Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol. 4, Suppl 1, 2011 ISSN - 0974-2441

Research Article

EFFECTS OF *LIPPIA NODIFLORA* EXTRACTS ON MOTOR COORDINATION, EXPLORATORY BEHAVIOR PATTERN, LOCOMOTOR ACTIVITY, ANXIETY AND CONVULSIONS ON ALBINO MICE

TURASKAR AO[1*], BHONGADE SL[1], MORE SM [1], DONGARWAR AS[1], SHENDE VS[2], PANDE VB[3]

[1]Manoharbhai Patel Institute of Pharmacy (B. Pharm), Kudwa, Gondia, MS- 441614, [2]Sharadchandra Pawar College of Pharmacy, Otur, Pune, MS- 412409, [3] Gurunanak College of Pharmacy, Nari Road, Nagpur, MS- 440026, Email: ashish_turaskar2000@yahoo.com

ABSTRACT

Background: *Lippia nodiflora* (Verbanaceae) is commonly known as *Poduthalai*, found mostly in southern parts of India. Literature survey revealed that no work has been evaluated for its therapeutic effects against behavioral disorders.

Objective: In this investigation, the neuropharmacological profile of petroleum ether, chloroform and ethanolic extracts of aerial part of *Lippia nodiflora* Linn. was evaluated.

Materials and Methods: By using models like; potentiation of diazepam-induced sleeping time, locomotor activity, motor coordination, exploratory behavior pattern, elevated plus maze and maximal electroshock convulsions; the neuropharmacological effects were observed for *Lippia nodiflora* extracts. Diazepam at doses of 5 mg/kg, 4 mg/kg and 1 mg/kg served as standard.

Results: It has been shown that ethanolic extract of *L. nodiflora* (LNEHE) at both doses (250 mg/kg and 500 mg/kg p.o.) and its chloroform extract (LNCEE) at higher dose of 500 mg/kg produce central inhibitory (sedative) effects, anticonvulsant effect and anxiolytic effect in mice. Values are statistically significant ($p < 0.05$ and $p < 0.01$) when compared to control group. The petroleum ether extract of plant (LNPEE) at both dose levels (250 mg/kg and 500 mg/kg p.o.) did not produce any central effects.

Conclusion: The observation and results obtained in this study indicated that LNEHE and LNCEE given the restrained central activity.

Keywords: *Lippia nodiflora*, CNS depressant, Antianxiety, Anticonvulsant, Flavonoids.

INTRODUCTION

Mental, neurological and behavioral disorders are common to all countries and cause immense suffering. People with these disorders are often subjected to social isolation poor quality of life and increased mortality. These disorders are the cause of staggering economic and social costs. [1] Habituation, dependence and the resulting potential for addiction are the greater disadvantages of the modern synthetic psychopharmacological agents.

The abrupt discontinuation of long term therapy with these drugs leads to serious withdrawal symptoms. Therefore modern society is now cautiously discovering traditional herbal medicine particularly those which have been proved to be effective in controlled studies and which in some cases, demonstrated even better galenic properties than conventional medicines. Unique opportunities for research exist in the field of CNS- active Indian medicinal plants. [2]

L. nodiflora is a creeping, much branched herb, found in the wet places, almost throughout India and traditionally used as anodyne, antibacterial, diuretic etc. Filipinos drink an infusion of the leaves instead of tea. [3, 4] Studies were earlier carried out on anti-inflammatory, analgesic and antipyretic activities and showed an analgesic activity which continued until 3 hrs using eddy's hot plate method. [5]

In previous studies, it is shown that *L. nodiflora* contains 15 flavonoids, 3 flavone glycosides, and 12 new flavones sulphates. [6] Researchers also indicated its gastro protective [7], antifungal [8], antibacterial [9], and its excellent effectiveness in cutaneous leishmeniasis. [10]

When we reviewed the literature, not detailed proof was given for central nervous effects of *L. nodiflora* Linn. and M. Zetola *et al* evaluated the CNS activities of Liquid and spray dried extracts from *Lippia alba* -Verbenaceae and concluded that *L. alba* ethanolic extract (80% v/v) present sedative activity which is related to non volatile components in the leaves and possibly to the flavonoids. [25] Based on the above facts the emphasis has been given on L. nodiflora, Linn. to evaluate its neuropharmacological profile.

MATERIALS AND METHODS

Plant collection

The aerial part of the plant L. nodiflora Linn. was collected locally, from the campus of Ultra College of Pharmacy Madurai. The sample of plant was identified and authenticated by Dr.Stephen, Taxonomist, American College, Madurai (TN), India.

Extraction

Freshly collected aerial parts of the plant L. nodiflora Linn. were washed, shade dried under room temperature for a period of three weeks. The dried plant material was made to a coarse powder and weighed quantity of the powder (800 g) was subjected to hot percolation in a soxhelt apparatus using, petroleum ether, chloroform and ethanol, at a temperature range of 40-80°C. Before and after every extraction, the marc was completely dried and weighed. The extracts were concentrated to a dry mass by concentrating on water bath and keeping it in desiccators.[11]

Preliminary Phytochemical Test

LNPEE, LPCEE and LNEHE obtained by the above methods from L. nodiflora Linn. were subjected to qualitative test for the identification of various plant constituents by the standard procedures. [11, 12]

Animals

Swiss albino male mice (20-25 g) were obtained from the animal house of Ultra College of Pharmacy, Madurai (TN), India and used for present study. The animals were housed in groups of six per polypropylene cages and maintained at 24°C ± 1°C with the relative humidity of 45-55 % and 12:12 h dark light cycle. The experiments were carried out between 10:00 to 17:00 h. The animals had free access to food (standard chew pallets, Amruth Rat feed, Banglore) and water ad libidum. The institutional animal ethics committee (UCP/IAEC/2007/011) of Ultra College of Pharmacy, Madurai (TN), India approved the Pharmacological and acute toxicity protocol.

Acute Oral Toxicity Study

Acute oral toxicity has been evaluated, for LNPEE and LNCEE by A.M. Forestieri et al[5]. They were examined that drug extract preparations showed a low toxicity, in fact the LD_{50} were more than 10 g/kg for LNPEE and LNEHE. Therefore the acute oral toxicity for the LNCEE is evaluated in present study.

As per the OECD guidelines a stepwise procedure with the use three female mice per step was followed. The animals were observed individually after dosing once in 30 minutes periodically during the first 2 hrs. The special attention given during the first four hours and observed for 24 hours. [13]

Pharmacological Evaluation

Potentiation of diazepam induced sleeping time

This test is used to elucidate CNS- active properties of drugs. Not only hypnotics, sedatives, and tranquilizers but also antidepressants at higher doses can be evaluated as they potentiate the sleeping time. Many of the pharmacological tests are based on the potentiation of sleeping time induced by barbiturates or other sedative agent. [14, 15]

The animals were divided into 8 groups each containing 6 mice and all Group received Diazepam (5 mg/kg i.p.) except Group I which received only vehicle.

Because usually in the day time mice will sleep, this negative control group is also observed. Group II - Treatment given with vehicle (2.5% Tween 80). Group III and Group IV received LNPEE at doses of 250 and 500 mg/kg p.o. Group V and Group VI were treated with 250 and 500 mg/kg p.o doses of LNCEE. Group VII and Group VIII received LNEHE at doses of 250 and 500 mg/kg p.o. Each animal was observed and onset of sleep and duration of sleep was recorded. Sleeping time in all cases was measured as the time interval between the loss and regaining of righting reflex.

Spontaneous motor activity (SMA)

Spontaneous motor activity was performed using Actophotometer (Inco, Ambala, India). The central nervous system depressant or stimulate property can be evaluated by considering locomotor activity of the animal after treating with drug. [16, 17] Mice were grouped of six each. Group I received vehicle orally (2.5% Tween 80).Group II received diazepam 1mg/ kg.i.p. Group III and Group IV treated with 250 and 500 mg/kg .p.o. of LNPEE. Group V and VI received LNCEE at doses of 250 and 500 mg/kg .p.o. Group VII and Group VIII received LNEHE 250 and 500 mg/kg .p.o. The locomotor activity for each animal was automatically recorded for 5 minutes before drug treatment and after the treatment at 30 minute interval for total 120 minutes. Results of the treated groups were compared with those of control group at each time interval.

Motor Coordination

Rotarod (Inco, Ambala, India), a biological research apparatus is using to evaluate the activity of drugs interfering with motor coordination. The instrument (a horizontal rotating device) (company name) was set at a rate of 16 revolutions per minute. Mice were placed on the horizontal rod and those that were able to remain on the rod longer than 3 min were selected for the study. [18, 19] Animals were grouped and treated as per treatment schedule given in SMA. Mouse unable to remain on the rod at least for three minutes was considered as positive test and the time of its fall was recorded.

Exploratory Behavior Pattern

The study was carried out using wooden board measuring 40 x 40 cm with 16 evenly spaced holes. [16, 20] 8 groups of mice containing 6 in each were used for study and they were treated as per treatment schedule given in SMA.

The total head dips for before any treatments, thirty minutes after diazepam treatment and 45 minutes after extract treatment were recorded for 5 min by placing the animal on a board with 16 evenly spaced holes. Results were expressed as means for the various treatment groups at different time intervals.

Elevated Plus Maze (EPM)

The EPM apparatus consists of two open arms (30 x 5 cm) and two closed arms (30 x 5x20 cm) emanating from a common central platform (5 x 5 cm). The two pairs of identical arms were opposite to each other. The entire apparatus was elevated to a height of 50 cm above the floor level. [19, 21] The animals received the treatment as per SMA treatment schedule, 45 minutes before the start of session. At the beginning of the session, a mouse was placed at the centre of the maze, its head facing the closed arm. It was allowed to explore the maze for 5 min. The time spent in open arm, percent entries in the open and closed arms and total entries were recorded. An entry was defined as the presence of all four paws in the arm. The EPM was carefully wiped, with 10% ethanol after each trial, to eliminate the possible bias due to the odor of the previous animals. The percentage time spent and open arm entries were calculated using following formulae.

Percent time spent in open arm =

$$\frac{Time\ in\ the\ open\ arm}{Time\ in\ the\ open\ arm + time\ in\ the\ closed\ arm} x100$$

Percentage of open arm entries =

$$\frac{Number\ of\ entries\ in\ open\ arm}{Total\ arm\ entries} x100$$

Maximal Electroshock Induced Convulsions

The electroshock assay in mice is used primarily as an indication for compounds which are effective in grandmal epilepsy. Tonic hind limb extensions are evoked by electric stimuli which are suppressed by anti-epileptics but also by other centrally acting drugs. [19, 22] Eight groups of mice containing 6 in each were used for study and they were treated as per treatment schedule given in SMA except group II which received diazepam in a dose of 5 mg/ kg. The animals received a current of 45 mA for 0.2 sec duration through electro convulsiometer (Techno, India) using corneal electrodes, after 60 min of oral administration of plant extract or vehicle or diazepam. The incidence and duration of extensor tonic was noted. A complete abolition of hind limb tonic extension was noted. A complete abolition of hind limb tonic extension was considered as 100% protection.

Statistical Analysis

The data obtained were analyzed using one way analysis of variance (ANOVA) followed by Dunnett's't' test. $P < 0.05$ was considered statistically significant. The results are expressed in mean $\pm$ SEM and three animals from each group.

RESULTS

Preliminary Phytochemical Test

The LNPEE extract of *L. nodiflora* showed the presence of phytosterols only. LNCEE showed the presence of alkaloids, phytosterols, flavonoids. In LNEHE phytosterols, carbohydrates, tannins and flavonoids were found to be present. .

Acute oral Toxicity

It was found that the administration of various doses of LNCEE of L. nodiflora to the dose of 5000 mg/kg body weight has not produced any signs of toxicity and mortality. One tenth and one twentieth of the half dose of the lethal dose were selected for further study.

Potentiation of Diazepam induced sleeping time

LNPEE of L. nodiflora did not showed any significant potentiation of sleeping time at both doses levels (250 mg /kg & 500 mg/kg) while the LNCEE at 500 mg/kg significantly ($p < 0.01$) potentiated the diazepam induced sleeping time. The LNEHE increased the duration of sleeping in dose dependant manner, both the doses levels of

LNEHE (250 mg /kg and 500 mg/kg) potentiated sleeping time significantly (p <0.01) when compared to vehicle treated group and the effects were dose dependant. . (Table 1)

Spontaneous Motor Activity

The LNPEE at 250 mg/kg and 500 mg/kg and the LNCEE at 250 mg/kg did not show any decrease in locomotor activity. LNCEE at a dose of 500 mg/kg showed significant (p<0.01) decrease in locomotor activity within 60 min. When the mice treated with LNEHE (250 mg/kg and 500 mg/kg), the locomotion was reduced with increase in the dose compared to control. Diazepam showed significantly decrease (p <0.01) in locomotor activity after 30 min of its treatment. Results are given in following (Table 2)

Motor Coordination

The LNPEE extract of plant L. nodiflora did not produced any reduction in motor coordination. LNCEE at 500 mg /kg dose showed a significant (p <0.01) reduction in motor coordination within 60 mins. It was found that, the LNEHE (250 mg/kg) and 500 mg/kg) exhibited a marked reduction (p < 0.01) in motor coordination in mice and they were unable to held on the rotating rod. These effects were dose dependent and observed within 60 min of extract administration and persisted for 120 min. Diazepam at a dose of 1 mg /kg showed same effects within 30 min of administration (Table 3)

Exploratory Behaviour (Head dip test)

The effect of LNPEE, LNCEE and LNEHE of L. nodiflora at dose level of 250 mg/kg and 500 mg/kg on the number of head poking in the head dip apparatus were shown in following table. During the test interval of 5 min, the LNEHE reduced the number of Head dipping in a dose dependent manner. Diazepam a well established sedative also exhibited decrease in number of head –dipping. LNPEE did not showed any significant effect on head dipping while LNCEE at dose of 500 mg/kg significantly (p <0.01) reduced the head dipping (Table 4).

Elevated Plus Maze (EPM)

In the EPM the behavior, which as observed, confirmed the anxiolytic activity of diazepam as reported previously[51]. The LNPEE and LNCEE at 250 mg/kg did not showed any significant effect. The LNCEE at 500 mg/kg significantly (p <0.01) increased time spent in open arm and percent open arm entries. The LNEHE at 250 mg/kg showed marked increase in time spent in open arm than effect of extract at 500 mg/kg. The total arm entries and percent open arm entries also were increased significantly in the LNEHE of L. nodiflora Linn. at a doses of 250mg/kg and 500 mg/kg (p <0.01). The LNEHE at a higher dose of 500 mg/kg, showed a significant (p< 0.01) decrease in time spent in the open arm, without any change in entries in open arm, entries in closed arm and total entries (Fig 1 and 2)

Maximal Electroshock Induced Convulsions

The LNEHE at 250 mg/kg and LNCEE at 500 mg/kg showed significant (p <0.05) decrease in the duration of hind limb extensor phase. The LNEHE at 500 mg /kg and diazepam at 4 mg/kg exhibited the significant (p <0.01) decrease in the duration of hind limb extensor phase and also the incidence of convulsions in mice also reduced. The LNPEE at both dose levels did not express any anticonvulsant activity (Table 5).

DISCUSSION

Insomnia, seizures, anxiety and mental health problems in general and senile neurological disorders in particular, are widely prevalent in modern fast-paced life with a multitude of stressful conditions. It is now becoming exceedingly apparent that available psychotherapeutics does not properly meet therapeutic demands of a vast majority of patients with mental health problems, and that herbal remedies remain to be the ultimate therapeutic hope for many such patients in the world. However, till now, very little attention has been paid to develop structurally and /or functionally novel CNS active drugs from psychoactive medicinal plants. [23]

The present study demonstrated that the LNEHE of *L. nodiflora* Linn. at both doses and LNCEE (at 500 mg/kg dose) produced central inhibitory effects in mice. These extracts of *L. nodiflora* significantly reduced spontaneous motor activity in mice. The decrease in spontaneous motor activity gives an indication of the level of excitability of the central nervous system and this decrease may be closely related to sedation resulting from depression of the central nervous system. [18]

The LNEHE significantly reduced the motor co-ordination in mice and prolonged diazepam-induced sleep. The prolongation of diazepam induced sleeping time may be attributed to an action of extracts on central mechanisms involved in regulation of sleep. The similar effects was also obtained with LNCEE at dose of 500 mg/kg while, LNPEE did not shown any central effect at both dose levels.Thus suggesting that the LNEHE at both doses (250 mg/kg and 500 mg/kg) and LNCEE at 500 mg/kg dose might be acting as a mild neurosedative drug. The reducing in motor coordination might be also being a result of neuromuscular blockage [18].

The LNCEE at 500 mg/kg dose and the LNEHE at both dose levels (250 mg/kg and 500 mg/kg), produced a significant decrease in exploratory behavior pattern as show by the result on head-pocking in the Head –dip test. This decrease in head dipping by plant extracts also reveals sedative behavior. The LNCEE at a dose of 500 mg/kg and LNEHE at a dose of 250 mg/kg increased the time spent and entries in the open arm. Results are suggesting that the increase in total arm entries is due to an increase in open arm entries rather than closed arm entries. At 500 mg/kg dose of LNEHE it was found that the time spent in open arm was decreased without any change in open arm entries, closed arm entries and total entries. This prominent effect shown by higher dose of LNEHE may be due to sedative effect of the extract rather than anxiolytic effect. At higher doses, diazepam (20 mg/kg p.o) in rats, chlordismethyldiazepem (benzodiazepine receptor full agonist) at 5 mg/kg. i.p. in mice showed decrease in activity in anxiolytic tests, which was concluded as sedative effect [19]. The LNCEE at a dose of 500 mg/kg and LNEHE at both dose levels (250 mg/kg and 500 mg/kg) inhibited the maximal electroshock induced convulsions. This may also suggest that the anticonvulsant action is mediated by the chloride channel of the GABA / benzodiazepine receptor complex. While the pet-ether extract at both doses and LNCEE at lower dose (250 mg/kg) did not shown any effect in electroshock induced convulsions. [23]

Sedation and anxiety are primarily mediated in the CNS by the GABA-A receptor complex, which is also involved in other physiological and neurological disorders such as epilepsy, depression, Parkinson syndrome and Alzeimer's disease. Diverse drugs such are used in these pathologies might modify the phenomena of GABA system at the level of the synthesis of GABA mediators, release or re-uptake or metabolism. [24]

Several experiments with some natural and synthetic flavones and flavanones have shown that they can modulate GABA-generated chloride current, either positively or negatively. [25] M. Zetola *et al* appraised the CNS activities of *Lippia alba* -Verbenaceae and concluded that ethanolic extract of *L. alba* present sedative activity which is related to non volatile components in the leaves and possibly to the flavonoids [26]. The preliminary phytochemical studies of the plant extracts of *L. nodiflora* indicated that the LNCEE and LNEHE contains flavonones, flavones etc. Literature review also indicated that *L. nodiflora* contains number of flavonoids namely, nepetine, jaceosidine and hispidulin aglycones; Hispiduline, jaceosidin,nepetin, hydroxyluteoline and nodiflorein mono and disulphates; Lippiflorin A and B glycosides nodifloretin A and B, nodiflorin A and B and nodifloridin A and B gluicosides. Therefore it may produce CNS depressant activity. However, along with flavonoids, number of other chemical constituents like alkaloids, resins, sugars, stigmasterol and β -sitosterol also present in *L. nodiflora*. Therefore further studies are planned to establish the exact mechanism of CNS depressant, anticonvulsant and anxiolytic activity of LNCEE and LNEHE of aerial part of *L. nodiflora* by using agonists and antagonists.

Table 1: Effect of L. nodiflora Linn on Diazepam induced sleeping time

Group	Treatment	Onset of action (min)	Duration of Action (min)
I	Negative Control 0.2 ml Vehicle	2.5 ± 0.26	41.61 ± 1.9
II	Control 0.2 ml Vehicle + Diazepam 5 mg/kg	6.5 ± 0.28	54.5 ± 1.7
III	LNPEE 250 mg/kg + Diazepam 5mg/kg	7 ± 0.40	52.5 ± 1.5
IV	LNPEE 500 mg/kg + Diazepam 5 mg/kg	6.5 ± 0.28	55.25 ± 1.7
V	LNCEE 250 mg/kg + Diazepam 5 mg/kg	6.75 ± 0.47	53.75 ± 1.5
VI	LNCHE 500 mg/kg + Diazepam 5 mg/kg	5.5 ± 0.8	67.75 ± 2.6**
VII	LNEHE 250mg/kg + Diazepam 5 mg/kg	5.28 ± 0.25	68.75 ± 2.7**
VIII	LNEHE 500 mg/kg + Diazepam 5 mg/kg	4.25 ± 0.25**	94.25 ± 1.2**

Each value represents the mean ± SEM (n=6) ** Values are significantly different at p <0.01

Table 2: Effect of L. nodiflora Linn on Spontaneous motor activity

Group I	Treatment	Experimental Mean Time (min)				
		0	30	60	90	120
I	Vehicle (Control)	374.75 ± 7.33	352.98 ± 18.23	338.5 ± 17.5	300 ± 4.79	274.25 ± 5.64
II	Diazepam 1mg/kg	382.75 ± 11.70	104 ± 4.14**	52.75 ± 4.32**	38 ± 1.87**	18 ± 1.2**
III	LNPEE 250 mg/kg	377.5 ± 13.52	354.25 ± 11.88	316.25 ± 22.25	295.75 ± 6.7	266 ± 9.4
IV	LNPEE 500 mg/kg	365.5 ± 8.89	333.75 ± 16.14	315.75 ± 7.28	285.75 ± 7.3	257 ± 10.15
V	LNCEE 250 mg/kg	389.75 ± 11.96	363.75 ± 16.80	289 ± 11.74	264.5 ± 5.69	255 ± 11.5
VI	LNCEE 500 mg/kg	372.25 ± 10.34	355.75 ± 16.67	237.5 ± 9.13**	185.25 ± 13.87**	143.5 ± 5.7**
VII	LNEHE 250 mg/kg	370.75 ± 5.87	343.25 ± 10.70	219 ± 8.35**	136.5 ± 12.08**	84.5 ± 5.3**
VIII	LNEHE 500 mg/kg	375.25 ± 14.09	332.5 ± 20.25	110 ± 6.32**	68 ± 6.01**	38.25 ± 2.87**

Each value represents the mean ± SEM (n=6) * values are significantly different at p <0.05 ** values are significantly different at p <0.01

Table 3: Effect of L. nodiflora Linn on Motor coordination

Group I	Treatment	Time Spent on Rods				
		0	30	60	90	120
I	Vehicle (Control)	210.0 ± 7.38	210.75 ± 3.86	213.75 ± 5.64	208.75 ± 5.39	210.2 ± 5.15
II	Diazepam 1mg/kg	207 ± 8.49	18.75 ± 1.49**	20.25 ± 1.79**	49.25 ± 3.56**	73.25 ± 2.28**
III	LNPEE 250 mg/kg	213.75 ± 12.57	214.75 ± 6.39	208.0 ± 8.52	213.5 ± 9.56	219.5 ± 7.00
IV	LNPEE 500 mg/kg	201.75 ± 4.66	210.5 ± 4.66	217.75 ± 3.88	206.5 ± 3.52	213.0 ± 6.17
V	LNCEE 250 mg/kg	208.2 ± 7.34	206.0 ± 5.93	202.0 ± 3.08	207.5 ± 2.72	205.25 ± 5.07
VI	LNCEE 500 mg/kg	217.75 ± 5.64	203.75 ± 3.86	69.25 ± 4.27**	100.5 ± 1.79**	140.5 ± 4.52**
VII	LNEHE 250 mg/kg	208.0 ± 3.87	197.75 ± 1.79	62.5 ± 3.86**	100.25 ± 1.79**	40.75 ± 3.17**
VIII	LNEHE 500 mg/kg	218.75 ± 3.35	204.5 ± 3.79	41.75 ± 1.49**	71.5 ± 3.06**	108.25 ± 2.81*

Each value represents the mean ± SEM (n=6) * Values are significantly different at p <0.05** Values are significantly different at p <0.01

Table 4: Effect of L. nodiflora Linn on Exploratory behavior (Head dip test)

Group	Treatment	Head dips in 5 min		Percent Activity (%)
		Before treatment	After treatment	
I	Vehicle (Control)	36.5 ± 2.53	37 ± 2.48	-
II	Diazepam 1mg/kg	36.5 ± 1.93	8 ± 0.91	78.08
III	LNPEE 250 mg/kg	38 ± 1.2	32.25 ± 1.31	15.13
IV	LNPEE 500 mg/kg	34 ± 2.27	33.5 ± 1.55	1.47
V	LNCEE 250 mg/kg	34.75 ± 1.93	30 ± 1.08*	13.66
VI	LNCEE 500 mg/kg	38 ± 2.16	22.25 ± 1.31**	41.44
VII	LNEHE 250 mg/kg	33.25 ± 3.06	22 ± 1.78**	33.83
VIII	LNEHE 500 mg/kg	34 ± 2.4	13.25 ± 1.109**	61.02

Each value represents the mean ± SEM (n=6) * Values are significantly different at p <0.05 ** Values are significantly different at p <0.01

Table 5: Effect of L. nodiflora Linn on Maximal electroshock convulsions

Group	Treatment	Duration of tonic hind limb extension	Incidence of convulsions
I	Vehicle (Control)	15.5 ± 1.32	6/6
II	Diazepam 5 mg/kg	1.25 ± 0.94**	2/6
III	LNPEE 250 mg/kg	14.75 ± 1.10	6/6
IV	LNPEE 500 mg/kg	14.25 ± 1.25	6/6
V	LNCEE 250 mg/kg	15.5 ± 1.04	6/6
VI	LNCEE 500 mg/kg	10.5 ± 0.64*	5/6
VII	LNEHE 250 mg/kg	10.25 ± 0.62*	5/6
VIII	LNEHE 500 mg/kg	2.5 ± 1.5**	2/6

Each value represents the mean ± SEM (n=6) * Values are significantly different at p <0.05 ** Values are significantly different at p <0.01

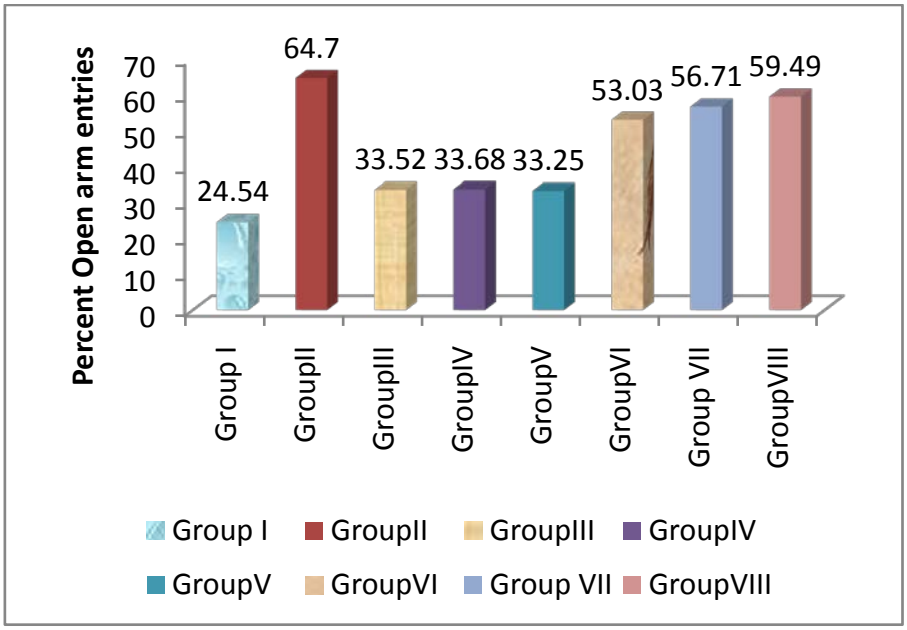

Fig. 1: Effect of L. nodiflora Linn. on percent open arm entries

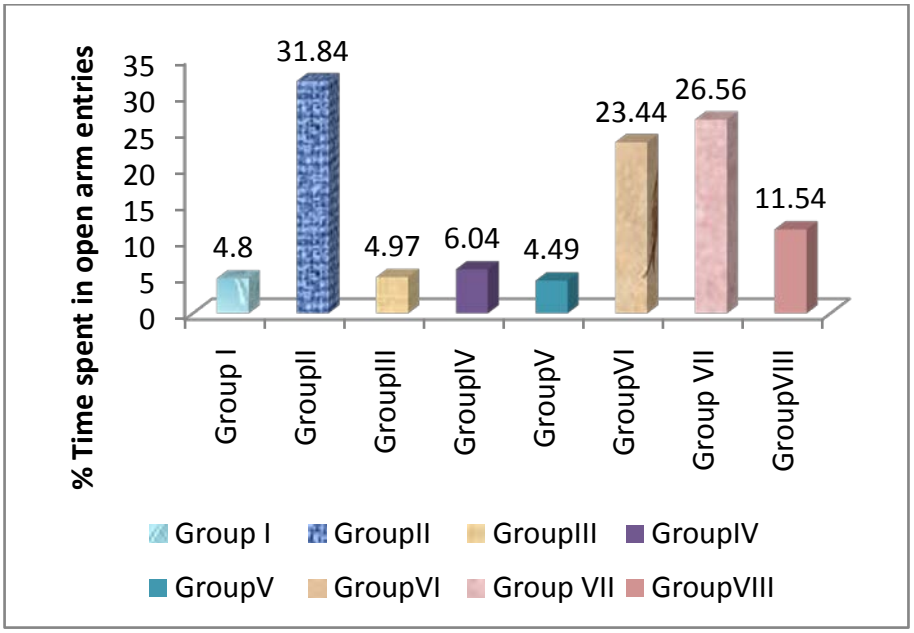

Fig. 2: Effect of L. nodiflora Linn percent time spent in open arm

REFERENCES

1. An Integrative approach to understanding brain related medical disorders. Available online at www.brain_dynamics.net/aboutus.php
2. Rudalf FW, Fintelmann V. Herbal medicine 2nd ed. Thiem publication 2000; 251-91.
3. Council of Scientific and Industrial Research (CSIR). The Wealth of India, A dictionary of Indian Raw Material and Industrial Products, New Delhi, India. 2003; Vol 5, 142.-143.
4. Pascual ME, Slowing K, Carretero E, Sanchez MD, Villar A. *Lippia:* traditional uses, chemistry and pharmacology: a review, J. Ethanopharmacol 2001;76:201-14.
5. Forestieri AM, Monforte MT, Ragusa S, Travoto A. Antiinflammatory, analgesic and antipyretic activity in rodents of plant extracts used in African medicine. Phytother. Res 1996;10:100-06 .
6. Franscisco A, Tomas B, Jeffery B., Harborne, Ron S. Twelve 6 oxygenated flarone sulphates from *L. nodiflora* and *L. canescens*. Phytochemical Unit Plant Sc 2001.
7. Khalil H, Ismail H, Taye A, Kamel N. Gastroprotective effect of *L. nodiflora* L. extract in ethanol-induced gastric lesions. Pharma Mag. 2007; 3 Suppl 12: 259 -262 .
8. Prizada AJ, Iqbal P, Shaikh W, Kazi TG, Ghani KU. Studies on the elemental composition and antifungal activity of medicinal plant *L. nodiflora* L. against skin fungi. J. Paksitan Association of Dermatologist. 2005;15:113-118.
9. Mako GA, Noor AA. Antibacterial activity of ethanolic and aqueous crude extracts of *L. nodiflora* of Kharpur mines, Sindh Pakistan. . J. Sci. Ser. 2006;38 Suppl 2: 01-04.
10. Farooq RS, Pathan GM, Abbasi P, Bhatti NS, Hussain J, Sarwar G, Bhutto, et al. Clinical trial of 20% mat lippia(buccan) topical ointment for cutaneous leishmaniasis; a preliminary trial. Sindh Univ. Res. J. Sci. Ser 2006; 38 Suppl 2: 108-12.
11. Khandewal KR. Practical pharmacognosy. 14th ed .Nirali prakashan pune. 2005.
12. Mukharjee PK. Extraction of herbal drug and quality control of herbal drug. 2nd ed. Business horizon publication New Delhi. 2005.
13. OECD guidelines 423 for acute oral toxicity: Environmental Health and Safety Monograph Series on testing and assessment number 24, 2000.
14. Khan A, Mosaddik MA, Rahman MM, Haque ME, Jahan, SS, Islam MS,et al. Neuropharmacological effects of *Laportea crenulata* Roots in mice. J. Appl. Sci. Res. 2007; 3 Suppl 7: 601-606
15. Sandabe UK, Onyeyili PA, Chibuzo GA. Sedative and anticonvulsant effects of aqueous extract of *Ficus sycomorus* L. (moracceae) Stembark. Vet. Arhiv. 2003; 73 Suppl 2:103-110.
16. Viswanatha Swamy AHM, Thippeswam AHM, Manjula DV, Mahendra Kumar CB.Some neuropharmacological effects of the methanolic root extract of *Cissus quadrangulasis* in mice. African J. Bio. Res. 2006; 9: 69-75.
17. Nagrajan NS, Soundari PG, Kumaresan PT. CNS depressant activity of *Dalsbergia malabarica.* Indian Drugs. 2003; 40 Supp 12: 716-17.
18. Samson A, Adzu B, Binda L, Wambebe C, Gamariel K. Neuropharmacological effect of the aqueous extract of *Sphaeranthus senegalensis* in mice. J. Ethanopharmacol. 2001; 78:33-37.
19. Kulkarni SK. Hand book of experimental pharmacology 3nd ed. Vallabh Prakashan, New Delhi, India. 2005.
20. Vogel HG. Drug Discovery and Evaluation Pharmacological Assays. 3nd ed, Springer, Verlag, Berlin Heialelberg, New York. 2002.
21. Ambavade SD, Mhetre NA, Tate VD, Badhankar SL. Pharmacological evaluation of the extracts of *sphaeranthus indicus* flowers on anxiolytic activity in mice. Ind. J. Pharmacol. 2006; 38 Suppl 4: 254-59.
22. Achliya, G.S., Dorse, A.K.: Evaluation of CNS activity of *Bramhi Grita*. Indian J. Pharmacol. 37(1): 33-36 (2005).
23. Husain G, Mishra D, Singh P, Rao C, Kumar V. Ethnopharmacological review of native traditional medicinal plants for brain disorders. Phcog. Rev. 2007; 1(1): 19-28.
24. Al-Naggar TB, Gomez-serranillos MP, Carretero ME. Villar AM. Neuropharmacological activity of *Nigella sativa* L.extracts. J. Ethanopharmacol. 2003; 88: 63-68.
25. Medina JH, Viola H, Wolfman C, Marder M, Wasowski C, Calvo D, et al Overview – Flavonoids: A new family of Benzodiazepine receptor Ligands. Neurochem. Res. 1997; 22 Suppl 4: 419-425.
26. Zetola, M., De Lima, T.C.M., Bonaglio, D., Gonzalez-Ortega, G., Limberger, R.P., Patrovick, P.R. and Bassani, V.L. CNS activities of Liquid and spray-dried extracts from *Lippia alba*-verbenaceae. J. Ethanopharmacol., 82(2-3): 207-215 (2002).