

Formulation and Characterization of Nanomedicine (Solid Lipid Nanoparticle) Associate with the Extract of *Pterospermum acerifolium* for the Screening of Neurochemicals and Neuroendocrine Effects

Abstract

Background: Nanotechnology has given the likelihood of conveying medications to particular cells utilizing nanoparticles. Nanosystems can convey the dynamic constituent at an adequate fixation amid the whole treatment time frame, guiding it to the fancied site of activity. Traditional medications do not meet these necessities. The fundamental motivation behind creating elective medication conveyance advancements is to expand effectiveness of medication conveyance and security during the time spent medication conveyance and give more accommodation to the patient. **Objectives:** *Pterospermum acerifolium*, basic plant in India, is viewed as carminative, stimulant, and emmenagogue. The improvement of control discharge conveyance systems could prompt huge preferences in the clinical employments of these medications to diminish the toxicities. The point of this study was to figure another conveyance framework for impacts of neurochemicals by the joining of concentrate of *P. acerifolium* into strong lipid nanoparticles (SLNs). **Methods:** SLN formulations were prepared by Ethanolic extract, lipid layer was liquefied by warming at 5°C above liquefying purpose of the lipid. After that, SLNs were separated and dried. Shape and surface morphology of the SLNs were pictured by checking scanning electron microscopy. Particle size and size distribution were dictated by photon connection spectroscopy. **Results and Discussion:** The change of molecule charge was contemplated by zeta potential estimations. Treatment with SLN with concentrate was found to altogether diminish the serum levels of adrenocorticotrophic hormone (ACTH), corticosterone and-endorphin and in addition the cerebrum and serum level of norepinephrine. Moreover, SLN with concentrate could essentially turn around the constant anxiety by diminishing the cerebrum and serum levels of the monoamine neurotransmitters dopamine, 5-hydroxytryptamine. **Conclusion:** The outcomes got from this study recommended that the memory-improving impact of SLN with concentrate was interceded through directions of neurochemical and neuroendocrine frameworks.

Keywords: 5-Hydroxytryptamine, adrenocorticotrophic, corticosterone, dopamine, nanotechnology, solid lipid nanoparticle

Introduction

Advances in nanoparticulate frameworks for enhanced medication conveyance show an incredible potential for the organization of critical dynamic particles. Strong lipid nanoparticles (SLNs) have risen as a contrasting option to other novel conveyance approaches because of different favorable circumstances, for example, plausibility of joining of lipophilic and hydrophilic medications, enhanced physical strength, minimal effort was contrasted with liposomes and simplicity of scaleup and producing. In addition, the capability of SLNs in epidermal focusing on, follicular conveyance, controlled medication conveyance, expanded skin hydration because of more noteworthy

occlusivity and photostability change of dynamic pharmaceutical fixings has been exceptionally settled. Solid lipid nanoparticles are colloidal transporter frameworks made out of high-dissolving point lipids as a strong center covered by surfactants. The term lipid in a more extensive sense incorporates triglycerides, halfway glycerides, unsaturated fats, hard fats, and waxes. A reasonable point of preference of SLNs is the way that the lipid lattice is produced using physiological lipids which diminish the threat of intense and ceaseless danger. Exceedingly refined normal strong lipids, for example, stearine portions of organic product bit, are minimal effort contrasting option to the business lipids utilized for SLN generation. This

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Table 1: Effects of strong lipid nanoparticles with extract on the serum level of adrenocorticotrophic hormone

Group	ACTH (pg/mL)
Group I	16.0±3.12
Group II	32.10±4.14*
Group III	24.20±4.29**

*Results are given as means±SEM, when compared to the normal group (Group I), $P < 0.01$. **Results are given as means±SEM, when compared to the model group (Group II), $P < 0.05$.
ACTH – Adrenocorticotrophic hormone; SEM – Standard error of mean

Table 2: Effects of *Pterospermum acerifolium* on the serum level of corticosterone

Group	CORT (pg/mL)
Group I	12.10±4.9
Group II	29.23±4.5*
Group III	20.18±4.6**

*Results are given as means±SEM, when compared to the normal group (Group I), $P < 0.01$. **Results are given as means±SEM, when compared to the model group (Group II), $P < 0.05$.
CORT – Corticosterone; SEM – Standard error of mean

Table 3: Effects of *Pterospermum acerifolium* on the serum level of β -endorphin

Group	β -EP (pg/mL)
Group I	154.17±27.19
Group II	256.21±24.12*
Group III	201.32±30.25**

*Results are given as means±SEM, when compared to the normal group (Group I), $P < 0.01$. **Results are given as means±SEM, when compared to the model group (Group II), $P < 0.05$.
 β -EP – β -endorphin

claim to fame material is gotten from indigenous source, accessible in wealth and supplemented with crucial bioactives. Plants have been used in the conventional human services framework from time immemorial, especially among tribal groups. In India, around 2000 medications utilized are of plant origin.^[1] Plant assets are draining all inclusive at a disturbing rate and various monetarily and therapeutically foremost plant species will anon be wiped out. Therapeutic plants are currently under awesome weight because of their excessive collection or exploitation.^[2]

It is entrenched that the disturbance of the hypothalamus-pituitary-adrenal (HPA) pivot, a focal pathway to the whole endocrine framework, is frequently key to most well-being issues, disorders, infections, and notwithstanding maturing itself. Hyperactive status of the HPA hub can bring about expanding levels of corticotropin-discharging hormone (CRH), adrenocorticotrophic hormone (ACTH), and glucocorticoids in the hypothalamus, pituitary, and adrenal cortex, separately. From one perspective, anxiety can likewise

Table 4: Effects of *Pterospermum acerifolium* on the serum level of norepinephrine (ng/mL)

Group	NE (ng/mL)
Group I	422.22±20.19
Group II	695.55±23.10*
Group III	628.22±28.20**

*Results are given as means±SEM, when compared to the normal group (Group I), $P < 0.01$. **Results are given as means±SEM, when compared to the model group (Group II), $P < 0.05$.
NE – Norepinephrine; SEM – Standard error of mean

Table 5: Effects of *Pterospermum acerifolium* on the serum level of 5-hydroxytryptamine (ng/mL)

Group	5-HT (ng/mL)
Group I	100.5±14.19
Group II	40.50±21.10*
Group III	105.5±30.20**

*Results are given as means ±SEM, when compared to the normal group (Group I), $P < 0.01$. **Results are given as means ±SEM, when compared to the model group (Group II), $P < 0.05$.
5-HT – 5-hydroxytryptamine

Table 6: Effects of *Pterospermum acerifolium* on the serum level of dopamine (ng/mg)

Group	DA (ng/mg)
Group I	2900.25±21.19
Group II	2212.50±18.10*
Group III	4650.5±37.25**

*Results are given as means ±SEM, when compared to the normal group (Group I), $P < 0.01$. **Results are given as means ±SEM, when compared to the model group (Group II), $P < 0.05$.
DA – Dopamine

adjust the physiological homeostasis which can bring about different neuronal, endocrine, and instinctive dysfunctions. Moreover, stretch is likewise known not psychological capacities, for example, memory, and it has been connected to the pathophysiology of inclination and tension issue.^[1,3-8] A focal component of the anxiety reaction is the actuation of the HPA pivot which can bring about an expansion in plasma levels of glucocorticoids. As a result of their significant consequences for neurons, glucocorticoids can impact conduct, state of mind, and memory process. Neurotransmitter frameworks are additionally required in learning and memory forms, and a considerable piece of learning and memory debilitations is because of changes in neurotransmission.^[9-18] It is entrenched that neurotransmitters can meddle with learning securing and memory. In this setting, the memory brokenness depicted in irregular Savda disorder could include an unnecessary creation of CRH, adrenocorticotrophic hormone (ACTH), and glucocorticoids in the hypothalamus, pituitary, and adrenal cortex, separately, under the anxiety condition.

SLN has gotten incredible consideration as of late for different applications, including the oral conveyance

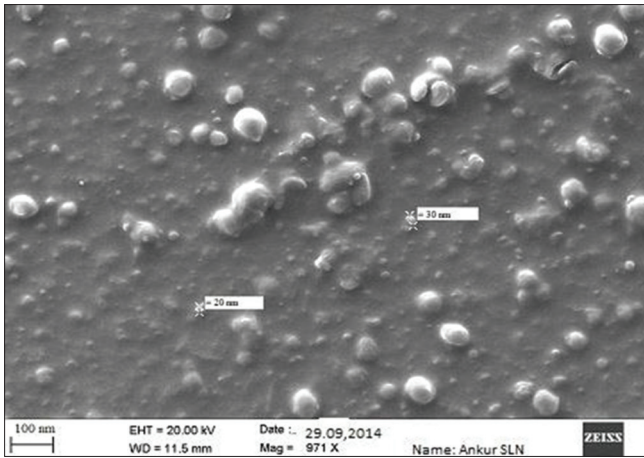


Figure 1: Scanning electron microscopy image

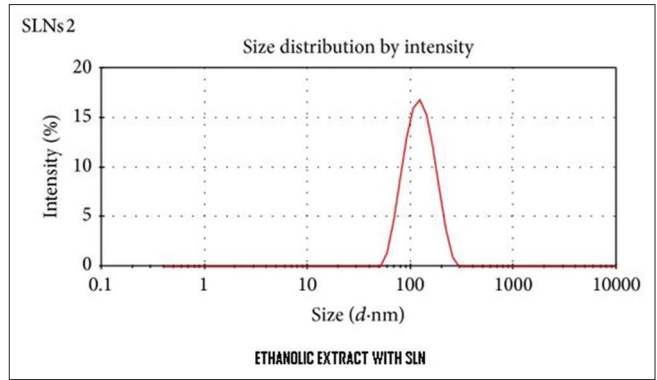


Figure 2: Size distribution

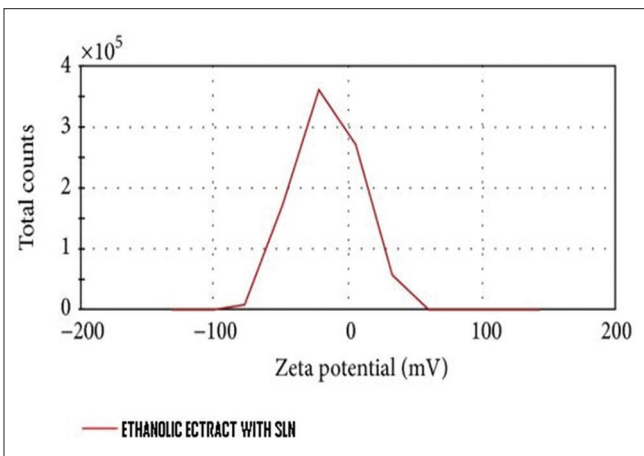


Figure 3: Zeta potential distribution

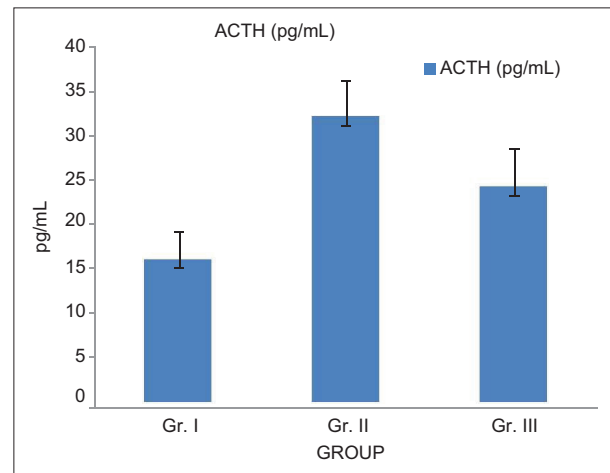


Figure 4: Effects of strong lipid nanoparticle with extract on the serum level of ACTH

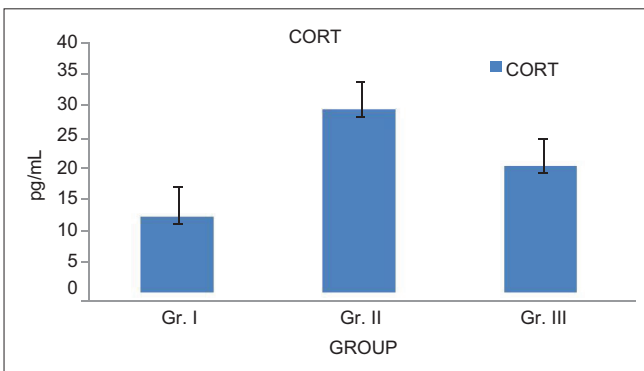


Figure 5: Effects of strong lipid nanoparticle with extract on the serum level of corticosterone

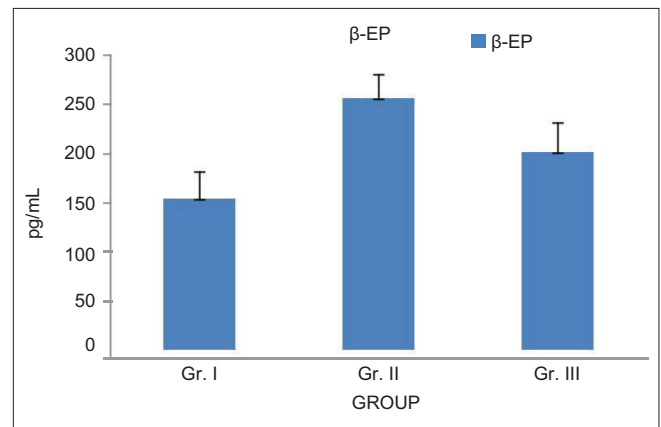


Figure 6: Effects of strong lipid nanoparticle with extract on the serum level of beta-endorphin 6.3 effects of *Pterospermum acerifolium* on the contents of monoamine neurotransmitters of brain and serum in the chronic stress mice

of medications. It has been guaranteed that SLN has numerous points of interest, for example, controlling medication discharge and medication focusing to diminish the medication harmfulness, expanding drug dependability, high medication payload, consolidation of lipophilic and hydrophilic and no biotoxicity of the transporter. The present venture is done to investigate the capability of natural medications for the treatment of central nervous

system issue with a perspective to perform phytochemical examination and survey neurochemical screening. The concentrate additionally includes improvement of SLN plan connected with the concentrates took after by portrayal and other assessment parameters utilizing *Pterospermum acerifolium*.

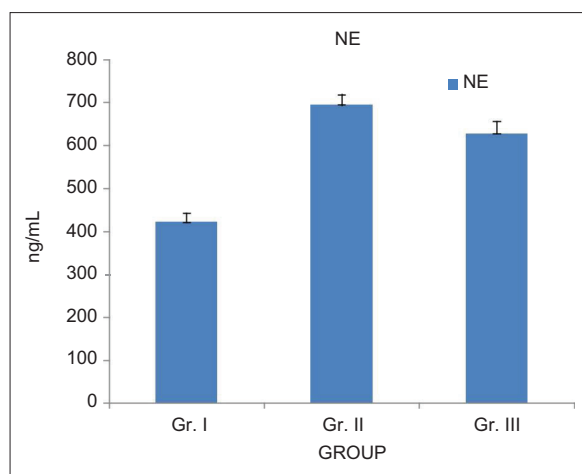


Figure 7: Effects of strong lipid nanoparticle with extract on the serum level of Norepinephrine (ng/mL)

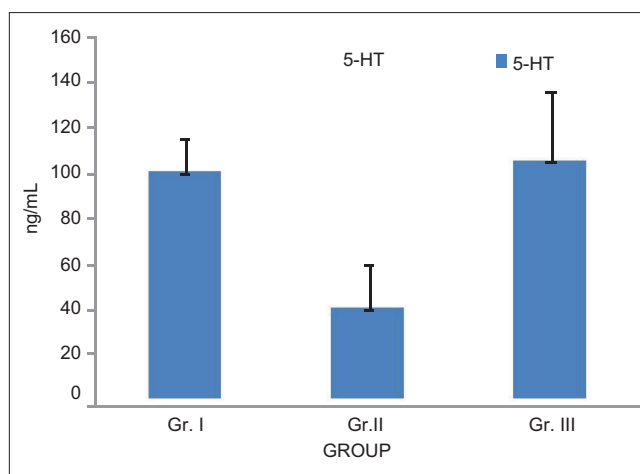


Figure 8: Effects of *Pterospermum acerifolium* on the serum level of 5-hydroxytryptamine (ng/mL)

Experimental design

Plant material

Plants materials, *P. acerifolium* bark, were collected from the local market of Bhopal (M. P.), during the month of May–July, 2012. The specimens were identified and authenticated by Dr. Zia ul Hassan, Assistant professor, Department of Botany, Saifia College of Science and Education, Bhopal, and their herbarium was deposited. The authentication number is safia/75. These collected specimens were chosen for the extraction process and assessment of neurochemical activity.

Extraction

Ethanol extraction

The plant materials so collected were cleaned properly and washed with distilled water to remove dust particles and dried in shade. The dried drugs were coarsely powdered and then exhaustively extracted with 50% ethanol in Soxhlet apparatus for 72 h. The ethanolic extracts so obtained were freed of solvent under vacuum (Yield: 9.33%).

Development and evaluation of strong lipid nanoparticle formulation

Chemicals

Glyceryl monostearate (1-stearoyl-rac-glycerol), stearic acid (octadecanoic acid), and Tween 80 (polysorbate 80) along with all the other chemicals were of analytical grade and were purchased from Sigma-Aldrich (New Delhi, India). Compritol ATO 888 and Precirol were the gift sample from Asoj Soft Caps, Baroda, India.

Preparation of strong lipid nanoparticle

A volume of 150 mg of GMS were dissolved in 10 mL organic solvent (1: 1 chloroform and methanol) and 50 mg of ethanolic extract was dispersed in this lipid solution. Organic solvent was removed using rotary evaporator. Ethanolic extract embedded lipid layer was melted by heating at 5°C

above melting point of the lipid. Simultaneously, an aqueous phase was prepared by dissolving Tween 80 in Milli-Q water and heated to same temperature. Hot aqueous phase was added to the lipid phase with continuous stirring at 3000 rpm for 30 min. The mixture was homogenized for 4 h. After that, SLNs were filtered and dried.

Characterization

Shape and surface morphology

Shape and surface morphology of the solid lipid nanoparticles were visualized by scanning electron microscopy (SEM). The samples for SEM were prepared by lightly sprinkling nanoparticles on a double adhesive carbon tape, which was stuck to an aluminum stub. The stubs were then coated with gold to a thickness of 200–500 Å under an argon atmosphere using gold sputter module in a high-vacuum evaporator. The samples were then randomly scanned and photomicrographs were taken at different magnifications.

Particle size and size distribution

Photon correlation spectroscopy (PCS) is the most powerful technique for the measurement of particle size. 1 mL of SLN suspension was diluted to 10 mL with distilled water and average particle size and polydispersity index were measured by PCS.

Zeta potential measurements

The surface charge of solid lipid nanoparticles is denoted as zeta potential (ZP). It was determined by the electrophoretic mobility of solid lipid nanoparticles in U type tube at 25°C, using Zetasizer (Malvern, UK).

In vivo experimental design

Animals for experiment

Swiss albino rats were obtained from animal house VNS Institute of Pharmacy with due permission from Institutional Animal Ethical Committee (Registration Number. 778/03/c/cpsa). Acute toxicity studies were conducted using albino

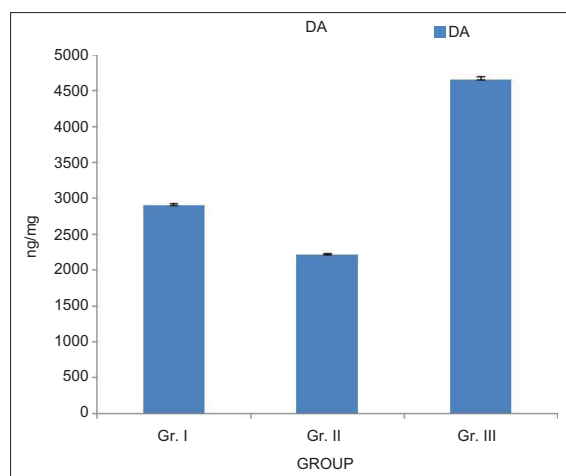


Figure 9: Effects of *Pterospermum acerifolium* on the serum level of dopamine (ng/mg)

mice of either sex weighing between 20 and 25 g and healthy adult male albino rats weighing between 150 and 200 g were selected for the neurochemical screening. The animals were acclimatized to standard laboratory conditions (temperature: $25 \pm 20^\circ\text{C}$) and maintained on 12-h light: 12-dark cycle. They were provided with regular rat chow (Lipton India Ltd., Mumbai, India) and drinking water *ad libitum*.

Acute toxicity

In an acute toxicity studies, *pterospermum acerifolium* extract were given single doses of drug. The Swiss albino rats were divided into groups. All animals fed with standard rat pelleted diet (Lipton India Ltd., pellets) and had free access to tap water *ad libitum*. Acute toxicity studies were performed according to the OECD guidelines. The doses selected for the study were 50 mg/kg, 100 mg/kg, 200 mg/kg, 300 mg/kg, and 400 mg/kg for 1 day. Three animals were taken for each dose. It was observed that the extract do not produce any significant effect on the behavior of rats. The animals were observed for 3 h after dose administration and also after 24 and 48 h.

Estimation of neurochemicals

Estimation of adrenocorticotropin, corticosterone and β endorphin

The blood was collected and centrifuged at 4°C ; the serum was stored at -80°C before assay. Serum levels of ACTH, corticosterone (CORT), and β -endorphin (β -EP) were determined using ELISA kit (obtained from R and D systems). The sensitivity of the assay was 1.0 ng/mL. Intra- and inter-assay coefficients of variation were $<4.85\%$ and 6.08% , respectively. The test was performed according to the manufacturer's specification.

Adrenocorticotropin (ACTH), CORT, and β -EP kits were obtained from R and D systems, USA. Norepinephrine (NE, purity $\geq 97\%$), dopamine (DA, purity $\geq 99\%$), 5-hydroxytryptamine (5-HT, purity $\geq 99\%$),

3,4-dihydroxyphenylamine (DOPAC, purity $\geq 99\%$), and 3,4-dihydroxybenzylamine (DHBA, purity $\geq 98\%$) were obtained from Sigma Co., Ltd., USA. All other reagents were of analytical grade.

Surgicals

Hemostatic sponge

AbGel, Absorbable gelatin sponge USP, Srikrishna Laboratories, Mumbai, India.

Sterile sutures

Ethicon 4-0, Nonabsorbable surgical sutures USP; Mersilk (Braided silk black); Ethicon 4-0, Absorbable surgical sutures USP (Catgut), Johnson and Johnson, India.

Surgical needle

Curved surgical needles were obtained from Pricon Surgical, New Delhi, India.

Drug administration

In the preliminary investigation, the animals were divided into three matched groups; Wistar rats were divided into three groups of six rats each. Group I served as normal group. Animals of Group II serves as model group and Group III were administered orally with respective test drugs, between 7:30 am and 9:30 am daily during 14 days. These doses were calculated according to the conversion table of equivalent effective dose ratios from human to animals based on the body surface area. Food was withdrawn from the animals 2 h before drug administration, but water was allowed freely. The pretreatment groups (Gr. III) received the same electric foot-shock 1 h after drug administration (8:30 am–10:30 am).

- Normal group (0.5% sodium carboxyl methyl cellulose (CMC-Na) solution (20 mL/kg, b. w.)
- Model group
- SLN with ethanolic extract of *P. acerifolium* (2.53 g/kg)

Measurements of monoamine neurotransmitters by high-performance liquid chromatography- fluorescence detector

Levels of monoamine neurotransmitters (NE, DA, 5-HT) in serum and brain were measured by high-performance liquid chromatography (HPLC) coupled with a fluorescence detector (FCD). Mice were sacrificed immediately after exposure to the stress. Blood was sampled into ethylenediamine tetraacetic acid-containing tubes at 10:00 am and separated in a refrigerated centrifuge at $10,000 \times g$ for 10 min at 4°C . The serum was stored at -80°C until assayed. After blood collection, the brains were quickly removed, frozen in liquid nitrogen, and stored at -80°C until assayed. To determine serum monoamine neurotransmitter levels, an equal volume of 0.1M HCl was added to the serum samples containing 200 $\mu\text{g}/\text{mL}$ of DHBA as an internal standard. The samples were then shaken and

mixed for 1.5 min in ice water. One drop of concentrated HCl was then added to the solution and mixed in ice water for another 1.5 min and then centrifuged at 3000 rpm, 4°C for 10 min. The samples of brain tissue were homogenized in ice water solution of 0.1M HCl. Then, 0.1M HCl solution was added to the samples (1 μ L/1 mg tissue) containing 200 μ g/mL of DHBA as an internal standard and centrifuged at 18000 rpm, 4°C for 10 min. The samples were filtered through 0.45 μ m microfilters (MFS Inc., USA). Aliquots (10 μ L) of supernatant were injected into a reverse-phase HPLC column (condition: Agilent 110180 high-voltage pump coupled to a FCD, chromatographic column ZORBAX ODB C18 4.6 mm \times 150 mm \times 5 mm, voltage 121V, and wavelength 360 nm). All the brain samples were weighed on an electronic scale before HPLC analysis, and the results were expressed as ng of monoamine/mg of wet weight tissue.^[19-22]

Statistical Analysis

Statistical evaluation of the data was done by Student's *t* test (Graph PAD InStat software, Kyplot). A value of $P < 0.05$ was considered statistically significant.

Results and Discussion

Characterization of solid lipid nanoparticle

The mean particle size of SLN formulations ranges from -20 nm to -30 nm. The particle size of the solid lipid nanoparticle was appreciably lower (nm) compared to other formulations. This result is in accordance with the report that the addition of surfactant to solid lipid nanoparticle systems causes the interfacial film to condense and stabilize. All the formulations had particles in the nano range which is well evident from the values of polydispersity. Polydispersity is basically the ratio of standard deviation to the mean particle size. All formulations had low values of polydispersity (0.230–0.450) indicating the uniformity of particle size.

The ZP indicates the degree of charge present on suspended particles in dispersion. A suitably high value of ZP (positive or negative) confers stability because particles resist aggregation. All the studied formulations have shown the value of ZP between -15.8 and -22.0. Result shows in Figures 1-3.

Effects of strong lipid nanoparticle with extract on the Serum Levels of ACTH, corticosterone, and β -EP in the chronic stress mice

Figures and table showed that the serum levels of ACTH, CORT, and β -EP were markedly increased ($P < 0.01$) in the chronic stress mice (Group II) when compared to the normal group (Group I). Oral administration of *P.acerifolium* at doses of SLN with extract 2.53 g/kg, (Group III) for 14 days caused a decrease of the levels of ACTH, CORT, and β -EP in the serum when compared to the model group (Group II). Result shows in Figures 4-6 and Tables 1-3.

Effects of *P.acerifolium* on the Contents of Monoamine Neurotransmitters of Brain and Serum in the Chronic Stress Mice

Figures and table showed an increase ($P < 0.05$) of NE level in the serum of the chronic stress mice (Group II) and a decrease of the serum levels of DA, when compared to the normal group (Group I). Oral administration of *P.acerifolium* during 14 days at doses of 2.53 g/kg SLN with extract (Group III) was able to decrease the NE levels ($P < 0.01$), while the levels of DA, Figures and table showed similar results with an increase of the NE level ($P < 0.01$) but a decrease in the levels of DA ($P < 0.01$) in the brain of the chronic stress mice (Gr.II), when compared to the normal group (Group I). SLN with extract doses of *P.acerifolium* (2.53 g/kg) were found to reduce the concentration of NE in the brain ($P < 0.01$) when compared to the stress mice (Group II). Result shows in Figures 6-9 and Tables 4-6.

Conclusion

Figures and table showed that the serum levels of ACTH, CORT, and β -EP were markedly increased ($P < 0.01$) in the chronic stress mice (Gr. II) when compared to the normal group (Gr. I). Oral administration of *P. acerifolium* at doses of SLN with extract 2.53 g/kg (Gr. III), for 14 days caused a decrease of the levels of ACTH, CORT, and β -EP in the serum when compared to the model group (Gr. II). Figures and table showed an increase ($P < 0.05$) of NE level in the serum of the chronic stress mice (Gr. II) and a decrease of the serum levels of DA, when compared to the normal group (Gr. I). Oral administration of *P. acerifolium* during 14 days at doses of 2.53 g/kg SLN with extract (Gr. III) was able to decrease the NE levels ($P < 0.01$), while the levels of DA, Figures and table showed similar results with an increase of the NE level ($P < 0.01$) but a decrease in the levels of DA ($P < 0.01$) in the brain of the chronic stress mice (Gr. II), when compared to the normal group (Gr. I). SLN with extract doses of *P. acerifolium* (2.53 g/kg) were found to reduce the concentration of NE in the brain ($P < 0.01$) when compared to the stress mice (Gr. II).

Glyceryl monostearate and Tween 80 were selected as main lipid component and surfactant, respectively. ELSN-3 showed smallest particle size and it contained highest surfactant content. It has been reported that Tween 80 promotes formation of smaller sized nanoparticles. It was observed that increased content of surfactant promotes formation of smaller nanoparticles. All the SLN formulations showed negative ZP values which indicate the stable nature of nanoparticles owing to electrostatic repulsion. As the surfactant content increased, entrapment efficiency increases which could be due to formation of stabilized nanoparticles.

The present study shows the applicability of lipid-based formulation in increasing the absorption of lipophilic drugs.

GMS was used in the study because it has shown highest solubility for the drug. However, the presence of surfactant is critical for formulating stable SLN formulation. In the present study, stable SLNs were formulated using appropriate proportions of GMS and Tween 80. The formulation has shown optimally stable physicochemical parameters with higher values and AUC.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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