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EVALUATION OF ANTI-ANXIETY ACTIVITY OF *ACHYRANTHES AASPERA* LEAVES

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Keywords:

Achyranthes aspera, Elevated plus maze model, Light dark model, Social interaction model and hole board technique

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ABSTRACT: Anxiety is one of the main psychiatric disorders worldwide. Furthermore, it is difficult to find out the patients suffering from various kinds of anxiety diseases. Numerous herbal plants have been utilized to treat the anxiety disease in traditional medical systems for thousands of years because of their safety, efficacy, and effectiveness. The present study was designed to evaluate the anti-anxiety activity of the hydroalcoholic extracts of the leaves of the *Achyranthes aspera* plant in rodents like mice. The anti-anxiety activity was assessed in swiss albino mice. The leaf of the *Achyranthes aspera* plant was air-dried and was reduced to coarse powders. The Soxhlet extraction method was used for this anti-anxiety activity. Qualitative phytochemical screening of hydroalcoholic extracts of *Achyranthes aspera* was carried out. Several models were used to evaluate the anti-anxiety activity of the leaves of the Achyranthes aspera plant: the elevated plus-maze model, the light-dark model, the social interaction model, and the hole board technique. For this, the dose of hydroalcoholic extract p.o. at 300 and 600 mg/kg, respectively, was selected, and diazepam was used as a standard anxiolytic agent. According to the findings, hydroalcoholic extracts of *Achyranthes aspera* leaves exhibited diminished aversion fear induces anti-anxiety activity. The hydroalcoholic extracts of *Achyranthes aspera* leaves were found to have anti-anxiety action. The data indicates that the hydroalcoholic extract of *Achyranthes aspera* leaves has more anti-anxiety action than the control.

INTRODUCTION: Anxiety is one of the most common mental diseases, affecting approximately 10-15% of the population [1]. The anxiety can be seen with a variety of behavioral and affective symptoms like avoidance of danger cues or situations escape, flight search for refuge, reassurance restlessness, anxiety, pacing, hyperventilation freezing, motionless, nervous, anxious, wound up scared, afraid, frightened, jittery, edgy, jumpy and restless, irritated [2].

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Anxiety can be triggered by a breakdown in the control of the central nervous system. Many individuals believe that low serotonin system activity and enhanced noradrenergic system activity induce its creation. Several studies have found that anxious persons have higher WBC counts and a negative association between red blood cells and mean corpuscular hemoglobin levels and anxiety symptoms [3].

Further, it is believed that stress is a major cause of anxiety. It is critical to identify anxiety symptoms early to prevent the development of an anxiety disorder [4]. Herbal plants are used to treat various diseases, and anxiety is one of them. Because of its unique agro-climatic conditions and regional topography, India has long been recognized as a

botanical paradise of plant genetic riches. Many medicinal plants grow in the country's tropical forests of the Western Ghats. According to existing information, over 1,800 species are used in classical Indian medical systems [5].

For medication, serotonin-norepinephrine reuptake inhibitors like venlafaxine and duloxetine are considered effective ones. However, they have some side effects, including an increase in systemic blood pressure. Other side effects are nervousness, somnolence, nausea, decreased appetite, weight loss, constipation, increased sweating, dry mouth, dizziness, difficulty sleeping, and sexual dysfunction are the most commonly reported side effects [6].

Benzodiazepines are now the most often given medicines for anxiety disorders. However, the therapeutic uses of benzodiazepines as anti-anxiety medications are restricted due to their undesirable side effects. As a result, novel pharmacological drugs derived from plants are well warranted. Many herbs are used to treat anxiety disorders. One of them is Achyranthes aspera. Traditionally, the plant has been used to treat asthma, cough, diuretic, purgative, and laxative disorders, as well as edema, piles, pneumonia, poisonous snake bites, night blindness in ulcers, and warts, liver complaints, rheumatism, scabies, and other skin illnesses. It also has calming effects [7, 8]. *Achyranthes aspera (Amaranthaceae)* is a valuable medicinal plant that grows like a weed across India. In India, it is found widely in Maharashtra, Kerala, Karnataka, Tamil Nadu, etc. [9, 10].

Though virtually all of its components are utilized in ancient systems of medicine, the seeds, roots, and shoots are the most significant medicinal portions. The plant has been used as anti-arthritic activity [10], antiparasitic activity [11], anti-cancerous [11], hypoglycaemic activity, wound healing activity [9], anti-inflammatory, anti-arthritic and anti-oxidant activity, nephroprotective activity, cardiac activity [9], spermicidal activity, anti-depressant activity [12], antimicrobial activity [13], antiviral and anti-carcinogenic [13] *etc*. The current study sought to assess the anti-anxiety efficacy of hydroalcoholic extracts of the leaves of the *Achyranthes aspera* plant. This study aimed to evaluate the anti-anxiety activity of the hydroalcoholic extracts of the leaves of the *Achyranthes aspera* plant in rodents like mice.

MATERIALS AND METHODS:

Plant Material:

Collection and Authentication of Plant Materials: The leaves of *Achyranthes aspera* belonging to the family Amaranthaceae were collected in the month of January 2020 from the local areas of the 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)).

Processing of Sample: The leaves of this plant were dried in the shade for one month and then powdered. This powder was stored in an air-tight closed container and used for successive extraction.

Preparation of extracts:

Preparation of Hydro Alcoholic Extract of *Achyranthes aspera* Leaves: The leaves powder of *Achyranthes aspera* was reduced to coarse powders (40 size mesh) were used for the Soxhlet extraction. The solvent used was 50% water and 50% alcohol. The coarse powder was placed uniformly in the Soxhlet device and extracted with a hydroalcoholic solvent. The extracts were collected and evaporated to dryness to give a dry crude extract. The extracted materials were submitted to phytochemical analysis.

Phytochemical Analysis: Qualitative phytochemical screening of hydroalcoholic extracts of *Achyranthes aspera* were carried out as per standard method [14].

Experimental Animals: For the research of anxiolytic activity, healthy adult male Swiss albino mice (20–30 g) were utilized. 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 natural light and dark cycle, and had unlimited access to food and water.

Treatment: The experimental animals were randomly allocated into four groups of six animals each. Group I served as vehicle-treated control, Groups. II received the standard drug diazepam (1 mg/kg i.p.), and Group III & IV received Hydroalcoholic extract p.o. at 300 and 600 mg/kg, respectively.

Ethical Approval: The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of B. N. University, Udaipur. The experiments were conducted according to the Committee for Control & Supervision on Experiments guidelines (15/BNCP/IAEC/2021).

Drugs and Chemicals: Diazepam was used as a standard anxiolytic agent, and all other chemicals and reagents were used of analytical grade.

Anti-anxiety Activity: The hydroalcoholic extracts of *Achyranthes aspera* leaves were tested for anti-anxiety activity using elevated plus maze, Light-dark test, Social interaction test, and Hole board test.

Anti-anxiety Activity:

Elevated Plus Maze (EPM) Model: The anxiolytic effect of hydroalcoholic extract of the plant was investigated in swiss albino mice utilizing an elevated plus-maze method. The plus-maze was in the shape of a cross or plus with two open (30 x 5 cm x 0.2 5 cm) and two closed (30 cm x 5 cm x 15 cm) arms extending from the center platform (5 cm x 5 cm) and lifted to 50 cm above the ground. The entire maze was constructed of wood. Swiss albino mice weighing 20-30 grams are divided into four groups of six. The standard medication (Diazepam) was given i.p. 30 minutes before testing, and the extracts were given p.o 45 minutes before testing. Mice were individually placed on the center of the maze facing an open arm, and the number of entries and the time spent in closed and open arms were recorded during a 5 min observation period. Arm entries were defined as the entry of all four paws into the arm. The procedure was conducted preferably in a sound-attenuated environment [15].

Light-dark test: The apparatus consisted of two 20cm×10cm×14cm plastic boxes; one was dark the other was transparent. The rat was allowed to move from one box to the other through an open door between the two boxes. A 100W bulb placed 30 cm above the floor of the transparent box was the only light source in the room. Swiss Albino mice weighing 20 -30 gm divided into four groups of six animals each. The standard medication (diazepam) was given intravenously 30 min before testing, and the extracts were given orally 45 minutes before testing. Mice were placed in the lightbox facing to the hole. The transitions between the light and dark boxes and the time spent in the lightbox were recorded for 10 minutes after the mice entered the dark box [16].

Social Interaction test: Swiss albino mice weighing 20-30 grams are divided into four groups of six mice each. The standard drug (Diazepam) was given 30 minutes before testing, and the extracts were given 45 minutes before testing. They were housed alone for 5 days before the trial and were given unlimited food and water. During this time, they were weighed and handled regularly. The location of the cages in the rack was adjusted to ensure that all rats had equivalent exposure to the various levels of illumination.

The mice were allocated to the ‘low light and unfamiliar' test settings at random. The text box was (22 *15 * 12 cm) in size, and pairs of mice were placed in it for 5 minutes, and their behaviour was monitored. Sniffing, nibbling, grooming, following, mounting, kicking, boxing, wrestling, and leaping on, under, or over the companion were all observed [17].

Hole Board test: The hole board test is designed to assess particular head dipping habits. The hole board equipment consisted of an open field with 16 evenly placed 3 cm diameter holes in the floor. Swiss Albino mice were split into four groups of six mice each.

The standard medication diazepam was given i.p. 30 minutes before testing, and the extracts were given p.o. 45 min before testing. Each mouse was placed in a different corner of the board, with the animal then wandering about and dipping its head into the holes. Individual mice had their head dips, and sectional crossings counted every 5 min [18].

Statistical Analysis: The results were expressed as mean ± S.E.M. The differences were compared using one-way analysis of variance (ANOVA) and subsequently followed by Dunnett's test.

RESULTS:

Physical Properties of the Extracts: The colour, texture, and type of the extracts of leaves of *Achyranthes aspera* were tabulated in **Table 1.**

TABLE 1: PHYSICAL PROPERTIES OF *ACHYRANTHES ASPERA* LEAVE EXTRACTS

Plant Part	Type of Extract	Texture	Colour
Leaves of *Achyranthes aspera*	Hydro alcoholic	Gummy	Dark Brown

Phytochemical Analysis: After the screening, hydroalcoholic extracts of *Achyranthes aspera* leaves indicated the presence of flavonoids, glycosides, tannins, and saponins. **Table 2** lists the phytochemical components in detail.

TABLE 2: PHYTOCHEMICAL ANALYSIS OF *ACHYRANTHES ASPERA* LEAVES EXTRACTS

S. no.	Phytochemical	Hydroalcoholic extract
1	Carbohydrates	+
2	Alkaloids	++
3	Flavonoids	++
4	Glycosides	+
5	Saponins	+
6	Tannins	–
7	Coumarins	–

"+" 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 hydroalcoholic extracts of *Achyranthes aspera* leaves at a dosage of 600 mg/kg p.o. performed well in the elevated plus-maze test (EPM) **Table 3**. It substantially increased the amount of entries and time spent in the open arm. The strength of the anti-anxiety effects 600mg/kg p.o. The pharmacokinetics of hydroalcoholic extracts of *Achyranthes aspera* were equivalent to those of diazepam 1 mg/kg i.p. **Fig. 1, 2, 3,** and **4**.

TABLE 3: ANXIOLYTIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *ACHYRANTHES ASPERA* 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 the 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 (300 mg/kg)	7.67±0.33ns	10.5±0.43*	93.17±0.60*	206.83±0.60*
4	Group IV	Hydro-alcoholic Extract (600 mg/kg)	8.67±0.33b	9.33±0.49*	135.17±1.08*	164.83±1.08*

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.

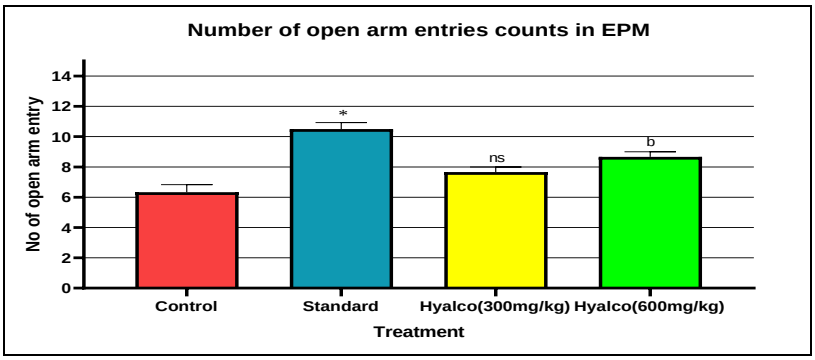

FIG. 1: RESULT OF EPM TEST OF HYDROALCOHOLIC EXTRACT OF *ACHYRANTHES ASPERA* LEAVES (NUMBER OF OPEN ARM ENTRIES)

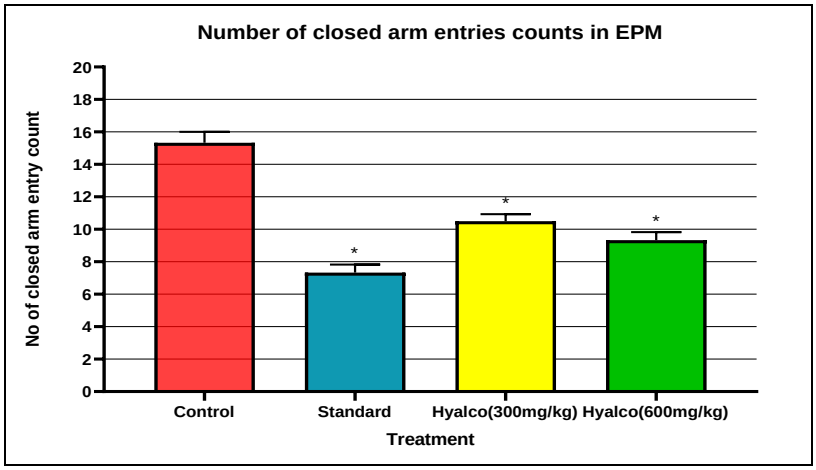

FIG. 2: RESULT OF EPM TEST OF HYDROALCOHOLIC EXTRACT OF *ACHYRANTHES ASPERA* LEAVES (NUMBER OF CLOSED ARM ENTRIES)

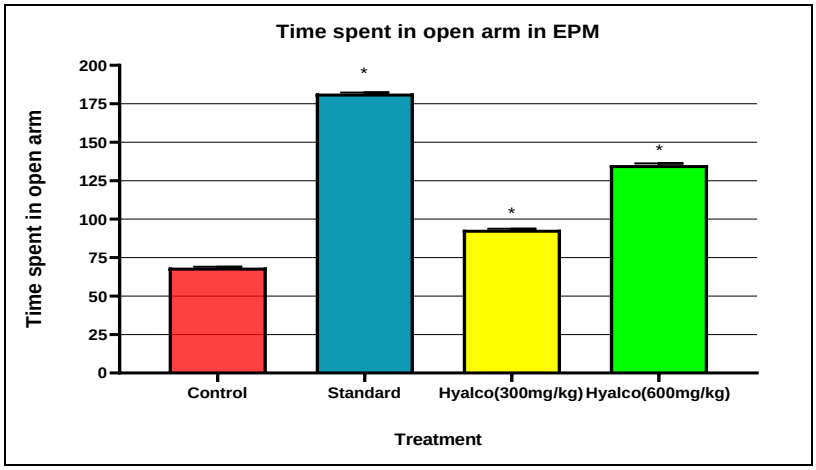

FIG. 3: RESULT OF EPM TEST OF HYDROALCOHOLIC EXTRACT OF *ACHYRANTHES ASPERA* LEAVES (TIME SPENT IN OPEN ARM)

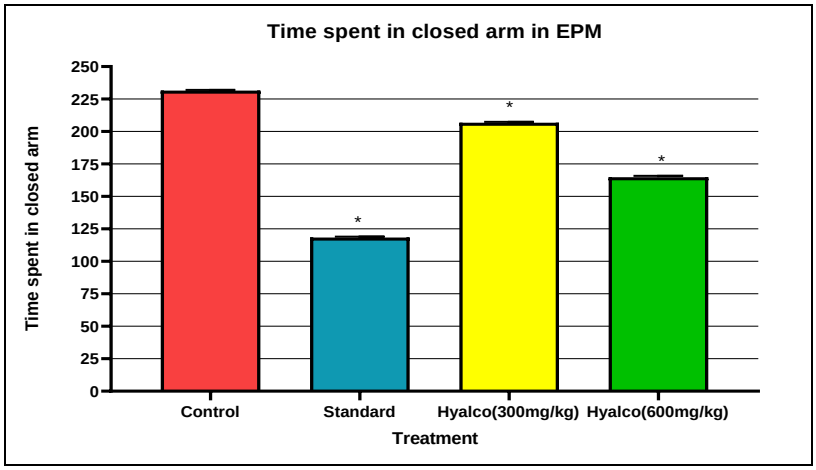

FIG. 4: RESULT OF EPM TEST OF HYDROALCOHOLIC EXTRACT OF *ACHYRANTHES ASPERA* LEAVES (TIME SPENT IN CLOSED ARM)

Light Dark test: In the light-dark test, the time spent(s) in the lighted box, numbers of crossing and transfer latency increased significantly ($p< 0.01$), and the time spent in the dark box decreased significantly ($p< 0.01$) in extract solution (300 & 600 mg/kg p.o.) and diazepam treated groups compared to the control group **Table 4**. The values of hydroalcoholic extract at 600 mg/kg p.o. treated group) were comparable with the standard treated group for all the parameters under study **Fig. 5, 6, 7,** and **8.**

TABLE 4: ANXIOLYTIC ACTIVITY OF HYDRO ALCOHOLIC EXTRACT OF *ACHYRANTHES ASPERA* 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 (300 mg/kg)	10.17±0.60*	7±0.37ns	150.7±0.92*	4.83±0.31ns
4	Group IV	Hydro-alcoholic Extract (600 mg/kg)	6.83±0.60*	8±0.37a	200.2±1.25*	5.67±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.

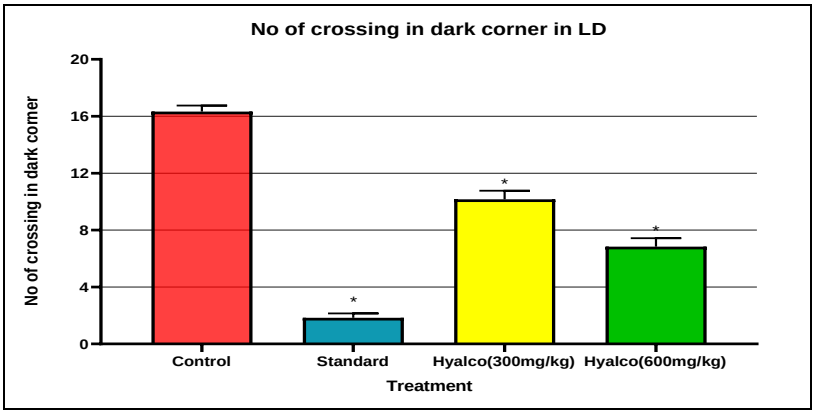

FIG. 5: RESULT OF LIGHT-DARK TEST OF HYDROALCOHOLIC EXTRACT OF *ACHYRANTHES ASPERA* LEAVES (NO OF CROSSING IN DARK CORNER)

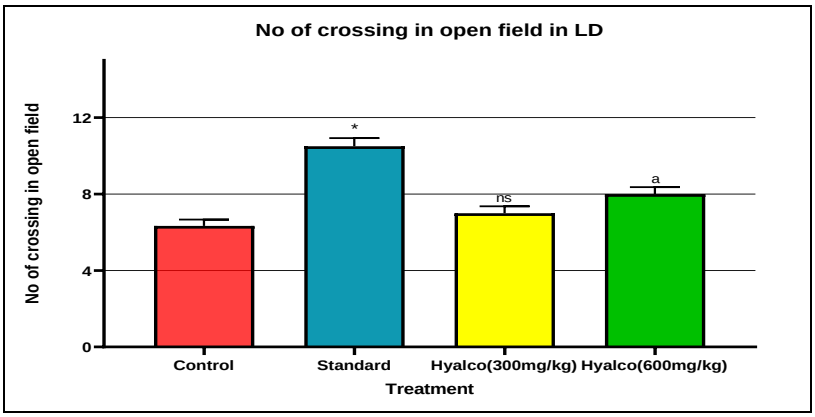

FIG. 6: RESULT OF LIGHT-DARK TEST OF HYDROALCOHOLIC EXTRACT OF *ACHYRANTHES ASPERA* LEAVES (NO OF CROSSING IN OPEN FIELD)

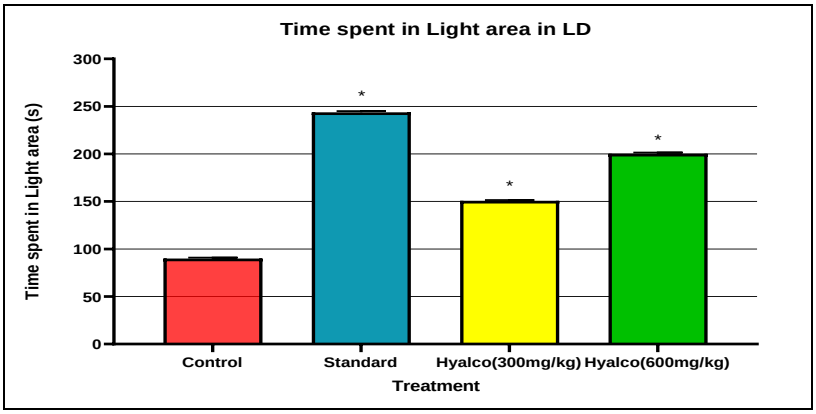

FIG. 7: RESULT OF LIGHT-DARK TEST OF HYDROALCOHOLIC EXTRACT OF *ACHYRANTHES ASPERA* LEAVES (TIME SPENT IN LIGHT AREA)

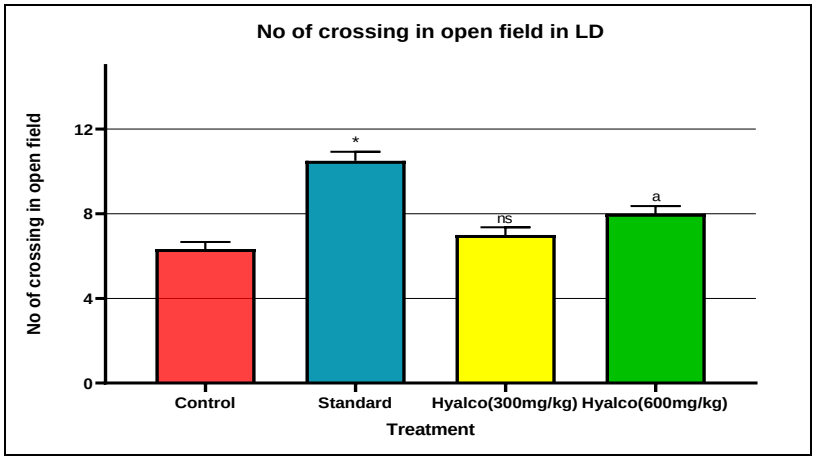

FIG. 8: RESULT OF LIGHT-DARK TEST OF HYDROALCOHOLIC EXTRACT OF *ACHYRANTHES ASPERA* LEAVES (NO OF CROSSING IN OPEN FIELD)

Social Interaction test: The effects of hydroalcoholic extract 300 mg/kg, hydroalcoholic extract 600 mg/kg, and diazepam on social interaction as shown in **Table 5**. Diazepam has increased in social interaction time significantly (p <0.01).

The hydroalcoholic extract 600 mg/kg has shown increased time in social interaction as compared to the control group. The hydroalcoholic extract 600 mg/kg, as shown, increased social interaction time significantly (p< 0.01) as compared to the control group **Fig. 9.**

TABLE 5: ANXIOLYTIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *ACHYRANTHES ASPERA* 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 (300 mg/kg)	428±2.61*
4	Group IV	Hydro-alcoholic Extract (600 mg/kg)	515.3±2.54*

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.

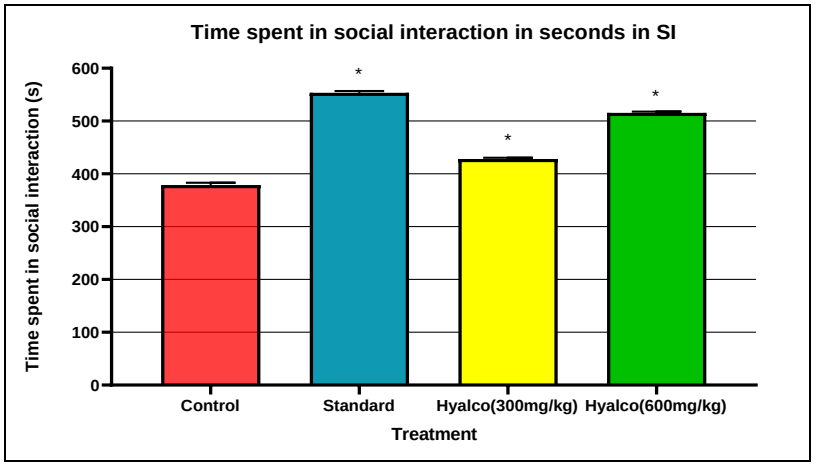

FIG. 9: RESULT OF SOCIAL INTERACTION TEST OF HYDROALCOHOLIC EXTRACT OF *ACHYRANTHES ASPERA* LEAVES (TIME SPENT IN SOCIAL INTERACTION)

Hole Board test: In the hole board test, the hydroalcoholic extract of a plant at dose 300 mg/kg and 600 mg/kg is shown in **Table 4**.

The hydroalcoholic extract 300 mg/kg showed increased head dipping (p< 0.05) and an increased number of line crossing, not significantly compared to the control group. The hydroalcoholic extract 600 mg/kg showed that significantly increased head dipping (p< 0.05) and increased number of line crossing did not significantly result as compared to control group **Fig. 10** and **11.**

TABLE 6: ANXIOLYTIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *ACHYRANTHES ASPERA* 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 (300 mg/kg)	30.17±0.48*	45.5±0.76*
4	Group IV	Hydro-alcoholic Extract (600 mg/kg)	40.67±0.68*	52.5±0.76*

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.

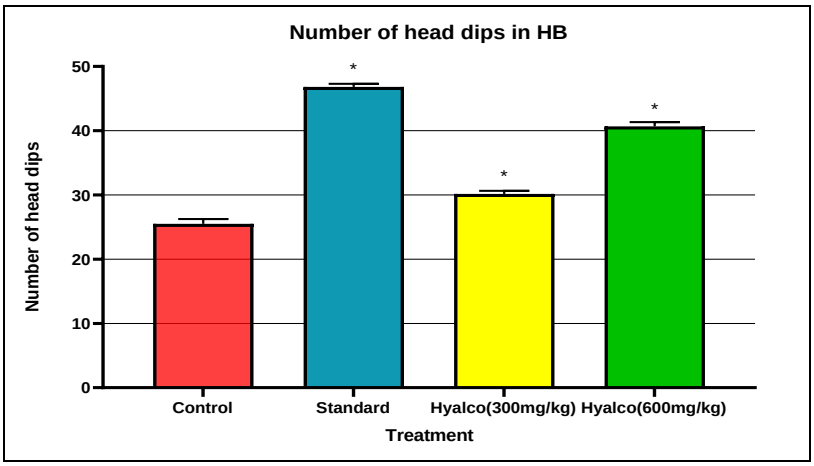

FIG. 10: RESULT OF WHOLE BOARD TEST OF HYDROALCOHOLIC EXTRACT OF *ACHYRANTHES ASPERA* LEAVES (NUMBER OF HEAD DIP)

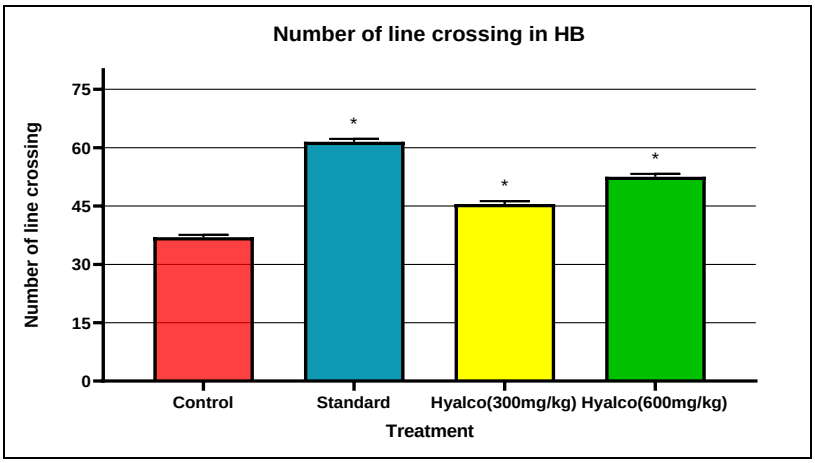

FIG. 11: RESULT OF WHOLE BOARD TEST OF HYDROALCOHOLIC EXTRACT OF *ACHYRANTHES ASPERA* LEAVES (NUMBER OF LINE CROSSING)

DISCUSSION: There are no reports of scientific evolution of *Achyranthes aspera* anxiolytic action, despite its historical usage for treating a variety of disorders. The presence of carbohydrate, tannin, phenolic compounds, flavonoids, steroids and coumerin was discovered in a hydroalcoholic extract of *Achyranthes aspera* leaf. The present work used raised pulse maze, light-dark test, social interaction test, and hole board test models to demonstrate the anxiolytic effect of a hydroalcoholic extract derived from the *Achyranthes aspera* plant in mice. Mice were chosen for the evolution of anxiolytic activity in an experimental model because mice exhibit robust anxiety-like behaviour when exposed to stressors (for example, novelty, bright light, or social confrontation). These phenotypes have obvious utility in testing the effects of psychotropic drugs [19]. The elevated plus-maze is used to assess mice's psychomotor performance and emotional aspects [20]. The results showed that hydroalcoholic extract of a plant at both doses 300 mg/kg and 600 mg/kg treated mice significantly increased the time spent and the number of entries in open arms. Still, they

decreased the time spent and the number of entries in close arms depending on dose when compared to diazepam as the standard drug reflects plants and anxiolytic properties. The light-dark test is also commonly employed in mice as an animal model for creating anxiolytic or angiogenic medicines. It is predicated on animals' natural aversion to lighted regions and spontaneous exploring behaviour in response to my stresses [21].

The results revealed that a hydroalcoholic extract of a plant's leaf at doses of 300 mg/kg and 600 mg/kg greatly enhanced the amount of time spent and the number of transactions in the light area.

When diazepam 1 mg/kg was employed as the reference medication in light and dark models, similar effects were seen. The social interaction test was the first animal anxiety test that employed natural behaviour as the dependent variable. In this test, low light and unfamiliar conditions are used, resulting in moderate anxiety [22].

The hydroalcoholic extract at 300 mg/kg and 600 mg/kg considerably increased the length of social contact in low light and unfamiliar conditions, indicating that the plant extract has an anxiolytic effect. The hydroalcoholic extract of plant leaves at doses of 300 mg/kg and 600 mg/kg significantly increased the number of head dipping and line crossing in the hole board test. This shows the anxiolytic effect of the plant's leaves extract. It is conceivable that the mechanism of anxiolytic action of the *Achyranthes aspera* plant's hydro alcoholic extract is related to any of these phytochemicals to the GABA A-BZD complex.

CONCLUSION: According to the findings, hydroalcoholic extracts of *Achyranthes aspera* leaves exhibited anti-anxiety action. These data indicate that the hydroalcoholic extract of *Achyranthes aspera* leaves has more anti-anxiety action than the control.

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CONFLICTS OF INTEREST: All authors declare no conflicts of interest.

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