CHAPTER 2: LITERATURE REVIEW

Research on CNS disorders and delivery of drug to brain were technologically limited till last few years. But with the advancement in technology, the global brain drug market is rising high although it is unable to meet the need of the patient. The prime reason for this underdevelopment is that most therapeutic agents available are unable to cross the BBB. Only a small class of drugs with low molecular size, high lipophilicity and low molecular mass are the candidates which cross the BBB. But there are only few diseases of the brain which respond to this small class of limited drugs. Also due to insufficient research in the field of novel drug delivery, the treatment is left back and most of the diseases in the brain remain still untreated. Research in the area of neurodegenerative disease such as Parkinson’s, Alzheimer’s and Huntington disease which affects higher percentage of global population is still in infancy. Research work is going on to overcome the challenges of brain delivery and neurodegenerative disease and to make the existing treatment system more effective and efficient. Although all the research conducted in this field cannot be included, some of the important and recent literatures are included below.

2.1 Nose to brain drug delivery system

Olanzapine nanoparticles were formulated by Seju and coworkers using poly(lactic-co-glycolic acid) (PLGA) for direct nose to brain delivery which showed the sustained action. NPs were formulated using nano-precipitation technique and characterized for particle size, zeta potential, and entrapment efficiency. The prepared NPs were also characterized using modulated temperature differential scanning calorimetry (MTDSC) and X-ray diffraction (XRD) analysis. The prepared NPs had an entrapment efficiency of 68.91 ± 2.31%. The in vitro drug release study showed a biphasic pattern with initial burst release and followed by sustained release, which showed Fickian diffusion based release. In vivo pharmacokinetic studies showed almost 11 times higher uptake of olanzapine into brain when delivered intranasally in comparison to intravenous route. Also, PLGA NPs showed no significant toxicity to nasal mucosa, indicating their suitability as carriers for nasal delivery of drugs. This study revealed that longer stay of
the drug in the brain when formulated as PLGA NPs can be a tool for delivering drugs for CNS disorders treatment (Seju et al., 2011).

Nanostructured lipid carriers (NLCs) of valproic acid were formulated by Eskandari and associates. They used emulsion-solvent diffusion method followed by ultrasonication to transport and maintain therapeutic concentrations in the brain via intranasal route to treat seizures. Further prepared formulation was characterized for size, zeta potential, drug loading percentage and release. Animal studies were carried out in rats stimulated by articular electrodes to produce tonic hind limb extension. Concentration of valproic acid in brain after 60 min of administration of NLC intranasally was 64.35 ± 5.7 µg/ml and that for intraperitoneal route was 19.85 ± 8.5 µg/ml. Conclusively the result demonstrated a better protection of seizure by valproic acid when administered as NLCs through nasal route (Eskandari et al., 2011).

Perez and coworkers formulated an in situ forming mucoadhesive gels of 32P-small interference RNA (siRNA) complexed with poly(amidoamine) G7 dendrimers (siRNA dendriduplexes) prepared by blending thermosensitive polaxomer (23 % w/w) with mucoadhesive chitosan (1% w/w) or carbopol (0.25 % w/w). The researcher compared the brain radioactivity of radiolabeled siRNA dendriplexes within in situ forming mucoadhesive gel with naked siRNA gel after intranasal administration and with siRNA dendriplexes after intravenous administration. The result conclusively demonstrated the increase in direct brain delivery of in situ forming mucoadhesive gel containing siRNA dendriplexes when in complexes with dedrimers (Perez et al., 2012).

Wu and associates prepared cubosomes by surface engineering technique of poly (ethylene glycol) (PEG) with Odorranalaectin (OL) to accomplish the delivery of streptavidin (SA) to the brain via nose. OL-Cubs were prepared using dilution-sonication technique and characterized for surface morphology, particle size, zeta potential, and liquid crystalline phase. Nasal ciliotoxicity of cubosomes were performed using both in situ toad palate model and in vivo rat nasal mucosa model. Evaluation of systemic absorption showed 1 hour earlier maximum blood concentration with OL-Cubs. The
study suggested that OL in the surface of cubosomes could accelerate the access of drugs to the brain via nasal route (Wu et al., 2012).

Magnetic nanoparticle (MNP) based carrier system to target brain derived neurotropic factor (BDNF) across the BBB by Kanthikeel and coworkers. The MNPs were prepared by co-precipitation of Fe$^{2+}$ and Fe$^{3+}$ ions in alkaline solution. The MNPs were evaluated on the basis of their efficacy and ability to transmigrate across the \textit{in vitro} BBB model and suppress the morphine induced apoptosis, induce cAMP response element binding (CREB) expression and restore spine density. Firstly, various morphological changes were induced in cells using morphine and effect of treatment using MNP-BDNF was studied. The result showed that approximately 73% of the MNP bound BDNF was able to transport across the BBB and compensate the changes caused by morphine. Cytotoxicity of the prepared formulation was also evaluated by flow cytometry using live or dead fixable dead cell stains which showed no significant decrease in the viability of cells indicating non-toxic formulation (Kanthikeel et al., 2013).

Liu and coworkers formulated lactoferrin (Lf) conjugated poly(ethyleneglycol)-poly (ε-caprolactone) (PEG-PCL) nanoparticles (Lf-NPs) to deliver neuroprotective agents to treat Alzheimer’s disease. Lf-NPs were characterized for particle size distribution and zeta potential. Fluorescent labeled nanoparticles were used to study the \textit{in vitro} cellular interaction and study \textit{in-vivo} biodistribution and brain targeting using thirty six rats after the intranasal administration. Cellular experiments showed enhanced cellular accumulation of Lf-NP than unmodified NPs. Significant concentration of NPs in olfactory tract, hippocampus, cerebellum and cerebrum with hippocampus removed showed a desirable brain biodistribution in rat. This research conclusively demonstrated an effective non-invasive approach to facilitate the access of neuropeptides to the CNS (Liu et al., 2013).

Naik and Nair used chitosan and glycerophosphate based thermoreversible systems for delivery of doxepin to brain through intranasal administration. Formulations were prepared by admixture of suitable dilutions of chitosan and glycerophosphate with or without polyethylene glycol, followed by addition of the antidepressant doxepin
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hydrochloride. Both systems were evaluated for gelling characteristics, rheology, mucoadhesion, in vitro release and ex vivo permeation through the forced swim test. Nasal tissues of mice subjected to repeated exposure to formulation were evaluated histopathology. Both formulations gelled rapidly at 37 °C, returned to sol state on cooling and exhibited thixotropy. Addition of polyethylene glycol decreased the glycerophosphate content required for gelation and rendered the formulation isotonic. Both gels showed good mucoadhesion, enhanced drug permeation and provided prolonged in vitro release at 37 °C. Efficacy of the formulation in treated groups was inferred from measured pharmacodynamic parameter and histopathology reports of formulation treated groups showed no significant local toxicity. The biogels could be potential systems for effective drug delivery to brain via nose (Naik et al., 2015).

Sharma and coworkers optimized diazepam (Dzp)-loaded poly(lactic-co-glycolic acid) nanoparticles (NP) to attain delivery in the brain via intranasal route. Dzp nanoparticles (DNP) were formulated by nanoprecipitation and optimized using Box-Behnken design. Optimized DNP showed z-average 148-337 d.nm, polydispersity index in the range of 0.04-0.45, drug entrapment 69-92%, and zeta potential in the range of -15 to -29.24 mV. Ex vivo drug release study via sheep nasal mucosa from DNP showed a controlled release of 64.4% for 24 h. 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay performed on Vero cell line showed less toxicity for DNP as compared to Dzp suspension (DS). Gamma scintigraphy and biodistribution study of DNP and DS was performed on Sprague-Dawley rats using technetium-99m-labeled ((99m)Tc) Dzp formulations to investigate the nose-to-brain drug delivery pathway. Scintigraphy images showed uptake of Dzp from nose-to-brain, and this observation was in agreement with the biodistribution results. These results suggest that the developed poly(D,L-lactide-co-glycolide) (PLGA) NP could serve as a potential carrier of Dzp for nose-to-brain delivery in outpatient management of status epilepticus (Sharma et al., 2015).

Bi and coworkers formulated biodegradable poly(ethylene glycol)–poly(lactic-co-glycolic acid) (PEG-PLGA) nanoparticles (NPs), which were surface-modified with lactoferrin (Lf), for efficient intranasal delivery of rotigotine to the brain for the treatment of PD. Rotigotine NPs were prepared by nanoprecipitation, and the effect of various
independent process variables on the resulting properties of NPs was investigated by a Box–Behnken experimental design. The physicochemical and pharmaceutical properties of the NPs and Lf-NPs were characterized, and the release kinetics suggested that both NPs and Lf-NPs provided continuous, slow release of rotigotine for 48 h. Neither rotigotine NPs nor Lf-NPs reduced the viability of 16HBE and SH-SY5Y cells; in contrast, free rotigotine was cytotoxic. Qualitative and quantitative cellular uptake studies demonstrated that accumulation of Lf-NPs was greater than that of NPs in 16HBE and SH-SY5Y cells. Following intranasal administration, brain delivery of rotigotine was much more effective with Lf-NPs than with NPs. The brain distribution of rotigotine was heterogeneous, with a higher concentration in the striatum, the primary region affected in PD. This strongly suggested that Lf-NPs enable the targeted delivery of rotigotine for the treatment of PD. Taken together, these results demonstrated that Lf-NPs have potential as a carrier for nose-to-brain delivery of rotigotine for the treatment of PD (Bi et al., 2016).

Guo and coworkers developed and characterized a nanovesicular formulation of ferric ammonium citrate (ferric ammonium citrate nanoliposomes, FAC-LIP) to increase brain iron levels in rats following nasal administration. FAC was incorporated into liposomes with high efficiency (97%) and the liposomes were small (40 nm) and stable. Following intranasal delivery in rats, FAC-LIP significantly increased the iron content in the olfactory bulb, cerebral cortex, striatum, cerebellum and hippocampus, and was more efficient at doing so than FAC alone. No signs of apoptosis or abnormal cell morphology were observed in the brain following FAC-LIP administration, and there were no significant changes in the levels of SOD and MDA, except in the cerebellum and hippocampus. No obvious morphological changes were observed in lung epithelial cells or tracheal mucosa after nasal delivery, suggesting that the formulation was not at all toxic (Guo et al., 2017).

Yan and coworkers formulated lactoferrin-modified rotigotine nanoparticles (Lf-R-NPs) for enhanced nose-to-brain delivery and studied their biodistribution, pharmacodynamics, and neuroprotective effects following nose-to-brain delivery in the rat 6-hydroxydopamine model of PD. Liquid extraction surface analysis coupled with tandem mass spectrometry analysis, used to examine rotigotine biodistribution, showed that Lf-
R-NPs more efficiently supplied rotigotine to the brain (with a greater sustained amount of the drug delivered to this organ, and with more effective targeting to the striatum) than R-NPs. The pharmacodynamic study revealed a significant difference (P<0.05) in contralateral rotations between rats treated with Lf-R-NPs and those treated with R-NPs. Furthermore, Lf-R-NPs significantly alleviated nigrostriatal dopaminergic neurodegeneration in the rat model of 6-hydroxydopamine-induced PD (Yan et al., 2018).

Meng and coworkers developed huperzine A (HupA)-loaded, mucoadhesive and targeted polylactide-co-glycoside (PLGA) nanoparticles (NPs) with surface modification by lactoferrin (Lf)-conjugated N-trimethylated chitosan (TMC) (HupA Lf-TMC NPs) for efficient intranasal delivery of HupA to the brain for the treatment of Alzheimer’s disease. HupA Lf-TMC NPs were prepared using the emulsion-solvent evaporation method and optimized using the Box-Behnken design. Optimized HupA Lf-TMC NPs were found to have a particle size of 153.2±13.7 nm, polydispersity index of 0.229±0.078, zeta potential of +35.6±5.2 mV, drug entrapment efficiency of 73.8%±5.7%, and sustained release in vitro over a 48 h period. Adsorption of mucin onto Lf-TMC NPs was 86.9%±1.8%, which was significantly higher than that onto PLGA NPs (32.1%±2.5%). HupA Lf-TMC NPs showed lower toxicity in the 16HBE cell line compared with HupA solution. Qualitative and quantitative cellular uptake experiments indicated that accumulation of Lf-TMC NPs was higher than nontargeted analogs in 16HBE and SH-SY5Y cells. In vivo imaging results showed that Lf-TMC NPs exhibited a higher fluorescence intensity in the brain and a longer residence time than nontargeted NPs. After intranasal administration, Lf-TMC NPs facilitated the distribution of HupA in the brain, and the values of the drug targeting index in the mouse olfactory bulb, cerebrum (with hippocampus removal), cerebellum, and hippocampus were about 2.0, 1.6, 1.9, and 1.9, respectively (Meng et al., 2018).

2.2 Nanoemulsion formulation via intranasal route for brain delivery

Thermodynamically stable and infinite dilutable nanoemulsion of ropinirole for the treatment of parkinson’s disease using a minimum concentration of surfactant by Mustafa and associates were prepared. Ternary phase diagrams were prepared using sefisol 218
(oil), Tween 80 (surfactant), Transcutol (co-surfactant) and water. Formulation were selected from the phase diagram and studied for physical stability. The optimized formulation was characterized for adequate drug release (72.23 ± 9.56 %), globule size (58.61 ± 5.18), polydispersity index (0.201), viscosity (31.42 ± 6.97 mPas). In vivo study was carried out using Wisatr rats which showed optimum concentration of drug in the brain. The research concluded the delivery of ropinirole nanoemulsion through the nasal route as the better approach for the treatment of Parkinson’s disease (Mustafa et al., 2012).

Mahajan and coworkers developed nanoemulsion of saquinavir mesylate (SQVM) for brain targeting by nasal route by spontaneous emulsification method. The prepared nanoemulsions were characterized for droplet size, zeta potential, pH, drug content. Ex vivo permeation study was also performed using sheep nasal mucosa. Drug permeation rate was significantly increased in optimized nanoemulsion formulation compared to drug suspension. Higher concentration of drug was observed in brain after intranasal administration of nanoemulsion compared to intravenously administered drug suspension. The study conclusively demonstrated the transport of SQVM into the CNS after intranasal administration as nanoemulsion (Mahajan et al., 2013).

Pangeni and coworkers formulated a kinetically stable nanoemulsion (o/w) using vitamin E:sefsol (1:1) as the oil phase, Tween 80 as the surfactant and Transcutol P as the cosurfactant for the better management of Parkinson’s disease. The nanoemulsion was prepared by a spontaneous emulsification method, followed by high-pressure homogenization. Ternary phase diagrams were constructed to locate the area of nanoemulsion. The prepared formulations were studied for globule size, zeta potential, refractive index, viscosity, surface morphology and in vitro and ex vivo release. The homogenized formulation, which contained 150 mg ml⁻¹ of resveratrol, showed spherical globules with an average globule diameter of 102 ± 1.46 nm, a least poly dispersity index of 0.158 ± 0.02 and optimal zeta potential values of −35 ± 0.02. The cumulative percentage drug release for the pre-homogenized resveratrol suspension, prehomogenized nanoemulsion and post-homogenized nanoemulsion were 24.18 ± 2.30%, 54.32 ± 0.95% and 88.57 ± 1.92%, respectively, after 24 h. The ex vivo release also showed the
cumulative percentage drug release of 85.48 ± 1.34% at 24 h. The antioxidant activity determined by using a DPPH assay showed high scavenging efficiency for the optimized formulation. Pharmacokinetic studies showed the higher concentration of the drug in the brain (brain/blood ratio: 2.86 ± 0.70) following intranasal administration of the optimized nanoemulsion. Histopathological studies showed decreased degenerative changes in the resveratrol nanoemulsion administered groups. The levels of GSH and SOD were significantly higher, and the level of MDA was significantly lower in the resveratrol nanoemulsion treated group (Pangeni et al., 2014).

Pandey and coworkers developed a paroxetine loaded nanoemulsion (o/w type) for direct nose-to-brain delivery. Nanoemulsions were prepared by the spontaneous emulsification technique using Capmul MCM, Solutol HS 15 and propylene glycol as oil phase, surfactant and co-surfactant, respectively, for delivery of drug directly to the brain through the nasal route for better management of depression. Formulations were studied for droplet size, polydispersity index (PDI), percentage transmittance, refractive index, viscosity, zeta potential, surface morphology and in vitro permeation study. TEM images of optimized formulation showed spherical droplets with a mean diameter of 58.47 ± 3.02 nm, PDI of 0.339 ± 0.007 and zeta potential values of -33 mV. The formulation showed good results for transmittance (100.60 ± 0.577%), refractive index (1.412 ± 0.003) and viscosity (40.85 ± 6.40 cP). Permeation studies revealed a 2.57-fold enhancement in permeation as compared to the paroxetine suspension. Behavioural studies such as the forced swimming test and locomotor activity test were done on Wistar rats to study the antidepressant effect of the optimized formulation. Treatment of depressed rats with paroxetine nanoemulsion (administered intranasally) significantly improved the behavioural activities in comparison to paroxetine suspension (orally administered). Biochemical estimation results revealed that the prepared nanoemulsion was effective in enhancing the depressed levels of glutathione and decreasing the elevated levels of TBARS (Pandey et al., 2015).

Kumar and coworkers formulated selegiline loaded nanoemulsion for direct nose-to-brain delivery for the better management of Parkinson’s disease. A quality by design (QbD) approach was used in a statistical multivariate method for the preparation and
optimization of nanoemulsion. The optimized formulation had a droplet size of 61.43±4.10 nm, polydispersity index of 0.203±0.005, refractive index of 1.30±0.01, transmittance of 99.80±0.04%, zeta potential of −34 mV and viscosity of 31.85±0.24 mPas. Surface characterization studies demonstrated a spherical shape of nanoemulsion which showed 3.7 times enhancement in drug permeation as compared to drug suspension. The results of behaviour studies showed that treatment of haloperidol induced Parkinson’s disease in rats with selegiline nanoemulsion (administered intranasally) showed significant improvement in behavioural activities in comparison to orally administered drug (Kumar et al., 2016).

Yadav and associates evaluated the therapeutic efficacy of intranasal cationic nanoemulsions encapsulating an anti-TNFα siRNA, for potential anti-inflammatory therapy tested in a LPS induced model of neuroinflammation. The strategy of developing a cationic nanoemulsion system for silencing the TNFα gene was to efficiently provide neuroprotection against inflammation. TNFα siRNA nanoemulsions were prepared and characterized for particle size, surface charge, morphology, and stability and encapsulation efficiency. Qualitative and quantitative intracellular uptake studies by confocal imaging and flow cytometry, respectively, showed higher uptake compared to Lipofectamine® transfected siRNA. Nanoemulsion significantly lowered TNFα levels in LPS-stimulated cells. Upon intranasal delivery of cationic nanoemulsions almost 5 fold higher uptake was observed in the rat brain compared to non-encapsulated siRNA. More importantly, intranasal delivery of TNFα siRNA nanoemulsions in vivo markedly reduced the unregulated levels of TNFα in an LPS-induced model of neuroinflammation. These results indicate that intranasal delivery of cationic nanoemulsions encapsulating TNFα siRNA offered an efficient means of gene knockdown and this approach has significant potential in prevention of neuroinflammation (Yadav et al., 2016).

2.3 Naringenin Formulations

Annadurai and associates estimated the presumed antihyperglycemic and antioxidant effects of NRG, in comparison with a standard drug for therapy of diabetes mellitus i.e., glyclazide. Untreated diabetic rats showed significantly higher mean levels of blood
glucose and glycosylated hemoglobin, significantly lower mean levels of serum insulin, pancreatic antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase), plasma non-enzymatic antioxidants (reduced glutathione, vitamin C, vitamin E) and significantly elevated mean levels of pancreatic malondialdehyde (MDA) and serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH). NRG (50 mg/kg b.w./day) was given via oral route to diabetic rats for 21 days, untreated diabetic rats showed significantly lower mean levels of fasting blood glucose and glycosylated hemoglobin, significantly elevated serum insulin levels, significantly higher mean activities of pancreatic enzymatic antioxidants, significantly higher mean levels of plasma non-enzymatic antioxidants, lower mean pancreatic tissue levels of MDA and lower mean activities of ALT, AST, ALP and LDH in serum. The values obtained in the naringenin-treated animals approximated those observed in glyclazide-treated animals (Annadurai et al., 2012).

Hermenean and coworkers evaluated NRG and its β-cyclodextrin formulation for its antioxidant and hepatoprotective effects. The assessment was done in male Swiss mice, by the investigation of serum-enzymatic and liver antioxidant activity, histopathological and ultrastructural changes. β-cyclodextrin formulation and free NRG were orally given to mice for 7 days. On the 8th day mice were intraperitoneally injected with 1.0 mL/kg of Carbon tetrachloride (CCl₄). They observed that after 24 h of CCl₄ administration, an increase in the levels of transaminases aspartate aminotransferase and alanine aminotransferase activities and malondialdehyde concentration occurred and a significant decrease in superoxide dismutase, catalase glutathione-peroxidase activities, and glutathione levels was detected as well. The changes in enzymatic levels were accompanied by extended centrilobular necrosis, steatosis, fibrosis, and an altered ultrastructure of hepatocytes. Pretreatment with formulated or free flavonoid retained the biochemical markers to control values. Histopathological and electron-microscopic examination confirmed the biochemical results. They concluded that both NRN and NRN/β-cyclodextrin complex showed antioxidant and hepatoprotective effects against injuries induced by CCl₄ (Hermeanean et al., 2013).
Sonia and associates confirmed the neuroprotective role of sesamol and NRG both individually on rotenone-induced rodent model of PD. This model exhibits a distinct dwindling in the expression of parkin, C terminus Hsp70 interacting protein (CHIP) and PARK 7 protein (DJ1) whereas improved levels of caspases and ubiquitin were observed. Rotenone administrated for 11 days to generate the PD model (Angeline et al., 2012) followed by individual doses of sesamol (15mg/kg) and NRG (10mg/kg) from 11th day onward individual doses of sesamol (15mg/kg) for 10 consecutive days. The studies showed marked improvement in motor skills, body weight, expression of parkin, DJ1, tyrosine hydroxylase and CHIP compared to the group treated with rotenone alone in the striatum and substantia nigra. These results were correlated with the reduction in caspase and ubiquitin levels by immunostaining and immunoblotting. Moreover, improved morphology and survivability of neurons were seen upon sesamol and NRG treatment in the same rat PD model. Restoration in muscle morphology, elevated level of parkin, DJ1, differential expression of heat shock proteins and reduced cell death were observed (Sonia et al., 2013).

Xu and associates prepared the β-cyclodextrin (β-CD) complex of NRG as a therapeutic option for choroidal neovascularization (CNV). The prepared β-CD complexes were characterized using infrared spectra and X-ray diffraction analyses. The content and solubility analysis in the complex showed that NRG accounted for 20.53% in the complex and its solubility was increased by more than 10-fold. Using a laser-induced CNV model in rats they demonstrated that NRG/ β-CD complex more significantly reduced CNV area than NRG alone in rats. Furthermore, NRG and its β-CD complex significantly inhibited the mRNA and protein and protein expression of VEGF, COX-2, PI3K, p38MAPK, MMP-2, and MMP-9 in retina and choroid tissues. NRG/ β-CD complex showed more significant inhibitory effect on VEGF and COX-2 expression than free NRG. Their results collectively indicated that NRG/ β-CD complex could be promising therapeutic option for CNV and that the beneficial effects may be well lined to the anti-inflammatory properties of NRG (Xu et al., 2014).

Semalty and coworkers prepared β- cyclodextrin complexes of NRG. The main aim of the study was to improve the solubility and amorphous nature of NRG along with
enhancing the dissolution profile. Solvent evaporation method was used for the formulation of complexes in three different molar ratios (1:1, 1:2 and 1:3) which were further characterized for the chemical interaction, drug content, its solubility, phase transition behaviour, in-vitro dissolution and other parameters. The results showed that the prepared β-cyclodextrin complexes of NRG were highly soluble in water (from 41.81 to 76.31 µg/ml in the complex with 1:3 ratio) and also showed high drug content (ranging from 69.53 to 84.38 %). The complexes showed irregular and rough surface in the SEM studies. It was also reported that after 60 min free NRG showed drug release of 48.75 % only however from the complex it was 98.0-100 %. Also, per dissolution study it was concluded that β-cyclodextrin complex of NRG can be potentially used for improving the bioavailability of poorly soluble drugs. β-cyclodextrin complex was critically compared with the phospholipid complex of NRG and it was concluded that both the techniques were almost equally successful in enhancing the solubility and dissolution performance of NRG (Semalty et al., 2014).

Khan and associates developed SNEDDS of NRG comprising of Triacetin (oily phase), Tween 80 (surfactant) and transcutol HP (co-surfactant) for enhancing the dissolution and BA of NRG. SNEDDS were optimized based on increased dissolution rate, optimum globule size and polydispersity, higher solubility as well as higher BA. Rapid and complete drug release was achieved from the optimized SNEDDS formulation which was significantly higher than NRG suspension. Furthermore, area under the drug concentration time-curve (AUC) of NRG from SNEDDS revealed a significant increase in NRG absorption compared to NRG suspension (Khan et al., 2014a).

Khan and coworkers developed and characterized solid dispersion of NRG which were prepared by kneading and solvent evaporation techniques. The dissolution of NRG up to 100 ± 2.6 % after 2 h was achieved by solvent evaporation method while in the case of kneading method the value was lower (53 ± 3 %). The difference is due to the efficiency of technique to convert drug to amorphous form as well as to produce a homogenous product. The use of soluplus brought about a remarkable enhancement in both the dissolution rate and solubility of NRG. On the basis of the results obtained, it was concluded that application of the solid dispersion technique markedly enhanced the in
**vitro** drug release and **in vivo** behavior of the grapefruit flavonoid NRG (Khan et al., 2014b).

Tsai and coworkers explored the potent antioxidant effect of NRG. Elastic liposomes or NRG for topical application was developed containing different amounts of Tween 80 and cholesterol. Different physicochemical properties like vesicle size, surface charge, encapsulation efficiency, and permeability capacity along with formulation’s stability were evaluated. Skin irritation caused due to drug-loaded elastic liposomes was also evaluated. Saturated aqueous solution of NRG and NRG dissolved in 10% Tween 80 solution (5 mg/mL) were treated as the control group. Results revealed that while using elastic liposomes as carrier, the deposition amounts in the skin of NRG were significantly increased (about 7.3~11.8-fold and 1.2~1.9-fold respectively) in comparison to the saturated aqueous solution and Tween 80 solution-treated groups. Drug’s level was more than 98.89±3.90% after 3 months of storage at 4°C. The skin irritation studies showed that experimental formulation exhibit considerably less irritating than the positive control (paraformaldehyde-treated) group, symptomatic of its potential therapeutic application (Tsai et al., 2015).

Song and associated developed a naringenin formulation to enhance its solubility and intestinal permeability overcoming its low bioavailability. The naringenin-loaded mixed micelle formulation with a naringenin: Pluronic F127: Tween 80 ratio of 1:10:0.2 (w/w/w) was prepared using a thin-film hydration method. The solubility, size distribution, and cell viability were characterized. Subsequently, **in vivo** pharmacokinetic parameters of the naringenin-loaded mixed-micelle formulation were investigated in rats. The formulation increased the solubility of naringenin by 27-fold without a significant decrease in the viability of treated cells. The absolute bioavailability of naringenin dramatically increased from 4.1 to 26.9 % following per oral administration of naringenin-loaded mixed-micelle form. Absorption permeability of naringenin from the developed formulation increased by 1.7-fold compared to that of naringenin administered alone. Conclusively, oral bioavailability of naringenin from the loaded mixed micelle formulation can be attributed to its increased solubility and intestinal permeation (Song et al., 2015).
Martinez and coworkers evaluated the physicochemical and functional antioxidant stability of NGN containing formulations, and the effects of selected NRG containing formulation on UVB irradiation-induced skin inflammation and oxidative damage in hairless mice. NRG presented ferric reducing power, ability to scavenge 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and hydroxyl radical, and inhibited iron-independent and dependent lipid peroxidation. Among the three formulations containing NGN, only the F3 kept its physicochemical and functional stability over 180 days. Topical application of F3 in mice protected from UVB-induced skin damage by inhibiting edema and cytokine production (TNF-α, IL-1β, IL-6, and IL-10). Furthermore, F3 inhibited superoxide anion and lipid hydroperoxides production and maintained ferric reducing and ABTS scavenging abilities, catalase activity, and reduced glutathione levels. In addition, F3 maintained mRNA expression of cellular antioxidants glutathione peroxidase 1, glutathione reductase and transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2), and induced mRNA expression of heme oxygenase-1 (Martinez et al., 2016).

Zhang and associates prepared and characterized the naringenin-loaded sulfobutylether-β-cyclodextrin/chitosan nanoparticles (Nag-CD/CS-NPs), which were further evaluated for their potential for the topical ophthalmic delivery. Firstly, naringenin was complexed with sulfobutylether-β-cyclodextrin (SBE-β-CD) for enhancing the solubility and then nanoparticles were prepared by ionic gelation of chitosan. The resulting nanoparticles showed an average size of 446.4 ± 112.8 nm and zeta potential of +22.5± 4.91 mV with predominant spherical in shape. The FT-IR and DSC confirmed the formation of Nag-CD/CS-NPs. The in vitro release study indicated that Nag-CD/CS-NPs achieved moderate sustained-release effect, and the in vivo study revealed that the prepared nanoparticles was nonirritating to rabbit’s eye and had better ability to prolong the residence time then the naringenin bioavailability in the aqueous humor (Zhang et al., 2016)
Wang and coworkers prepared naringenin-loaded liposome for oral administered and were evaluated for pharmacokinetic and tissue distribution studies in animal models. The liposomal system, consisting of phospholipid, cholesterol, sodium cholate, and isopropyl myristate, was prepared using the thin-film hydration method.

Physicochemical characterization of naringenin-loaded liposome such as particle size, zeta potential, and encapsulation efficiency produced 70.53 ± 1.71 nm, -37.4 ± 7.3 mV, and 72.2 ± 0.8%, respectively. The in vitro release profile of naringenin from the formulation in three different media (HCl solution, pH 1.2; acetate buffer solution, pH 4.5; phosphate buffer solution, pH 6.8) was significantly higher than the free drug. The in vivo studies also revealed an increase in AUC of the naringenin-loaded liposome from 16648.48 to 223754.0 ng·mL⁻¹·h as compared with the free naringenin. Thus, approximately 13.44-fold increase in relative bioavailability was observed in mice after oral administration. The tissue distribution further showed that the formulation was very predominant in the liver (Wang et al., 2017).

Sandhu and associates formulated nano-miceller drug delivery carriers of tamoxifen (TMX) having natural ingredients like polyunsaturated fatty acid (PUFA) with self-nano-emulsifying properties was developed with naringenin (NG) in a synergistic manner i.e., TMX-NG-SNEDDS. The optimized nano-formulation revealed complete drug release in 30 min and >80% permeation in 45 min. Superior cellular uptake potential (4.6-6.5-fold) of the TMX-NG-SNEDDS using Caco-2 cells while cytotoxicity study on MCF-7 cells indicated significant results (P<0.05) of TMX-NG-SNEDDS. The in vivo pharmacokinetic study also construed remarkable improvement (7.3 and 11.4-fold increase in Cmax and AUC) in rate of drug absorption and 2-fold reduction in Tmax by optimized TMX-NG-SNEDDS. In vivo DMBA model construed superior efficacy of the formulation by reducing tumor size, and improved survival rate of the animals justifies its safety aspect as well (Sandhu et al., 2017).

Prashar and coworkers formulated naringenin loaded poly caprolactone (PCL) nanoparticles to expand the functionality of naringenin in terms of release, chemoprevention and therapeutics. The designing of Hyaluronic acid (HA) decorated
PCL nanoparticles were prepared by utilizing self-assembling LBL technique, where a polycationic layer of a polymer was used as a linker for modification between two polyanionic layers. Additionally, an attempt has been made to strengthen the therapeutic efficacy of PCL nanocarriers by active targeting and overcoming the extracellular matrix associated barriers of tumors using HA targeting cluster determinant 44 receptor (CD44). Cell cytotoxicity study on A549 cells and J774 macrophage cells depicted enhanced anticancer effect of NAR-HA and CH-PCL-NP with safe profile on macrophages. Uptake study on A549 cells advocated enhanced drug uptake by cancer cells. Cell cycle arrest analysis (A549 cell lines) demonstrated the superior cytotoxic effect and active targeting of NAR-HA and CH-PCL-NP. Further chemopreventive treatment with NAR-HA and CH-PCL-NP was found effective in tumor growth inhibitory effect against urethane-induced lung cancer in rat. In conclusion, developed formulation possesses a promising potential as a therapeutic and chemopreventive agent against urethane-induced lung carcinoma in albino wistar rats (Prasahr et al., 2018).

2.4 Patent Search

Virk et al., (US9622984B1) in their patent evaluation described a method of preparing naringenin nanoparticles by dissolving naringenin in an organic solvent to form a solution and adding that solution to boiling water under ultrasonic conditions to form a mixture. The mixture was stirred to obtain the naringenin nanoparticles, where the organic solvent can be at least one of methanol, ethanol, dichloromethane and chloroform and Ultrasonic conditions can include applying ultrasonic energy at a frequency of 30-60 kHz and a power of 100 watts for about 20-30 minutes to the mixture.

Sieg (WO2007011595A2) in his invention includes compositions for treating conditions in which neurons would otherwise degenerate or die as a result of an insult to the nervous system. In his study, compositions include a neural regeneration peptide (NRP) and an antioxidant. The method comprised of administering a composition comprising a neural regeneration peptide and an antioxidant to a subject. Composition includes one or more NRPs and one or more antioxidants that can also be used in vitro, to promote growth and differentiation of neural cell cultures.