

Formulation and Evaluation of Negatively Charged Liposomes of Sertraline Hydrochloride for Antidepressant Activity

Neelam, Varsha Rani, Ruchi Chawla

Department of Pharmaceutical Engineering and Technology, Indian Institute of Technology (Banaras Hindu University), Varanasi, Uttar Pradesh, India

Abstract

Introduction: In the present study, liposomes of sertraline hydrochloride were formulated for use in depression. **Materials and Methods:** The liposomes were formulated by film hydration technique and were characterized for surface morphology, particle size, drug entrapment, and drug release potential. The antidepressant-like activity was studied using chronic unpredictable mild stress procedure and then further, the responsiveness toward treatment as measured by changes in swimming, immobility, and struggling behavior of rats. **Results:** Negatively charged liposomes with an average size of 154 nm showed controlled drug release. Behavioral studies on stress-induced rat model showed a significant increase in the swimming and immobility time and a decrease in the struggling behavior of animals in comparison to a marketed sertraline hydrochloride formulation. **Conclusions:** The antidepressant-like activity was studied using chronic unpredictable mild stress procedure and then further, the responsiveness toward treatment as measured by changes in swimming, immobility, and struggling behavior of rats.

Key words: Antidepressant, blood–brain barrier, drug release, liposome, surface morphology

INTRODUCTION

Depression is a serious mental health disorder and a psychiatric syndrome characterized by loss of interest in activities, causing significant impairment in daily life. A report on the Global Burden of Disease based on 1-year prevalence estimates that depression is more common in women than men, i.e., 5.8% for men and 9.5% for women.^[1]

Various classes of drug are being used for the treatment of psychiatry disorders, but sertraline is the drug of choice for depression. Sertraline is a selective serotonin reuptake inhibitors (SSRIs) used as major therapeutic drug in psychiatry. It shows antidepressant activity by enhancing serotonergic transmission and its elimination half-life (approximately 26 h) makes it suitable for once a day administration. Sertraline has a wide therapeutic index and has no direct effect on central noradrenergic and dopaminergic systems, but can cause downregulation of central β -adrenoceptors. Further, sertraline has no anticholinergic and sedative activity due to lack of significant affinity for central muscarinic and H1 histaminic receptors.^[2]

The vascularization of central nervous system (CNS) possesses unique properties. Distinctively termed as blood–brain barrier (BBB), it is composed of brain capillary endothelial cells (BCECs) which tightly regulate the movement of ions, molecules, and cells across the brain. The homeostasis is responsible for healthy neuronal function and also for the protection of neurons against toxins and pathogens. Alterations in the barrier properties lead to pathology and progression of various neurological conditions.^[3] A number of transport mechanisms regulate the transport of essential nutrients into CNS, such as passive diffusion of small lipophilic molecules, carrier-mediated transport, and receptor-mediated transport and absorptive transport.^[4] Small lipophilic molecules O_2 , CO_2 , and ethanol passively diffuse across BBB, while large molecules such as glucose and amino acids are carried through specific solute transporters expressed on both the luminal and abluminal sides of BCECs, while the transport of macromolecules

Address for correspondence:

Ruchi Chawla, Department of Pharmaceutical Engineering and Technology, Indian Institute of Technology (Banaras Hindu University), Varanasi - 221 005, Uttar Pradesh, India.

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such as insulin and transferrin across BBB occurs through receptor-mediated endocytosis. ATP-dependent efflux pumps such as breast cancer resistance protein and P-glycoproteins characteristic of BCECs are capable of transporting a diverse range of substances to brain.^[5,6]

Liposomes are lipid bilayered membrane structures formulated with relatively biocompatible and biodegradable lipids, providing the ease of encapsulation of lipophilic drugs, either in the phospholipid bilayer or at the bilayer interface.^[7] The biodistribution of liposomes is influenced by surface hydration and charge density, size, and fluidity of bilayer membrane.^[8,9] Serotonin hydrochloride exhibits poor permeability across BBB, so drug carriers which can exploit the transcellular transport through circulating mononuclear phagocytic cells and transfer it to brain can play a potential role in increasing the availability of drug in brain.^[10,11] With this idea, negatively charged liposomes of sertraline hydrochloride were prepared using cholesterol, HSPC (hydrogenated soy phosphatidylcholine), and DMPG (dimyristoylphosphatidylglycerol sodium salt) for delivery to brain for the treatment of depression.

phosphatidylcholine-L- α -phosphatidylcholine, hydrogenated (Soy) (HSPC), and 1,2-dimyristoyl-sn-glycero-3-phosphoglycerol sodium salt (DMPG). Cholesterol, HSPC, and DMPG were provided as a gift sample by Lipoid GmbH, Germany. Sertraline hydrochloride was obtained as a gift sample from Matrix Laboratories Ltd., India. Chloroform and methanol were purchased from Merck, India. Dialysis bag membrane (cellulose membrane mol. wt. cutoff 12–14,000 Da) was purchased from HiMedia, India. All solvents used were of HPLC grade. Sertraline hydrochloride tablets (dose 25 mg) were purchased from the market.

Atomic force microscopic (AFM) studies were performed by NT-MDT AFM instrument present in Central Instrument Facility Centre, IIT (BHU), Varanasi. The samples were prepared by adding a drop of liposomal formulation on a microscopic slide which was then air dried and examined in 40x resolution mode in AFM.

Preparation of liposomes of sertraline hydrochloride

Liposomes of sertraline hydrochloride were prepared by lipid film hydration technique with modification.^[12] Mixture of chloroform and methanol taken in a ratio of 9:1 was used to dissolve 20 mg drug along with 137.7 mg of DMPG, 146.95 mg of HSPC, and 54.37 mg of cholesterol in a round bottom flask (250 ml, Quickfit neck B-24). Organic solvent was then removed using a rotary evaporator (IKA, China)

MATERIALS AND METHODS

Materials

Materials required for the preparation of liposomes were cholesterol, hydrogenated soy

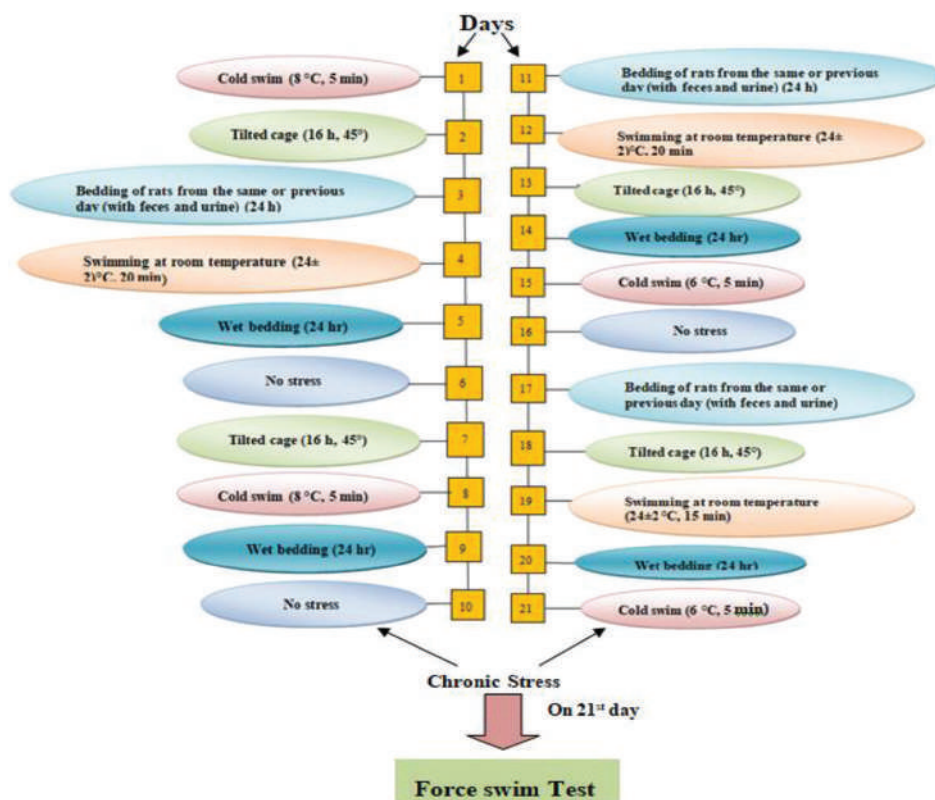


Figure 1: Chronic unpredictable mild stress procedure for the induction of depression in animal

operated at 50°C, 125 rpm for 45 h under reduced pressure. The lipid film formed was hydrated with 20 ml of phosphate buffer (pH 7.4) and rotated again for 45 min. The particle size of the liposomes formed was reduced by sonication for 3 min on an ice bath. The resultant liposomal suspension was lyophilized at -40°C for 24 h and stored. Lyophilization was performed using a Benchtop freeze dryer (Labconco Corp., USA).

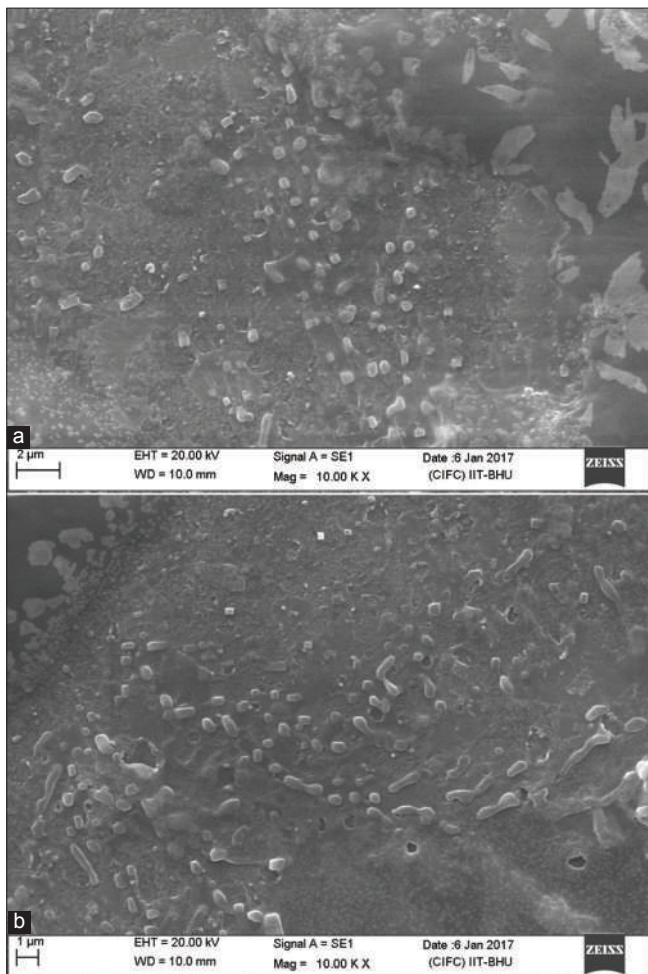


Figure 2: Scanning electron microscopic images of liposomes (a) scale of 2 μm and (b) scale of 1 μm .

Physicochemical characterization of SRT liposomes

Measurement of particle size

The vesicle size of liposomes was determined using DELSA™ NANO (Beckman Coulter, Inc.). The samples were diluted with ultrapurified water and the estimations were made at 20°C.

Solid-state characterization studies (SEM, FTIR, XRD and AFM)

The surface morphology of prepared liposomes was assessed using environmental scanning electron microscopy (SEM) (FEI Quanta™ 200, USA) having a secondary electron detector at accelerated voltage of 20 kV. The samples were sputter coated with aluminum pre-coated with silver glue, under vacuum and observed in SEM.

Further characterizations were performed by Fourier-transform infrared (FTIR) spectroscopy. FTIR spectra of lyophilized liposomes, individual excipients, and the physical mixture at the same loading ratios were collected using FT-IR spectrometer (Shimadzu, Model 8400S, Japan).

The scanning electron microscopic studies showed that liposomes exhibited spherical shape in the nano-size range as shown in Figure 2.

In general, morphological information like surface roughness can be obtained by AFM by analyzing the isolated single particles, which provide the shape and surface roughness of particles. Two dimensional and corresponding three dimensional views of liposomes have been shown in Figure 3a and b, respectively. The average height of liposomes, as obtained through AFM was 53.459 nm. Further, roughness of liposomes was also measured by using Nova Px 3.1.0 Rev 3880 software. The average roughness of liposomes was found to be 15.425 nm which reflects liposomes have a smooth surface. Estimation of roughness of particles gives an idea about the mechanism of interaction with cells and tissues.

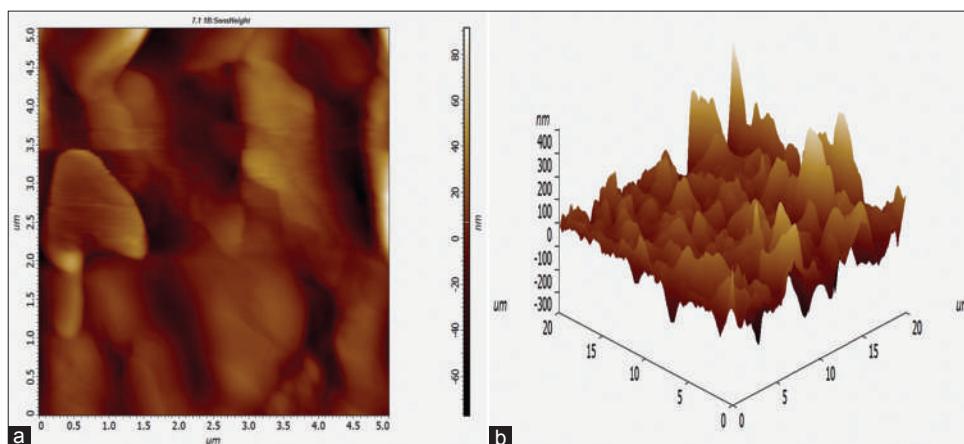


Figure 3: AFM image of liposomes in a) Two dimension and b) Three dimension

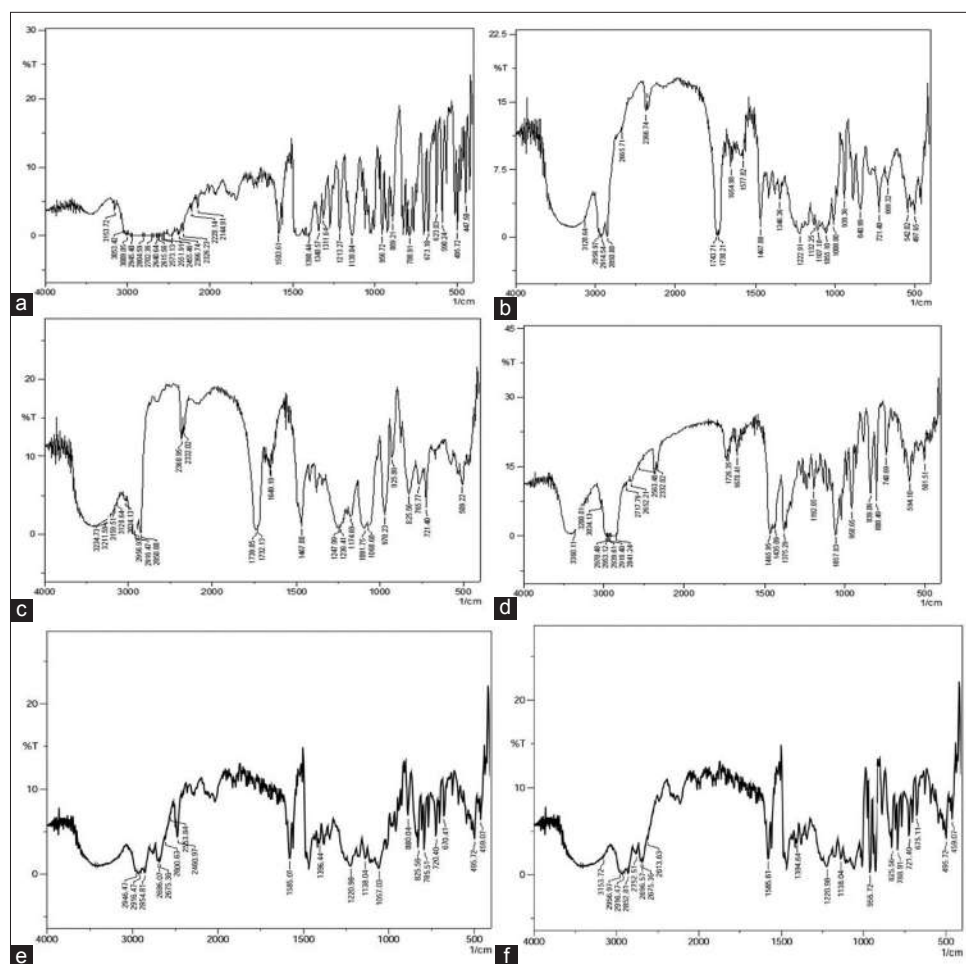


Figure 4: FTIR spectrum of (a) sertraline hydrochloride (SRT); (b) DMPG; (c) HSPC; (d) Cholesterol; (e) physical mixture; and (f) liposomes

The crystallographic structure of SRT liposomes was also determined by X-ray diffraction (XRD) analysis. Powder XRD patterns of SRT (pure drug), physical mixture of drug and excipients, and SRT-loaded liposomes were collected in transmission using a X-ray diffractometer (PANalytical's X'Pert PRO MRD) with monochromatic $\text{CuK}\alpha_1$ radiation ($\lambda = 1.5406 \text{ nm}$) generated at a voltage of 45 kV and current of 40 mA. The scanned angles were set from $10^\circ < 2\theta > 70^\circ$ with a step size of $0.001^\circ/\text{min}$ at 25°C temperature.

Entrapment efficiency of the liposomal formulation

The entrapment efficiency is a ratio of the amount of drug entrapped in liposomes to total drug present in liposomal suspension. The suspension was ultracentrifuged at 5000 rpm for 15 min at 4°C in cooling centrifuge (REMI). After centrifugation, the supernatant containing free drug was separated. The supernatant was centrifuged again at 15,000 rpm at 4°C for 30 min. After the removal of pelleted liposomes, the supernatants so obtained were pooled and drug content was estimated by UV-visible spectrophotometric method at 273 nm.^[12,13] The entrapment efficiency was then calculated using the following equation:

$$\text{EE (\%)} = \left(\frac{\text{Total Drug Content} - \text{Free dissolved drug}}{\text{Theoretical Drug Content}} \right) \times 100$$

In vitro drug release studies and drug release kinetics

The *in vitro* drug release studies were performed using dialysis bag diffusion method^[14-16] in phosphate buffer (pH 7.4). Dialysis bag (cellulose membrane mol. wt. cutoff 12–14,000 Da, HiMedia, India) was hydrated overnight in dissolution media. A 2 ml of liposomal suspension was placed in the dialysis bag with the two ends tied and firmly clamped. The bag was immersed in a release chamber containing 100 ml of dissolution media under continuous stirring at 75 rpm at $37 \pm 0.5^\circ\text{C}$. Two-milliliter samples were withdrawn at fixed time intervals (0, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 18, and 24 h), with the replacement of equivalent volume of fresh media (to maintain sink conditions). The drug content was analyzed after filtration through $0.2 \mu\text{m}$ syringe filter. The experimental data were fit into kinetic models: Zero order, first order, Higuchi, and Peppas–Korsmeyer, and based on correlation coefficient (R^2) values, drug release pattern was predicted.

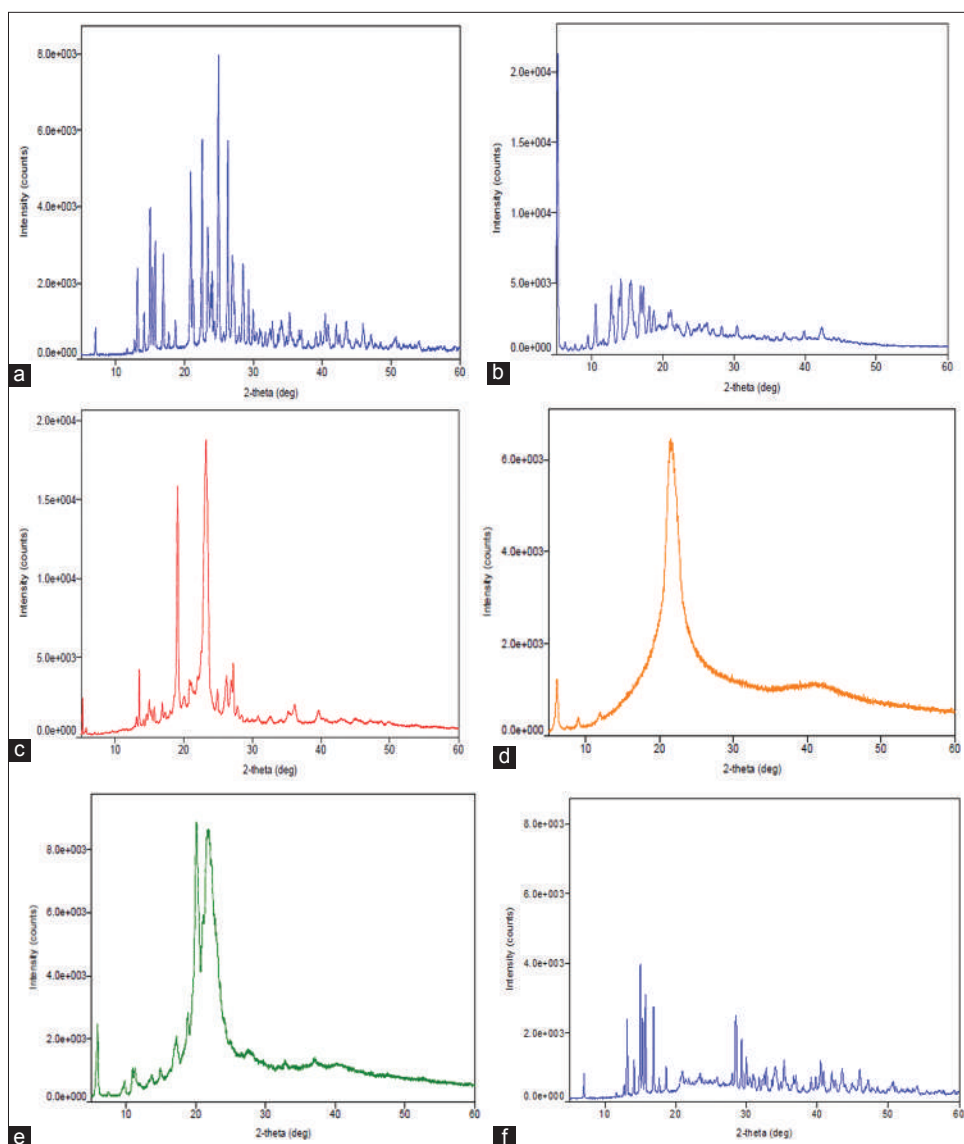


Figure 5: X-ray diffractograms of (a) sertraline hydrochloride (SRT); (b) cholesterol (CHOL); (c) HSPC; (d) DMPG; (e) physical mixture of SRT, CHOL, HSPC, and DMPG; (f) liposomes

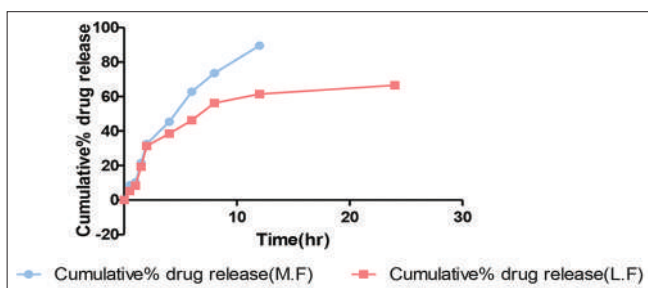


Figure 6: *In vitro* release profile of sertraline hydrochloride from liposomes and free drug suspension in pH 7.4 phosphate buffer studied up to 24 h. Values represent Mean \pm SD ($n = 6$)

Animal experiments

The study protocol for animal studies was approved by the Animal Ethical Committee, Institute of Medical Science,

Banaras Hindu University (Varanasi, India). Female Wistar rats weighing 200 ± 20 g and 6–8 weeks old were housed in the animal house facility conditioned at $20 \pm 2^\circ\text{C}$ and 60–70% RH with alternate 12 h dark and light cycle. The animals were provided free access to pelleted food and distilled water *ad libitum*. The dose of sertraline hydrochloride used for study 5 mg/kg body weight.^[17]

Study of antidepressant-like activity

Chronic unpredictable mild stress (CUMS) procedure

Animals were subjected to various unpredictable stresses as per the modified procedure previously described elsewhere.^[18,19] Stress was given to animals once per day over a period of 21 days between 09:00 and 13:00 h. The procedures applied have been schematically represented in Figure 1.

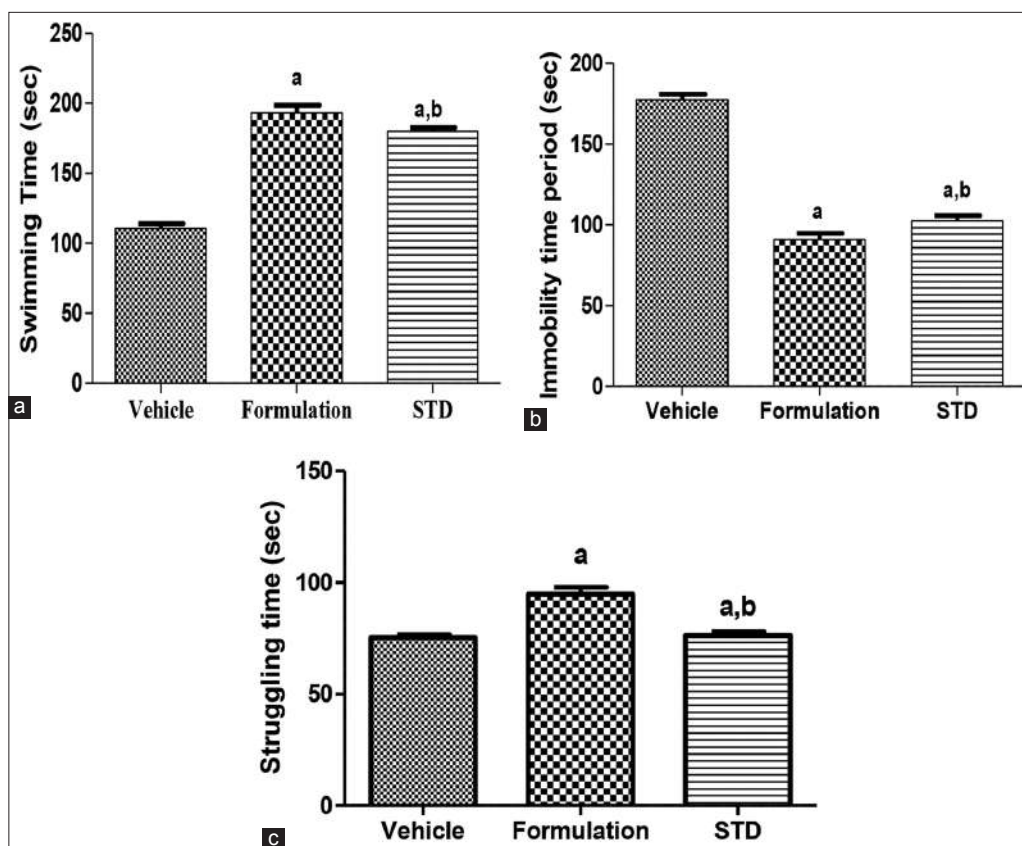


Figure 7: Effect of treatment on (a) swimming time; (b) immobility time; and (c) struggling time of rats. Data are expressed as mean and standard error (n=6); ^aP<0.05 versus Group I (control group); and ^bP<0.05 versus Group II (treatment group) in one-way ANOVA followed by Newman-Keuls *post hoc* test

Table 1: Kinetic modeling of drug release data

Regression coefficient	Zero-order release	First-order release	Higuchi model	Korsmeyer-Peppas model
R ²	0.6914	0.936	0.9769	0.9086

Forced swim test

Briefly, the animals were placed individually in a glass cylinder 30 cm diameter × 60 cm height, filled with 40 cm of water at 25°C. Initially, the rats were placed in water for a training session (forced swim) for 15 min. After 24 h, rats were placed in the cylinder again for a 5 min test session. During the test session, rats were scored for immobility time (floating with only small movements necessary to keep their head above water), swimming time (pedaling or making circular movements), and struggling time (climbing walls or diving attempts to escape). The animals were scored by an observer blind to the treatment given to the animal.^[20]

Experimental treatment groups

The animals were divided into three groups (12 animals/group): Group I was administered with normal saline solution (control group), Group II was administered liposomal formulation through intravenous route (treatment group),

and Group III (standard group) was administered sertraline hydrochloride tablets (marketed formulation) dispersed in pH 7.4 buffer through intraoral gavage. Groups II and III were administered dose equivalent to 5 mg/kg body weight. After subjecting the animals for 21 days of chronic stress, on day 21, 1 h after the administration of vehicle, test, and standard drug to the animals, immobility time, swimming time, and struggling time were scored and recorded.^[20]

RESULTS AND DISCUSSION

Physicochemical characterization

Measurement of vesicle size

The liposomes vesicle sizes ranging from 30 nm to several micrometers.^[21] The mean vesicle size of the liposomal formulation as measured by DELSA™ NANO was found to be 154.10 nm.

The drug-excipient compatibility studies were evaluated by FTIR. The FT-IR spectra of lyophilized liposomes, individual drug and excipients, and their physical mixture at the same loading ratios were taken and interpreted [Figure 4a-f]. The spectra of physical mixture of SRT, DMPG, HSPC,

and CHOL [Figure 4e] and liposomes [Figure 4f] showed the presence of all the characteristic peaks of individual components indicating no interaction between them.

X-ray diffractograms of SRT, CHOL, HSPC, and DMPG are shown in Figure 5a-f. Appearance of no new peak or absence/shift in original characteristic peak was observed in the case of liposomes. The XRD data revealed crystalline nature of drug and lipids indicating compatibility among them.

Entrapment efficiency of the liposomal formulation

Approximately $69.46\% \pm 1$ of SRT was found to be entrapped in the liposomes. Liposomes were formulated using DMPG and HSPC. HSPC, which is a soy phospholipid, forms less rigid and permeable lipid films, whereas DMPG (glycerol-based phospholipid) forms rigid and stable layers. Addition of DMPG adds firmness to lipid bilayer, thereby enhancing the stability of liposomes.^[22,23]

In vitro drug release studies and drug release kinetics

The *in vitro* release of SRT from liposomes was studied in phosphate buffer pH 7.4, using *in vitro* dialysis bag technique. The *in vitro* release pattern of SRT from pure drug suspension was also evaluated. A comparison of drug release pattern from liposomal formulation and pure drug suspension showed more than 80% release of drug from pure drug suspension within 12 h, however, the liposomal formulation showed a controlled release over a period of 24 h, releasing approximately 66.56% of the entrapped drug. The liposomal formulation releasing close to 30% of surface-bound drug within 5 h and remaining drug were released from the entrapped drug within the matrix [Figure 6].

The *in vitro* kinetic modeling [Table 1] showed that Higuchi model was the best fit model, indicating diffusion controlled release mechanism from liposomal vesicles.

Study of antidepressant-like activity

Chronic unpredictable mild stress procedure

After consecutive 21 days of chronic unpredictable mild stress procedure, antidepressant-like activity was evaluated by forced swim test, as shown in Figure 7a-c. Significant increase in the swimming time was observed in animals of treated with SRT liposomes (Group II) in comparison to Group I (control group) and Group III (standard group). Similarly, the immobility period was significantly reduced in Group II in comparison to Group I and Group III. Further, the struggling time observed for Group I and Group III were comparable, though significantly different from the formulation treated group (Group II). All values presented in Figure 7 are mean \pm SEM ($n = 6$).

CONCLUSIONS

The present study was aimed at investigating behavioral effects of rats exposed to chronic mild stress procedure and thereafter evaluating their response to treatment with selective serotonin reuptake inhibitor sertraline hydrochloride. CUMS-induced depressive-like behavior was evaluated through forced swim test. FST was used to study the responsiveness toward treatment as measured by changes in swimming, immobility, and struggling behavior of rats. Our study showed decrease in helplessness of animals administered with negatively charged sertraline hydrochloride liposomes. The effects observed were significantly better than marketed formulation. The present study provides potential insight into the use of targeted drug delivery systems for the treatment of depression, thus providing the basis for future studies.

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ETHICAL DISCLOSURE

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations.

The study protocol for animal studies was conducted according to CPCSEA guidelines and approved by the Animal Ethical Committee, Institute of Medical Science, Banaras Hindu University (Varanasi, India).

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APPENDIX

SRT	Sertraline hydrochloride
HSPC	Hydrogenated soy phosphatidylcholine
CHOL	Cholesterol
DMPG	Dimyristoylphosphatidylglycerol sodium salt
FDA	Food and Drug Administration
PEG	Polyethylene glycol
CPR	Cumulative percentage release
AFM	Atomic force microscopy
SEM	Scanning electron microscope
X-RD	X-ray diffraction
RPM	Revolutions per minute
Hrs	Hours
P. S	Particle size
E. E	Percentage entrapment efficiency
µg	Microgram
RH	Relative humidity
