



## Research Article

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### ACUTE ANXIOLYTIC ACTIVITY OF AQUEOUS EXTRACT OF *TERMINALIA CHEBULA* FRUIT PULP IN RATS

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#### ABSTRACT

Anxiety is a general term for several disorders that cause nervousness, fear, apprehension and worry. These disorders affect how we feel and behave and they can even manifest as real physical symptoms. Mild anxiety is vague and unsettling, while severe anxiety can be extremely debilitating, having a serious impact on daily life. When anxiety becomes excessive, it may fall under the classification of an anxiety disorder. Most of the times, benzodiazepines (BZDs) remains the major class of compounds used in anxiety. Although BZDs are safe in short term treatment for anxiety, on long term therapy they cause many adverse psychological and physical effects. In the present study, we have attempted to evaluate the acute anxiolytic activity of aqueous extract of *Terminalia chebula* Fruit Pulp (AETCFP) by using Elevated Plus Maze model in rats. The rats weighing 150–200gm were divided into 5 groups containing 6 animals for each dose and were housed for 10 days prior to experiment. For acute study, the control (1% Gum acacia), test drug AETCFP (9, 18 and 36mg/ kg) and standard drug Diazepam (1.0mg/kg) were administered orally one hour before behavioral experiment using Elevated Plus Maze. The results suggest that, behavioral disinhibitory effects of AETCFP exhibited anxiolytic activity at the dose of 18mg/kg comparable to diazepam.

**Keywords:** Anxiety, Anxiolytic, Elevated Plus Maze, *Terminalia chebula* fruit pulp, Tannic acid

#### INTRODUCTION

Anxiety is considered as a common and significant psychiatric disorder which affects about 10–30% of the general population.<sup>1-3</sup> Till date, BZDs are the most preferred treatment for the management of anxiety disorders, but they have side-effects such as sedation, muscle relaxation, amnesia, and dependence.<sup>4,5</sup> About 43% of patients with anxiety use some form of complimentary therapy like herbal medicines, massages, folk remedies and homeopathy etc.<sup>6</sup> Among complementary therapy, the most popular treatments include herbal medicines.<sup>7</sup>

Considerable adverse effects of BZDs have lead to grow interest in the use of natural remedies or herbal products, to treat anxiety. Currently, several plants have been reported to possess anxiolytic effects in different animal models of anxiety. Herbs such as St. John's wort and ginseng have been clinically introduced for the treatment of anxiety.<sup>8-11</sup> One of the best-established equipment for observing anxiolytic behavior in rodents is Elevated Plus Maze. Several plants increase the exploration of open arms in the elevated plus-maze test and are used to diminish anxiety in folk medicine, for example *Panax ginseng*,<sup>12</sup> *Passiflora coerulea*,<sup>13</sup> *Matricaria recutita*,<sup>14</sup> *Azadirachta indica*,<sup>15</sup> *Cassia siamea*,<sup>16</sup> *Tilia tomentosa*,<sup>17</sup> *Sesbania grandiflora*,<sup>18</sup> *Zingiber officinale*, *Ginkgo biloba* (Ginkgoaceae),<sup>19, 20</sup> *Casimiroa edulis*,<sup>21, 22</sup> and especially *Terminalia chebula* in chronic study.<sup>23</sup>

Triphala is an Ayurvedic herbal formula consisting of equal parts of three myrobalans (dried plumlike fruits) taken without seed: Amalaki (*Embllica officinalis*), Vibhitaki (*Terminalia bellerica*), and Haritaki (*Terminalia chebula*).<sup>24</sup> The Sanskrit word Triphala literally means "three fruits".<sup>25</sup> *Terminalia chebula*, one

of the ingredients in triphala belongs to family Combretaceae. It is a medium to large sized tree distributed throughout tropical and sub tropical Asia, including China and Tibet.<sup>26</sup> This tree wildly grows in the forests of Northern India, Uttar Pradesh, Bengal and is common in Tamil Nadu, Karnataka and in Southern Maharashtra. The fruit of *Terminalia chebula* is used medicinally in Indian system of medicine. In Ayurveda, this drug is considered to be a *rasayana* with literal meaning Path (ayana) of the Juice (rasa), or Elixir vitae for *Vata*, balances all three *doshas* (*tridosham*), enhances digestion (*dipanapachana*), sharpens the senses (*medhyam*), and displays alterative, astringent, expectorant, anti-inflammatory, anodyne, cardiogenic, laxative, antiseptic, and antiemetic properties.<sup>27-29</sup> *Terminalia chebula* exhibits a variety of biological activities such as anti-diabetic,<sup>30</sup> anti-cancer,<sup>31</sup> antimutagenic<sup>32</sup> and anti-viral activity.<sup>33</sup>

#### MATERIALS AND METHODS

The experimental project was approved by the Institutional Animal Ethics Committee (IAEC) of Yenepoya Medical College, Yenepoya University, Mangalore, India, dated 6<sup>th</sup> May 2010. The herbarium voucher specimen of the test drug has been deposited in the museum of the Dept. of Pharmacology, Yenepoya Medical College, Yenepoya University, Mangalore, India (Ref. no YU/TC/23/2010).

#### Animals

Adult male and female Wistar Albino rats (equal number) weighing 150-200 g from our breeding stock were used in this study. The animals were housed at 24±2°C with 12:12 hr light and dark cycle. They had free access to food and water *ad libitum*. The animals were acclimatized for a

period of 10 days before the study. The study was conducted according to CPCSEA guidelines.

**Authentication**

Fruit pulp of *Terminalia chebula* was authenticated by Dr. Krishna Kumar G., Chairman, Dept. of Applied Botany, Mangalore University, Mangalore, India

**Extraction**

About 1000g of air dried crude powder material of fruit pulp of *Terminalia chebula* was extracted with water in a Soxhlet extractor for 36 hours. It was concentrated to dryness under reduced pressure and controlled temperature (40-50°C) using rotary evaporator. The aqueous extract yielded a brownish mass weighing 145g. The yield obtained was 14.5% w/w with respect to dried powder.

**Sample Size, Grouping and Dose of the Drugs**

Animals were divided into 5 groups (Control, Standard & Test drug) containing 6 animals, making a total number of 30 animals (Table 1).

**Drugs and Chemicals**

The standard anti-anxiety drug Diazepam (Cipla) was obtained from our institutional pharmacy. The dried fruit of *Terminalia chebula* was provided by Sri Lakshmi Ayurvedic Dispensary, Central market, Mangalore and

1% gum acacia from Dept. of Pharmacology, YMC, Mangalore, India

**Apparatus**

**Elevated Plus Maze**

The wooden maze consisted of two open arms (length 50 cm X breadth 10 cm) and two closed arms of the same size (height 40 cm). The arms of the same type are opposite to each other, with a central square of 10 cm. The maze was elevated to a height of 50 cm above the floor. The apparatus consisted of an open top wooden box.

**Behavioral Assessment**

One hour after drug or vehicle administration, each animal was placed in the centre square of the Elevated Plus Maze, facing one of the closed arms. The numbers of entries into open and closed arms, the time spent in open and closed arms were recorded. The number of rears in each arm was also recorded in a five-minute period.

**Statistical Analysis**

The data were analyzed by one-way ANOVA and Post-hoc comparisons were performed by applying Dunnett's multiple comparison test. P <0.05 was considered statistically significant.

**Table 1: Drugs/Dose of the drugs, groups and number of rats in each group**

Drugs / Dose	Groups	Number of Rats (n=6)
1% Gum acacia (10.0ml/kg)	I	6
Diazepam (1.0 mg/kg)	II	6
AETCFP (9mg/kg)	III	6
AETCFP (18 mg/kg)	IV	6
AETCFP (36 mg/kg)	V	6

**Table 2: Effect of acute administration of AETCFP on rat behaviour in elevated plus maze**

Drugs/ Groups	Time spent in open arm (Sec)	Time spent in closed arm (Sec)	Number of rears open arms (Sec)
1% Gum acacia (10.0ml/kg)	15.27±0.93	267.78±6.61	4.66±0.98
Diazepam (1.0 mg/kg)	105.93±4.57***	188.72±2.47***	19.83±1.44***
AETCFP (9mg/kg)	94.79±7.50*	205.21±7.50*	6.16±1.79*
AETCFP (18 mg/kg)	134.15±7.81***	172.51±2.27*	12.33±0.76***
AETCFP (36 mg/kg)	170.44±5.43***	129.54±5.43***	4±0.96*

n=6. The observation are mean ± SEM \*p>0.05, \*\* <0.05, \*\*\* p< 0.01, as compared to control (ANOVA followed by Dunnett's multiple comparison test), AETCFP - Aqueous Extract of *Terminalia chebula* fruit pulp

**Table 3: Effect of acute administration of AETCFP on rat behavior in elevated plus maze**

Drugs /Groups	Number of open arm entries	Number of total arm entries	Percentage ratio of open/total arms
1% Gum acacia (10.0ml/kg)	0.83±0.30	3.83±0.30	31±4.32
Diazepam (1.0 mg/kg)	3.5±0.34***	5.33±0.42***	68.5±8.16***
AETCFP (9mg/kg)	1.33±0.42*	5.50±0.42*	29±7.26*
AETCFP (18 mg/kg)	3±0.36***	8±0.51***	64.1±8.65**
AETCFP (36 mg/kg)	1±0.25***	4.83±0.30*	24±2.23*

n=6. The observation are mean ± SEM \*p>0.05, \*\* <0.05, \*\*\* p< 0.01, as compared to control (ANOVA followed by Dunnett's multiple comparison test), AETCFP - Aqueous Extract of *Terminalia chebula* fruit pulp

**RESULTS**

Results obtained were studied statistically by calculating standard error of mean (SEM). Results in Table 2 and 3 indicate that AETCFP in the dose of 18mg/kg significantly increased the number of open arm entries, number of total arm entries, percentile ratio of open arm to total arm entries, time spent in the open arms and number of rears in the open arms when compared to vehicle treated group.

**DISCUSSION**

Anxiety has become a very important area of research in psychopharmacology in this decade. This increase of

interest is the result of a rapid growth of scientific studies and the discovery of new drugs that alter anxiety in animal models.<sup>34</sup>

The elevated plus-maze is a test for the identification of anxiolytic or anxiogenic effect of a drug in rodents. In the elevated plus maze, the open arms are more fear provoking than the closed arms. The total ratio of entries, time spent and rearing behaviour in open arms to closed arms reflects the safety of closed arms with relative fearfulness of open arms.<sup>35</sup>

In the present study, group IV that received AETCFP at the dose of 18mg/kg showed a significant increase in the time spent (Table II) and the rears in open arms (Table II)

and the percentage ratio of open/ total arm entries (Table III). They showed a decrease in time spent in closed arms (Table II) of elevated plus maze. All these suggest that decreased fear, an increased exploratory behaviour and the behavioral disinhibitory effect of AETCFP at the dose of 18mg/kg comparable to diazepam, the standard anxiolytic. These agents are known to act via the BZD-GABA receptors; the role of GABA in anxiety is well established.<sup>36</sup>

*Terminalia chebula* contains total phenolic and tannin content.<sup>37</sup> Tannins have neuroprotective functions capable of reversing 6-hydroxydopamine-induced toxicity. Tannic acid, a potential therapeutic agent, which has shown beneficial effect in patients with neurological disease.<sup>38</sup> The aqueous extract appeared to have good antioxidant activities.<sup>39</sup> Exact mechanism underlying the anxiolytic action of AETCFP can not be concluded at the moment due to the presence of large number of phytochemicals in the *Terminalia chebula* fruit pulp. However, the anxiolytic activity may be attributed to the presence of tannic acid and polyphenols in the AETCFP and it may also be due to attenuation of oxidative stress produced during anxiety.

However, anxiolytic action of AETCFP through the BZD-GABA receptors can not be excluded. Hence further studies are needed to know the exact mechanism of action and as a potential therapeutic agent in the treatment of anxiety.

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