e - ISSN - 2249-7668
Print ISSN - 2249-7676

International Journal of
Pharmacology & Toxicology

www.ijpt.org

EFFECT OF *Artocarpus heterophyllus* PHENOLIC SEED EXTRACT IN ANIMAL MODELS OF ANXIETY: A PRECLINICAL STUDY

Prabhakar Adake[1], Mahalaxmi S Petimani[2], Nagapati Prabhakar Bhat[3*]

[1]Associate Professor of Pharmacology, Yenepoya Medical College, Yenepoya (Deemed to be University), Deralakatte, Mangalore-575018, Karnataka, India.
[2]Assistant Professor of Biochemistry, Yenepoya Medical College, Yenepoya (Deemed to be University), Deralakatte, Mangalore-575018, Karnataka, India.
[3]Assistant Professor of Pharmacology, Yenepoya Medical College, Yenepoya (Deemed to be University), Deralakatte, Mangalore-575018, Karnataka, India.

ABSTRACT

Background: Many indigenous plants with anti oxidant property have shown to be beneficial in many behavioral disorders like anxiety. **Objective:** To evaluate anxiolytic activity of *Artocarpus Heterophyllus* phenolic seed extract (AHPSE) and to screen for it's the possible potentiating anxiolytic activity with Diazepam in Swiss albino mice. **Methodology:** A total of 84 healthy male Swiss albino mice weighing 25-35 g. were used and they were divided into fourteen groups of six mice in each. First seven groups (1^{st} -7^{th}) were evaluated by Light and Dark Arena (LDA) and remaining by Elevated Plus Maze (EPM) for anxiolytic action. 1^{st} group (control) received normal saline 10mg/kg, 2^{nd} group (standard) Diazepam 1mg/kg, 3^{rd} , 4^{th}, 5^{th} , 6^{th} and 7^{th} groups (test) respectively received AHPSE in different doses 100mg, 200mg, 400mg, 800 mg and 800mg/kg + 1mg/Kg Diazepam per orally. They were evaluated for anxiolytic activity using LDA after 60 minutes of drug administration. Similarly, remaining seven groups received the same drugs and evaluated using EPM after 60 minutes of drug administration. The duration of time spent and numbers of entries in light compartment/open arm and dark compartment/closed arm were noted for five minutes for each mouse. **Results:** One way ANOVA followed by Tukey-Kramer multiple comparison test clearly showed that AHPSE in all the doses (100-800mg/kg) has shown increase in the time spent in light compartment/open arm and decrease in the time spent in the dark compartment/closed arm when compared to control and standard groups ($p<0.001$) in both LDA and EPM models. The study also proved potentiating activity of AHPSE in both the models when combined with Diazepam with p value <0.001. **Conclusion:** The current study has demonstrated an anxiolytic and potentiating effect of AHPSE in animal models of anxiety.

Keywords: *Artocarpus Heterophyllus* phenolic seed extract, Elevated plus maze, light and dark arena.

INTRODUCTION

Anxiety disorders are the most common psychiatric illnesses in the general population. Around 15–20% patients in general practice present with anxiety disorders. It is characterized by excessive rumination, worrying, uneasiness, apprehension and fear about future uncertainties either based on real or imagined events, which may affect both physical and psychological health.

Numerous studies have shown that patients with anxiety disorders over-estimate the dangerousness of various stimuli.

Current psychiatric diagnostic criteria recognize a wide variety of anxiety disorders. Recent surveys have found that as many as 18% of Americans, 20.7% of Indians and 14% of Europeans may be affected by one or more of them [1].

Corresponding Author:- **Dr. Nagapati Prabhakar Bhat** Email:- npbhat17@gmail.com

The DSM-IV (American Psychiatric Association) includes the following major categories of anxiety disorders: Panic disorder (with or without agoraphobia), agoraphobia without panic, social phobia (social anxiety disorder), specific phobia, generalized anxiety disorder (GAD), acute stress disorder, posttraumatic stress disorder, obsessive compulsive disorder, and anxiety disorder not otherwise specified [2].
Low levels of GABA, an inhibitory neurotransmitter in the central nervous system, contribute to anxiety. A number of anxiolytics achieve their effect by modulating the GABA receptors. Benzodiazepines are most commonly used, safe and effective drug in the short term management of anxiety. Long-term use of benzodiazepines has adverse psychological and physical effects. They are also associated with tolerance, physical dependence and withdrawal syndrome on sudden discontinuation [3]. Hence need for newer, better-tolerated and more efficacious treatments remains high.
Oxidative stress mechanisms underlying anxiety disorder has been in existence since long, with the earlier suggestion that NO and peroxy-nitrite might play a major role in setting up a vicious etiological cycle involving free radicals and inflammatory cytokines in post-traumatic stress disorder [4].
Association of vitamin E depletion and increased oxidative stress markers, anxiety behaviors in phospholipid transfer protein (PLTP) knock-out mice has further enhanced oxidative role in pathogenesis of anxiety. Even clinical studies have reported elevated lipid peroxidation products and antioxidant changes in obsessive–compulsive disorder, panic disorder and social phobia. Following treatment with Citalopram for social phobia, there was a reversal of these disturbances [5,6].
Thus anti-oxidants could have anxiolytic potential by preventing oxidative pathway. Green tea polyphenol (–) epigallocatechin gallate (EGCG), and chlorogenic acid, a dietary polyphenol, showed anxiolytic effects on mice model with a dose-dependent manner due to their potent antioxidant property [7].
The jackfruit (*Artocarpus heterophyllus Lam.)* belonging to family Moraceae is an integral part of common Indian diet and commonly known as "Kathal". Jackfruit appears in the Indian market in spring and is available till summer. Jackfruit pulp is eaten afresh and used in fruit salads and possesses high nutritional value [8]. Jackfruit also has been reported to contain antioxidant prenyl flavonoids [9]. However, jackfruit seeds are less popular as vegetable and are eaten when boiled or roasted. These are believed to be digested with difficulty. The composition of jackfruit seeds has been reported and found to contain similar compositions as that of grains. The seeds are also rich source of carbohydrates and proteins and good source of fibre and vitamins. A major protein, Jacalin has been isolated from jackfruit seeds and possessed immunological properties [10].
Phytochemical studies have shown the presence of many valuable compounds such as saponins, alkaloids, polyphenols, and flavonoids. The results of different in vitro antioxidant activity assays indicated that these seed extracts possessed appreciable free radical scavenging effects and metal ion chelating activity in a concentration-dependent manner [11].

So the present study carried out to elucidate the possible anxiolytic and potentiating activity of *Artocarpus Heterophyllus* phenolic seed in Swiss albino mice.

OBJECTIVE:

- To evaluate the possible anxiolytic activity of *Artocarpus Heterophyllus* phenolic seed extract in Swiss albino mice.
- To screen the possible potentiating anxiolytic activity of *Artocarpus Heterophyllus* phenolic seed extract with Diazepam in Swiss albino mice.

MATERIALS AND METHODS:

Animals: Institutional Animal Ethical clearance was obtained before conducting the study. Male Swiss albino mice weighing 25-35 g. were used for the study. The mice were housed in the central animal house of the Department of Pharmacology, Yenepoya Medical College, Yenepoya Deemed to be University, Mangalore, Karnataka, India under suitable conditions of housing, temperature, ventilation and nutrition. The study was conducted in accordance with standard CPCSEA guidelines.

Collection of plant material: *Artocarpus heterophyllus* (Jackfruit) was collected from the places around the Mangalore, Karnataka, India**.** *A. heterophyllus* seeds were cleaned and sliced with around 2mm thickness and sun dried for 7 days without removing the thin brown spermoderm covers the fleshy white cotyledons. The dried seeds were grinded uniformly for 10 minutes with highest precaution to avoid any contamination and made them as particle-sized powder (<0.5mm). The powdered material was packed in plastic pouch and stored in normal room temperature until use. The procedure for jackfruit seed powder extraction was followed based on previous reports in the literature [12]. Powder was subjected to solvent extraction with phenol in a soxhlet apparatus. After exhaustive extraction, the phenolic extract was dried at low temperature under reduced pressure in a rotary evaporator to obtain greenish-black colored residue which was used for anxiolytic studies.
Drugs: The standard antianxiety drug Diazepam tablet 5mg was purchased from institutional pharmacy store.

Inclusion criteria:

- Male Swiss albino mice weighing between 25-35g.
- Age 3-4 months.
- Healthy with normal behavior and activity.

Exclusion criteria:

- Mice <25g and >35 and age <3 months and > 4 months.
- Female mice were excluded
- Animals previously used in other experiments.

A total of 84 animals (n=84) were used. Mice were divided into fourteen groups of six animals each.

Acute oral toxicity test: Acute oral toxicity test was carried out in male *Swiss albino* mice according to Organization of Economic Co-operation and Development (OECD) guidelines, ANNEX- 423 [13]. Mice were administered AHPSE in a dose of 5, 50, 300 and 2000 mg/kg per orally to find out safe dose range in animals. Mice were observed for 48 hours from the time of drug administration and looked for general behavior and mortality.

Anxiolytic activity of AHPSE was evaluated by two models –Elevated plus maze and light and dark arena. The experiment was conducted in Ethanopharmacology laboratory of the Department of Pharmacology, Yenepoya Medical College, Yenepoya University, between 8:00 A.M. to 2:00 P.M. The food and water will be removed during study period. Appropriate calculated dose of drug was given to each mouse according to its body weight, per orally (p.o). The experiment was conducted sixty minutes after the administration of the drug.

Elevated plus maze: It is a novel test for the evaluation of anxiolytic drug effects in rodents. The wooden plus maze consists of two open arms (length 16 cm X breadth 5 cm) and two closed arms of the same size (height 12 cm). The arms of the same type are opposite to each other, with a central square of 5 cm. The maze is elevated to a height of 25 cm above the floor. The apparatus consists of an open top wooden box. The mouse is placed individually at the centre of the elevated maze with their head facing towards open arm during 5min test period. The preference of the mouse for first entry, the number of entries into the open and closed arms reflects the relative safety of closed arms as compared with the relative fearfulness of open arms. Mouse being rodent feel safe in dark, hence normal rodents prefer dark arm first. Anxiolytics would be expected to increase the proportion of entries into and time spent in open arms [14].

Light and dark arena: The apparatus consisted a rectangular box (45 × 27 × 27 cm), partitioned into two compartments connected by a 7.5 × 7.5 cm opening in the wall between compartments. Each mouse is placed in the center of the light compartment and observed for 5 minutes for the time spent in open (white/light) compartment [15].

RESULTS:

One way ANOVA was used for multiple comparisons followed by Tukey- Kramer multiple comparison tests for comparison between groups. The results are expressed in Mean ± SD.

Table 1: Effect of AHPSE on mice behavior in LDA model

Group	No of Entry into light box	No of Entry into dark box	Time spent in light box	Time spent in dark box
Group I: NS 10 ml/kg	10.35± 0.01	20.63 ± 1.28	87± 1.4	212.98±1.3
Group II: Diazepam 1 mg/kg	18.55± 0.01*	16.13±0.44*	183.91±2.13*	114.51±1.79*
Group III: AHPSE 100 mg/kg	8.25± 0.01	8.5±0.47*[a]	76.44±0.98	222.98±3.07
Group IV: AHPSE 200 mg/kg	7.25± 0.02	7.07±0.40*[a]	102.05±2.8*	197.68±2.34*
Group V: AHPSE 400 mg/kg	9.6±0.12	9.1±0.7*[a]	128.95±1.25*	171.03±2.45*
Group VI AHPSE 800mg/Kg	6.60± 0.16	5.45±0.62*[a]	144.51±2.8*	153.68±0.01*
Group VII AHPSE 800mg/kg+ 1mg Diazepam	7.53± 0.10	8.07±0.7*[a]	92.11±2.14*	206.93±2.50*

AHPSE= *Artocarpus Heterophyllus* phenolic seed extract; N=6; Mean ± SD,
* p<0.001 on comparing G 2-7 with G1 ; a: p<0.001 on comparing with G2

Table 2: Effect of AHPSE on mice behavior in EPM model

Group	No of Entry into open arm	No of Entry into closed arm	Time spent in open arm	Time spent in closed arm
Group I: NS 10 ml/kg	11.62±1.78	21.46±1.53	84.38±3.17	213.41±2.4
Group II: Diazepam 1 mg/kg	25.71±2.06*	15.45±0.7*	146.5±2.5*	147.5±2.73*
Group III: AHPSE 100 mg/kg	18.88±0.84*	13.7±2.64*	99.03±0.96*	195.81±4.9*
Group IV: AHPSE 200 mg/kg	18.33±0.91*	9.3±0.57*[a]	112.43±3.54*	186.26±1.6*
Group V: AHPSE 400 mg/kg	16.4±0.57*	13.15±2.8*	137.5±1.79*	161.4±2.93*
Group VI AHPSE 800 mg/kg	14.72±0.48*	14.04±10*	147.20±1.70*	146.51±4.07*
Group VII AHPSE 800 mg/kg+ 1mg diazepam	17.2±1.6*	14.68±1.67*	146.38±2.18*	152.0±3.3*

AH= *Artocarpus Heterophyllus* N=6; Mean ± SD, * p<0.001 on comparing G 2-7 with G1
a: p<0.001 on comparing with G2

DISCUSSION:

Artocarpus Heterophyllus also known as jack tree [16]. There are about 100-500 seeds per fruit. The seed coat consists of a thin parchment like husk and a brownish membranous tegmen. The cotyledons are unequal in size and endosperm is minimally present. Previous studies showed that *Artocarpus Heterophyllus* has antibacterial, anti-inflammatory, antiangiogenic, antidiabetic, anti-carcinogenic, antifungal, anti-oxidative and wound healing properties [17,18]. Present study was conducted to evaluate the possible anxiolytic and potentiating anxiolytic activity of *Artocarpus Heterophyllus* phenolic seed extract in Swiss albino mice.

In this study, we have performed acute oral toxicity test in male *Swiss albino* mice according to Organization of Economic Co-operation and Development (OECD) guidelines, ANNEX- 423.[13] Mice were administered AHPSE in a dose of 5, 50, 300 and 2000 mg/kg per orally to find out safe dose range in animals. Mice were observed for 48 hours from the time of drug administration and looked for general behavior and mortality. There was no morbidity or mortality observed. So the dose of 100, 200, 400 and 800 mg/kg of AHPSE was chosen for the present study. Then mice were evaluated for anxiolytic activity of AHPSE by using Light and dark arena (LDA) test and Elevated plus maze (EPM) tests.

In LDA**,** Mice treated with standard drug Diazepam have shown increase in the time spent in light box and decrease in the time spent in dark box (table 1). AHPSE in all the doses (100-800mg/kg) have shown increase in the time spent in light box and decreased in the time spent in the dark box when compared to control and Diazepam with p value <0.001. There is statistically significant potentiating activity of AHPSE was noted in LDA model when combined with Diazepam with p value <0.001.

Similarly, in EPM test, AHPSE in all the doses (100-800mg/kg) have shown increase in the time spent in open arm and decrease in the time spent in the closed arm along with increase in the number of entries in the open arm and decrease in the number of entries in the closed arm when compared to control with p value <0.001 (table 2). However, only one dose of AH (200mg/kg) has shown statistically significant anxiolytic action when compared to standard drug Diazepam with p value <0.001. Even the combined effect of AH 800mg/Kg with diazepam has shown statistically significant anxiolytic action when compared to control group with p value <0.001. The result clearly showed the anxiolytic property of *Artocarpus Heterophyllus* phenolic seed extract in light dark arena and elevated plus maze models. Phytochemical studies have shown the presence of many valuable compounds such as saponins, alkaloids, polyphenols, and flavonoids. Further studies are required to evaluate the exact mechanism of *Artocarpus Heterophyllus* as an anxiolytic drug.

CONCLUSION:

The current study has demonstrated an antianxiety effect of *Artocarpus Heterophyllus* phenolic seed extract. This could probably be due to its antioxidant property.

REFERENCES

1. Kessler RC, Chiu WT, Demler O, Merikangas KR, Walters EE. Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. *Arch. Gen. Psychiatry*, 62 (6), 2005, 617–627.
2. Diagnostic and statistical manual for the assessment of mental disorders. 4th ed. Washington, DC: American Psychiatric Association; 1994. American Psychiatric Association.
3. Brody TM, Larner J, Minneman KP, and Neu HC. Human Pharmacology: *Molecular to Clinical*, 2nd ed. St. Louis: Mosby Year-Book, 1998.
4. Miller CS. Are we on the threshold of a new theory of disease? Toxicant-induced loss of tolerance and its relationship to addiction and abdiction. Toxicology and Industrial Health 1999;15: 284–294.
5. Desrumaux C, Risold PY, Schroeder H, Deckert V, Masson D, Athias A, Laplanche H, Le Guern N, Blache D, Jiang XC, et al. Phospholipid transfer protein (PLTP) deficiency reduces brain vitamin E content and increases anxiety in mice. *FASEB Journal*, 19, 2005, 296–297.
6. Ersan S, Bakir S, Erdal Ersan E, Dogan O. Examination of free radical metabolism and antioxidant defence system elements in patients with obsessive-compulsive disorder. *Progress in Neuropsychopharmacology and Biological Psychiatry*, 30, 2006, 1039–1042.
7. Vignes M, Maurice T, Lante F, Nedjar M, Thethi K, Guiramand J, Recasens M. Anxiolytic properties of green tea polyphenol (x)-epigallocatechin gallate (EGCG). *Brain Research*, 1110, 2006, 102–115.
8. Samaddar HM. Jackfruit. In: T. K. Bose,and S. K. Mishra. Fruits of India: tropical and subtropical, Naya Prokash/Calcutta, India, 1985, pp. 638- 64
9. Jagtap UB, Panaskar SN and Bapat VA, Evaluation of antioxidant capacity and phenol content in jackfruit (Artocarpus heterophyllus) fruit pulp. *Plant Foods Hum Nutr*, 65, 2010, 99-104.
10. Selvaraj Y and Pal DK: Biochemical changes during ripening of jackfruit (Artocarpus heterophyllus L). *J Food Sci Technol*, 26, 1989, 304-307.
11. Deepika gupta, sonia mann, avijit sood and rajinder k. Gupta. Phytochemical, nutritional and antioxidant activity evaluation of seeds of jackfruit (*Artocarpous Heterolphyllus* Lam.). *International Journal of Pharma and Bio Sciences*, 2(4), 2011, 336-345.
12. Kabir S. The Isolation and characterization of jacalin *Artocarpus heterophyllus* (Jackfruit) Lectin] based on its charge properties. *International J. Biochem Cell Biol*, 27(2), 1995, 147–156.
13. Abrar Hussain Mir, Manjusha Sexena and Mohd Yousuf Malla. An acute oral toxicity study of methanolic extract from Tridex procumbens in Sprague Dawley's Rats as per OECD guidelines 423. *Asian Journal of Plant Science and Research*, 3(1), 2013, 16-20.
14. Kulkarni SK. Delhi: Vallabh Prakashan. Handbook of Experimental Pharmacology 1999, pp. 135–37.
15. Crawley J, Goodwin FK. Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. *Pharmacol Biochem Behav*, 13, 1980, 167–70.
16. Rehman MA, Nahar N, Mian AJ, Mosihuzzman M. Variation of carbohydrate composition of two forms of fruit from jack tree (*Artocarpus heterophyllus* L.) with maturity and climatic condition. *Food chemistry*, 65, 1999, 91-97.
17. 17. Khan MR, Omolso AAD, Kihara M. Antibacterial activity of *Actocarpus heterophyllus*. *Fitoterapia.* 74(5), 2003, 501-5
18. Oktavia S, Wijayanti N, Retnoaji B. Anti-angiogenic effect of *Actocarpus heterophyllus* seed methanolic extract in ex ovo chicken chorioallantoic membrane. *Asian Pacific Journal of Tropical Biomedicine*, 7(3), 2017, 240-4.