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ANXIOLYTIC ACTIVITY OF LEAVES EXTRACTS OF *CORDIA MYXA* L. IN MICE AS EXPERIMENTAL MODELS OF ANXIETY.

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ABSTRACT

Cordia myxa L. belonging to family Boraginaceae has been used traditionally for treatment of various neuro disorders. Yet, the plant has never been subjected to systematic biological investigation. In the present investigation, various extracts of *Cordia myxa* leaves were prepared by successive Soxhlet extraction and were evaluated for anti-anxiety activity in mice using elevated plus maze test (EPMT) and light and dark test (L and DT). Each extract were administered orally in three different doses *i.e.* 100mg/kg, 200mg/kg and 400mg/kg, and the anti-anxity effect was compared with standard drug diazepam (2.0mg/kg, orally). Among all the extracts, only methanol extract exhibited significant anti-anxiety activity at a dose of 200 mg/kg with respect to control as well as standard drug.

KEYWORDS: *Cordia myxa*; Boraginaceae; Anxiolytic; Diazepam; Elevated plus maze.

INTRODUCTION

Nature has bestowed India with an enormous wealth of medicinal plants. Therefore, India has been referred to as the medicinal garden of the world. Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. In the western world, as the people are becoming aware of the potency and side effects of synthetic drugs, there is an increasing interest in the natural product remedies with a basic approach towards the nature.[1] Traditional medicines have served as a source of alternative medicine, new pharmaceuticals and healthcare products.

Anxiety affects most of the population nearly one-eighth of the total population world-wide.[2] Benzodiazepines, being major class of compounds used for treatment of anxiety[3], present a narrow margin of safety between the anxiolytic effect and unwanted side effects, has prompted researchers to evaluate new compounds specially plant based drugs having less undesirable effects.[4] Plants like *Valeriana officinalis*, *Nardostachys jatamansi*, *Withania somnifera* and *Panax ginseng* have been used extensively in various traditional systems of therapy because of their adaptogenic and psychotropic properties. Inclusion of these well established CNS affecting plants in the arsenal of modern therapeutics has revived the faith of researchers in the plants.[5]

Cordia myxa L. (Boraginaceae) is tree of tropical and subtropical regions, commonly known as Lasaura /Lasura. It is a medium sized tree with short crooked trunk, leaves simple, entire and slightly dentate, elliptical-lanceolate to broad ovate with round and cordate base, flower white, fruit drupe, yellowish brown, pink or nearly black when ripe with viscid sweetish transparent pulp surrounding a central stony part.[6] It grows in sub-Himalayan tract and outer ranges, ascending up to about 1500m elevation. It is used as immunomodulator, antidiabetic, anthelminitic, diuretic and hepatoprotective in folklore medicine.[7] Despite a long tradition of use, no work has been carried out to justify its traditional claims, specially, CNS depressant properties. Thus, the goal of the present study was to investigate the anti anxiety activity of various extract of *Cordia myxa* leaves by elevated plus maze test (EPMT) and light and dark test (L and DT).

MATERIALS AND METHODS

Plant Material

Leaves of *Cordia myxa* L. were collected from the Herbal Nature Park, Forest Division, Chuharpur, Yamuna Nagar and was authenticated at National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. Fresh leaves were washed under running tap water, air dried in shade and then homogenized to make fine powder. The voucher specimen of the leaves was preserved in the herbarium of Department of Pharmacognosy, M.M. College of Pharmacy, Mullana, Ambala.

Drugs and Chemicals

Diazepam used as a standard drug was obtained from Ranbaxy Laboratories Ltd., Poanta Sahib (HP) as a gift sample for evaluation of anti-anxiety activity. Aqueous solution of Tween 80 (5%) was used as vehicle for preparing the suspension of various test doses of

different extracts. The solvents Petroleum ether (60-80°C), chloroform and methanol, were employed for extraction of the plant material are of LR grade.

Preparation of crude extracts

Air dried leaf powder of *Cordia myxa* L. (100g) was subjected to successive Soxhlet extraction by solvents in increasing order of polarity *viz.* petroleum ether (60-80°C), chloroform, methanol and water. Before each extraction the powdered material (marc) was air dried in shade. Each extract was concentrated by using Buchi rotary vacuum evaporator (Gupta scientific store, Ambala) and then evaporating to dryness on the water-bath. All the extracts were weighed and percentage was calculated in terms of the air-dried weight of the plant material and preserved in vacuum desiccators.

Phytochemical Screening

All the extracts were dissolved in respective solvents and were screened for different classes of phytoconstituents.[8] Table 1 shows the results of phytochemical screening of various extracts of P. nigricans aerial parts.

Test Animals

Swiss albino mice (25-30 gm) of either sex were procured from the animal house of M.M. College of Pharmacy, Mullana (Ambala) and were housed in polypropylene cages with paddy house bedding under standard laboratory conditions with 12 h dark and 12 h light cycle. The animals were given standard laboratory feed in the form of dry pellets and water *ad libitum*. The experiments were performed in a sound proof laboratory between 8.00 am to 11.00 am. All the experimental procedures and protocols used in this study were approved by the Institutional Animal Ethics Committee of M.M. College of Pharmacy, Mullana (Ambala).

Treatments

The test animals were randomly divided into six groups (I-VI) of six mice each. Group I was served as control which was given only vehicle, in a dose of 0.25ml. Group II was given standard drug diazepam (2mg/kg, orally), suspended in the vehicle. Group III-VI were treated as test groups and were given petroleum ether (60-80°C), chloroform, methanol and aqueous extracts of *Cordia myxa* L. leaves respectively, at different doses *viz.*100, 200 and 400mg/kg. All the test solutions, standard drug and control were administered orally 45 minutes prior to elevated plus maze test (EPMT) and light and dark test (L and DT).

Elevated plus maze test

The elevated plus-maze test is well established animal model for testing anxiolytic drugs. The model was chosen because it is effective, cheap, simple, less time consuming, requires no preliminary training for the mice and does not cause much discomfort to the animals while handling. Elevated plus-maze apparatus comprised of two open arms (16 x 5 cm) and two closed arms (16 x 5 x 12 cm) having an open roof, with the entire maze elevated (25 cm) from the floor.[9] Animals were fasted 18 h prior to the experiment. Extracts of *Cordia myxa* L. were administered orally using a tuberculin syringe fitted with oral canula. The dose administration schedule was so adjusted that each mouse was having its turn on the elevated plus-maze apparatus 45 min after the administration of the dose. The animals were placed individually in the centre of the elevated plus-maze, with its head facing towards open arms and the stop watch was started and following parameters were noted for 5 min. a) First preference of mice to open and closed arm. b) Number of entries in open and closed arms (an arm entry defined as the entry of four paws into the arm). c) Average time each animal spends in each arm (average time = total duration in the arm/number of entries). During the entire experiment, precaution was taken to ensure that no external stimuli could invoke anxiety in the animals. Similar observations were recorded for the standard group (Diazepam 2 mg/kg) as well as the control group (vehicle, 0.25 ml).

Light and Dark Exploration test

The light/dark apparatus consisted of a rectangular box (46×27×30 cm), divided into one small (18× 30 × 30 cm), one large (27×30 × 30 cm), with an opening door (8 × 8 cm) located in the centre of the partition at floor level. The small compartment was painted in black, whereas the large compartment was painted in white and brightly illuminated with 60 W cold light source. Each mouse was individually placed in the centre of the light compartment (facing towards the door). During 5-min test period, number of the crossing from light to dark compartment and time spent in light zone were noted.

Statistics

The results have been expressed as mean ± standard error of mean (S.E.M.). The statistical analysis of data was done using the one way analysis of varianc (ANOVA) followed by Turkey's multiple range test. A probability level (p value) less than 0.05 is considered statically significant.

RESULTS AND DISCUSSION

The percentage yields (w/w) of various extracts viz. petroleum ether (60-80°C), chloroform, methanol and water extracts were 3.90, 4.30, 12.50 and 4.80 respectively. **Table 1** shows results of phytochemical screening of various extracts of *Cordia myxa* L. leaves. The mean number of entries and time spent by mice in open arms and in light compartment after oral administration of three doses *viz.* 100, 200 and 400 mg/kg of all extracts are given in **Table 2**. The results obtained from the EPM model and L and DT, indicates that only methanolic extract of *Cordia myxa* L. leaves markedly increased the percentage of average time spent by the animals in the open arms of EPM and in light compartment of L and DT. The anxiolytic effect of the plant extract was more prominent at 200mg/kg and doses higher or lower than this did not exhibit significant anxiolytic effects. The lack of significant anxiolytic effects at doses higher than 200mg/kg may be due to strong sedative properties of the plant extracts. None of other extracts showed anti-anxiety activity at any dose tested.

Table 1: Phytochemical screening of various extracts of *Cordia myxa* L. leaves.

Plant Constituents	**Extracts**				
Test	**Reagent Used**	**Pet ether extract**	**Chloroform extract**	**Methanol extract**	**Aqueous extract**
Carbohydrates	Molisch Reagent	-	-	-	+
	Feheling Solution	-	-	-	-
	Barfoed reagent	-	-	-	-
Proteins and amino acids	Millions Reagent	-	-	-	-
	Ninhydrin Reagent	-	-	-	-
Alkaloids	Hager's Reagent	-	+	-	-
	Wagner Reagent	-	+	-	-
	Mayer's Reagent	-	+	-	-
Phenolic Compound & Tannins	Lead acetate Test	-	-	+	-
	$FeCl_3$ Solution	-	-	+	-
	Bromine Water Test	-	-	+	-
Flavonoids	Shinoda Test	-	-	+	-
	Lead Acetate Test	-	-	+	-
Saponins	Foam Test	-	-	+	+

In addition, Phytochemical screening of *Cordia myxa* L. leaves showed the presence of alkaloids, Phenolic compound, Tannins, flavonoids and saponins. Flavonoids are proven to possess remarkable anti-anxiety activity in various studies. Further, the anxiolytic effect of flavonoids has been attributed to its effect on central nervous system[10-11] and benzodiazepine receptors.[12-13] Therefore, flavonoids of methanolic extract of *Cordia myxa* L. may be responsible for the anti-anxiety activity.

Table 2 Anti Anxiety Activity of Various Extract of *Cordia myxa* L. on EPM and L and DT.

Sr. No.	Treatment	Dose mg/kg	Mean time spent in open arms (sec ± SEM)	Mean time spent in light comp. (sec ± SEM)
I.	Control (Vehicle)	0.25 ml	10.308 ± 0.572	79.8 ± 8.4
II.	Standard (Diazepam)	2	23.217 ± 0.427	157.39 ± 8.2
III.	Petroleum Ether Extract	100 200 400	10.976 ± 0.449 11.102 ± 0.607 11.142 ± 0.304	86.3 ± 7.3 94.7 ± 9.1 113.7 ± 3.52
IV.	Chloroform Extract	100 200 400	11.7 ± 0.754 12.767 ± 1.496 11.192 ± 1.018	12.7±0.754 12.767±1.496 11.192 ± 1.018
V.	Methanol Extract	100 200 400	17.15± 0.749 22.102 ± 0.307 19.142 ± 0.604	106.0 ± 10.3 135.35 ± 7.3 120.63 ± 10.1
VI.	Aqueous Extract	100 200 400	9.65 ± 0.834 11.05 ± 0.992 11.785 ± 1.165	81.23 ± 6.3 98.76 ± 7.4 112.6 ± 8.1

Values are Mean ± SEM (n=6). *p<0.05 compared to Control; One way ANOVA followed by *post hoc* Tukey's multiple range test. a= $p < 0.05$ vs. Control (Vehicle); b= $p < 0.05$ vs. diazepam (Standard Drug).

However, it is concluded from the present study that mathanolic extract of *Cordia myxa* L. leaves exhibits anti-anxiety activity at the dose of 200 mg/kg in mice using EPM and L and DT model of anxiety. In addition, further studies are required to identify the phyto-constituent responsible for the observed anxiolytic effect of methanol extract and to explain anxiolytic mechanism.

REFERENCES

1. Verma S, Singh SP. Current and future status of herbal medicines. Veterinary World, 2008; 1(11): 347-350.
2. Eisenberg DM, Davis RB, Ettner SL, Appel S, Wilkey S, Van Rompay M, Kessler RC. Trends in alternative medicine use in the United States, 1990–1997: results of a follow-up national survey. JAMA, 1998; 280: 1569-1575.
3. Lader M, Morton S. Benzodiazepine problems. Br J Addict, 1991; 86: 823-828.
4. Griffiths RR, Ator NA, Roache JD, Lamb RJ. Abuse liability of triazolam: experimental measurements in animals and humans. Psychopharmacol Res, 1987; 3: 83-87.

5. Bloom FE, Kupfer DJ. Psychopharmacology: The Fourth Generation of Progress. Raven Press, New York., 1994; 1301-1310.
6. The Wealth of India, Raw Materials, A Dictionary of Indian Raw Material and Industrial Products, Vol 9, Council of Scientific & Industrial Research, New Delhi., 1950; 293-295.
7. Kirtikar KR, Basu BD, Indian Medicinal Plants, Edition 11, Vol 3, Orient enterprises, 1935; 1029-1030.
8. Trease EG and Evans WC. Textbook of Pharmacognosy, 12th edn. Alden Press, Singapore., 1983.
9. Kulkarni SK, Reddy DS. Animal Behaviour Models for Testing Anti-anxiety Agents. Methods and Findings in Experimental and Clinical Pharmacology., 1996; 18: 219-240.
10. Al Awadi FM, Srikumar TS, Anim JT, Khan I. Anti-inflammatory effects of Cordia myxa fruit on experimentally induced colitis in rats. Nutrition, 2001; 17(5): 391–396.
11. Paladini AC, Marder M, Viola H, Wolfman C, Wasowaki C and Medina JH. Flavonoids and the central nervous system: from forgotten factors to potent anxiolytic compounds. J. Pharm. Pharmacol., 1999; 51: 519-526.
12. Soulimani R, Younos C, Jarmouni S, Bousta D, Misslin R, Mortier F. Behavioural effects of Passiflora incarnata L. and its indole alkaloid and flavonoid derivatives and maltol in the mouse. J Ethnopharmacol., 1997; 57(1): 11-20.
13. Medina JH, Viola H, Wolfman C, Marder M, Wasowski C, Clavo D, Paladini AC. Neuroactive flavonoids: new ligands for the benzodiazepine receptors. Phytomed., 1997; 5: 235-243.
14. Wolfman C, Viola H, Paladini A, Dajas F, Medina JH. Possible anxiolytic effects of chrysin, a central benzodiazepine receptor ligand isolated from Passiflora coerulea. Pharmacol Biochem Behav, 1994; 47: 1-4.