Psychopharmacological profile of hydro-alcoholic extract of *Euphorbia neriifolia* leaves in mice and rats

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The leaf extract of *E. neriifolia* significantly reduced apomorphine-induced stereotypy in mice at all doses (100, 200, 400 mg/kg body weight) in mice and rats and was devoid of cataleptic effect thereby, suggesting specific dopaminergic receptor modulating activity. The extract (400 mg/kg) potentiated pentobarbitone-induced hypnosis. It showed protection against maximal electro-shock-induced convulsion at 400 mg/kg. *E. neriifolia* leaf extract had anxiolytic action at 400 mg/kg by increasing the percentage of time spent in open arm in elevated plus-maze. The extract did not reverse scopolamine-induced amnesia on elevated plus-maze. It increased transfer latency at 200 and 400 mg/kg and also in combination with scopolamine. These results indicated anti-anxiety, anti-psychotic and anti-convulsant activity of *E. neriifolia* leaf extract in mice and rats. Phytochemical study showed the presence of steroidal saponin, reducing sugar, tannins, flavonoids in the crude leaf extract.

Keywords: Anxiolytic; Anti-psychotic, anti-convulsant, *Euphorbia neriifolia*, Steroidal saponin

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There are over 1500 species of Euphorbias in the world ranging from annual weeds to trees. Methanolic extracts of *Mallotus peltatus (Geist)* Muell Arg.ver leaf, *Tragia involucrate* root and *Euphorbia calyprate* root cause significant reduction in general behaviour profile, potentiate phenobarbitone-induced sleeping time and reduction in spontaneous motor activity. *Euphorbia esula* a leafy spurge in Western North America produces food taste aversion to sheep that relates to their reluctance to graze it, suggests its activity on hypothalamic-pituitary-adrenal axis increasing cortisol levels. *Euphorbia neriifolia* leaves are used as aphrodisiacs, diuretic, bronchitis, bleeding piles and in ano-rectal fistula. The tribal population of Chattishgarh region uses the milky latex as an ingredient of aphrodisiac mixture. The present study was undertaken to find out the possible actions of *Euphorbia neriifolia* leaves on central nervous system in rats and mice.

Materials and Methods

**Plant material**—Leaves of *Euphorbia neriifolia* Linn. were collected from Hoshangabad, India, in the month of September 2003. The plant was identified with the help of available literature and authenticated by Dr A P Shrivastava, Principal, Pandit Khushilal Sharma Govt. Ayurveda College and Institute, Bhopal, India. A voucher specimen was deposited in the herbarium of department (No. 1085).

**Drugs**—The drugs used in the study were obtained from the following sources—diazepam (Ranbaxy, Dewas, India), pentobarbitone sodium and apomorphin (Sigma, St. Louis, U.S.A), scopolamine hydrobromide (Merck, U.S.A) and chlorpromazine injection (Intas, Ahmedabad). All the drugs were dissolved in water for injection (ip). Experimental animals—Swiss albino mice (weighing 18-25 g) and Wister albino rats (weighing 150-200 g) of either sex bred in Animal House facility at Department of Pharmacy, Sagar University, Sagar were used. The animals housed under standard laboratory conditions maintained at 24°±1°C and under 12:12 hr light dark cycle. Food and water were given *ad libitum*. All the experiments were performed between 0900 and 1700 hours. Ethical Committee approval was obtained before carrying out these experiments on 90 rats and 156 mice. Animals were fasted over night before the experiment and randomly divided into different groups containing six in each group.

**Preparation of hydro-alcoholic extract**—Ethanolic extract of air dried coarse powder was prepared by macerating sample (500 g) in 1.5 l of ethanol (70%) for one week. The macerated mixture was filtered through muslin cloth and evaporated at 40°C up to one third of initial volume, remaining solvent was
completely evaporated at 40°C using a rotary vacuum evaporator (Superfit, India). The residue was designated as hydro-alcoholic extract and used for further studies. Doses at 400, 200 and 100 mg/kg were selected (ranges from 1/6 to 1/15 of LD₅₀) based on the preliminary study conducted at our laboratory. Crude extract was suspended in 2% of carboxymethyl cellulose prepared in distilled water and administered orally to the experimental animals 45 min prior to the experiment.

**Effect of E. neriifolia leaf extract on central nervous system**

**Effect on pentobarbitone-induced hypnosis in mice**—Onset of sleep (loss of righting reflex) was noted and duration of sleep measured, that is the period between loss of righting reflex and its revival after pentobarbital (45 mg/kg, ip) administration.

**Effect on spontaneous motor activity in mice**—Spontaneous motor activity was measured by placing the animals individually in the digital photobeam counter (Allwin MFG and Marketing, New Delhi) for 5 min. Locomotor activity was expressed in terms of total photobeam counts per mouse per 5 min.

**Effect on motor co-ordination in mice**—Falling off time was recorded by placing the animals individually on a Rota-rod apparatus (Allwin MFG and Marketing, New Delhi) at 20 rpm and recorded as pre-test session. Those animals that stayed on the rod for not less than 3 min were selected for the test session.11

**Screening of psychotropic action of E. neriifolia leaf extract**

**Anti-anxiety activity on elevated plus-maze in mice**—The elevated plus-maze consisted of two open arms (16 × 5 cm) and two enclosed arms (16 × 5 × 12 cm) with an open roof, elevated to a height of 25 cm12,13 as validated by Lister12. During the 5 min test session, the following parameters were noted—(i) preference of open or enclosed arm as the first arm entry, (ii) number of entries the animal made in open and enclosed arms, and (iii) total time spent in each arm.

**Taming effect on apomorphin induced stereotype in rat**—Rats were placed individually in a glass beaker (1000 ml) and intensity of compulsive behaviour i.e., continuous rearing, sniffing, licking, baring, gnawing and grooming were observed 15 min after apomorphin (2.5 mg/kg ip) administration. Intensity of stereotypy behaviour was recorded as described by Costall et al.15

**Study of condition avoidance response in rats**—Avoidance of conditioned response was examined on pole climbing apparatus (Techno, India) consisting a transparent chamber with an electric grid floor and a pole attached to the roof with a buzzer.16 Rats were trained to climb the pole at the sound of buzzer within 30 sec. Initially a shock of 20 V was given, then preceded by a buzzer for 15 sec.

**Effect on extrapyramidal system in rats**—Extrapyramidal effects on rats were measured by placing the animals individually on wooden blocks (3 or 9 cm high) after test drug and chlorpromazine (3 mg/kg, ip) administration. Catatonic effects were observed and cumulative cataleptic score at 60 min was calculated.16

**Measurement of transfer latency on elevated plus-maze in mice**—Measure of transfer latency (TL) on elevated plus-maze was essentially same as described by Itoh et al.17 In the first trial the mouse was allowed to explore the maze for 20 sec after the measurement of TL. Transfer latency was the time taken by the animal to fully enter (with all four paws) any of the enclosed arms from the open arm end. TL measured on 1st and 2nd day served as parameters for acquisition and retrieval, respectively. Test drug and scopolamine (0.3 mg/kg ip) was given 45 and 30 min before the first trial, respectively.

**Screening of anticonvulsant activity of E. neriifolia leaf extract in rats**—Maximum current of 150 mA for 0.2 sec duration was employed on rats using eye electrode of electro convulsimeter (Techno, India). Test drug was given to the animals 1 hr before electro shock and time taken for each phase observed.

**Statistical analysis**—Experimental data was analyzed using one-way ANOVA followed by Turkey-Kramer multiple comparison test. Difference at P < 0.05 was considered statistically significant.

**Results and Discussion**

Crude plant extract was subjected to qualitative phytochemical investigation following the methods of Libermann’s Burchard Test18 for steroidal sapogenins, Benedict’s Test and Fehling’s Test19 for reducing sugar, Lead Acetate Test and Vanillin-hydrochloride test20 for tannins and Shinoda test and Alkaline reagent test21 for flavonoids.

E. neriifolia leaf extract was found to be mild depressant on central nervous system at higher doses. E. neriifolia leaf extract at 400 mg/kg dose potentiates...
pentobarbital-induced duration of sleep. Leaf extract did not have any motor incoordination or ataxia on muscle grip performance in mice in rota rod test and showed statistically insignificant reduction in locomotor activity. The elevated plus-maze introduced by Lister for mice is based on the apparent natural aversion of rodent to open and high spaces which forms the basis for its use in the measurement of anxiety as well as short-term memory. E. neriifolia at 400 mg/kg dose exhibited pronounced antianxiety activity by significantly increasing preference to open arm per cent number of open arm entries and per cent time spent in open arm as in Fig. 1. The results of the present study showed that the mice spent a significantly higher time in the open arm and also entered them more frequently signifying the anti-anxiety activity.

E. neriifolia leaf extract significantly reduced apomorphine induced stereotype in mice at all the tested dose level compared to apomorphine (Fig. 2) suggesting that the extract might have dopamine receptor modulating activity. Leaf extract did not block conditioned or unconditioned response. Leaf extract did not show catatonic effect, as scoring of leaf extract at 200 and 400 mg/kg doses were 0.41 ± 0.11 and 0.82 ± 0.68, respectively in comparison to chlorpromazine 8.58 ± 2.18. E. neriifolia leaf extract caused significant potentiation of scopolamine induced amnesia at 200 and 400 mg/kg on elevated plus-maze employed for the evaluation of short-term memories as shown in Table 1. Transfer latency on second day was significantly higher in co-administered group suggesting lack of retrieval of memory. These findings suggested that the extract

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**Fig. 1**—Antianxiety effect of E. neriifolia leaf extract on elevated plus-maze in mice. [Data expresses mean ± SEM. *P<0.05, **P<0.01, ***P<0.001 as compared to control. ns = not significant as compared to diazepam. Ctrl – control, Dia – diazepam.]

**Fig. 2**—Effect of E. neriifolia leaf extract on apomorphine induced stereotypy behaviour in mice. [Data expressed as mean ± SEM, **P<0.01, ***P<0.001 as compared to control values. ns = not significant as compared to Apomorphin group. ANOVA, F-ratio (5,30) = 32.199 for cumulative score in 15 minutes. Apo – Apomorphin]
might have interaction with cholinergic system or with central norepinephrine as depletion of which in striatum coincide with retrieval process. E. neriifolia leaf extract at 400 mg/kg dose significantly reduced tonic (both flexor and extensor), clonic convulsion phase time and stupor phase.

In conclusion, the psychopharmacological profile suggested that E. neriifolia leaf extract exhibited an anxiolytic action without affecting motor coordination and spontaneous activity. The finding of the earlier workers also substantiates CNS depressant effect of plants of Euphorbiaceae family.

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References