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Original Research Article

ANXIOLYTIC POTENTIAL OF METHANOLIC SEED EXTRACT OF ARTOCARPUS HETEROPHYLLUS IN SWISS ALBINO MICE: EVIDENCE FROM DARK–LIGHT ARENA

Article History:

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Abstract: ***Background:*** Anxiety disorders represent one of the most prevalent neuropsychiatric conditions globally. Although benzodiazepines remain effective, their long-term use is associated with sedation, tolerance, and dependence, prompting the search for safer alternatives from medicinal plants. Artocarpus heterophyllus (jackfruit) has been traditionally used for various central nervous system–related ailments, but its anxiolytic potential remains inadequately explored. Objective: To evaluate the anxiolytic activity of methanolic extract of Artocarpus heterophyllus seeds (MEAH) using validated behavioural models in mice. ***Methods:*** Adult Swiss albino mice (n = 30) were divided into five groups (n = 6). Animals received normal saline (control), diazepam (1 mg/kg), or MEAH at doses of 50, 100, and 200 mg/kg orally. Anxiolytic activity was assessed using the Dark–Light Arena (DLA). Behavioural parameters reflecting exploratory behaviour were recorded. Statistical analysis was performed using one-way ANOVA followed by Dunnett's post hoc test. ***Results:*** MEAH significantly increased time spent in the illuminated compartment, number of entries, and rearing behaviour in the DLA compared with control animals ($p < 0.05$–0.001). The anxiolytic effect was dose-dependent, with the 100 mg/kg dose demonstrating maximal efficacy, comparable to diazepam. ***Conclusion:*** The methanolic seed extract of Artocarpus heterophyllus exhibits significant anxiolytic activity in experimental mice. These findings support its potential as a plant-based anxiolytic agent and warrant further mechanistic and clinical investigations.

Keywords: Anxiolytic activity; Dark–Light Arena; Artocarpus heterophyllus; Diazepam

INTRODUCTION

Anxiety is a fundamental emotional response that enables organisms to cope with perceived threats. However, when anxiety becomes excessive, persistent, or disproportionate to stimuli, it manifests as a pathological condition requiring medical intervention. Anxiety disorders constitute a major global health burden, affecting nearly 300 million individuals worldwide and significantly impairing quality of life [1,2].

Pharmacotherapy for anxiety primarily includes benzodiazepines, selective serotonin reuptake inhibitors, and serotonin–norepinephrine reuptake inhibitors. Despite their efficacy, benzodiazepines are associated with adverse effects such as sedation,

cognitive impairment, tolerance, and dependence, limiting their long-term utility [3,4]. This has driven increasing interest in identifying safer anxiolytic agents from medicinal plants with favourable safety profiles.

Artocarpus heterophyllus Lam. (family Moraceae), commonly known as jackfruit, is widely distributed in tropical regions and has been used in traditional medicine for its anti-inflammatory, antioxidant, antidiabetic, and neuroprotective properties [5–7]. Phytochemical analyses of its seeds have revealed the presence of flavonoids, phenolic compounds, saponins, and alkaloids, many of which are known to modulate central nervous system activity [8,9].

While earlier studies have explored the antioxidant and anticonvulsant effects of A. heterophyllus, systematic evaluation of its anxiolytic potential remains limited. Therefore, the present study aimed to investigate the anxiolytic activity of the methanolic extract of Artocarpus heterophyllus seeds using validated behavioural paradigms—the Dark–Light Arena in Swiss albino mice.

MATERIALS AND METHODS

Ethical Approval

The study was approved by the Institutional Animal Ethical Committee, A.J. Institute of Medical Sciences, Mangalore (IAEC No: AJIMS/IAEC/18-19/02; dated 12/11/2018) and conducted in accordance with CPCSEA guidelines.

Experimental Animals

Adult Swiss albino mice of either sex (3 months old; n = 30) were housed under controlled laboratory conditions (26–28°C, 12-h light/dark cycle) with free access to food and water. Animals were acclimatized for one week prior to experimentation.

Drugs and Chemicals

Methanolic extract of Artocarpus heterophyllus seeds (MEAH) was procured from the Department of Pharmacognosy, Srinivas College of Pharmacy, Mangalore. Diazepam (Valium®, Roche Healthcare) was used as the standard anxiolytic drug.

Preparation of Methanolic Extract

Dried jackfruit seeds were powdered and subjected to successive solvent extraction using petroleum ether, chloroform, and methanol (1:6 ratio). The methanolic extract was concentrated under reduced pressure and stored at <10°C until use.

Study Design

Animals were randomly divided into five groups (n = 6):

- Group I: Normal saline (10 ml/kg)
- Group II: Diazepam (1 mg/kg)
- Group III: MEAH (50 mg/kg)
- Group IV: MEAH (100 mg/kg)
- Group V: MEAH (200 mg/kg)

All treatments were administered orally 60 minutes prior to behavioural testing.

Behavioural Assessment

Anxiolytic activity was evaluated using:

1. Dark–Light Arena (DLA): Time spent in the bright chamber, number of entries, and rearing behaviour were recorded over 5 minutes.

Statistical Analysis

Data were expressed as Mean ± SEM and analysed using one-way ANOVA followed by Dunnett's post hoc test. A p-value < 0.05 was considered statistically significant.

RESULTS

In the Dark–Light Arena test, mice in the control group spent a mean duration of 100.83 ± 1.04 seconds in the bright arena. Treatment with the standard anxiolytic drug diazepam (1 mg/kg) produced a significant increase in the time spent in the bright arena (108.00 ± 1.06 seconds; $p < 0.001$), indicating reduced anxiety levels. Similarly, administration of methanolic extract of *Artocarpus heterophyllus* seeds (MEAH) at doses of 50, 100, and 200 mg/kg resulted in a highly significant increase in time spent in the bright arena compared to the control group ($p < 0.001$). Among the test doses, MEAH at 100 mg/kg produced the maximum anxiolytic effect (123.83 ± 1.10 seconds), which was greater than both the lower and higher doses, suggesting an optimal dose-dependent response.

NUMBER OF ENTRIES INTO THE BRIGHT ARENA OF THE DLA (ACUTE STUDY):

Mean number of entries into the bright arena for the control group was observed to be 3.33 ± 0.49 and for the group that received standard drug Diazepam was 7.16 ± 0.47. The mean number of entries for the group pre-treated with a single dose of MEAH 50mg/kg was 8.66 ± 0.42, for MEAH 100 mg/kg was 12.0 ± 0.57, for MEAH 200 mg/kg was 9.0 ± 0.36.

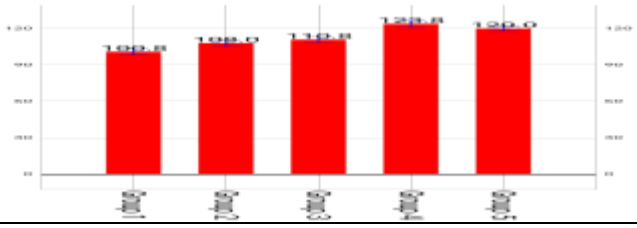
The differences in the time spent in the bright arena for various groups of the DLA are as shown in **Table 2 & Fig 1.**

Table 2: Effect of acute treatment of MEAH on Time spent in the bright arena of the DLA

Groups (n=6)	**Treatment (Dose in mg/kg)**	**Duration of Time spent in the bright arena in seconds (mean ± SEM)**	**p value of different groups when compared with the control**	**Significance**
I	Control (normal saline)	100.83 ± 1.04	-	-
II	Diazepam (1.0)	108.00 ± 1.06	<0.001**	Highly Significant
III	MEAH (50)	110.83 ± 0.87	<0.001**	Highly Significant
IV	MEAH (100)	123.83 ± 1.10	<0.001**	Highly Significant
V	MEAH (200)	120 ± 0.85	<0.001**	Highly significant

All values are Mean ± SEM; statistical analysis by one-way ANOVA followed by Dunnett's test; p value > 0.05 = Not significant, < 0.05 = Significant, < 0.01 = Highly significant compared with control.

Fig 1: Effect of acute treatment of MEAH on Time spent in the bright arena of the DLA



X – axis: Groups of experimental animals
Y – axis: Mean time spent in bright arena

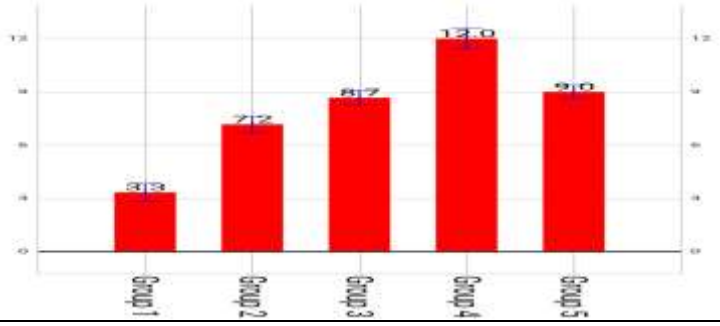
The differences in the number of entries into the bright arena for various groups of the DLA are as shown in **Table 3 & Fig 2.**

Table 3: Effect of acute treatment of MEAH on Number of entries into the Bright arena of the DLA:

Groups (n=6)	**Treatment (dose in mg/kg)**	**Number of entries into the Bright arena (mean ± SEM)**	**p value of different groups when compared with the control**	**Significance**
I	Control (normal saline)	3.33 ± 0.49	-	-
II	Diazepam (1.0)	7.16 ± 0.47	<0.001**	Highly Significant
III	MEAH (50)	8.66 ± 0.42	<0.001**	Highly Significant
IV	MEAH (100)	12.0 ± 0.57	<0.001**	Highly significant
V	MEAH (200)	9.0 ± 0.36	<0.001**	Highly significant

All values are Mean ± SEM; statistical analysis by one-way ANOVA followed by Dunnett's test; p value > 0.05 = Not significant, < 0.05 = Significant, < 0.01 = Highly significant compared with control.

Fig 2: Effect of acute treatment of MEAH on Number of entries into the Bright arena of the DLA



X – axis: Groups of experimental animals
Y – axis: Mean time spent in bright arena

NUMBER OF REARS IN THE BRIGHT ARENA (ACUTE STUDY):

Mean number of rears in the Bright arena for the control group was found to be 0.83 ± 0.30. The standard group treated with diazepam showed a mean number of 2.66 ± 0.33. For the group pre-treated with a single dose of MEAH 50 mg/kg, it was 2.33 ± 0.49, for MEAH 100 mg/kg was 2.83 ± 0.54, for MEAH 200 mg/kg was 2.5 ± 0.42.

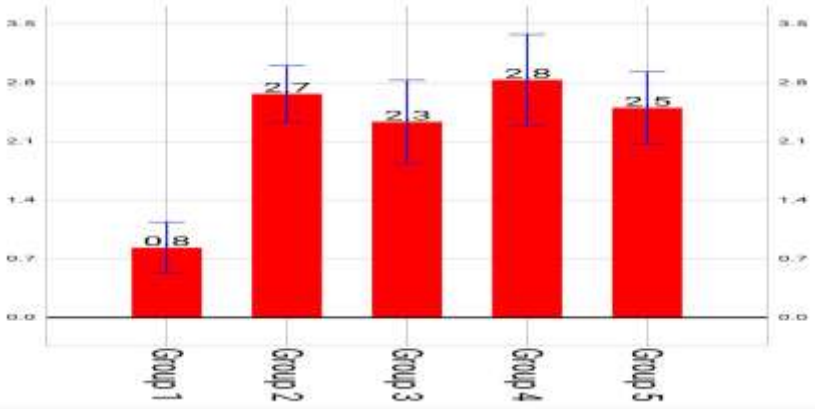
The differences in the number of rears in the bright arena for all groups of the DLA are as shown in **Table 4 & Fig 3.**

Table 4: Effect of acute treatment of MEAH on Number of rears in the Bright arena of the DLA

Groups (n=6)	**Treatment (dose in mg/kg)**	**Number of Rears in Bright Arena (mean ± SEM)**	**p value of different groups when compared with the control**	**Significance**
I	Control (normal saline)	0.83 ± 0.30	-	-
II	Diazepam (1.0)	2.66 ± 0.33	0.02*	Significant
III	MEAH (50)	2.33 ± 0.49	0.068	Not Significant
IV	MEAH (100)	2.83 ± 0.54	0.011*	Significant
V	MEAH (200)	2.5 ± 0.42	0.038*	Significant

All values are Mean ± SEM; statistical analysis by one-way ANOVA followed by Dunnett's test; p value > 0.05 = Not significant, < 0.05 = Significant, < 0.01 = Highly significant compared with control.

Fig 3: Effect of acute treatment of MEAH on Number of rears in the Bright arena of the DLA



X – axis: Groups of experimental animals
Y – axis: Mean time spent in bright arena

MEAH produced a significant increase in exploratory behaviour compared to control animals.

- **Time spent in bright arena:** MEAH at 100 mg/kg showed the highest increase (123.83 ± 1.10 s), surpassing diazepam and other doses ($p < 0.001$).
- **Number of entries:** A dose-dependent increase was observed, with the 100 mg/kg dose demonstrating maximal effect (12.0 ± 0.57 entries).

- **Rearing behaviour:** Significant increases were observed at 100 and 200 mg/kg doses, indicating enhanced exploratory activity.

Overall, MEAH exhibited anxiolytic activity comparable to diazepam, with optimal efficacy at 100 mg/kg.

DISCUSSION

The present study demonstrates that methanolic extract of Artocarpus heterophyllus seeds possesses significant anxiolytic activity in Swiss albino mice. Increased exploration of aversive environments in both the Dark–Light Arena is a well-established indicator of reduced anxiety levels [10,11].

The anxiolytic effect of MEAH may be attributed to its rich phytochemical composition, particularly flavonoids and phenolic compounds. Flavonoids are known to interact with the GABA_A receptor complex, producing benzodiazepine-like anxiolytic effects without pronounced sedation [12–14]. The observed dose-dependent response, with maximal activity at 100 mg/kg, suggests receptor-mediated modulation rather than nonspecific CNS stimulation.

Interestingly, the anxiolytic efficacy of MEAH was comparable to diazepam, indicating its potential as a natural alternative with possibly fewer adverse effects. These findings align with previous studies demonstrating CNS-modulatory properties of plant-derived flavonoids and phenolics [15–18].

However, the study is limited by the absence of neurochemical and molecular analyses. Further studies exploring neurotransmitter modulation, receptor binding, and chronic toxicity are warranted.

CONCLUSION

The methanolic extract of Artocarpus heterophyllus seeds exhibits significant anxiolytic activity in validated animal models of anxiety. The extract enhanced exploratory behaviour in a manner comparable to diazepam, particularly at a dose of 100 mg/kg. These findings support the traditional use of A. heterophyllus and highlight its potential as a promising natural anxiolytic agent. Further mechanistic and clinical studies are recommended to establish its therapeutic utility.

REFERENCES

1. Stein MB, Sareen J. Generalized anxiety disorder. N Engl J Med. 2015;373(21):2059–2068.
2. Bandelow B, Michaelis S. Epidemiology of anxiety disorders in the 21st century. Dialogues Clin Neurosci. 2015;17(3):327–335.
3. Baldwin DS, Anderson IM, Nutt DJ, et al. Evidence-based pharmacological treatment of anxiety disorders, post-traumatic stress disorder and obsessive-compulsive disorder. CNS Drugs. 2011;25(1):1–15.
4. Lader M. Benzodiazepines revisited—will we ever learn? CNS Drugs. 2011;25(1):19–29.
5. Jagtap UB, Bapat VA. Artocarpus: A review of its traditional uses, phytochemistry and pharmacology. Food Chem. 2010;122(3):702–707.
6. Arung ET, Wicaksono BD, Handoko YA, et al. Anti-melanogenesis properties of phenolic compounds from Artocarpus heterophyllus. J Nat Med. 2009;63(4):384–388.
7. Vazhacharickal PJ, Sajeshkumar NK, Mathew JJ, et al. Neuroprotective and antioxidant potential of Artocarpus heterophyllus seed extract. J Ethnopharmacol. 2018; 222:205–213.
8. Shyamal S, Latha PG, Shine VJ, et al. Phytochemical and pharmacological evaluation of Artocarpus heterophyllus. Pharmacogn Rev. 2015;9(17):78–82.
9. Islam MS, Nasrin S, Khan MA, et al. Evaluation of antioxidant and CNS depressant activities of Artocarpus heterophyllus seed extract. BMC Complement Altern Med. 2016; 16:220.
10. Bourin M, Hascoët M. The mouse light/dark box test. Eur J Pharmacol. 2003;463(1–3):55–65.
11. Walf AA, Frye CA. The use of the elevated plus maze as an assay of anxiety-related behaviour in rodents. Nat Protoc. 2007;2(2):322–328.
12. Paladini AC, Marder M, Viola H, et al. Flavonoids and the central nervous system: from forgotten factors to potent anxiolytic compounds. CNS Neurol Disord Drug Targets. 2013;12(1):27–48.
13. Fernández SP, Wasowski C, Loscalzo LM, et al. Central nervous system depressant action of flavonoid glycosides. J Neurosci. 2006;26(20):5360–5367.
14. Griebel G, Belzung C, Perrault G, et al. Differences in anxiety-related behaviours and sensitivity to diazepam in inbred mouse strains. Prog Neuropsychopharmacol Biol Psychiatry. 2003;27(4):555–567.
15. Kumar S, Sharma A. Anti-anxiety activity of plant-derived bioactive compounds: A review. J Ethnopharmacol. 2016; 193:159–170.
16. Gupta G, Kazmi I, Afzal M, et al. Anxiolytic and antidepressant-like activities of flavonoids: A review. Biomed Pharmacother. 2018; 97:183–193.
17. Zhang ZJ. Therapeutic effects of herbal extracts and constituents in animal models of psychiatric disorders. CNS Drugs. 2004;18(8):495–513.
18. Ernst E. Herbal remedies for anxiety—A systematic review of controlled clinical trials. J Psychopharmacol. 2006;20(1):84–90.

