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PREPARATION, CHARACTERIZATION AND *IN-VIVO* RELEASE OF NANOMEDICINE (SOLID LIPID NANOPARTICLE) ASSOCIATE WITH THE EXTRACT OF *PTEROSPERMUM ACERIFOLIUM* FOR THE SCREENING OF NEUROBEHAVIOURAL

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Keywords:

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ABSTRACT: Advances in nanoparticulate frameworks for enhanced medication conveyance show an extraordinary potential for the organization of urgent dynamic particles. The fundamental motivation behind creating elective medication conveyance advances is to build proficiency of medication conveyance. *Pterospermum acerifolium* is normal plant in India is viewed as carminative, stimulant and emmenagogue. The point of this study was to figure another conveyance framework for impacts of neurobehavioural by the joining of concentrate of *Pterospermum acerifolium* into strong lipid nanoparticles SLN. SLN plans were set up by ethanolic extricate implanted lipid layer was softened by warming at 5°C above liquefying purpose of the lipid. After that, SLNs were sifted and dried .Shape and surface morphology of the strong lipid nanoparticles were envisioned by filtering electron microscopy (SEM).Particle Size and Size Distribution were dictated by Photon relationship spectroscopy (PCS). The change of molecule charge was considered by Zeta Potential (ZP) estimations. The CNS action was examined in a few test models for Epilepsy i.e. Limit Pentylenetetrazole prompted seizure, Models for anxiolytic study i.e. Hoisted in addition to labyrinth, Models for nootropic study i.e. Object acknowledgment test, Models for stimulant study i.e. Constrained swim test, Tail suspension test, Models for Muscle relaxant study i.e. Hold quality test, The ethanol extricate essentially and in dosage subordinate way lessen the nociception impelled by acidic corrosive. From the present study it was inferred that the natural medications can be possibly used to control the condition of CNS issue.

INTRODUCTION: Present day ways of life have brought about anxiety related scatters, and different methodologies, for instance, yoga, contemplation and antistress medications, are utilized to check aversive anxiety impacts. Plant drugs have acted the hero to humankind in numerous diseases and may offer acceptable answer for anxiety impelled annoyances.

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The significance of plant following up on the CNS has been looked into, underlining the part of adaptogens from plant starting points. The advancement of pharmaceutical for CNS issue has been a fortunate procedure. Regardless of advances in the treatment of CNS issue, that is a requirement for medicines with more prominent adequacy and less reactions.[1-9]

A few nanotechnological techniques, for example, polymeric nanoparticles, strong lipid nanoparticles (SLNs), fluid gem (LC) frameworks, forerunners frameworks for fluid precious stones (PSLCs), liposomes, and microemulsions, have endeavored to break this obstruction; they permit substances

with various properties to be utilized as a part of the same plan, and may even change a substance's properties and conduct in a biological situation. These mechanical revelations have reformed medication conveyance. The new medication conveyance frameworks have the capacity not just to build the adequacy of dynamic segments, additionally to reintroduce different parts that were disposed of in light of the fact that they were not valuable in plan. Additionally, the capacity to enhance new substances, for example, by expanding selectivity and adequacy, securing against warm or photograph corruption, decreasing symptoms, and controlling the arrival of dynamic constituents, before they are acquainted with the business sector or utilized restoratively, makes this methodology considerably more appealing. [10-13]

Various studies have inspected psycho-pharmacologic treatment approaches for discouraged patients with nervousness side effects, which incorporate the utilization of antidepressants, anxiolytics and other novel mixes. Psycho-remedial methodologies have likewise been tried and incorporate the utilization of conventional subjective conduct based methodologies and also between by and by situated psychotherapies. While some result examines propose that standard proof based medicines are compelling in diminishing both depressive and tension manifestations, different studies prescribe alterations to customary treatment approaches so as to ideally oversee gloom with comorbity.

To date, in any case, no psycho-pharmacologic or psycho-remedial treatment has been set up in randomized controlled trials as unrivaled, in the treatment of melancholy with comorbid tension. Keeping in mind the end goal to better comprehend what medications are best in overseeing discouraged patients with comorbid tension issue, it is first basic to comprehend the different side effects of comorbid issue. In this way there is a requirement for models that evaluate regular pathogenic instrument, hazard element, manifestations and comorbidity connected with these scatters. SSRIs in illustration are compelling in the treatment of the sorrow and tension, distinction in measurement, time of onset of activity and at times, instrument might be distinctive. Benzodiazepines, azapirones, SSRI, barbiturates medications are accessible for the treatment of CNS issue, yet numerous patients experience the ill effects of noteworthy unfriendly occasions; in this manner, there is a requirement for new drugs. Right now accessible antipsychotics are connected with assortment of autonomic, endocrine, hypersensitive, hematopoietic and neurological reactions. Thus there is high predominance of use of correlative and option medications for treatment of psychiatric issue. In the quest for new helpful items for the treatment of neurological issue, therapeutic plant research, around the world, has advanced continually, exhibiting the pharmacological adequacy of various plant species in an assortment of creature models. [14-19]

Pterospermum acerifolium is customarily used to diminish CNS issue. By and large, plants have numerous pharmacological activities, for example, Hepatoprotective, cancer prevention agent, calming, anthelmintic, antimicrobial since they contain various constituents of dynamic chemicals in it Based on the case by customary healers that the plant is successful in the treatment of focal sensory system (CNS) sicknesses. Some plant show intense danger connected with the renal, heart, hematopoietic and regenerative frameworks. The advancement of controlled discharge conveyance systems would prompt noteworthy points of interest in the clinical utilization of these medications to diminishing the harmfulness.

In the event that the chose plant having harmfulness and terrible bioavailability, definition of strong lipid nanoparticle connected with the separate reductions the lethality with expanding bioavailability.

The present project is done to explore the potential of herbal drugs for the treatment of CNS disorders with a view to perform phytochemical investigation and assess Neurochemical screening. The study also involves development of solid lipid nanoparticle formulation associated with the extracts followed by characterization and other evaluation parameters employing *Pterospermum acerifolium*. [20-29]

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 & Education, Bhopal and their herbarium was deposited. These collected specimens were chosen for the extraction process and assessment of Neurobehavioural activity.

Ethanolic 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 SLN 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 SLN:
150 mg of GMS was 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 by 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 hours. After that, SLNs were filtered and dried. [30-36]

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 to 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. 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/cpcsa). Acute toxicity studies were conducted by using albino mice of either sex weighing between 20 and 25 gms and healthy adult male albino rats weighing between150 and 200 gms were selected for the Neurochemical screening. The animals were acclimatized to standard laboratory conditions (temperature: 25±20C) 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 study of *pterospermum acerifolium* plant 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, 400 mg/kg for one day. Three animals were taken for each dose. It was observed that the extract don't produce any significant effect on the behaviour of rats. The animals were observed for 3 hours after dose administration and also after 24 and 48 hours.

Pharmacological evaluation of formulation for Neurobehavioural and Neurochemical screening

Screening for Neurobehavioural and Neurochemical screening:

Screening for Neurobehavioural and Neurochemical screening was carried out in Wistar albino mice of either sex weighing 100-120 g.

Assessment of Epileptic activity:

Wistar albino mice (25-35 g) bred in Central Animal facility of the Institute, were used. They were housed under standard conditions, maintained on a 12 h light/dark cycle and had free access to food and water up to the time of experimentation. The mice were acclimatized to the laboratory environment 1 h before the experiments. All experiments were conducted during the light period (08.00-16.00 h). During the experiments animals were free access to water only. All the protocols were approved by the Institutional Animal Ethical Committee (IAEC) and conducted according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Pentylenetetrazol (PTZ)-induced convulsion test:

Animal groups:

Four groups of mice (n = 10) were used. Group I was administered the vehicle, i.e., normal saline (1 ml/100g body weight) and served as control, Group II received reference standard (diazepam, 2 mg/kg, i.p.) while Groups III and IV were administered SLN with extract 100 and 200 mg/kg, p.o., respectively, of the extracts. Two hours later, PTZ was administered (60 mg/kg, i.p.) to all four groups. The animals were observed for 30 min and the onset and duration of convulsion noted.

Assessment of anxiolytic study:

Elevated plus – Maze test:

Animals were randomly allocated to four experimental groups (n= 5 each). Group 1 and 2 were named as negative and positive control and 2 other groups were termed as treated group. The test groups received SLN with ethanolic extract of *P. acerifolium* at the dose of 100 and 200mg/kg body weight respectively. Group 1 received 1% Tween 80 solution. Group 2 got administration of Diazepam (as a standard drug) at 1mg/kg bodyweight. Drug or vehicle was injected intraperitoneally in a volume of 0.5ml/kg. Tests were performed 30 min after injections.

Assessment of nootropic study:

Object recognition test:

A plastic chamber (35cm×35cm×35 cm) was used in low light condition (about 40 lx) during the light phase of the light/dark cycle. The general procedure, as described elsewhere, consisted of three different phases: a habituation phase, an acquisition phase, and a retention phase. On the 1st day (habituation phase), mice were individually subjected to a single familiarization session of 10 min, during which they were introduced in the empty arena, in order to become familiar with the apparatus. On the 2nd day (acquisition phase) animals were subjected to a single 10-min session, during which floor-fixed two objects (A and B) were placed in a symmetric position in the central line of the arena, 10cm from each and 8 cm from the nearest wall (each object occupies approximately 5 cm space by its size).

The two objects, made of the same wooden material with the similar color and smell, were different in shape but identical in size. Mice were allowed to explore the objects in the open field. The exploration time on each object was shown (as seconds) to indicate the exploring activity of mice. On the 3rd day (retention phase), mice were allowed to explore the open field in the presence of two objects: the familiar object A and a novel object C in different shapes but in similar color and size (A and C). A recognition index (for retention session), calculated for each mouse, was expressed as the ratio (TC×100)/(TA + TC), where TA and TC are the time spent during retention phase on object A and object C, respectively. The time spent exploring any object (nose pointing toward the object at a distance≤1 cm, but not mounting on the object or playing with the object) was recorded (using stopwatch) by hand.

Assessment of antidepressant study:

Forced Swimming Test (FST):

Mice of either sex were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water at 25±1°C. The immobility time, defined as the absence of escape oriented behaviors, such as swimming, was scored during 6min with the help of stop-watch, as described previously by.

All the mice of either sex were divided in five different groups. The first group assigned as control receiving only vehicle (NaCl 5 mL kg-1). The other groups received acute dose of extracts. The total duration of immobility was recorded during the last 6 min of the 10 min period, where the activity in the two first minutes is discarded. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant like effect. For the next exposure of crude extracts, FST of repetitive doses of crude extracts were assessed after 3 days of treatment within 30 min after the last dose of administration. During the test session, the immobility time was recorded.

The mice were considered immobile when neither hind leg was moving; the mice were slightly hunched forward. Another reason for choosing this animal model is the correlation which is observed between results in this model and clinical potency, which is not found in any other models. A different interpretation of the FST holds that rats or mice, in this behavioral paradigm, learn to be immobile. Immobility is considered to be an adaptive response to the situation consisting of animals learning to keep their heads out of water with a minimum of energy expenditure.

Tail Suspension Test (TST):

The total duration of immobility induced by tail suspension test was measured. Mice both acoustically and visually isolated were suspended 70 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. The total immobility period was scored manually during 6 min test session with the help of stopwatch. Immobility was defined as the absence of any limb or body movements, except for those caused by respiration or when they hung passively and completely motionless. The parameter obtained was the number of seconds spent immobile. Parameter used was the number of seconds spent immobile.

Assessment of Muscle relaxant study:

Grip strength test:

A Rota-rod (Inco- Ambala, Instruments and chemicals Pvt. Ltd. Model town, Ambala City -03) was used to measure the grip strength in mice. The instrument (a horizontal rotation device) was set at a rate of 16 revolutions per minute5,6. The animals were placed on the rotating rod and fall off time i.e, when the animal falls from the rotating rod, was recorded, which was taken as grip strength. Diazepam (26 mg/kg i.p.) was used as the standard drug. Grip strength in all the groups was measured before and at 30 minutes, 1 h, 2 h, 4 h, and 6 h of the administration of the dose.

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 to be significant.

RESULTS AND DISCUSSION:

Characterization of Solid Lipid Nanoparticle:

The mean particle size of SLN formulations ranges from nm– 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 zeta potential indicates the degree of charge present on suspended particles in dispersion. A suitably high value of zeta potential (positive or negative) confers stability because particles resist aggregation. All the studied formulations have shown the value of zeta potential between −15.8 to −22.0.

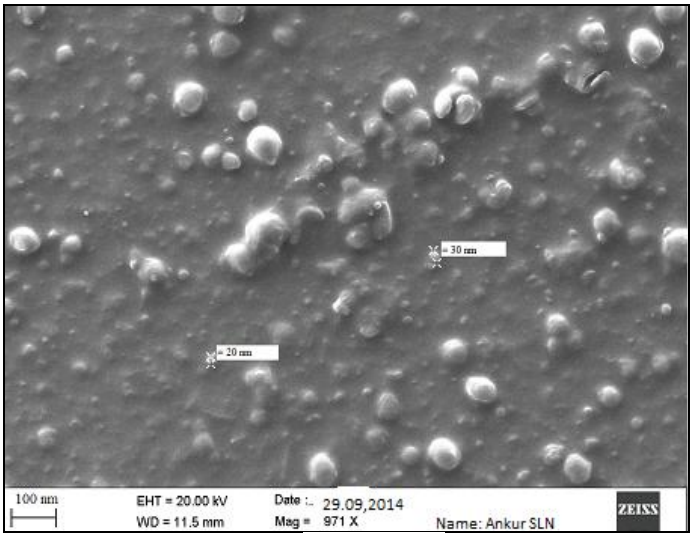

FIG.1: SEM IMAGE

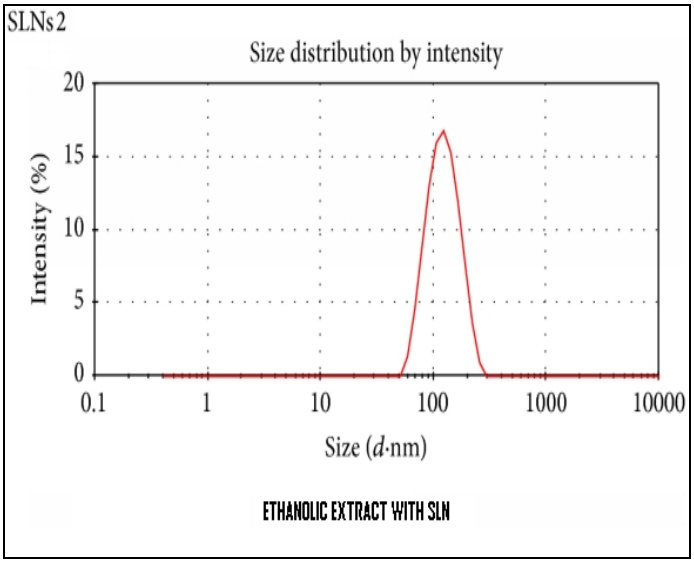

FIG.2: SIZE DISTRIBUTION

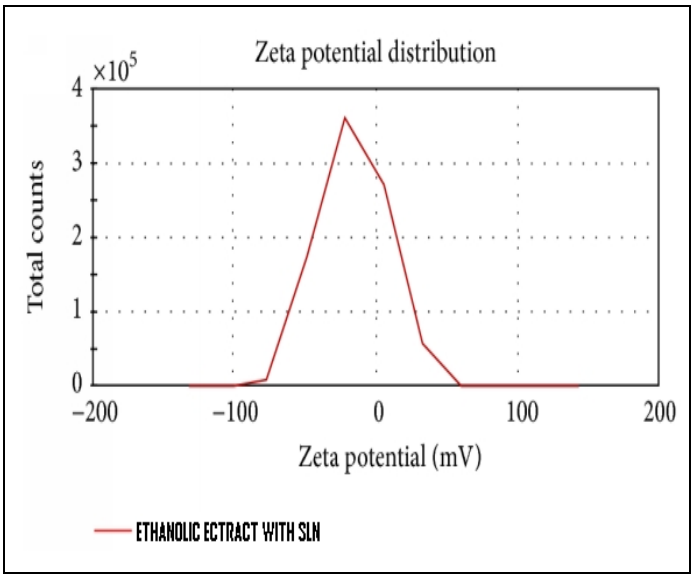

FIG. 3: ZETA POTENTIAL DISTRIBUTION

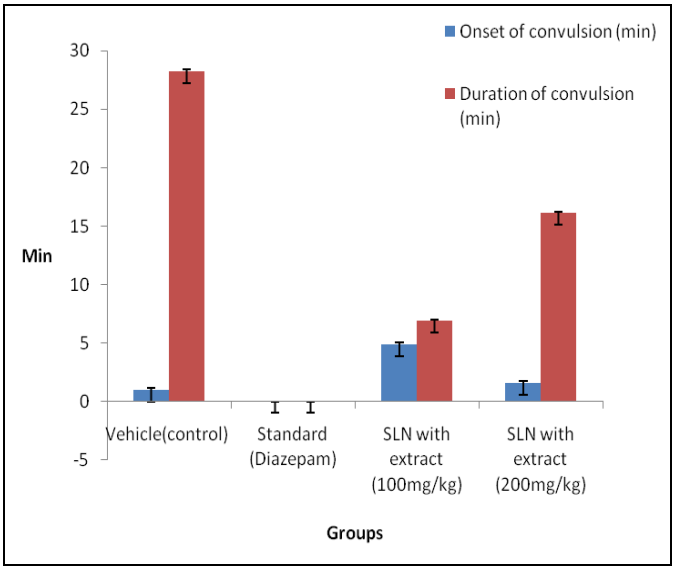

FIG. 4: EFFECT OF SLN WITH EXTRACT ON PENTYLENETETRAZOL (PTZ)-INDUCED SEIZURES IN MICE (n= 10)

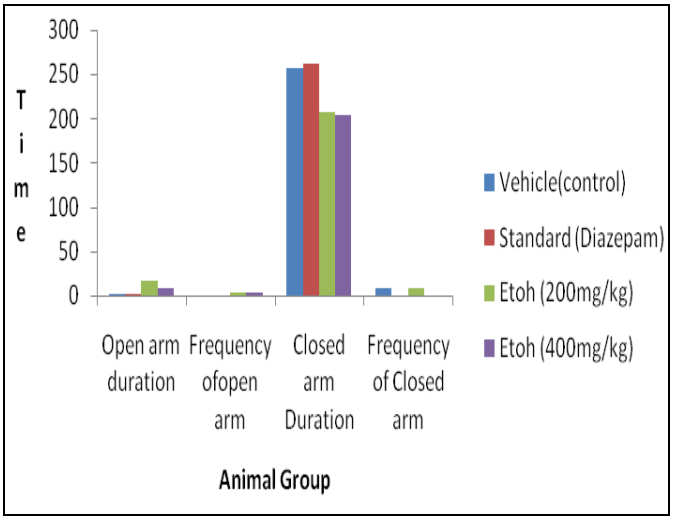

FIG.5: EFFECT OF SLN WITH EXTRACT ON ELEVATED PLUS MAZE EXPERIMENT

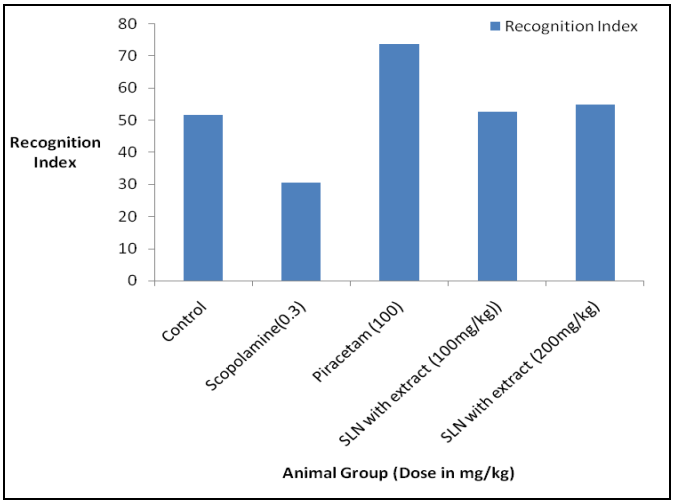

FIG. 6: EFFECT OF SLN WITH EXTRACT ON OBJECT RECOGNITION TEST

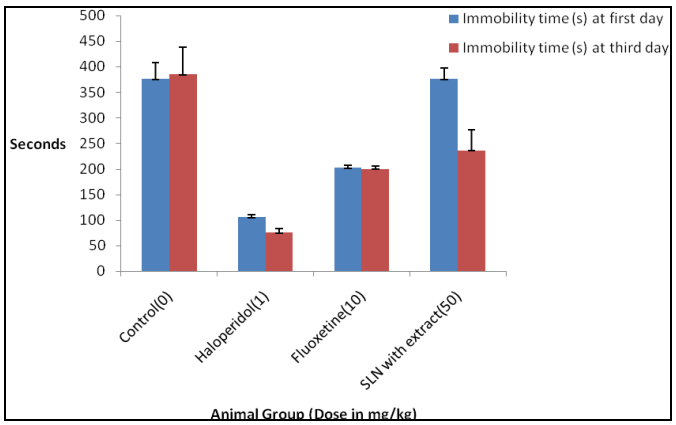

FIG. 7: EFFECT OF SLN WITH EXTRACT ON FORCED SWIMMING TEST (FST)

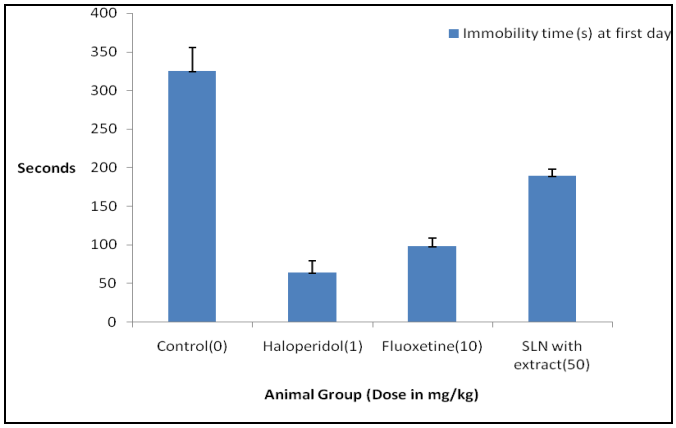

FIG. 8: EFFECT OF SLN WITH EXTRACT ON TAIL SUSPENSION TEST (TST)

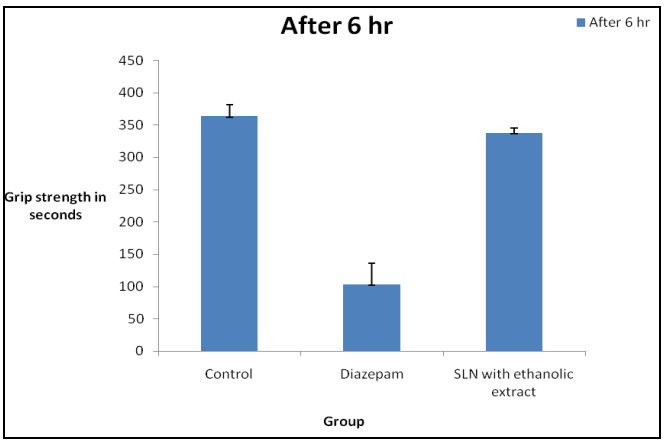

FIG. 9: EFFECT OF SLN WITH EXTRACT ON GRIP STRENGTH TEST

TABLE 1: EFFECT OF SLN WITH EXTRACT ON PENTYLENETETRAZOL (PTZ)-INDUCED SEIZURES IN MICE (n = 10)

Groups	Onset of convulsion (min)	Duration of convulsion (min)
Vehicle(control)	1.01 ± 0.11	28.2 ± 0.2
Standard (Diazepam)	0 ± 0†	0 ± 0 †
SLN with extract (100mg/kg)	4.90 ± 0.17#	6.5 ± 0.1#
SLN with extract (200mg/kg)	1.60 ± 0.13*	16.4 ± 0.1#

†P < 0.001; #p < 0.01; *p < 0.05

TABLE 2: EFFECT OF SLN WITH EXTRACT ON ELEVATED PLUS MAZE EXPERIMENT

Animal Group	Open arm Duration	Frequency of open arm	Closed arm Duration	Frequency of Closed arm
Vehicle (control)	2.6	0.8	257.8±11.3939	8.4±1.719
Standard (Diazepam)	2.4	0.2	261.8±11.3939	7.6±1.719
SLN with extract (100mg/kg)	15.33	3.1	204.6±11.3600	12.9±1.209
SLN with extract (200mg/kg)	8.7	2.9	206.2±11.3500	14.1±1.223

TABLE 3: EFFECT OF SLN WITH EXTRACT ON OBJECT RECOGNITION TEST

Animal Group (Dose in mg/kg)	Trial-1	Trial-2 (sec)		Recognition Index
	Time spent (sec)	Time spent familiar object	Time spent new object	
Control	20.00±3.05	10.10±0.47	11.10±1.27	51.58±1.84
Scopolamine(0.3)	31.11±3.84	31.98±20.98**	14.45±2.48	30.60±3.24*
Piracetam (100)	36.80±4.04	15.80±2.60	42.80±2.27**	73.89±2.62**
SLN with extract (100mg/kg))	21.40±5.09	19.45±2.90	23.30±4.50	52.70±3.29
SLN with extract (200mg/kg)	24.40±5.11	23.45±2.11	25.30±4.44	54.90±3.22

TABLE 4: EFFECT OF SLN WITH EXTRACT ON FORCED SWIMMING TEST (FST)

Animal Group (Dose in mg/kg)	Immobility time (s) at first day	Change (%)	Immobility time (s) at third day	Change (%)
Control(0)	376.0 ± 31.5	-	385.0 ± 52.8	-
Haloperidol(1)	105.7 ± 4.5***	-71.84	74.8 ± 8.2***	-80.62
Fluoxetine(10)	201.7 ± 6.1***	-46.25	200.0 ± 6.0***	-48.06
SLN with extract(50)	376.3 ± 21.5ns	0.80	236.3 ± 40.8*	-37.17

TABLE 5: EFFECT OF SLN WITH EXTRACT ON TAIL SUSPENSION TEST (TST)

Animal Group (Dose in mg/kg)	Immobility time (s) at first day	Change (%)
Control(0)	325.3 ± 30.4	42.39
Haloperidol(1)	63.8 ± 15.1***	-80.39
Fluoxetine(10)	97.8 ± 11.2***	-69.90
SLN with extract (50)	189.0 ± 9.0***	-44.30

TABLE 6: EFFECT OF SLN WITH EXTRACT ON GRIP STRENGTH TEST

Grip Strength Test

Grip strength in seconds

Group	Treatment	Dose (mg/kg)	0 hr	30 min	1 hr	2 hr	4 hr	6 hr
1	Control	--	358.17 ± 12.620	370.33 ± 18.416	379.23 ± 12.456	381.50 ± 12.132	376.33 ± 11.071	363.17 ± 18.511
2	Diazepam	26	370.50 ± 17.936	* 202.17 ± 14.893	* 153.17 + 12.946	** 125.23 ± 12.923	** 103 ± 33 ± 6.310	** 103 ± 33 ± 5.310
3	SLN with ethanolic extract	200	332.50 ± 11.233	* 309.17 ± 11.778	* 326.23 ± 8.127	ns 362.01 ± 10.923	359.50 ± 11.987	337.23 ± 7.940

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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