting, and delivering poorly soluble phytopharmaceuticals and drugs. The encapsulation of bioactive compounds in NEs offers different advantages in comparison with conventional delivery systems such as stabilization in aqueous environment of stomach and foodstuffs of lipophilic bioactive compounds with scarce solubility in water, protection of bioactive compounds against gastric degradation. The goal of novel delivery system is to enhancing absorption/permeation and bioavailability of low bioavailable phytopharmaceuticals by improving its solubility using nanoemulsion carrier system. The effectiveness of phytopharmaceuticals is dependent upon delivering an effective level of the active compounds. Lipid solubility and molecular size are the major limiting factors for molecules to cross the biological membrane to be absorbed systematically through oral. The improvement of bioavailability of drugs with such properties presents one of the greatest challenges in novel drug formulations.

NEs are an important nanocarriers for poorly soluble phytopharmaceuticals; it also offers facility for both hydrophobic and hydrophilic molecules. This carrier system not only avoids the adverse effects but also increases the availability of the drug concentration to the systemic circulation and increases the patient compliance. To achieve these objectives, the NEs of some potent phytomolecules based dosage form are prepared & evaluated for controlled and site specific delivery to treat diseases and maintains the plasma drug level for a extended period of time. Oral lipid/oil based formulations (nano-encapsulation) are attracting considerable attention due to their capacity to increase the solubility, facilitating gastrointestinal absorption and reduce or eliminate the effect of food on the absorption of poorly water soluble drug and thus increasing the bioavailability.

Nanoemulsions are isotropic mixtures of natural or synthetic oils, solid or liquid surfactants, or alternatively, one or more hydrophilic solvents and co-solvents/co-surfactants. Oil-in-water (o/w) nanoemulsions can be prepared by solubilizing the lipophilic bioactive components within the oil phase and then homogenizing this phase with an aqueous phase containing a water-soluble emulsifier. The size of the droplets produced depends on the composition of the system and the homogenization method used. Nanoemulsions are thermodynamically stable systems that consist of emulsifier coated oil droplets dispersed within an aqueous medium. The aim of the study was to prepare NEs of some potent phytomolecules using nanoemulsification technique to enhance the bioavailability with an aim to give stable formulation. Generally, nanoemulsions are either administered as liquid dosage forms, encapsulated either in hard or soft gelatin capsules. In case of topical or transdermal application, nanoemulsions are administered in the form of gel after incorporating them into suitable gel matrix. This is an emerging nanotechnology applied to phytopharmaceutical for the enhancement of bioavailability of potent plant secondary metabolites for fulfillment of several therapeutic effects.

2. Work performed

The main objective of this study is to develop nanoemulsions of some therapeutically potent phytopharmaceuticals having low bioavailability profiles. The molecules were selected based on their chemical properties, solubility and bioavailability problems and therapeutic potential. In order to overcome the shortcomings of those molecules, the present study has been undertaken to develop a novel formulation. This includes the preparation, characterization of the nanoemulsions and evaluation of the bioavailability as well as therapeutic efficacy. In the present study four potent phytomolecules namely ferulic acid, catechin and genistein were selected according to their therapeutic potential and poor bioavailability profiles. Different nanoemulsions of these phytomolecules were prepared, evaluated for their physicochemical characteristics, stability studies, *in vivo* biological activities and *in vivo* pharmacokinetics studies. The detailed studies of these four phytomolecules have been described in the various chapters of this thesis.

Phytopharmaceuticals are associated with several health benefits. However their bioavailability is restricted due to their low aqueous solubility hence low bioavailability moreover therapeutic efficacy. The pharmacokinetics of phytomolecules are mainly depends on the various factors including chemical nature of the drug, solubility, pH, absorption, metabolism, distribution, biliary and colonic excretion etc. Pharmacokinetic and bioavailability profiles of some important phytopharmaceuticals have been described in chapter 1 of this thesis. The various approaches and strategies for improving pharmacokinetic, bioavailability, therapeutic efficacy, formulation and development of phytopharmaceuticals have been discussed in this chapter.

Evolution of NDDS into a suitable pharmaceutical formulation for delivery of certain phytoconstituents is of premier importance. The promotion of novel technologies is providing a large platform for novel delivery systems of phytopharmaceuticals to improve their therapeutic activity along with bioavailability that has poor aqueous solubility. In the past decade, several novel herbal dosage forms and delivery systems have been developed by the researchers. Various aspects of the NDDS for herbal drugs along with the more emphasis on the nanoemulsion as a novel nanocarrier for development of phytopharmaceuitcal based NEs techniques have been discussed in chapter 1 of this thesis. Based on the study following book chapter has been published.

- Bioavailability of herbal products: approach toward improved pharmacokinetics, in: P.K. Mukherjee (Eds.), Evidence-based validation of herbal medicine, Elsevier, Amsterdam, 2015, pp. 217-245.

Chapter 2 of the thesis discusses the scope, objective and plan of work of the study to develop the nanoemulsions of some therapeutically potent phytomolecules like ferulic acid, catechin and genistein are having poor permeation/absorption or early elimination profile thereby poor bioavailability.

Chapter 3 described the formulation & development, characterization, pharmacological evaluation and bioavailability study of ferulic acid based different nanoemulsions and their nano-gel formulation. Ferulic acid (FA) is a potent polyphenolic antioxidant compound naturally occur in food plants i.e. wheat, rice, barley, oats, citrus fruits, tomatoes, etc. (Alias et al., 2009). FA has been proven to afford significant protection to the skin against UV-B-induced oxidative stress in human lymphocytes and erythema (Prasad et al., 2007). Staniforth et al. have been reported that FA inhibited the UVB-induced matrix metalloproteinases and attenuates the degradation of collagen fibers, abnormal accumulation of elastic fibers and epidermal hyperplasia through posttranslational mechanisms (Staniforth et al., 2012). Other pharmacological activities of FA are reported as antiageing, hepatoprotective, antiatherogenic, antimutagenic, anti-inflammatory, anticancer, antidiabetic, neuroprotective and cardioprotective (Staniforth et al., 2012; Prasad et al., 2007). Therefore it is utmost important that FA should be incorporated in some novel delivery system which can increase the concentration of FA and maintain its minimum effective level in blood for a longer period of time to enhance its therapeutic efficacy. Nanoemulsion of FA was developed with several excipients like oil, surfactant, co-surfactant and aqueous system by using spontaneous nanoemulsification method. Solubility profiles of FA in different excipients were studied and found to be maximum soluble in isostearyl isostearate (ISIS) as an oil phase. Developed FA loaded NEs were characterized by measuring different parameters, including electrical conductivity, % transmittance, viscosity, pH, refractive index, drug entrapment efficiency (%EE), drug loading (%DL), droplet size, PDI and zeta potential. The favorable conditions were observed for FA-NEs. The drug-excipients compatibility of FA encapsulated NEs in comparison with pure FA were evaluated through UV-spectrophotometry, HPTLC, FTIR analysis and it was observed that FA was

quite compatible with the excipients used for development of formulations. The shape and surface morphology of the optimized formulations, FA-NE3 was evaluated through TEM analysis and it was observed that the droplets appeared as dark spot due to the dispersed oil droplets. The size of droplet (~100 nm) was similar to that obtained by Zetasizer. Also the accelerated stability of the optimized formulation was studied and found stable for longer periods. The different FA-NE1-5, FA-CG and FA-NE3 based nano-gel (FA-NG) was evaluated for *ex vivo* skin permeation studies through rat's skin. Skin permeability studies were resulted that the nano-gel formulation significantly ($P < 0.01$) released the drug for extended period of time in a sustained manner in comparison with its conventional gel. The UV-protection efficacy of various gel formulations were evaluated on the rats skin against UVA mediated oxidative stress. Among the different gel formulations, nano-gel exhibited better antioxidant activity by improving the cutaneous antioxidant enzyme levels of skin against UVA mediated oxidative stress. It may be due to its enhanced permeability profile (96.95%) for longer periods. Skin irritation study of nano-gel was also evaluated along with its placebo gel. There were no substantial clinical signs of irritation, erythema or oedema observed throughout the study period. These results indicated that the nano-gel formulation of FA (FA-NG3) was non-irritant and safe for topical application.

In vivo bioavailability study of ferulic acid was performed in rats after administration of its oral suspension (5 mg FA) and FA-NG3 (equivalent to 5 mg FA). Result stated that FA has been well released and permeated from FA-NG3 as compared to the oral suspension for longer periods (48 h). The C_{max} of FA was found to be 80.98 ± 6.93 and 96.21 ± 5.49 $ngmL^{-1}$ after administration of oral suspension and FA-NG3 respectively. The T_{max} and MRT were higher for transdermal nano-gel than the oral administration ($P < 0.05$). The mean value of AUC_{0-t} by transdermal route was 3.96 times higher than that of oral route, and the difference was found to be statistically significant ($P < 0.05$). This indicated enhanced bioavailability of FA from the transdermal nano-gel. This could be due to avoidance of first-pass hepatic metabolism by transdermal route. The reported oral bioavailability of FA was 9% because of hepatic first-pass metabolism. In the current investigation, the relative bioavailability (F) of FA by transdermal route was found to be 396.26. Thus, FA loaded nano-gel could provide an effective strategy for the management of skin damage by extreme exposure of the UV radiations. Thus a thorough study of FA based NEs have been done and reported in this chapter. Based on the study following articles have been published and presented.

- Enhanced permeability of ferulic acid loaded nanoemulsion based gel through skin against UVA mediated oxidative stress. Life Sciences, Elsevier, 2015, 141, 202-211, doi: 10.1016/j.lfs.2015.10.001.

- Enhanced bioavailability of ferulic acid through development of a novel drug delivery system using nanoemulsion based nanogel. 2nd National Convention of

SFE-India on *"Integrated Approaches for Promotion and Development of Herbal Medicine"* at the Jadavpur University, Kolkata, India, December 5-6, 2015.

- Enhancing topical effect of ferulic acid against UVA induced oxidative stress via nanoemulsion based novel delivery system. 53rd National Pharmacy Week Celebration, *"IPA's Platinum Jubilee Celebration"*, Indian Pharmaceutical Association (Bengal Branch) at the Jadavpur University, Kolkata, India, November 16, 2014.

Formulation, characterization, pharmacological evaluation and bioavailability studies of catechin based nanoemulsions and their nano-gel formulation have been described in chapter 4. Catechin (CA) is a flavanol type polyphenolic antioxidant molecule which is present in green tea, red wine, coffee, apple, chocolate and several nutritional and functional food products (Pomponio et al., 2003; Dube et al., 2010). CA possesses potent antioxidant potential and is capable of scavenging free reactive oxygen radicals by virtue of its reducing properties arising from the multiple hydroxyl groups attached to the aromatic rings. CA has been proven to afford significant photoprotection to the skin against UV-mediated oxidative stress (Levin and Maibach, 2002), photo-damage, basal cell carcinoma, melanoma and sunburn (Fang et al., 2007). It inhibits the expression of inflammation-associated enzymes, matrix metalloproteinases (MMPs) and restores levels of cutaneous antioxidant enzymes (Pinnell, 2003). Other different activities of CA have been reported including antiaging, antidiabetic, neuroprotective, anti-obesity, antibacterial, hypolipidemic, anti-HIV and anti-inflammatory. Despite their exciting array of therapeutic effects, CA has a short (1.25 h) half-life ($t_{1/2}$) (Manach et al., 2005) and less than 5% oral bioavailability because of their extensive first-pass hepatic metabolism and elimination (Chen et al., 2011). Poor oral bioavailability of CA is also associated with its digestive instability and poor intestinal uptake (Tang et al., 2013). Therefore to enhance the bioavailability of CA is necessary for fulfillment of the optimum therapeutic activity. It should be made by encapsulating in suitable novel delivery system which can increase the solubility of CA and thereby the bioavailability for an extended periods in a sustained manner.

CA loaded different nanoemulsions are prepared with several excipients by using spontaneous nanoemulsification process. CA solubility was performed in different oil components and optimum solubility was observed in ethyl oleate (EO). CA loaded different NEs were characterized by measuring different parameters, including electrical conductivity, % transmittance, viscosity, pH, refractive index, %EE, %DL, droplet size, PDI and zeta potential. The favorable conditions were observed for CA-NEs. The drug-excipients compatibility of CA loaded NEs along with pure CA were evaluated through UV-spectrophotometry, HPTLC, FTIR analysis and it was observed that CA was quite compatible with the excipients. The TEM morphology of the optimized formulations (CA-NE4) was evaluated and it was observed that the surface of the droplet was observed as a dark spot in a photograph, which may be due to the dispersed oil droplets. Droplet

size (100 nm) produced by TEM was quite similar to that obtained by Zetasizer. The accelerated stability of the optimized formulation was also studied and found stable at 40 ºC for longer periods. Shelf life (t_{90}) of CA based nano-gel was found to be 3.23 years. *In vitro* skin permeation studies of different formulations (CA-NE1-5, CA-CG and CA-NE4 based nano-gel (CA-NG4) were evaluated through rat's skin. From result it can be revealed that the nano-gel formulation significantly ($P < 0.01$) released the drug for 24 h in a sustained manner in comparison with its conventional gel. *In vivo* UV-protection efficacy of CA based different gel formulations were studied on the rat's skin against UVA induced oxidative stress. Nano-gel of CA showed enhanced photoprotection activity by improving the cutaneous antioxidant enzyme levels of skin against UVA induced oxidative stress. This may be due to its enhanced permeability profile (96.62%) for longer periods. Evaluation of skin irritation study of CA nano-gel was also performed along with its placebo gel. There were no substantial clinical signs of irritation, erythema or oedema observed throughout the study period. These results indicated that the nano-gel was found to be non-irritant and safe for skin application.

The bioavailability study of CA was performed after administration of its oral suspension (5 mg CA) and CA-NG4 (≈5 mg CA). CA has been well released and permeated from CA-NG4 as compared to the oral suspension for longer periods (72 h). Statistically significant C_{max}, $T_{max,}$ MRT and AUC profiles were observed with oral suspension and CA-NG4 ($P < 0.05$). The C_{max} of CA was found to be 87.52 ± 8.56 and 93.79 ± 6.19 $ngmL^{-1}$ after administration of oral suspension and CA-NG4 respectively. The $AUC_{0\text{-}t\infty}$ (2653.99 ± 515.02 $nghmL^{-1}$), T_{max} (12.05 ± 0.02 h) and $MRT_{0\text{-}t\infty}$ (35.98 ± 10.34 h) were higher for CA-NG4 comparatively to the oral suspension ($P < 0.05$). The $t_{1/2}$el of CA was increased when it was in the nano-gel (24.75 ± 13.60 h) form and eventually the Kel (0.028 ± 0.02 h^{-1}) and Cl (0.0021 ± 0.04 Lh^{-1}) of the molecule in nano-gel form were also lowered. The mean value of $AUC_{0\text{-}t}$ by transdermal route was 8.94 times higher than that of oral route, and the difference was found to be statistically significant ($P < 0.05$). This could be due to avoidance of first-pass hepatic metabolism by the transdermal route. The reported oral bioavailability of CA was 5% because of hepatic first-pass metabolism (Chen et al., 2011). In the current investigation, the relative bioavailability (*F*) of CA (CA-NG4) by transdermal route was found to be 894.73. This indicated enhanced bioavailability of CA nano-gel through transdermal route. This may be due to that the CA-NG4 releases drug in a sustained manner for extended periods (72 h). For transdermal route, *stratum corneum* acts as a permeation barrier and thereby the sustained-release activity of CA was found with CA-NG4 in comparison to orally administered suspension is an immediate release dosage form. Therefore, CA based nano-gel could provide an effective treatment for the management of skin damage mediated by the UV exposure. The study explained in this chapter thus provided an approach and strategy to overcome the limitation of the phytomolecule related to its poor bioavailability and to enhance its therapeutic efficacy. Based on the study following articles have been published and presented.

- Enhancement of photoprotection potential of catechin loaded nanoemulsion gel against UVA induced oxidative stress. Journal of Photochemistry and Photobiology B: Biology, Elsevier, 2016, 160, 318-329, doi: 10.1016/j.jphotobiol.2016.03.026.
- Enhanced bioavailability of catechin through nanoemulsion based nano-gel via transdermal route. UGC Sponsored National Seminar on *"Prospects of Pharmacy Education"* at the Pt. Ravishankar Shukla University, Raipur, India, November 19-21, 2015.
- Enhancing transdermal delivery of catechin via nanoemulsion based novel delivery system. 1st National Convention of SFE-India on *"Opportunities in Medicinal Plant Research"*, at the Jadavpur University, Kolkata, India, November 29-30, 2014.

Chapter 5 described the development, characterization, evaluation of therapeutic efficacy and bioavailability study of genistein-nanoemulsions and their nano-gel formulation. Genistein (GN) occurrs manly in dietary and food plants. GN is a phytoestrogen, which belongs to the isoflavone class of flavonoids, naturally occurring in soybean seeds. Soybean is the main source of isoflavones in the human diet, it contains between 0.6 and 3.8 g isoflavones/kg fresh weight (Manach et al., 2004; Cassidy et al., 2000). GN is a potent tyrosinase enzyme inhibitor. GN has been used as a sunscreening and photoprotective agent against UV induced oxidative stress leading to skin damage (Wang et al., 2010). GN has been proven for their other potential therapeutic benefits like antioxidant, anti-inflammatory, hepatoprotective, antiobesity, antidiabetic, anti-cancer and anti-osteoporosis. It is proposed as a potent phytopharmaceutica for the treatment of metabolic disorders. GN is BCS II molecule exhibited poor solubility as well as low bioavailability due to its rapid absorption and clearance from the small intestine and liver. There are several studies reported on absorption, distribution, metabolism and excretion of GN in rats and humans. Pharmacokinetics of GN has been studied in rats and humans and demonstrated low bioavailability due first pass effect (Coldham and Sauer, 2000). Therefore it is necessary to formulate novel drug delivery system of GN which can increase the solubility by using nanocarrier system which can enhance their therapeutic activity and bioavailability by improving permeation profiles in a sustained manner for longer action. In this context nanoemulsion of GN was developed with several excipients like oil, surfactant, co-surfactant and aqueous system by using spontaneous nanoemulsification method. Solubility of GN in different excipients were performed and found to be maximum solubility in labrafac™ lipophile WL1349 (LLW), as an oil phase. GN loaded NEs were characterized by measuring different parameters like electrical conductivity, % transmittance, viscosity, pH, refractive index, %EE, %DL, droplet size, PDI and zeta potential. The favorable conditions were observed for GN-NE2. The drug-excipients compatibility of GN encapsulated NEs were analyzed through UV-spectrophotometry, HPTLC, FTIR in comparison with pure GN and it was observed that GN was pretty

compatible with the excipients used for NEs formulation. TEM was performed to analyze the shape and surface morphology of the optimized formulations, GN-NE2 and it was observed that the droplets seen as dark spot in photograph which may be due to the dispersed oil droplets. Droplet size (~110 nm) measured through TEM was quite similar to that obtained by Zetasizer. An accelerated stability of the optimized formulation was studied and found stable for longer periods at 40 ºC. *In vitro* skin permeation of GN-NE1-4, GN-CG and GN-NE2 based nano-gel (GN–NG2) was studied through rat's skin. Result demonstrated that the nano-gel formulation significantly (P < 0.01) released the drug in a sustained manner in comparison with its conventional gel for extended period of time (24 h). Photoprotection efficacy of various gel formulations were evaluated on the rats skin against UVA mediated oxidative stress. Nano-gel of GN exhibited better antioxidant activity among the different gel formulations by improving the cutaneous antioxidant enzyme levels of skin against UVA mediated oxidative stress. This may be due to its enhanced permeability profile (95.99 ± 2.70%) for longer periods. Skin irritation of nano-gel was also studied along with its placebo gel. There were no serious clinical signs of irritation, erythema or oedema observed throughout the study periods. These results indicated that the GN based nano-gel formulation was non-irritant and safe for skin application.

The bioavailability of GN was studied after administration of its oral suspension (20 mg GN) and GN-NG2 (≈20 mg GN). GN has been well released and permeated from GN-NG2 in comparison with oral suspension for extended periods (24 h). Statistically significant C_{max}, $T_{max,}$ MRT and AUC profiles were observed with oral suspension and GN-NG2 (P < 0.05). The C_{max} of GN was found to be 57.48 ± 4.42 and 95.06 ± 4.09 $ngmL^{-1}$ for oral suspension and GN-NG2 respectively. The $AUC_{0\text{-}t\infty}$ (911.55 ± 69.11 $nghmL^{-1}$), T_{max} (6.53 ± 0.19 h) and $MRT_{0\text{-}t\infty}$ (8.52 ± 0.38 h) were higher for GN-NG2 comparatively to the pure GN-oral suspension (P < 0.05). The $t_{1/2}$el of GN was increased with nano-gel (3.81 ± 0.25 h) while the Kel (0.18150 ± 0.01 h^{-1}) and Cl (0.02259 ± 0.02 Lh^{-1}) were also lowered. The mean value of $AUC_{0\text{-}t}$ by transdermal route was 3.52 times higher than oral route, and the difference was found to be statistically significant (P < 0.05). This could be due to avoidance of hepatic first-pass metabolism by the transdermal route. The reported oral bioavailability of GN (20 mg/kg) was ~24% because of its extreme hepatic first-pass metabolism (Kwon et al., 2007). In the present study, relative bioavailability (F) of GN was found to be 352.58 by transdermal route. This indicates enhanced bioavailability of nano-gel through transdermal route and avoidance of hepatic first-pass metabolism. This may be also due to that the nano-gel releases drug in a sustained manner for 24 h periods. For transdermal route, *stratum corneum* acts as a permeation barrier and thereby the sustained-release activity was produced with nano-gel in comparison with orally administered suspension which is an immediate release dosage form. Therefore, GN based nano-gel could provide an effective approach for the treatment and management of skin damage caused by intemperate exposure of the UV radiations. Thus the study showed that development of genistein-nanoemulsion can overcomes the limitation of

the molecule related to low bioavailability and enhances the therapeutic efficacy of the molecule. Based on the study following articles have been communicated and presented.

- Nanoemulsion based nano-gel of genistein enhancing UVA protection efficacy and bioavailability by improving its pharmacokinetics through transdermal route. *Journal of* Pharmaceutical and Biomedical Analysis, Elsevier, 2016, (Communicated).
- Nanoemulsion based nano-gel of genistein enhancing UVA protection efficacy and bioavailability by improving its pharmacokinetics through transdermal delivery. 3rd International Congress of Society for Ethnopharmacology (SFEC - 2016) on *"Ethnopharmacology and Evaluation of Medicinal Plants – Global Perspective"*, at the Pt. Ravishankar Shukla University, Raipur, India, February 19-21, 2016.

3. Conclusion

In order to enhance the therapeutic importance of several useful phytopharmaceuticals, this work was undertaken for developing novel drug delivery system by using nanoemulsion as carriers. The investigational works performed and produced in this thesis encompasses with various aspects of development and evaluation of nanoemulsion with several therapeutically potent phytomolecules like ferulic acid, catechin and genistein. These bioactive molecules from plant origin have been found to possess enormous health benefits in various disease conditions. But due to their various shortcomings related to poor solubility, low oral bioavailability and residence time in the body due to faster elimination rates, these phytopharmaceuticals find less attention as an effective drug molecule. Through the development of science and technology, it is possible to fabricate this phytopharmaceuticals with different nanocarrier system imparting value addition through enhanced efficacy, targeted drug delivery and fewer side effects. Also it increases the patient compliance. Also these approaches of value additions to herbal medicinal products are very much essential in commercial point of view as well as the medicinal values.

Excessive UV exposure interferes with the cellular defense system of human skin and causes oxidative stress. ROS as a result of UV irradiation may oxidize and damage cellular lipids, proteins and DNA. This leads to the destruction of skin structures and results in hindrance of regular function of the cutaneous antioxidant defense system of skin. A topical nano-gel of ferulic acid was developed, which showed sustained-release effect against UVA exposure in skin. The optimized gel formulation showed improved drug permeability as well as bioavailability and enhanced UV protection activity, which might be due to the potent antioxidant activity of FA in opposition to oxidative stress mediated by UVA. It significantly elevates the level of the antioxidant markers and

arrested the unwanted effects generated by ultraviolet radiation. This phenomenon attributed towards the encapsulated FA in NE having a nano size range of droplets provided large surface area, which possess superior skin penetration potential when compared with its conventional gel. The enhanced relative bioavailability of ferulic acid (396.26%) was obtained with nano-gel formulation after its transdermal administration for extended periods. The nanoemulsion based FA nano-gel was found to be stable, safe and effective for topical application against UV. The enhanced relative bioavailability of catechin (894.73%) was achieved with nano-gel formulation after its transdermal application for 72 h. The nano-gel increased antioxidant potential of catechin against UVA-induced oxidative stress in a sustained manner. The CA-NG4 formulation was found to be stable, safe and effective for transdermal delivery. Thus, nanoemulsion based gel formulation of catechin may be a promising nanocarrier for skin delivery. The enhanced relative bioavailability of genistein (352.58%) was obtained with nano-gel formulation. The nano-gel significantly improved antioxidant potential of genistein by enhancing their skin permeability against UVA-induced oxidative stress in a sustained manner. The GN-NG2 was found to be stable, safe and effective for transdermal delivery. Thus, nanoemulsion based gel formulation of genistein may be a novel approach for skin delivery. Result concluded that the nano-gel formulation of ferulic acid, catechin and genistein was developed with nanoemulsion that could be promising as a useful strategy for sun or photoprotection against UVA-induced oxidative stress.

Thus, nanoemulsion is a promising nano-carrier for increasing drug solubility and stability, thereby enhancing its therapeutic efficacy and bioavailability. This novel drug delivery system can modulate the pharmacokinetics of existing drugs, and it may be helpful to enhance delivery of phytopharmaceuticals to target sites. In this regard, the discussion on some of the delivery systems has made an impact either by enhancing the delivery of phytopharmaceuticals to their target tissues or by increasing their bioavailability by manifold.