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Research Article

Assessment of Anxiolytic and Anti-Amnestic Activity of Mundi (*Sphaeranthus indicus* Linn) Whole Plant Extracts in Swiss Albino Mice

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ABSTRACT

A science can start from any set of contradictory axioms and come up with a working hypothesis of practical use. A good scientific theory takes into account certain known facts and allows us to predict new ones, which can be verified by observation and experiment. Although Ayurvedic medicines have been used for centuries; however, for practical benefits, it is imperative to check the effectiveness of these drugs with modern parameters, and also to elucidate the possible mode of action of the drug. One way to verify such claims is to test them experimentally on animals, as there are limitations in conducting experiments on humans. Chemical analysis was the only method used to estimate the potency of Ayurvedic formulations. A biological apparatus with spectacular capacities has become more important due to the living body. The main objective of the experimental study is to give a pharmacological basis to the medicines used in Ayurveda to expand their therapeutic application.

Keywords: Ayurvedic medicines; Chemical; Drug; Biological; Plant; Pathological

INTRODUCTION

Anxiety is a physiological emotion that is aroused when there is an imminent threat and it is precipitated in situations where there is no overt danger, but when the individual considers danger as a possibility [1]. It is pathological when it is unreasonable, exaggerated, recurrent and causes significant psycho-physiological distress. According to the demographic studies done in 2017, it has been observed that 197.3 million (95% UI 178.4-216.4) people had mental disorders in India, including 45.7 million (42.4-49.8) with depressive disorders and 44.9 million (41.2-48.9) with anxiety disorders [2]. It appears that loss of memory is the initial problem with the consequent development of anxiety. Therefore, anxiety is probably an early predictor of future cognitive decline and even possible future cognitive impairment [3].

Mundi (*Sphaeranthus indicus* Linn.); whole plant, which is widely mentioned for its medhya and rasayan properties in various ayurveda classics medhya karma has various components when compared with conventional concepts like anti-amnestic, no tropic, anti-parkinsonism, anti-anxiety activity [4-6]. *Sphaeranthus indicus* Linn is a seasonal herb belonging to the Asteraceae family. Previous reports have suggested that the plant possesses anxiolytic and sedative effect [7].

Evaluation of any drug on experimental models is important to find out the probable pharmacodynamics and kinetics of the intended action of the drug. Various activities of different plant parts have been reported, but no scientific data is available regarding effects of the whole plant of *Sphaeranthus indicus* Linn. Thus, experimental study was designed to assess the effect on anxiety as well as learning memory in scopolamine induced amnesia.

MATERIALS AND METHODS

Selection of animals

The experimental study was conducted in animal house of Datta Meghe college of pharmacy, Salod (H), Wardha, Maharashtra. Adult Swiss albino mice of either sex, weighing in the range of 18-24 gm were obtained and kept in the animal house under controlled temperate conditions.

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The experimental mice were fed with a rodent pellet diet obtained from golden feeds India Ltd. They were having access to food and water ad libidum. They were exposed to ambient temperature, humidity, and natural day and night cycles. All the experiments were carried out between 8:00 am to 12:00 noon hrs of the day.

Chemicals used in the study

- Diazepam (procured from cipla pharmaceuticals).
- Piracetam (procured from Dr. Reddy's pharmaceuticals).
- Scopolamine (procured from sigma-aldrich).

Collection of plant material

The plant of *Sphaeranthus indicus* Linn. was collected from natural habitat (A/p tal Mogarne, Dist-Sindhudurg, Maharashtra, India, having coordinates 16.0566°C N, 73.5874°C E) during the months of October to December. Proper care was taken during the collection and preservation of the sample. Herbarium specimens are stored at the department of dravyaguna, all India institute of ayurveda, New Delhi. Authentication was done from the botanical garden of the Indian republic, botanical survey of India, Noida [8]. The collected sample was shade dried and stored in air tight baggage. Aqueous and hydro-alcoholic extracts of the whole plant of Mundi (*Sphaeranthus indicus* Linn), were taken.

Aqueous extract: The dried *Sphaeranthus indicus* Linn. (oarse powder) was extracted with 6 parts boiling water for 5 hrs and filtered to yield extract. The extract was concentrated and dried to powder form and stored in an airtight container for further research work.

Hydroalcoholic extract: The hydroalcoholic extract was prepared by using 50:50 ethanol to distilled water using the soxhlet apparatus. The extract was concentrated and dried to powder form and was stored in an airtight container for further research work.

Experimental models

For assessment of anxiolytic activity

Locomotor activity using actophotometer: Actophotometer was used to assess locomotor activity. The apparatus was placed in a darkened, sound attenuated, and ventilated testing room during the assessment. Locomotor activity was assessed by comparing the number of beam breaks across a 20 min session divided into two 10 min intervals by animals.

Elevated plus maze test: The anxiety behavior was assessed by elevated plus-maze. The elevated plus-maze was made of wood and consisted of two opposite open arms (50 × 10 cm), enclosed by 40 cm high walls. The maze was elevated 50 cm above the floor. Each mouse was placed for 5 min in a pre-test arena (45 × 45 × 45) before exposure to the maze. This step facilitates exploratory behavior. An investigator sitting approximately 2 m apart from the apparatus observed the mice. Immediately after the pre-test, exposed mice were placed in the center of the elevated plus-maze facing one of the open arms. During the 5 min test period, the following measurements were taken: The number of entries into the open and closed arms and the time spent in the open and closed arms. An entry was defined as entering into one of the arms with all four paws.

For assessment of anti-amnestic activity

Assessment of learning memory using morris water maze test: Morris water maze is one of the most commonly used assessment model, which is used for learning and memory. It is a circular pool (150 cm in diameter, 45 cm in height, filled to a depth of 30 cm with water at 28°C. The procedure of morris water maze was based on the principle that animals dislike swimming and thus have a tendency to escape from the water. Morris water maze was divided into four equal quadrants such as Q1, Q2, Q3 and Q4, a submerged platform (10 cm^2), painted white was placed inside the target quadrants of this pool, 1 cm below the surface of the water. Each animal was subjected to four such trials and each trial was started from a different quadrant, and animals were allowed 120 sec for searching the submerged platform. If the animal fails to find the platform within 120 sec then it was guided gently towards the platform. Day 4 Escape Latency Time (ELT) to locate the hidden platform in water maze was noted as an index of acquisition or learning. In this whole test Quadrant 4 (Q4) was maintained as a target quadrant in all acquisition trials. On the fifth day, the platform was removed and animals was allowed to explore the pool for 120 sec. The time spent in each quadrant was noted as a mean Time Spent in the Target Quadrant (TSTQ), which was served as an index of retrieval or memory. The experimenter always stood in the same position. Care was taken that the relative location of morris water maze concerning other objects in the laboratory serving, as prominent visual clues was not disturbed during the total duration of the study.

RESULTS

Histopathology of brain

2 animals from each group of morris water maze were euthanized by giving excessive dosage of succinylcholine and brain tissue was removed as per standard procedure. The tissue was then preserved in formalin solution and later sent to pharmacologist for detailed observation and conclusion (Tables 1-7 and Figure 1).

Table 1: Grouping of animals and dose regimen.

Sr. No	Group	No. of animals	Drug treatments	Dose (for seven consecutive days)	Route
1	Group A	6	Vehicle (distilled water)	10 ml/kg body wt.	Oral

2	Group B	6	*S. indicus* (aqueous extract)	200 mg/kg body wt.	Oral
3	Group C	6	*S. indicus* (hydroalcoholic extract)	200 mg/kg body wt.	Oral
4	Group D	6	Diazepam (anxiolytic activity) piracetam (anti-amnestic activity)	4 mg/kg body wt.	Oral

Locomotor activity

Table 2: Assessment of beams crossed per 20 mins.

Treatment	A	B	C	D
Observations	6	6	6	6
Mean ± S.E.	594.50 ± 32.07	323.66 ± 43.00	410.66 ± 70.91	520.5 ± 18.56

Turkey HSD results

Table 3: Statistical analysis of observations of locomotor activity.

Treatment	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
A *vs.* B	5.9631	0.0022	$^{**}p<0.01$
A *vs.* C	4.0476	0.0439	$^{*}p<0.05$
A *vs.* D	1.6293	0.648	Insignificant
B *vs.* C	1.9155	0.5376	Insignificant
B *vs.* D	4.3338	0.0287	$^{*}p<0.05$
C *vs.* D	2.4183	0.3451	Insignificant

Anxiolytic activity using elevated plus maze test

Table 4: Percentage time spent in open arm of elevated plus maze.

Treatment	A	B	C	D
Observations	6	6	6	6
Mean ± S.E.	4.22 ± 0.97	11.94 ± 4.45	11.10 ± 5.18	14.66 ± 5.15

Table 5: Statistical analysis of observations of locomotor activity.

Group	Bonferroni inference	Holm inference
A *vs.* B	$^{**}p<0.01$	$^{*}p<0.01$
A *vs.* C	$^{*}p<0.05$	$^{*}p<0.05$
A *vs.* D	Insignificant	Insignificant
B *vs.* C	Insignificant	Insignificant
B *vs.* D	$^{*}p<0.05$	$^{*}p<0.05$

C *vs.* D	Insignificant	Insignificant

Assessment of escape latency time (per second) using morris water maze test

Table 6: Escape latency time in morris water maze test.

Treatment	A	B	C	D
Number of observations	6	6	6	6
Mean ± S.E.	26.66 ± 6.89	8.33 ± 1.70	21.20 ± 10.02	15.40 ± 3.74

Table 7: Statistical analysis of observations of morris water maze test.

	A	B	C	D
Observations	6	6	6	6
Mean ± SE	26.66 ± 6.89	8.33 ± 1.70	21.2 ± 10.02	15.4 ± 3.74

Histopathological examination of brain tissue

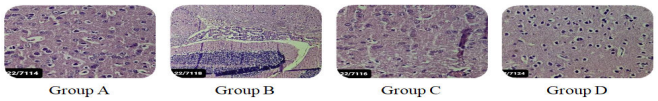

Figure 1: Histopathological examination of brain tissue.

DISCUSSION

Locomotor activity is directly related to the level of anxiety in the animal. The number of beams of actophotometer crossed in the stipulated time interval of 10 minutes was checked to compare level of anxiety. Reduction in number of beams crossed gives anxiolytic activity of the drug. From the observations of this study, it can be noted that, both the extracts have shown significant anxiolytic activity when compared with standard drug diazepam.

The EPM test is based on the premise that exposure to an EPM induced an approach avoidance conflict that was significantly stronger than that induced by exposure to a closed arm. The decrease in open arm aversion is the result of an anxiolytic effect, expressed as increase in open arm residence time and entries. SIHA (*S. indicus* whole plant hydroalcoholic extract) and SIA (*S. indicus* whole plant aqueous extract) increased the time spent and the percentage of entries in the open arm, with a percentage decrease in the closed arm. The increase in the percentage of entries in the open arm shown by SIA (200 mg/kg) was greater, but the time spent in the open arm was less than the SIHA (200 mg/kg), suggesting that the mean time was spent in the open arm per entry was lower for the latter. This finding may correlate with the previous finding that closed arm penetration correlates with locomotor activity, also indicating the extract's unique anti-stress effects [9]. Therefore, behavioural changes induced by hyroalcoholic extract (200 mg/kg) and aqueous extract (200 mg/kg) are consistent with anxiolytic activity. But, when compared statistically, both of them has shown weak anxiolytic activity ($p<0.1$) compared to diazepam (4 mg/kg).

Previous studies of the chemical constituents and their pharmacology indicate that saponins possess anxiolytic activity [10]. Tannins have also been shown to be effective against many CNS disorders [11]. The hydroalcoholic extract of *S. indicus* whole plant has shown the presence of tannins and steroids due to their higher solubility in alcoholic media. There are several fatty acids, oils, tannins and steroids that have been isolated from *Sphaeranthus indicus*, but there is little scientific data on their anxiolytic activity. Therefore, we cannot assign anxiolytic activity to any of them without isolating the specific component. Similarly, SIA showed the presence of saponins but showed weak anxiolytic activity because there are a large number of saponins, namely, steroid saponins, triterpene saponins, and all saponins do not possess anxiolytic activity [12].

Scopolamine induced reversible amnesia model was used to assess the anti-amnestic effect of the whole plant of *S. indicus*. Scopolamine acts as a competitive antagonist of muscarinic receptors. It damages central cholinergic functions, resulting in learning and memory disorders in rodents. This mimics the loss of cortical cholinergic neurons and impaired central cholinergic functions seen in AD brains. Because scopolamine is an anticholinergic, it blocks ACh binding sites, resulting in high ACh concentrations. This leads to nerve damage in the hippocampus, eventually leading to memory loss and learning problems.

Piracetam is used a standard control as it is widely used as a nootropic agent. It influences neuronal and vascular functions and influences cognitive function without acting as a sedative or stimulant. Piracetam is a positive allosteric modulator of the AMPA receptor, although this action is very weak and its clinical

effects may not necessarily be mediated by this action. It is hypothesized to act on ion channels or ion carriers, thus leading to increased neuron excitability GABA brain metabolism and GABA receptors are not affected by piracetam.

Morris water maze is generally used for the assessment of learning memory. The hippocampus is involved in spatial/ relational memory. The water maze specifically tests spatial memory. However, there are many other components of the task that do not involve spatial memory: Task related stress, understanding of the task rules (that to "escape", the animal must find a hidden platform and get on it remain to be "saved"), and the realization that there is a way to escape the task. Learned helplessness also includes a water tank, but the rules (no escape) are very different. The three pre-training trials "teach" the animals these characteristics of the task. They learn that they will be placed in a basin of warm water and will swim for a minute, but will then be removed. They are taught to find the platform (because it is visible) and that staying on it will lead to “escape" from the maze. And they are taught that homework comes to an end. Therefore, this hippocampus independent learning does not confound the analysis of the water maze test data.

From the observations made in the experimental study, it is evident that, there are significant results in all the groups, but when statistical significance between the groups was assessed, it is observed that the reduction in escape latency is more in hydroalcoholic extract group than in aqueous extract group. But, as both the extracts has shown significant result in comparison with piracetam, it can be stated that *S. indicus* possess anti-amnestic activity. There is no single pathway mentioned for occurrence of amnesia, thus it is difficult to justify this activity. Amongst the proposed pathways, GABA pathway is the highest accepted pathway. Reduction in the activity of GABA causes irreversible amnestic changes and leads to memory loss. The previous studies conducted for anti-epileptic activity have proven that extract of *S. indicus* has shown agonistic activity on GABA thus enhancing its efficacy. So, its action on GABA receptors can be studied in detail to find the possible pharmacokinetics of the drug, *S. indicus* Linn.

CONCLUSION

The anxiolytic effect produced by aqueous extract is highly significant ($p<0.05$) while, that by hydroalcoholic extract is statistically non-significant when compared to standard drug diazepam at the dosage of 200 mg/kg body weight. Anti-amnestic activity produced by both extracts is significant ($p<0.05$) when compared to the standard drug piracetam, but there is no difference in efficacy of both extracts at the dosage of 200 mg/kg body weight. Histopathology of the brain showed no change in all four groups.

FURTHER SCOPE

In the present study only a single dose of extracts was studied for its pharmacological activity, hence in further studies different extracts of Mundi can be studied at different dosage to find out dose response curve and to define the mechanism behind the anxiolytic activity of it.

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REFERENCES

1. Galani VJ, Patel BG, Rana DG. *Sphaeranthus indicus* Linn.: A phytopharmacological review. Int J Ayurveda Res. 2010;1(4): 247-253.
2. Sagar R, Dandona R, Gururaj G, Dhaliwal RS, Singh A, Ferrari A, et al. The burden of mental disorders across the states of India: The global burden of disease study 1990-2017. Lancet Psychiatry. 2020;7(2):148-161.
3. Sinoff G, Werner P. Anxiety disorder and accompanying subjective memory loss in the elderly as a predictor of future cognitive decline. Int J Geriatr Psychiatry. 2003;18(10):951-959.
4. Ramachandran S. Review on *Sphaeranthus indicus* Linn. (koṭṭaikkarantai). Pharmacogn Rev. 2013;7(14):157-169.
5. Jain A, Basal E. Inhibition of *Propionibacterium acnes* induced mediators of inflammation by Indian herbs. Phytomedicine. 2003;10(1):34-38.
6. Singh RH, Narsimhamurthy K, Singh G. Neuronutrient impact of ayurvedic rasayana therapy in brain aging. Biogerontology. 2008;9(6): 369-374.
7. Ambavade SD, Mhetre NA, Tate VD, Bodhankar SL. Pharmacological evaluation of the extracts of *Sphaeranthus indicus* flowers on anxiolytic activity in mice. Indian J Pharmacol. 2021;38(4):254-259.
8. Sahu TR. Less known uses of weeds as medicinal plants. Anc Sci Life. 1984;3(4):245.
9. Cha HY, Seo JJ, Park JH, Choi KJ, Hong JT, Oh JK. Anxiolytic effects of total saponin fraction from ginseng radix rubra on the elevated plus-maze model in mice. Ginseng Res. 2004;28(3):132-135.
10. Bhat SD, Ashok BK, Acharya RN, Ravishankar B. Anticonvulsant activity of raw and classically processed vacha (*Acorus calamus* Linn.) rhizomes. Ayu. 2012;33(1):119-122.
11. Galani VJ, Patel BG. Effect of hydroalcoholic extract of *Sphaeranthus indicus* against experimentally induced anxiety, depression and convulsions in rodents. Int J Ayurveda Res. 2010;1(2):87-92.
12. Gupta VK, Kaushik A, Chauhan DS, Ahirwar RK, Sharma S, Bisht D. Anti-mycobacterial activity of some medicinal plants used traditionally by tribes from Madhya Pradesh, India for treating tuberculosis related symptoms. J Ethnopharmacol. 2018;227:113-120.