

## PHARMACOLOGICAL STUDY AND EVALUATION OF ANTI-ANXIETY ACTIVITY OF LEAVES OF *AZIMA TETRACANTHA*

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Article Received on  
19 March 2018,

Revised on 09 April 2018,  
Accepted on 29 April 2018

DOI: 10.20959/wjpr20189-12097

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### ABSTRACT

Pathological anxiety is one of the most common mental disorders in humans. Anxiolytic drugs, mostly belonging to benzodiazepines (BDZ) group & serotonergic groups are widely used to treat anti-anxiety. However the clinical use of these established drugs are associated with a lot of adverse effects.<sup>[1]</sup> Therefore the development of new drug agent possessing anxiolytic effect with minimal or no adverse effect would be great importance in the treatment of anxiety related disorder. Very high performance is given to the phytoconstituents by the scientific community. for a new anxiolytic agent administered orally for 10 days to Wistar rats for screening effects of anxiolytic effect by using Elevated plus maze (EPM) &

Light Dark arnea (LAD) test.<sup>[3]</sup> The result of our study demonstrated that the ethanolic extract of *azima tetracantha* leaves has significant anxiolytic activity in EPM & LAD models of anxiety and supports the traditional use of this plant.<sup>[4]</sup>

**KEYWORDS:** ethanolic extract, elevated plus maze, light dark arnea, BDZ group.

### INTRODUCTION

Anxiety is a kind of emotional reaction it is an unpleasant emotion that most people feel when they face challenges. Anxiety is like some sort of worry. Mild anxiety can help people perform at their best. Best when anxiety becomes more intense, it causes distress, lasts for a longer time & interferes with daily activity. Which is a medical concern Anxiety disorder is one of the most common mental disorders in human. Drugs belonging to the benzodiazepines group have a prominent position among anxiolytic drugs. Currently the drugs affecting the

serotonergic system are concerned as first choice. In anxiety disorder only a small percentage of people attain a symptomatic free state during the course of therapy. Remission rates with serotonergic drugs are lower than the condensational benzodiazepins<sup>2</sup>. Therefore the treatment of anti-anxiety disorder, a novel agent with good therapeutic effect and with a better compliance is needed. *Anima tetraacantha* is a branching herb found in southern & eastern region of India. This plant belonging to the family Salvadoraceae was traditionally used for the treatment of analgesic, anti-inflammatory.<sup>[2]</sup> The leaves and roots have emetic, expectorant, diaphoretic, laxative and purgative properties. It has also been used for the treatment of asthma, allergic, cold, dysentery. There was no report on the anxiolytic activity of this indigenous plant. So in this pre-clinical study we have evaluated anxiolytic role of *Azima Tetraacantha* by using rat models.

## MATERIALS AND METHODS

**Animals:** Young adult wistar rats of either sex weighing 175- 200gm were used in this study after obtaining animal ethical committee clearance. The rats were maintained under standard condition in the animal house (CPCSEA approved regdno1960/PO/Re/S/16/CPCSEA) in dept of pharmacology of jeypore college of pharmacy, jeypore. The rats were kept in polypropylene cages (mfgd by POLYLAB LTD) under standard condition of pellet diet, water, and ad libitum. Animals were acclimatized under standard laboratory condition and were kept in 12hrs day and night cycle for seven days before conducting experiment.

**Drugs/Dose/Route of administration:** Diazepam (cipra ltd) was obtained from jeypore sub-divisional hospital, jeypore. It was administered at a dose of 1mg/kg in intra peritoneal.

**Instruments:** Soxhlet apparatus was used to prepare the plant extract. Elevated plus maze (EPM) apparatus and light dark arena (LDA) apparatus was used for screening anxiolytic activity.

**Plant Material:** *Azima tetraacantha* plant was cultivated during the month of June. The fresh leaves were collected in the month of September. Authentication of this plant was done by Swaminathan research center jeypore. The leaves were shade dried, grinded into coarse powder and used for extraction.<sup>[2]</sup>

**Preparation of Extract:** *Azima tetraacantha* ethanolic extract (ATEE). A weighed quantity (500mg) of the coarse powder was taken and extracted with ethanol (90%) in a Soxhlet

apparatus. The extract was concerned on a water bath at a temp not exceeding 60 degree centigrade. The % of yield of the extract was 10%. The ethanolic extract was suspended in distilled water ATEE was administred at a dose of 300mg /kg/bodyweight /day I n orally.

**Experimental Design:** 36 no of animals were used in this study. The animals were divided in to 3groups. Each group consisting of 6males and 6 females (n= 12).

**Group 1:** Normal saline (0.1ml) i.p for 10 days.

**Group 2:** ATEE (300mg /kg/day orally) for 10 days.

**Group 3:** Diazepam (1mg/kg/i.p) for 10 days.

On the 14<sup>th</sup> day after half an hour of the administration of test compounds the animals were taken for the following tests for screening of anxiolytic activity.

**Elevated Plus Maze (Epm):** This test has been widely validated to measure anxiety in rodents. The plus maze combines 3potential anxiolytic factors, novelty, height, and open space. Briefly the cross shaped maze consist of four arms that are connected by a central platform. Two opposing arms are surrounded by side and end walls, where the remaining two arms are unprotected. The setup consist of a maze of 2 open arms (25cm\*5cm) and crossed walls (35cm height and central plat form facing one of the enclosed arms and observed for 5 mints. During the 5 mints test period, the time spent in open and enclosed arms were recorded.<sup>[5]</sup>

**Light Dark Arnea (Lda):** Light dark exploration test is one of the few tests specifically designed for use in rats. The original maze is divided in to two parts, 1/3 with opaque walls and a cover (dark compartment) where as remaining 2/3 was open and illuminated (light compartment). The door between the two compartment permit rats to move from one side to another. Each rat was released in the light compartment and observed for 5 mints. Time spent in light and dark compartment are recorded.<sup>[6]</sup>

**Stastical Analysis:** Result were expressed as mean + or – SD. One way analysis of variance (ANOVA) was carried out & the statically comparison among the group were performed with Tukey Krammer test using a statistical package using a statistical package programme.

P< 0.05 was considered as significant.

**Table 1: (Anxiolytic Effect of Atee By Elevated Plus Maze Test).**

Groups	Drugs	Time spend in sec in open area	Time spend in sec in closed area
1	Normal saline	9.06+_1.04	264.33+_ 6.21
2	ATEE	55.13+_6.90	154.46+_ 11.53
3	Diazepam	51.58+_ 7.68	159.27+_ 7.21

One way ANOVA followed by Turkey Kramer multiple comparison test, Result are expressed as mean +\_SD; n=12. P<0.001 extremely significant, ATEE (Azima tetracantha ethanolic extract).

**Table-2: (Anxiolytic Effect of Atee By Light And Dark Arnea Test).**

Groups	Drugs	Time spend in sec in light area	Time spend in sec in dark area
1	Normal saline	34.01+_ 7.332	254+_6.44
2	Atee	139.27+_3.32	162.85+_9.51
3	Diazepam	123.53+_3.44	170.73+_ 4.35

One way ANOVA followed by Tukey Kramer multiple comparison test, Result are expressed as mean +\_SD; n=12. P<0.001 extremely significant, ATEE (Azima tetracantha ethanolic extract).

## RESULT

**Elevated Plus Maze:** ATEE treated animals (**GROUP -2**) showed a significant ( $p < 0.001$ ) increase in the mean time spent in open arms(**TABLE -1**) by EPM test on comprising between the ATEE treated animals (**GROUP -2**) and Diazepam treated ones (**GROUP -3**).

**Light And Dark Arnea:** ATEE treated animals (**GROUP -2**) showed a significant ( $p < 0.001$ ) increase in the time spent in bright arnea (**TABLE-1**) by LDA test on comprising with normal (GROUP-1). But there is no significant difference between ATEE treated animals (**GROUP -2**) & Diazepam treated animal (**GROUP-3**). |The above observation suggest that AZIMA TETRACANTHA has anxiolytic activity.

## DISCUSSION

The neurobiology of anxiety disorder is not fully known. Low level of GABA in CNS is most frequently associated with anxiety disorders. In addition to GABA, 5-HT plays an important role in the development and the persistence of anxiety disorders. Many studies have shown that patient with anxiety disorder have genetic polymorphism in the 5-HT transporter. Anxiety disorder can also be due to free radical induced damage to GABA nergic and serotonergic systems. Relatively little information exist on the CNS activity of AZIMA

**TETRACANTHA.** In our study the ethanolic extract of Azima Tetracantha showed significant anxiolytic activity. Its role on other neurotransmitter like serotonin, acetylcholine and nor-epinephrine can not be ruled out. Further studies are going to elucidate the extract mechanism. By which this plant extract exerts the anxiolytic activity.

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