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Original Research Article

Attenuation of anxiety on acute administration of aqueous extract of *Terminalia belerica* fruit pulp in Swiss albino mice

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ABSTRACT

Background: The objective of this study was to evaluate the attenuation of anxiety on acute administration of aqueous extract of *Terminalia belerica* fruit pulp (AETBFP) by using elevated plus maze test and dark and light arena models.

Methods: Thirty Swiss albino mice were divided into five groups, Group I received vehicle (1% Gum acacia suspension, 3ml/kg, orally), Group II received standard drug Diazepam (1mg/kg, orally) and Group III, IV and V received AETBFP 9, 18 and 36 mg/kg, orally respectively. In elevated plus maze test, the mouse was placed on the central platform facing towards open arm. The percentage of time spent and frequency of entries and number of rears in open arm was counted for a period of 5 min. In dark and light arena, the time spent, number of entries and number of rears in light arena was counted for a period of 5 min. The mean±SEM values were calculated for each group. The data was analyzed using one-way ANOVA followed by Dunnet's multiple comparison tests; $p< 0.05$ was considered as statistically significant.

Results: Significant ($p<0.05$) reduction in anxiety was noted in experimental animals when given at a dose of AETBFP (36mg/kg), where number of entries and duration of stay in open arm and light arena increased in elevated plus maze and light and dark arena respectively when compared with control animals.

Conclusions: Our study reveals that AETBFP at a dose of 36mg/kg has significant attenuation of anxiety in Swiss albino mice.

Keywords: Aqueous extract of *Terminalia belerica* fruit pulp, Dark and light arena, Elevated plus-maze, Gum acacia, Swiss albino mice

INTRODUCTION

Anxiety is a normal human emotion that serves an adaptive function from a psychobiological perspective. However, in case of psychiatric setting, feelings of fear that are unfocused or out of scale with the perceived threat often require treatment.[1] In United States the most prevalent lifetime disorder is anxiety disorders. About 28.8% out of 9,282 people aged 18 years and older had anxiety disorders.[2] Between 1990 and 2013, the number of people suffering from depression, anxiety or both increased by nearly 50%, from 416 million to 615 million globally.[3] We have two groups of drugs used for anxiety one is Benzodiazepine and second group is Non BZD's Azapirones agents such as Buspirone, Gepirone, and Ipsapiron whose effect is focused upon 5-HT_{1A} receptor.[4] Disadvantage of using Benzodiazepines are its adverse effects like psychomotor impairment, potentiation of other central depressant drugs and addiction liability and that of Non BZD's are its therapeutic effects are delayed for about 3-4 weeks.[5,6] Therefore screening and development of drugs for anti-anxiety activity without these drawbacks is the need of the hour.

Terminalia is a large genus of deciduous trees of the flowering plant which belong to family *Combretaceae* comprising of approximately 250 species distributed in the tropical regions of the world, out of these, about 16 species occur in India.[7] *Terminalia belerica* grows wild up to 1,000m, all over India except dry and marshy areas.[8] Phytoconstituents like gallotannic acid, bellericanin, ellagic acid, gallic acid, termilignan,

thannilignan, flavone, anolignanB, tannins, ellargic acid, ethyl gallate, galloyl, glucose, chebulaginic acid, phenyllemblin, sitisrerol, mannitol, fructose and rhamnose are found to be present in the fruit of *Terminalia belerica*.[9] The fruit pulp of *Terminalia bellerica* has been used for the treatment of anaemia, asthma, cancer, colicky pain, constipation, diarrhoea, dysuria, headache, hypertension, inflammation, and rheumatism.[10] This plant exhibits several pharmacological like anti-bacterial, anti-malarial, anti-fungal, anti-HIV, anti-oxidant, and anti-mutagenic effects.[11] Aqueous extract of *Terminalia belerica* fruit pulp also has antidepressant and antinociceptive property.[12-14] The constituents of Triphala (three fruit pulp) are *Terminalia belerica*, *Terminalia chebula* and *Emblica officinalis*. *Terminalia chebula* and *Emblica officinalis* individually in other studies had shown anxiolytic effect, but no data are available regarding anxiolytic activity of *Terminalia belerica*.[14,15] In the view of this, the present was undertaken to find the effect of aqueous extract of *Terminalia belerica* fruit pulp on anxiety in Swiss albino mice.

METHODS

The study was conducted according to Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA) guidelines.

Animals

Male swiss albino mice (weighing 25-30grams) were used from our breeding stock Central animal house, AJIMS and RC, Mangaluru, Karnataka, India. The animals were housed at 24±2^{o}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.

Authentication

Terminalia belerica fruit pulp was authenticated by Dr. Krishna Kumar G, Chairman, Department of Applied Botany, Mangaluru University, Mangaluru, Karnataka, India.

Extraction

About 1000 g of air dried crude powder of *Terminalia belerica* fruit pulp was extracted with water in Soxhlet extractor for 36 hours. It was dried and reduced under controlled pressure and temperature (40-50°C) using a rotator evaporator. The aqueous extract yielded a brownish mass weighing 145g. The yield obtained was 14.5% w/w with respect to dried powder.[16]

Sample size, grouping and dose of the drugs

A total of thirty animals were divided into five groups, containing six animals in each group (Table 1).

Table 1: Groups, treatment and dose.

Group n=6	Drug	Dose (Route)
Group I	Control 1% Gum Acacia	10ml/kg (Per oral)
Group II	Standard drug Diazepam	1 mg/kg (Per oral)
Group III	AETBFP	9mg/kg (Per oral)
Group IV	AETBFP	18mg/kg (Per oral)
Group V	AETBFP	36mg/kg (Per oral)

AETBFP- Aqueous Extract of *Terminalia Belerica* Fruit Pulp

Drugs and chemicals

The standard anti-anxiety drug Diazepam was obtained from institutional pharmacy. The dried fruit of *Terminalia belerica* was provided by Sri Lakshmi ayurvedic dispensary, Mangalore and 1% Gum acacia from Department of Pharmacology, A.J. Institute of Medical Sciences, Mangaluru, Karnataka, India.

Procedure

Behavioural assessment

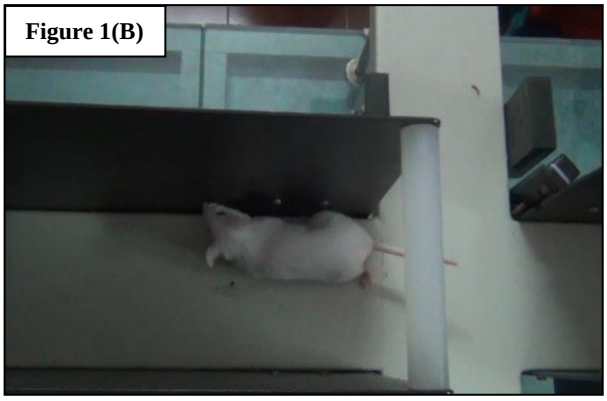
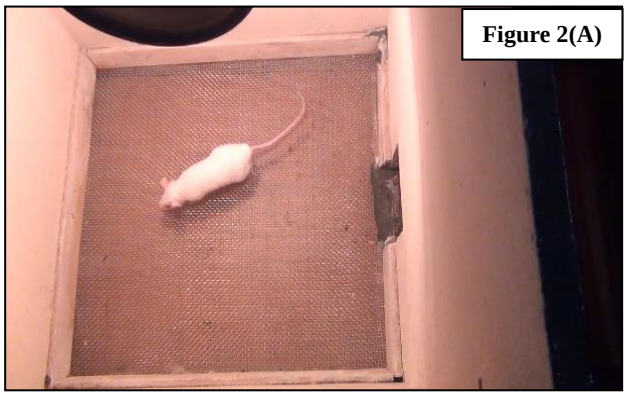


Figure 1: Entry of mouse into (A) Open arm and (B) Closed arm.

Each animal was first tested in Elevated plus maze and then in Light and dark apparatus in a single setting. One hour after drug administration, each animal was placed

on the central platform into the Elevated plus maze facing towards open arm. The time spent and frequency of entries in open arm and closed arm, number of rears in open arm was counted and Percentage Ratio of open/ total arm entries was calculated for a period of 5 min (Figure 1).

The mice were considered to be in an arm, only when all the four limbs cross the border. The cage was cleaned with cotton and spirit each time before placing mice, to give them a new environment which is not explored by any other mice before. In dark and light arena, the animal was placed at the centre of the brightly lit arena, the time spent; number of entries and number of rears in Light arena was counted for 5 minutes (Figure 2).[17,18]



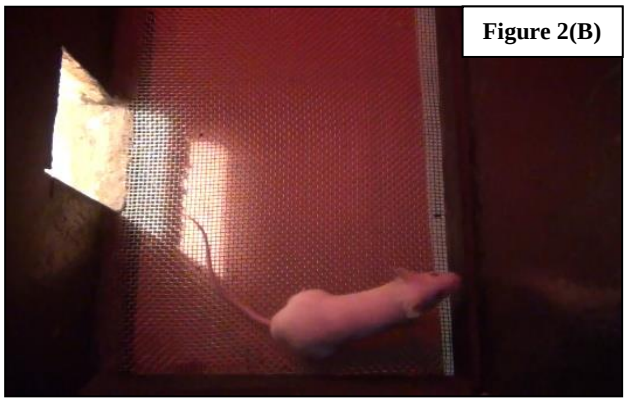


Figure 2: Entry of mouse into (A) Light and (B) Dark arena.

Statistical analysis

The mean ± SEM values were calculated for each group. The data was analyzed using one-way ANOVA followed by Dunnet's multiple comparison tests; $p<0.05$ was considered as statistically significant.

RESULTS

Results obtained statistically are tabulated. In the Elevated Plus Maze model, AETBFP at a dose of 36mg/ kg there was significant increase in the time spent in open arm (36±0.8 seconds), number of rears in open arm (0.5±0.2) (Table 2).

Table 2: Effect of acute administration of AETBFP on mice behaviour in elevated plus maze in terms of time spent in open arm, close arm and number of rears.

Dose/ group	Time spent in open arm (Sec)	Time spent in closed arm (Sec)	Number of rears in open Arm
1% Gum Acacia 10ml/kg	29.1±0.7	203.3±4.2	1.3±0.2
Diazepam 1mg/kg	36.8±1.2***	169.8±5.4***	0.5±2**
AETBFP 9mg/kg	31±1.3*	223.8±6.0*	1.3±0.2*
AETBFP 18mg/kg	29.8±1.6*	185.16±10.1*	1.3±0.2*
AETBFP 36mg/kg	36±0.8***	172.5±7.9**	0.5±0.2**

AETBFP - Aqueous Extract of *Terminalia Belerica* Fruit Pulp. n= 6. All values are mean±SEM; statistical analysis by ANOVA followed by Dunnet's Multiple Comparison tests; *P>0.05- not significant, **P<0.05- significant, ***P <0.01- highly significant as compared with control.

Number of open arm entries (9.1±0.4) and percentage ratio of open/total arm entries (35.2±3.0) when compared to control (Table 3).

Table 3: Effect of acute administration of AETBFP on mice behaviour in elevated plus maze in terms of number of open / total arm entries and percentage ratio.

Dose/ group	Number of open arm entries	Number of total arm entries	Percentage Ratio of open/total arm entries
1% Gumacacia 10ml/kg	5.6±0.7	28.6±1.2	20.4±3.4
Diazepam 1mg/kg	11.3±0.8***	25±1.9*	46.25±3.9***
AETBFP 9mg/kg	6.1±0.7*	32.9±3.7*	20.06±2.1*
AETBFP 18mg/kg	8.1±1.0*	25.8±1.1*	32.2±5.4*
AETBFP 36mg/kg	9.1±0.4**	26.5±1.4*	35.2±3.0**

AETBFP - Aqueous Extract of Terminalia Belerica Fruit Pulp. n= 6. All values are mean±SEM; statistical analysis by ANOVA followed by Dunnet's Multiple Comparison tests; *P>0.05- not significant, **P<0.05- significant, ***P <0.01- highly significant as compared with control.

In dark and light arena model AETBFP at a dose of 36mg/kg, there was a significant increase in the time spent in light arena (192.3±15.9) and number of entries into light arena (13.8±1.0) (Table 4).

Table 4: Effect of acute administration of AETBFP on mice behaviour in terms of time spent, number of entries and rears in light and dark arena.

Drugs/ Groups	Time spent in light arena (sec)	Number of entries into light arena	Number of rears in light arena
1% Gum acacia 10ml/kg	91.3±7.6	5.8±0.6	0.3±0.2
Diazepam 1mg/kg	181.6±21.0**	15.6±0.7***	1±0.5*
AETBFP 9mg/kg	75.6±20.1*	6.6±1.3*	2±0.4*
AETBFP 18mg/kg	122.5±30.1*	8±0.7*	0.3±0.2*
AETBFP 36mg/kg	192.3±15.9***	13.8±1.0***	1±0.4*

AETBFP - Aqueous Extract of Terminalia Belerica Fruit Pulp. n= 6. All values are mean±SEM; statistical analysis by ANOVA followed by Dunnet's Multiple Comparison tests; *P>0.05- not significant, **P<0.05- significant, ***P <0.01- highly significant as compared with control

DISCUSSION

Anxiety is the cardinal symptom of many psychiatric disorders and an almost inevitable component of many surgical and medical conditions. Elevated plus maze model is being used for identifying both anxiogenic and anxiolytic drugs. The closed and open arms must evoke same exploratory behaviour in mice. But because of elevation and open spaces, the mice develop anxiety and avoid the open arms and tend to be in closed arms. Anxiolytic drugs reduce anxiety and increases the open arm exploration. Rear is an exploratory behaviour in mice. Valproate and Benzodiazepines show increase in open arm exploration. Here AETBFP at a dose of 36mg/ kg has shown significant increase in the time spent in open arm, number of rears in open arm, number of open arm entries and percentage ratio of open/total arm entries comparable to standard drug Diazepam.

In Light and dark arena model, when the animals are allowed to move between dark and brightly-lit arena, the exploration to brightly-lit arena is decreased because of anxiety caused by the bright light. When AETBFP at a dose of 36mg/kg administered, there was a significant increase in the time spent in light arena and number of entries into light arena comparable to diazepam. Diazepam increases γ-aminobutyric acid binding to the $GABA_A$ receptors. AETBFP might can also exert its action in similar manner.[19] Tannins, Flavonoids and Phenolic compounds which are present in AETBFP are known to have action against many CNS disorders.

The present study indicates that acute administration of aqueous extract of *Terminalia belerica* fruit pulp in male mice has anxiolytic activity. Significant positive result was obtained in Elevated plus maze and light and dark arena models. However, further studies must be done to find the structure, pharmacokinetic and pharmacodynamic of aqueous extract of *Terminalia bellerica* fruit pulp in the near future for its anxiolytic activity.

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