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Original Article

Anxiolytic Activity of Ethanolic Extracts of *Tridax procumbens* using Different Experimental Anxiety Models in Mice

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ABSTRACT

The purposes of this study was to characterize the anxiolytic-like activity of an ethanolic extract of *Tridax procumbens* (ETP) using Elevated plus maze, Actophotometer and Marble burying behavior model. The efficiency of extract (250 and 500mg\kg) was compared with standard anxiolytic drugs diazepam (2mg\kg) and fluoxetine(10mg\kg). Extract administered animal showed exploratory behavior with all tests similar to diazepam. The result showed that the extract significantly increased the number of entries and time spent in the open arm in the elevated plus maze apparatus. The results also showed that the extract significantly decreased locomotor score and number of marble-buried in Actophotometer and Marble-burying behavior models respectively suggesting that the extract showed significant anxiolytic activity at both dose levels which is comparable with standard anxiolytic Diazepam and Fluoxetine. Phytochemical screening revealed that the presence of alkaloids, tannins, polyphenols, steroids, triterpenoids and flavanoids in ethanolic extract. We speculate that possible mechanism of anxiolytic action of ETP could be due to the binding of any of these phytochemicals to the gamma-aminobutyric acid-benzodiazepins (GABA-BZD) complex and/or effects on serotonergic transmission.

Key words: - *Tridax procumbens*, Elevated plus maze(EPM), Actophotometer, Marble-burying behavior(MBB)

1. INTRODUCTION

Anxiety disorders are among the most common psychiatric disorders that affects one-eighth of the population in world. Anxiety, a state of excessive fear, is characterized by motor tension, sympathetic hyperactivity, and apprehension and vigilance syndromes. Anxiety disorders include separation anxiety disorder, agoraphobia, specific phobia, social anxiety disorder, panic disorder and selective mutism.[1] Benzodiazepines are the compounds commonly used in the treatment of anxiety, despite the unwanted side effects that they produce such as sedation, muscle relaxation, lack of

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coordination, confusion, difficulty breathing, Confusion and barbiturate potentiation and tolerance.[2] Various herbal medicines have been used for treatment of anxiety disorder. The different pharmacological activities of *Tridax procumbens* are reported such as a Immunomodulatory effects[3], antioxidant[4], anti-hepatotoxic[5], wound healing activity[6] and blood pressure lowering activity[7] which are used as a folklore medicines but has not established through any scientific report. The present study is an attempt to prove the pharmacological evaluation for anxiolytic activity of Tridax procumbens in rodents. This makes me to evaluate the anxiolytic activity of Tridax procumbens.

2. MATERIALS AND METHODS

Experimental Animals

Albino Mice (15-30g) of either sex were used for the study. Animals were obtained from (Bhaskar medical college, Hyderabad) in house facility and housed in the room on an artificial light/dark cycle(12/12 hr, light on from 7 a.m. to 7 p.m.), under standard conditions with free access to food and water. The study was performed in accordance with the guidelines issued by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) an authority regulating animal experiments.

Plant material

The whole plant of *Tridax procumbens* were collected from local areas of Rangareddy, Hyderabad, India and authenticated by Department of Botany, Osmania university, Hyderabad. The whole plant was dried in shade and ground to get a coarse powder.

Preparation of extract

The Ethanol extract of *Tridax procumbens* (TPE) was prepared by maceration method using Ethanol solvent for **72** hrs at room temperature. A suspension of *EECAL* in 5% (w/v) Carboxy Methyl Cellulose was prepared for oral administration.

Chemicals

Diazepam (Ranbaxy Laboratories Ltd. Mumbai) and Fluoxetine (Pfizer Ltd., Mumbai) were used as the standard anxiolytic drugs. Ethanol was purchased locally and it was of analytical grade. Distilled water was used as a vehicle.

Preliminary phytochemical screening

A portion residue from extract was subjected for phytochemical analysis in order to see the presence of glycosides, polyphenols, saponins, flavonoids, tannins and steroids[8].

Acute toxicity study

The plant extract was administered orally at a dose of up to 5000 mg/kg body weight. Mice observed for 13 days to possible mortality and study their behavioral neurological toxicity. The acute toxicity study was followed as per OECD 423 guidelines[9].

Experimental Anxiety Models

Elevated Plus Maze Model:

The plus-maze apparatus, consisting of two open arms (16 x 5 cm) and two closed arms (16 x5 x 12 cm) having an open roof. The *TPE* (250and 500 mg/kg) and vehicle were administered for 5 days once daily p.o. and the last dose was given on the 5th day, 60 min prior to experiment. The standard drug was given at a dose of 2 mg/kg p.o.60 min before starting the experiment. After proper treatment each mouse was placed at the center of the maze with its head facing the open arm. During the 5 min experiment, the behavior of the mouse was recorded as: the number of entries into the open or closed arms and time spent by the mouse in each of the arms. An arm entry was defined as the entry of all four paws into the arm.

Spontaneous motor activity:

The locomotor activity was measured by using an Actophotometer. The movement of the animal interrupts a beam of light falling on a photocell, at which a count was recorded and displayed digitally. The *TPE* (250 and 500 mg/kg)and vehicle were administered for 5 days once daily p.o. and the last dose was given on the5th day, 60 min before starting the experiment. The standard drug was given at a dose of 2 mg/kg p.o.60 min before starting the experiment and the animals were kept in the Actophotometer individually. The locomotor activity was measured for a period of 10 min.

Marble-Burying Behavior Model:

In this method animals were individually placed in transparent; poly carbonate cages (22 x32 x 13.5 cm) containing a 5 cm layer of saw dust and 24 glass marbles (1.5 cm in diameter) were evenly distributed on the saw dust in the cages. *TPE* (250 and 500 mg/kg) and the vehicle were administered once daily p.o. for 5 days and the last dose was given on the 5th day, 60 min prior to experiment. The standard drug was Fluoxetine was given at a dose of 10 mg/kg p.o.60 min prior to the experiment and kept in the cages for a period of 30 min. and the number of marbles at least two-third buried in the saw dust was recorded.

Statistical Analysis:

The data were expressed as mean ±standard error mean (SEM). The significance of differences among the groups was assessed using one way analysis of variance (ANOVA). The test was followed by Dunnett' s 't"-test, p values less than 0.05 were considered as significance.

3. RESULTS

Preliminary Phytochemical Analysis:

The result of preliminary phytochemical screening is presented in Table:1. Qualitative phytochemical studies were performed on *Tridax procumbens* extract using suitable chemicals and reagents to confirm the presence of alkaloids, carbohydrates, polyphenols, steroids, flavoniods, lipids, tannins and triterpenoids.

Acute toxicity study:

Following oral administration aqueous extracts of *TPE* at a dose of 5000 mg/kg, P.O. animals were observed that no toxicity and no significant changes in their body weight, hence it was confirmed that the test drug *TPE* is practically non-toxic in normal mice.

ANTIANXIETY STUDIES

Elevated Plus Maze Model (EPM):

The mean number of entries and time spent by mice in open & closed arms after the drug administration are given in Table:2. The results showed that the number of open arm entries and time spent in the open arms were increased and number of closed arm entries and time spent in the closed arms were decreased significantly in the extract treated groups which was comparable with control group. The *TPE* at the dose of 250 &500 mg/kg exhibited significant increase of open arms entries and meantime spent in the open arms. The standard drug diazepam treated mice's showed significant increase ($P < 0.001$) in the number of open arm entries, time spent in open arms and reduction in the time spent in closed arm. The average time spent in open arms increased from 15.1±0.41 (sec) in control to 29.1±0.49*and 48.9±0.81**(sec) in the dose of TPE 250 mg/kg and 500 mg/kg respectively. TPE 250 mg/kg treated rats showed significant ($p<0.05$) in the time spent in open arm compared to control. TPE 500 mg/kg treated rats showed significant ($p<0.01$) in the time spent in open arm compared to control. The average no. of entries in open arm increased from 2.7 ±1.26 in control 8.2±0.34* and 11.2±0.59** in the dose of TPE 250 mg/kg and 500 mg/kg respectively. TPE 250 mg/kg treated rats showed significant increase ($p<0.05$) in the no. of entries in open arm compared to control. TPE 500 mg/kg treated rats showed highly significant ($p<0.01$) in the no. of entries in open arm compared to control.

Spontaneous motor activity:

The results showed that decrease locomotor score were observed in TPE compared to the control animals in table:3. The TPE at the dose of 250 and 500 mg/kg exhibited significant decrease locomotor score. The average locomotor score was decreased from 521±10.5 in control to 437.3±6.2*and 389.5±2.34*** in the dose of TPE 250 mg/kg and 500 mg/kg respectively. TPE 250 mg/kg treated rats showed significant ($p<0.05$) decrease in locomotor score compared to control. TPE 500 mg/kg treated rats showed highly significant ($p<0.001$) decrease in locomotor score compared to control. The standard drug diazepam treated mice's showed highly significant decreased locomotor score ($P < 0.001$).

Marble-Burying Behavior Model:

The results showed that decrease in the number of marble buried was observed in TPE compared to the control animals. Table:4. The TPE at the dose of 250 and 500 mg/kg exhibited significant decrease in the number of marble buried was observed for the standard Fluoxetine compared to the control animals. The TPE at both dose levels showed significant decrease in the number of marble buried which was comparable with the standard Fluoxetine. The average in the number of marble buried was decreased from 17.8±0.58 in control to 12.3±2.16*and 10.4±1.83** in the dose of TPE 250 mg/kg and 500 mg/kg respectively. TPE 250 mg/kg treated rats showed significant ($p<0.05$) in decreased number of marble buried compared to control. TPE 500 mg/kg treated rats showed highly significant ($p<0.01$) in decreased number of marble buried compared to control. The standard drug diazepam treated mice's showed highly significant decreased locomotor score ($P < 0.001$).

4. DISCUSSION

Many herbal products are used to treat anxiety and cognitive disorder. The present work demonstrated that the Tridax procumbens had anxiolytic activity in mice was evaluated by Elevated plus maze, Actophotometer and Marble-burying Behavior models. The various studies has shown that involvement of Gamma-aminobutyric acid-benzodiazepines(GABA-BZD)complex, serotonergic,[10] adrenergic and dopaminergic neurotransmission to play a role in anxiety. [11] The elevated plus maze is used to evaluate psychomotor performance and emotional accepts of rodents. Results showed that the plant extract treated mice exhibited significance increase in the number of open arms entries but decrease in time spent in closed arm reflects plant anxiolytic properties[12]. Anxiolytic compounds by decreasing anxiety, increase the open arm exploration.[13] The conventional plus maze is highly sensitive to the influence of both anxiolytic and anxiogenic drugs acting at the $GABA_A$ - benzodiazepine complex [14]. In this study, we observed that TPE (250 and 500mg//kg) induced mice shows significant increase in the both number of entries and time spent in the open arms and in the closed arms were reduced in the EPM model. Locomotor activity is considered as an index of alertness and their decrease leads to sedation as a result of reduced excitability of the central nervous system.[15]

The locomotor activity in mice is determined by using actophotometer. When the ethanolic extract of Tridax procumbens was administered to mice, the locomotor activity is decreased. Thus indicating the sedative effect. The reduction in locomotor activity was evident during activity measurement. The marble-burying behavior model has been suggested as a useful model for evaluating anti-obsessive-compulsive disorder drugs because no change in the intensity of marble burying behavior occurred during repeated testing (this is considered as compulsive behavior) [16] .Both the doses 250 and 500 mg/kg of the extract decreased significantly the number of marble buried.

Phytochemical screening of Tridax procumbens revealed the presence of components such as saponins, tannins, steroids, triterpenoids, lipids and flavonoids. These compounds along with secondary metabolites present in plants shows additive or synergistic action. This fact reveals that medicinal activities of plants are distinctive to particular plant species

or groups, dependable with the combinations of secondary metabolites in a particular plant are generally taxonomically diverse. The results of this study showed that the Tridax procumbens extract possess anxiolytic effects at therapeutically acceptable dose. This effects may be due to the interaction of the extracts with the neurotransmitters or chemical mediators like noradrenaline, serotonin, GABA and BZD which are responsible for anxiolytic activity. There also be an interaction of extract with serotonergic pathway, since serotonin is involved in aggressive behavior. The mechanism of anxiolytic action is due binding of phytochemical constituents with GABA-BZD complex. Among these phytochemical constituents flavonoids have high affinity to bind with BZD site of GABA receptor responsible for anxiolytic activity. The effect of ethanolic extract of *Tridax procumbens* might be on $GABA_A$ receptor is a positive allosteric modulator, causes an increasing the opening of the chloride ion channel when GABA binds to an allosteric site on the $GABA_A$ receptor, leading to more chloride ions entering the neuron, which in turn leads to enhanced central nervous system anxiolytic effects and on serotonin receptor (5HT receptors) in serotonergic transmission that inhibitory action on those receptors. So the anxiolytic activity of TPE might involve an action on GABAergic transmission or effects on serotonergic transmission or due to its mixed aminergic potentiating effect. Some synthetic and natural flavonoids have been bind exclusively and competitively to benzodiazepine receptors and reveal anti-anxiety property in the EPM test in rat and mice, these extracts induce anxiolytic like effect. These findings indicate a remarkable sedative effect of this plant. In summary the present results demonstrated an anxiolytic effect from *Tridax procumbens* extract. Further pharmacological investigations are underway to identity the active constituents of the plant extract responsible for the reported activities.

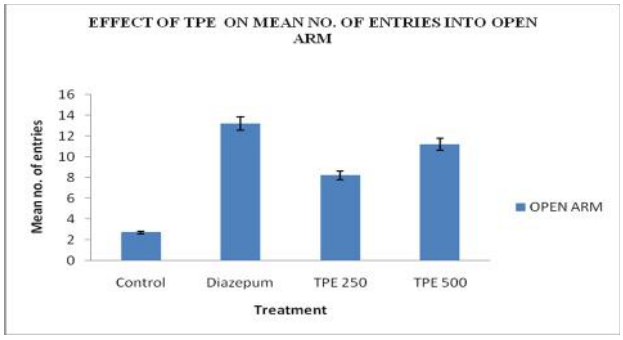

Fig 1: Effect of TPE on Mean No. of Entries into Open Arm

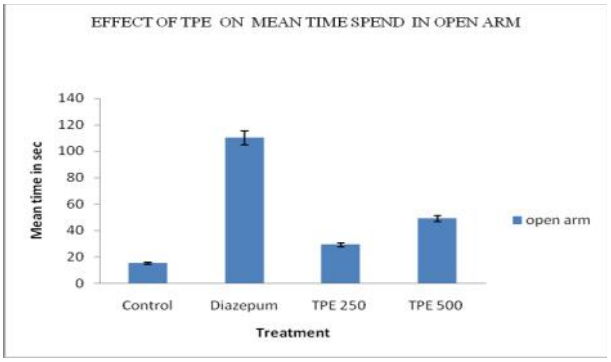

Fig 2: Effect of TPE on Mean time Spend in open arm

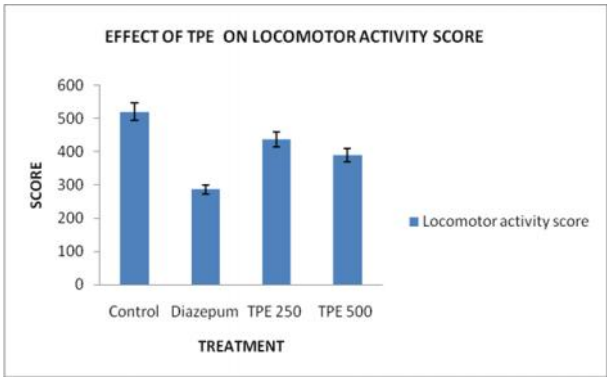

Fig 3: Effect of TPE on Locomotor Activity Score.

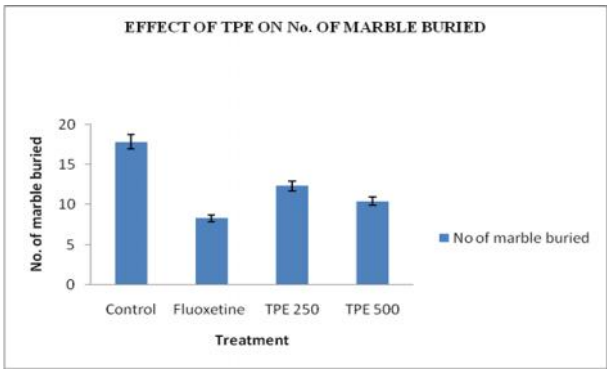

Fig 4: Effect of TPE on No. of Marble Buried

Table 1: Phytochemical Analysis of *Tridax procumbens* extract

Extract	Alkaloids	Glycosides	Polyphenols	Carbhydrates	Tannins	Flavonoids	Steroid	Triterpenoids	Lignins	Proteins	Aminoacids
Ethanol	+	-	+	+	+	+	+	+	-	-	-

"+" indicates the presence.
"-" indicates the absence.

Table 2: Effect of *Tridax procumbens extract* on animals in Elevated Plus Maze Model

Group	Treatment	Mean no. of entries in		Mean time spent in (sec)	
		Open arm	Closed arm	Open arm	Closed arm
I	Control-water	2.7 ±1.26	16.4±0.59	15.1±0.41	239±3.11
II	Diazepam-2mg/kg. i.o.	13.2±1.57**	6.02±1.53**	110±0.86**	137±2.49*
III	*TPE* 250 mg/kg. p.o.	8.2±0.34*	10.9±0.16*	29.1±0.49*	212±0.69*

IV	*TPE* 500 mg/kg. p.o.	11.2±0.59**	8.2±1.24*	48.9±0.81**	192.1±0.89*

*n = 6, *p < 0.05, **p < 0.01, ***p < 0.001 (one way ANOVA followed by Dunnett's 't' test)*

Table 3: Effect of *Tridax procumbens extract* on animal in Locomotor Activity

Group	Treatment	Locomotor activity score
I	Control- water	521±10.5
II	Diazepam- 2mg/kg. i.o.	286.4±4.6**
III	*TPE* 250 mg/kg. p.o.	437.3±6.2*
IV	*TPE* 500 mg/kg. p.o.	389.5±2.34***

*n = 6, *p < 0.05, **p < 0.01, ***p < 0.001 (one way ANOVA followed by Dunnett's 't' test)*

Table 4: Effect of *Tridax procumbens extract* on animal in Marble-burying Behavior model

Group	Treatment	No of marble buried
I	Control- water	17.8±0.58
II	Fluoxetine 10mg/Kg. i.o.	8.31±1.75**
III	*TPE* 250 mg/kg. p.o.	12.3±2.16*
IV	*TPE* 500 mg/kg. p.o.	10.4±1.83**

*n = 6, *p < 0.05, **p < 0.01, ***p < 0.001 (one way ANOVA followed by Dunnett's 't' test)*

5. CONCLUSION

The results suggest that possible mechanism of anxiolytic action of TPE could be due to the binding of any of these phytochemicals to the GABA-BZD complex. So the anxiolytic activity of TPE might involve an action on GABAergic transmission or effects on serotonergic transmission or due to its mixed aminergic potentiating effect. Further, studies with purified isolated phytochemical constituents are needed to understand the complete mechanism of Anxiolytic activity of *Tridax procumbens.*

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