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Evaluation of antianxiety activity of "*Mimosa pudica*" leaves

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Abstract
One of the most crucial aspects of living a healthy life is human health. Although many diseases are found in human being, however the anxiety disease, which is a psychiatric disorder, is most commonly found in human being. To treat this anxiety disease, many herbal plants plays very significant role.
The purpose of this study was to evaluate the anxiolytic action of hydro-alcoholic extract of laves of the *Mimosa Pudica* plant in mice animals.
To evaluate the anxiolytic action of this plant in mice, various animal models like elevated plus maze, light and dark test, social interaction test and hole board test were used. To obtained hydro alcoholic extraction of leaves of *Mimosa Pudica* plant, soxhlet extraction method was used, in which air dried leaves were used. Preliminary phytochemical investigations of abve extraction were also carried out.
The standard drug diazepam (1 mg/kg) was used as anxiolytic agent and the doses of hydro-alcoholic extract of leaves of *Mimosa Pudica* plant were p.o. 200, and 400 mg/kg.
Results recommended that hydro alcoholic extract of leaves of *Mimosa Pudica* at 400 mg/kg dose produced antianxiety effect consequences nearly much like diazepam. The data indicates that the hydro alcoholic extract of *Mimosa Pudica* leaves has more anti-anxiety action than the control.
Further studies are needed to identify the anxiolytic mechanism(s) and the phytoconstituents responsible for the observed central effects of the hydro-alcoholic extract of *Mimosa Pudica*.

Keywords: *Mimosa pudica,* elevated plus maze, light and dark test, social interaction test, hole board test

Introduction
Anxiety affects one-eighth of the world's population and has grown in importance as a study topic in psychopharmacology over the last decade. Alternative medicine and plant-based treatments that impact the "mind" are gaining popularity. Motor tension, sympathetic hyperactivity, apprehension, and vigilance syndromes are all symptoms of anxiety, which is a condition of excessive dread [1].
Anxiety is a condition of the Central Nervous System [2, 3]. Anxiety is a very prevalent psychological occurrence in humans [4].
Anxiety is a type of mental disorder. Anxiety is an unpleasant emotional state connected with unease, discomfort, and anxiety or fear about some definite or indeterminate future threat [5].
The anxiety can be treated by many ways, and one of them is by medication. Among them Benzodiazepines are the most often prescribed anxiety medication, despite the significant negative side effects such as drowsiness, muscular relaxation, ataxia, dementia, ethanol and barbiturate potentiating and tolerance [6].
Various sorts of herbal medicines are used as anxiolytic medication in numerous elements of the world. Self-administration of herbal medicines is among the foremost widespread of the alternative therapies that additionally embody massage therapy, megavitamins, and homeopathy. Plants have long been accustomed to treat central nervous system (CNS) disorders. Folks medicines have specific values, for example, plants that "calm down", tranquilize, and lift mood, similar to passiflora coerulea, Valeriana officinalis, Matricaria recutita, Jatropa cilliata, salvia guaranitica, tilia tormentosa, and Tilia europeae. One another plant is *Mimosa Pudica*, belonging to family *Mimosaceae* is a wild tropical plant. It has been identified as lajjalu in Ayurveda.
The herb has been used traditionally in the treatment of many diseases. It has been used as diuretic activity [7], antidiabetic activity [8], wound healing activity, analgesic and anti-inflammatory activity, antimalarial activity, antidiarrhoeal activity, anti-oxidant activity, antimicrobial activity [9], anticonvulsant activity [10], antidepressant activity [11], muscle relaxant activity [12], anxiolytic activity [13] etc. M. pudica is known to possess sedative, emetic, and tonic properties etc. [7].
The aim of the current study was to explore the anti-anxiety activity of hydro alcoholic extract of leaves of Mimosa Pudica plant using totally various animal models like EPM, light and dark test, hole board test and social interaction test in mice.

Materials and Methods

Plant material

Collection and authentication of plant materials

The leaves of *Mimosa pudica* belonging to the family *Mimosaceae* were collected in the month of January 2020 from the local areas of Jodhpur district, Rajasthan, India. The plant material was identified and authenticated by Sanjay Mishra, Scientist – C & officer in-charge, Government of India, Ministry of Environment & Forest, Botanical Survey of India, Arid zone Regional Centre, Jodhpur (Reference No.BSI / AZRC/Tech. /2019-20(pl. ld./650)).

Animals and Ethical approval

Swiss albino mice (females; 20–30 gm) were utilized in the current study. They were provided usual diet and water ad libitum and were exposed to 12-h light and 12-h dark cycle. All animals were acclimatized to laboratory conditions prior to the experiments. Experimental protocol was approved by Institutional Animal ethics committee of B. N. University, Udaipur. Care of the animals was taken as per tips of the Committee for the aim of Control and Supervision of Experiments on Animals (CPCSEA).

Preparation of Hydroalcoholic Extract

Leaves of *Mimosa pudica* were collected, washed completely in water, shredded and air dried at 20–25°C for a month. Dried leaves were fine-grained in grinder to get a fine powder. The powder obtained was placed in thimble of soxhlet apparatus. The solvent used was 50% water and 50% alcohol. The extracted material was subjected to phyochemical analysis.

Phytochemical analysis

Qualitative phytochemical screening of hydro alcoholic extracts of Mimosa Pudica were carried out as per standard method [14].

Drugs

Diazepam drug was used as a reference standard. It had been diluted with saline to the specified strength before use.

Completely different concentrations of the Mimosa pudica extract were made by serial dilution from a stock solution of 100 mg/ml of the extract in sterile water. All the solutions were prepared freshly on check days and administered intraperitoneal (i.p.) in a volume of 0.1 ml/10 g weight of mice.

Antianxiety Activity

The hydro alcoholic extracts of *Mimosa Pudica* leaves were tested for antianxiety activity using elevated plus maze, Light-dark test, Social interaction test and Hole board test.

Elevated Plus Maze

The plus maze equipment consisted of two open arms, measuring (30 x 5 cm x 0.2 5 cm) and two closed (30 cm x 5 cm x 15 cm) arms, linked to a central platform (5 × 5 cm). The maze was extended to a top of 50 cm above the floor. Each mice was positioned individually on the middle of increased plus maze with its head facing toward an open arm and discovered for five min to report the range of entries into open arm, closed arm and time spent in every arm [15].

Light and Dark Box Test

The equipment consisted a cuboidal box (20cm×10cm×14cm), partitioned into compartments linked through a 7.5 × 7.5 cm commencing with inside the wall among compartments. An animal was positioned in the middle of the mild compartment and was observed for 10 min for the transitions between the light and dark boxes, as well as the time spent in the light box [16].

Social Interaction Test

The social interplay area was an open topped box (22 × 15 × 12 cm). Mice have been isolated for 1 h earlier than the test. After advent to the check area, the mice were observed for 5 minutes for their cumulative time spent in genital investigation, sniffing a partner, following, grooming, kicking, biting, wrestling, hiking over and under, neck licking and boxing [17].

Hole board Test

The hole board test is intended to evaluate certain head dipping behaviours. The equipment for the hole board consisted of an open field with 16 uniformly spaced 3 cm diameter holes in the floor. Each mouse was placed in a separate corner of the board, and the animal was then allowed to go around and dip its head into the holes. Every 5 minutes, individual mice's head dips and sectional crossings were counted [18].

Experimental Protocol

Experimental animal groups used with inside the present study consisted of six mice in foyr group. Group I served as vehicle treated control, Groups. II received the standard drug diazepam (1 mg/kg i.p.) and Group III & IV received Hydro alcoholic extract p.o. at 200 and 400 mg/kg, respectively.

The animals were housed in the animal house of Bhupal Nobles College of Pharmacy near Old Station Road, Sevashra Choraha, Kumharon Ka Bhatta, Central Area, Udaipur- 313001, Rajasthan under normal laboratory conditions (India). The animals were fed a normal pellet diet, were kept on a natural light and dark cycle, and had unlimited access to food and water. Mice were exposed to EPM and light–dark check for ordinary duration (5 min & 10 mins respectively), enough to evaluate the anxiety stages in rodents [19].

Behavioral checks were carried out in impartial groups of mice. Drugs had been administered 30 min earlier than the evaluations with inside the equipment. The equipment was very well wiped clean using 5% ethanol earlier than placing every mouse in the cage.

Statistical Analysis

All the effects had been expressed as Mean ± SEM. Data had been analyzed through analysis of variance (ANOVA) and Dunnett's test in Graphpad prism 9 software. $P < 0.05$ was taken into consideration as significant.

Results

Physical properties of the extracts

The colour, texture and type of the extracts of leaves of *Mimosa Pudica* were tabulated in table 1.

Table 1: Physical properties of *Mimosa Pudica* leave Extracts

Plant Part	Type of Extract	Texture	Colour
Leaves of *Mimosa Pudica*	Hydro alcoholic	Semi-solid	Dark Brown

Phytochemical analysis

Hydro alcoholic extracts of *Mimosa Pudica* leaves revealed the presence of alkaloids, flavonoids, glycosides, tannins, and saponins after screening. Table 2 details the phytochemical components.

Table 2: Phytochemical analysis of *Mimosa Pudica* leaves extracts

S. No	Phytochemical	Hydro alcoholic extract
1.	Alkaloids	++
2.	Flavonoids	++
3.	Carbohydrates	-
4.	Glycosides	+
5.	Saponins	+
6.	Tannins	+
7.	Protein	-
8.	Amino acids	-

"+" indicates presence and "–" indicates absence of the phytochemical constituents which were screened using various identification tests.

Assessment of antianxiety activity

Elevated plus-maze model

The hydro alcoholic extracts of *Mimosa Pudica* leaves at a dosage of 400 mg/kg p.o. performed well in the elevated plus-maze test (EPM) (Table 3). It raised the amount of entries and time spent in the open arm substantially. The strength of the anti-anxiety effects 400mg/kg p.o. The pharmacokinetics of hydro alcoholic extracts of Mimosa Pudica were equivalent to those of diazepam 1 mg/kg i.p. (See Figures 1, 2, 3 and 4). Plant extract at 200 mg/kg had no vast effects on any of the parameters that have been measured at the EPM.

Table 3: Anxiolytic activity of hydro alcoholic extract of *Mimosa Pudica* plant on mice EPM test

S. No.	Group	Treatment	Number of open arm entries counts)	Number of closed arm entries (counts)	Time spent in open arm (s)	Time spent in closed arm (s)
1	Group I	Control (Saline)	6.33±0.49	15.33±0.67	68.5±0.56	231.5±0.56
2	Group II	Diazepam (1 mg/kg)	10.50± 0.43*	7.33±0.49*	181.67±0.67*	118.33±0.67*
3	Group III	Hydro-alcoholic Extract (200 mg/kg)	8.17±0.48[a]	9.5±0.43*	95.5±0.76*	204.5±0.76*
4	Group IV	Hydro-alcoholic Extract (400 mg/kg)	9.17±0.48[b]	8.33±0.49*	137.67±0.88*	162.33±0.88*

Values reported as Mean ± SEM (n=6). The data were analyzed by one way ANOVA followed by and Dunnett's test. a- P<0.05; b- P<0.01, #- P<0.001; *- P<0.0001; ns- not significant when compared with control group.

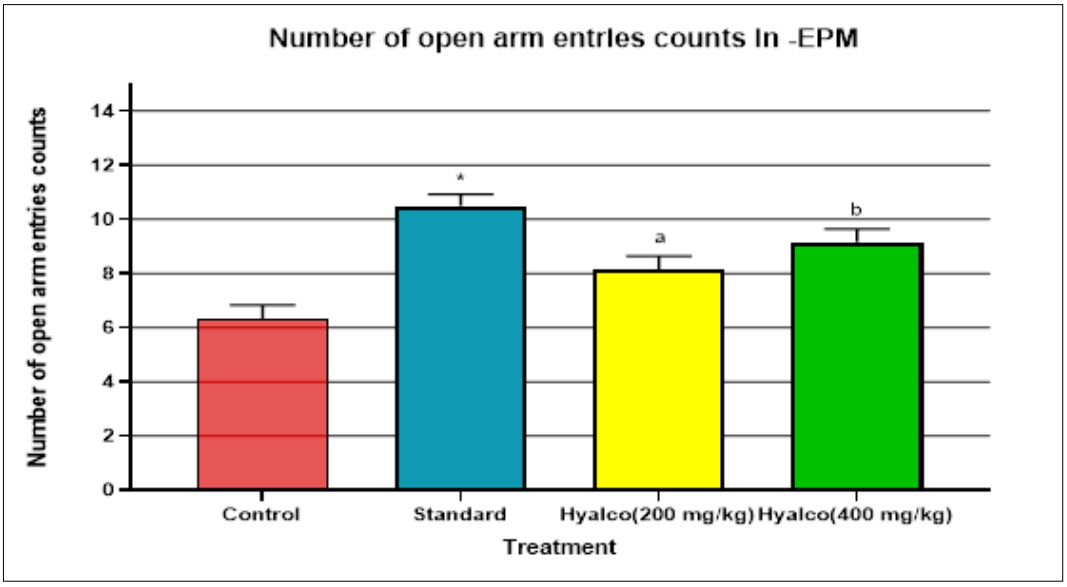

Fig 1: Result of EPM test of hydro alcoholic extract of *Mimosa pudica* leaves (number of open arm entries)

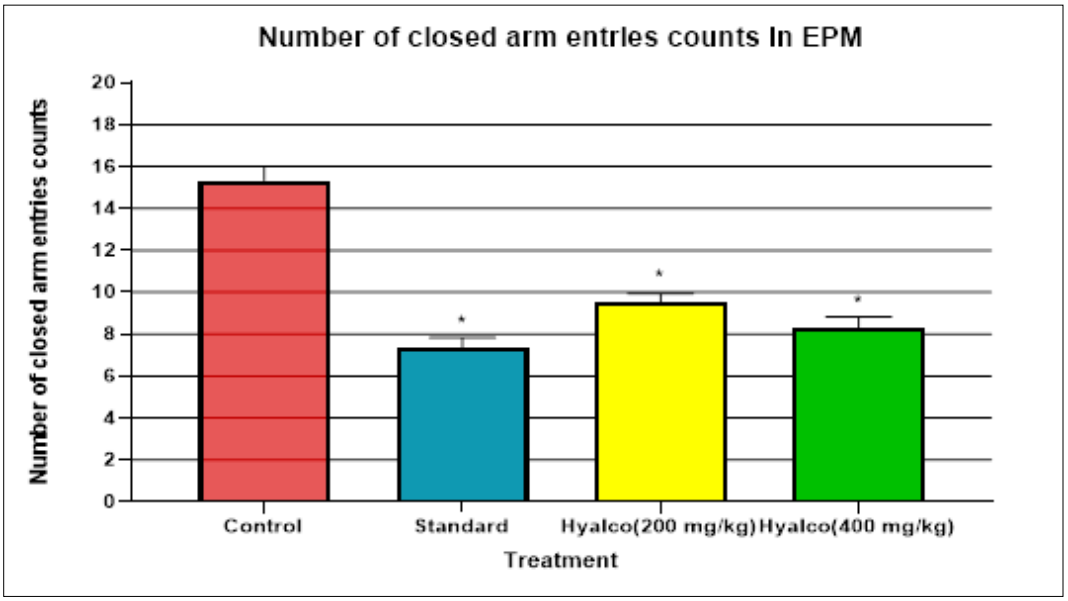

Fig 2: Result of EPM test of hydro alcoholic extract of *mimosa pudica* leaves (number of closed arm entries)

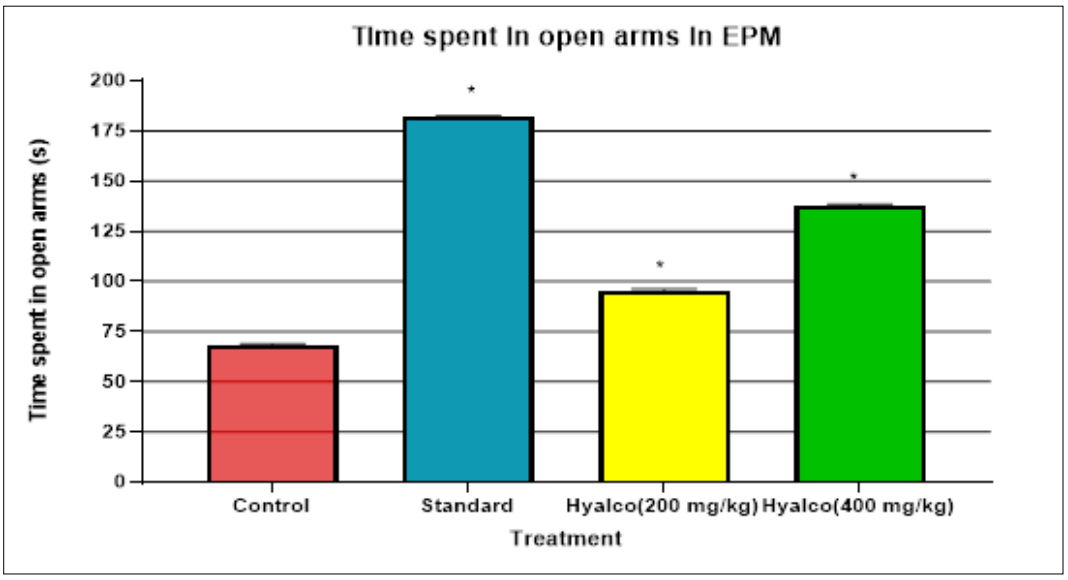

Fig 3: Result of EPM test of hydro alcoholic extract of *Mimosa pudica* leaves (time spent in open arm)

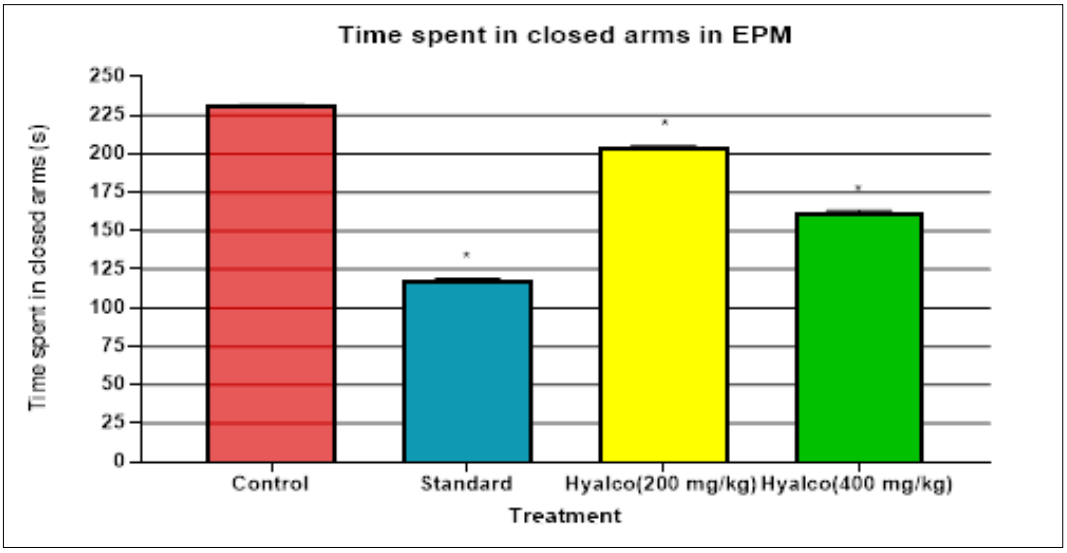

Fig 4: Result of EPM test of hydro alcoholic extract of *mimosa pudica* leaves (time spent in closed arm)

Light dark test

In light dark test, when compared to the saline-treated group, diazepam (1.0 mg/kg) substantially increased the duration spent in the light compartment and numbers of transition (P<0.001) [Table 4]. When the dose of hydro alcoholic extract of 200 and 400 mg/kg p.o. given, then the dose of 400 mg/kg p.o. resulted in significant increase in the time spent in the light compartment (P 0.05) compared to the saline-treated group of Mimosa Pudica leaf [Table 4]. At 200 mg/kg p.o., plant extract had no significant effects were assessed using the light and dark box test (See Figures 5,6,7 and 8).

Table 4: Anxiolytic activity of hydro alcoholic extract of *Mimosa pudica* plant on mice light dark test

S. No.	Group	Treatment	No of crossing in dark corner	No of crossing in open field	Time spent in Light area	No of Transition
1	Group I	Control(Saline)	16.33± 0.42	6.33± 0.33	90±0.97	3.83±0.31
2	Group II	Diazepam (1 mg/kg)	1.83± 0.31*	10.50± 0.43*	244±1.25*	8.16±0.31*
3	Group III	Hydro-alcoholic Extract (200 mg/kg)	8±0.68*	8.17±0.65[a]	156.7 ±1.75*	5.33±0.21[b]
4	Group IV	Hydro-alcoholic Extract (400 mg/kg)	5.5±0.43*	9.17±0.48[b]	204.3±1.69*	6.67±0.21*

Values reported as Mean ± SEM (n=6). The data were analyzed by one way ANOVA followed by and Dunnett's test. a- P<0.05; b- P<0.01, #- P<0.001; *- P<0.0001; ns- not significant when compared with control group.

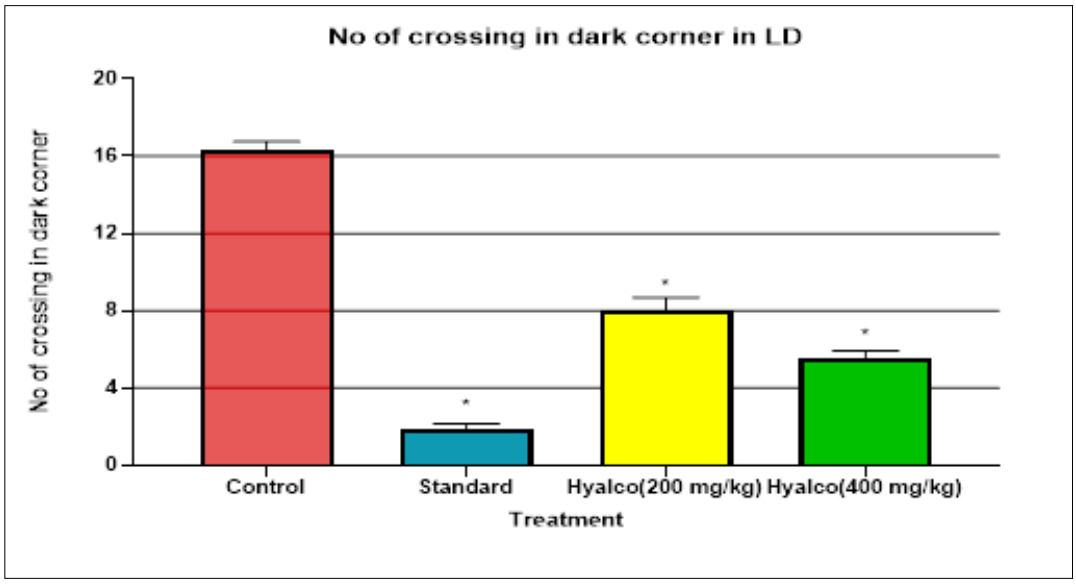

Fig 5: Result of light dark test of hydro alcoholic extract of *Mimosa pudica* leaves (no of crossing in dark corner)

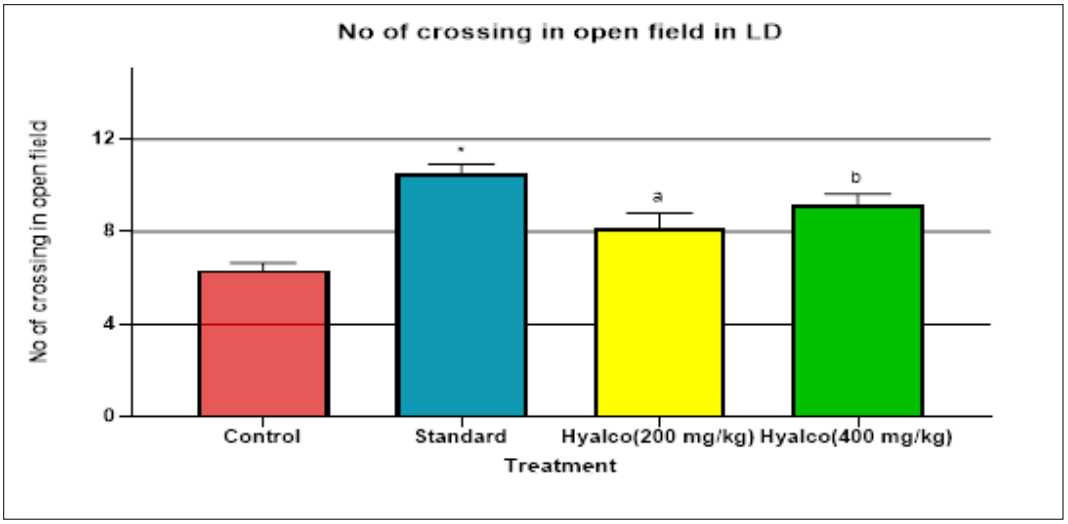

Fig 6: Result of light dark test of hydro alcoholic extract of *Mimosa pudica* leaves (no of crossing in open field)

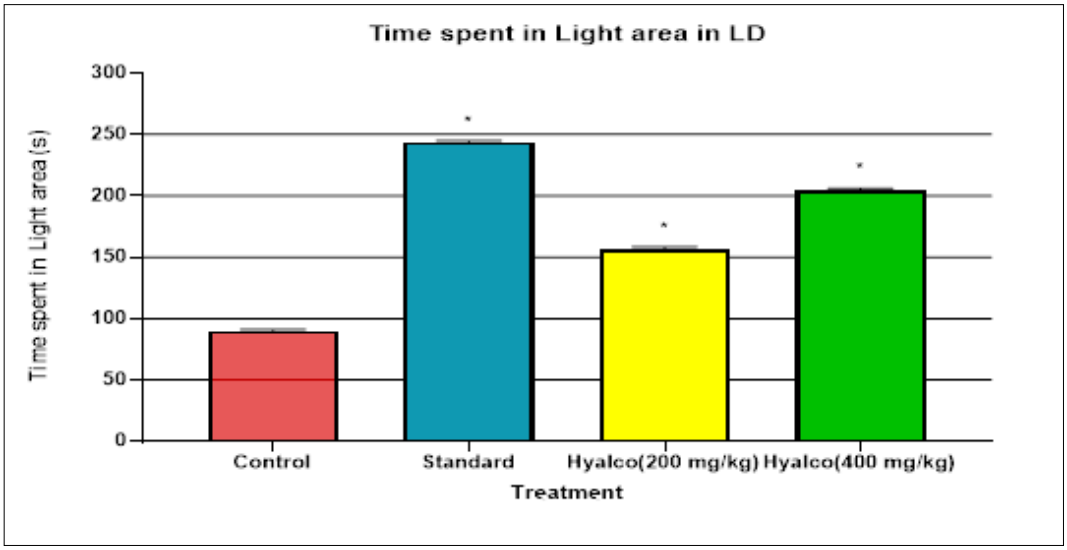

Fig 7: Result of light dark test of hydro alcoholic extract of *Mimosa pudica* leaves (time spent in light area)

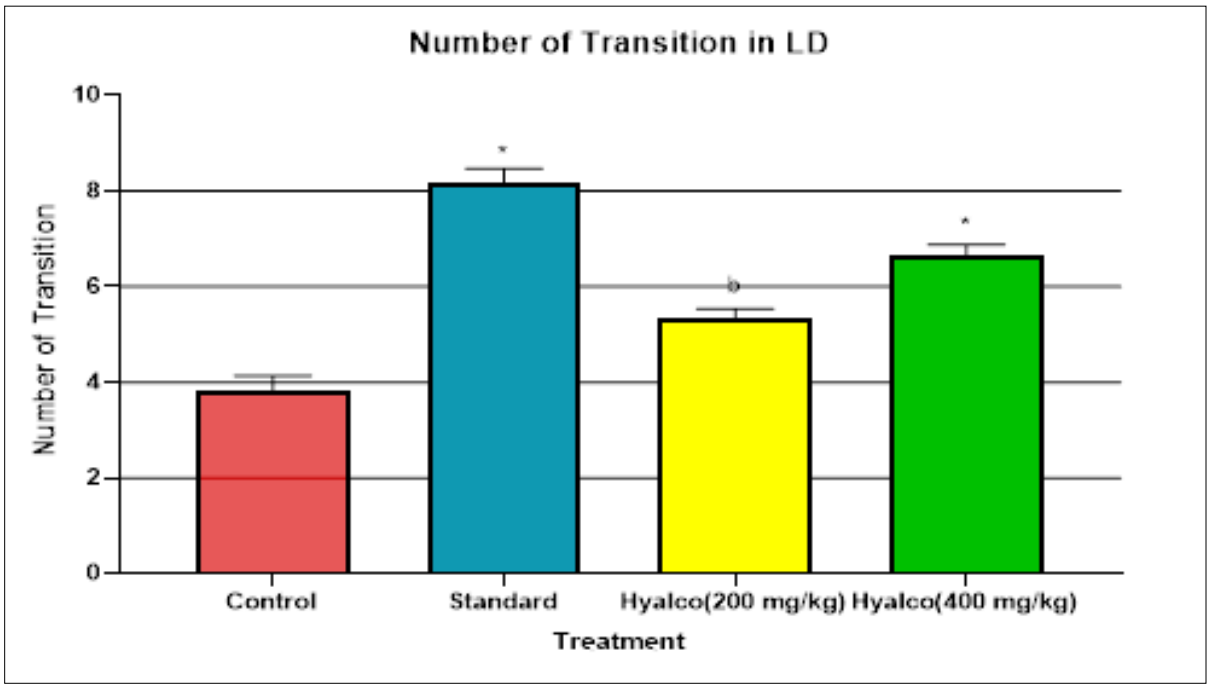

Fig 8: Result of light dark test of hydro alcoholic extract of *Mimosa pudica* leaves (no of crossing in open field)

Social interaction test

When compared to the saline-treated group, diazepam (1.0 mg/kg) substantially (P<0.001) enhanced the time spent in social contact among mice [Table 5]. The 200 mg/kg & 400 mg/kg oral dose of hydro alcoholic extract was used. At 400 mg/kg (P<0.001), *Mimosa Pudica* hydro alcoholic extract significantly enhanced the amount of time spent in social situations. In comparison to the saline-treated group [Table 5], at 200 mg/kg dose of *Mimosa Pudica*, no substantial impacts were observed. (See Figures 9)

Table 5: Anxiolytic activity of hydro alcoholic extract of *Mimosa pudica* plant on mice social interaction test

S. No.	Group	Treatment	Time spent in Social interaction
1	Group I	Control(Saline)	378.7±4.37
2	Group II	Diazepam (1 mg/kg)	554±3.29*
3	Group III	Hydro-alcoholic Extract (200 mg/kg)	429.5±3.34*
4	Group IV	Hydro-alcoholic Extract (400 mg/kg)	520.8±3.01*

Values reported as Mean ± SEM (n=6). The data were analyzed by one way ANOVA followed by and Dunnett's test. a- P<0.05; b- P<0.01, #- P<0.001; *- P<0.0001; ns- not significant when compared with control group.

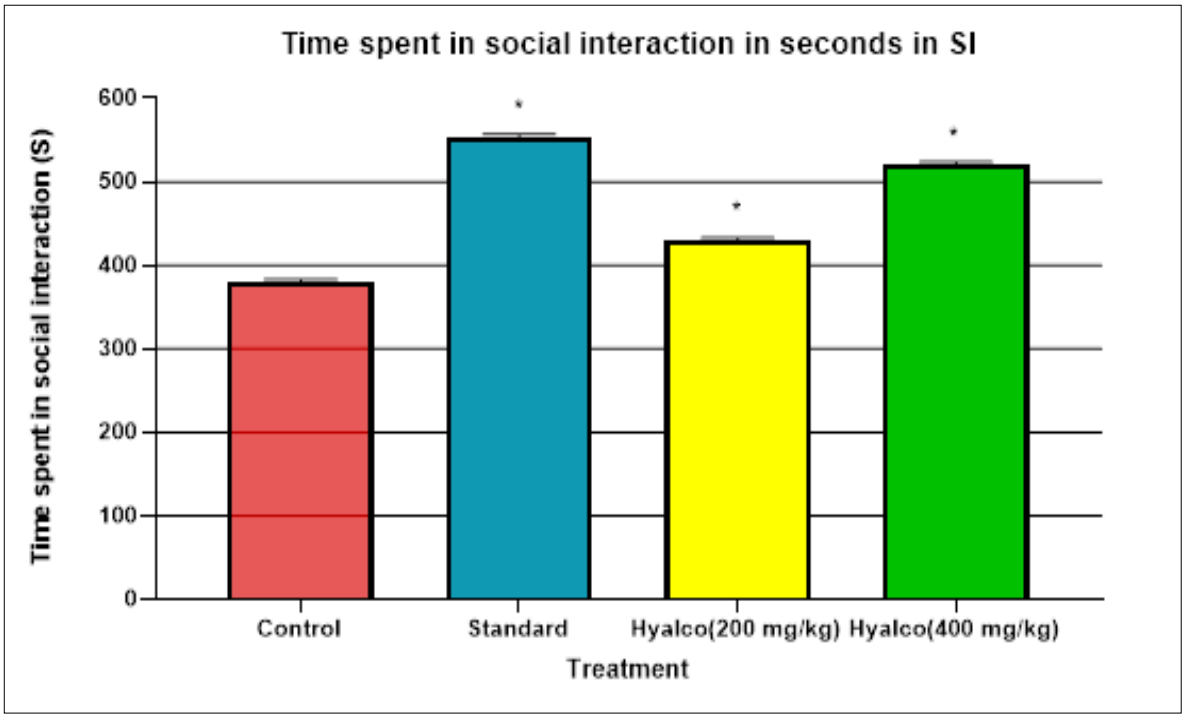

Fig 9: Result of social interaction test of hydro alcoholic extract of *Mimosa pudica* leaves (time spent in social interaction)

Hole board test

In this context, the hydro alcoholic extract of plant at dose 200 mg/kg and 400 mg/kg shown in Table 6. When compared to the control group, the hydro alcoholic extract 200 mg/kg enhanced the number of head dipping (p< 0.0001) and also the number of line crossing. The hydro alcoholic extract 400 mg/kg substantially increased the number of head dips (p< 0.0001) and also significantly increase the number of line crossings when compared to the control group and also with 200 mg/kg. (See Figures 10 and 11.)

Table 6: Anxiolytic activity of hydro alcoholic extract of *Mimosa pudica* plant on mice hole board test

S. No.	Group	Treatment	Number of head dips	No. of Line crossing
1	Group I	Control(Saline)	25.5± 0.76	37± 0.58
2	Group II	Diazepam (1 mg/kg)	46.8± 0.48*	61.5± 0.76*
3	Group III	Hydro-alcoholic Extract (200 mg/kg)	32.67±0.88*	45.33±0.67 *
4	Group IV	Hydro-alcoholic Extract (400 mg/kg)	41.67±0.67*	54.83±1.25 *

Values reported as Mean ± SEM (n=6). The data were analyzed by one way ANOVA followed by and Dunnett's test. a- P<0.05; b- P<0.01, #- P<0.001; *- P<0.0001; ns- not significant when compared with control group.

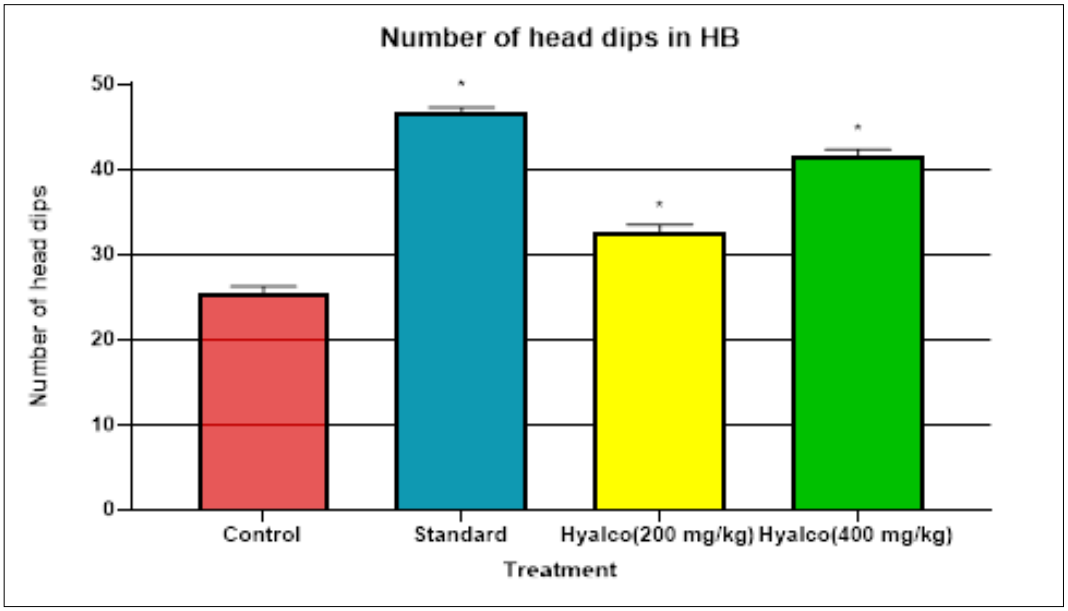

Fig 10: Result of hole board test of hydro alcoholic extract of *Mimosa pudica a* leaves (number of head dip)

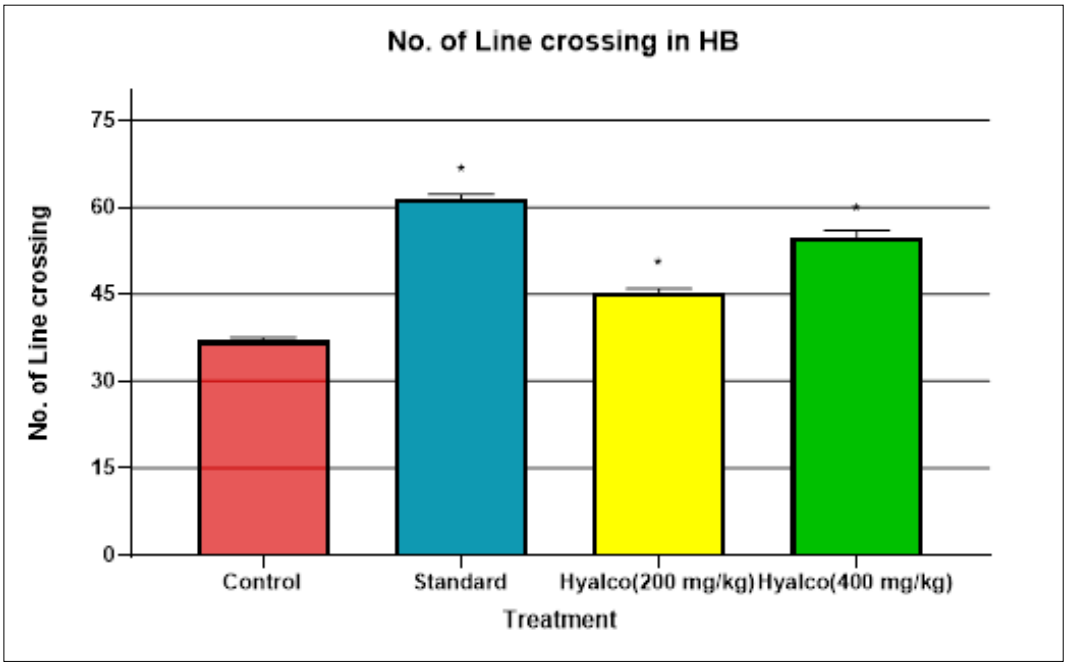

Fig 11: Result of hole board test of hydro alcoholic extract of *Mimosa pudica* leaves (number of line crossing)

Discussion

Alternative treatment options with favorable side-effect profiles, genuine benefits, and affordable costs are of interest, especially in primary care settings, because benzodiazepines have been widely utilized over the last 40 years to treat a variety of anxiety disorders. Medicinal plants are a great place to look for novel treatments for various illnesses. [20] A hydro alcoholic extract of Achyranthes aspera leaf was found to include carbohydrate, tannin, phenolic compounds, flavonoids, steroid, and coumerin. Carbohydrates, protein, glycosides, alkaloids, tannins, saponins, flavoides, lignin, flavonoids, steroids, terpenoids, and fatty acids were among the substances i dentified. [21, 22]

On the EPM, light–dark test, social interaction test and hole board test, the effects of 400 mg/kg hydro alcoholic extract of leaves of Mimosa Pudica were almost identical to those of 1.0 mg/kg diazepam.

In EPM test, when compared to diazepam, the hydro alcoholic extract of plant at dosages of 200 mg/kg and 400 mg/kg significantly increased the time spent and number of entries in open arms while decreasing the time spent and number of entries in closed arms, depending on dose.

For light dark test, the results revealed that at doses of 200 mg/kg and 400 mg/kg, a hydro alcoholic extract of a plant's leaf greatly enhanced the length of time spent and the number of transactions in the light region.

The hydro alcoholic extract at 200 mg/kg and 400 mg/kg significantly increased the length of social engagement in low light and unfamiliar conditions in social interaction test, suggesting that the plant extract has an anxiolytic effect. In the hole board test, the hydro alcoholic extract of plant leaves at doses of 200 mg/kg and 400 mg/kg

significantly increased the number of head dipping and line crossing, demonstrating that the plant extract has anxiolytic properties.

Conclusion

Based on the findings, we can conclude that hydro alcoholic extracts of Mimosa pudica plant at doses of 200mg/kg and 400mg/kg p.o. had significant anxiolytic activity comparable to the standard drug diazepam (1 mg/kg; i.p.). Further dose dependant action was found as 400mg/kg p.o has greater anxiolytic activity as compared to 200mg/kg p.o. dose in all experimental models.

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Conflicts of Interest

All authors declare that there are no conflicts of interest.

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