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Study of Phytochemical and Neuropharmacological Activity of Leaves Extract of Tridax Procumbens., Linn

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ABSTRACT: **Background:**Tridax procumbens Linn, is common weed and found throughout year. It is commonly known as "Coat buttons"belongs to the family Asteraceae. Tridax procumbens Linn is posses for several potential therapeutic activites like anti-inflammatory, analgesic, anti- oxidant, insecticidal and anti –viral. It is also used in Indian traditional medicine as an anticoagulant, hair tonic and insect repellent and treatment of diarrhoea,dysentery and wound healing. Leaf juice can be used to cure fresh wounds and to stop bleeding.

Along with extract of Allium sativum it is used to treat Leishmania Mexicana [1, 2] .We wanted to study its neuropharmacological effects, which may throw light on understanding the underlying mechanism for its central activity.

Purpose: The present study was designed to evaluate CNS depressant and behavioral effects of aqueous extract of T.Procumbens and to study the phytochemical responsible for these activities with possible mode of action.

Methods: The effect of CNS depressant was studied using Rota rod and Actophotometr test and the effects on behavioral activity was studied using open field test (OFT). The aqueous extract was given intraperitoneally at a dose of 200 mg/kg. Diazepam (0.4 mg/kg body weight i.p.) was used as standard.

Data was analyzed by ANOVA test followed by Dunnett's test. Values indicate Mean±SEM.(n=6)(Anova followed by Dennett s post- hoc test). Values are significantly different *P <0.001 compared with normal control.

Results: Phytochemical screening revealed the presence of alkaloids , carbohydrates, saponins, triterpenoids, tannins, flavonoids and phytosterols as major constituents. The result of the study demonstrated that aqueous extract of T.Procumbens (200 mg/kg i.p.) decreased locomotor activity, produced muscle relaxation and showed antianxiety activity.

Conclusions: Aqueous extract of T.Procumbens exhibit CNS depressant action and significant anxiolytic activity comparable to diazepam.

KEYWORDS:
Tridax procumbens (AETP)
Actophotometer
OFT (Open field method)
Rota-rod

I. INTRODUCTION

Tridax procumbens Linn is a common weedy perennial herb and it is commonly known as Gharma(Hindi), Vettukaya poondu (Tamil), Tridax daisy (English) , Javanthi (Kanada), Jayanthi veda (Sanskrit), Gaddi chemanthi (Telugu) and popularly called as "Coat buttons" because of the appearance of its flowers. It has been extensively used in Indian traditional medicine as an anticoagulant, hair tonic and insect repellent and treatment of diarrhoea, dysentery and wound healing.

NEUROPHARMACOLOGY

Neuropharmacology is the study of how drugs affect cellular function in the nervous system, and the neural mechanisms through which they influence behavior .There are two main branches of neuropharmacology behavioral and molecular. Behavioral neuropharmacology focuses on the study of how drugs affect human behavior (neuropsychopharmacology), including the study of how drug dependence and addiction affect the human brain.[3]

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MOLECULAR NEUROPHARMACOLOGY

Molecular neuropharmacology involves the study of neurons and their neurochemical interactions, and receptors on neurons, with the goal of developing new drugs that will treat neurological disorders such as pain, neurodegenerative diseases, and psychological disorders (also known in this case as neuropsychopharmacology).This article will focus on both behavioral and molecular neuropharmacology the major receptors, ion channels, and neurotransmitter manipulated through drug action and how people with a neurological disorder benefit from this drug action.

II. MATERIALS AND METHOD

Collection and authenification of plant material

The whole plant was collected from the medicinal garden of Madurai Medical College, Madurai and it was identified taxonomically and authenticated by Dr.Stephan, Botanist of the American college,Madurai, Tamilnadu.

Preparation of plant material

The leaves are separated and washed after then shade dried for 8to 10 days. The dried leaves were powdered in a mixer and the fine powder was collected by sieve no: 40. About 500 grams of the dried powdered leaves of Tridax procumbens Linn was defatted with 1.5 liter per ether by maceration. The solvent was removed by filtration and the marc was dried .Then the dried marc with 1.5 liter distilled water was added and the extraction was performed by maceration (72 hours process). It was then filtered and the filtrate was evaporated to cohesive mass using rota vapour.[4]

METHODS

Preliminary phytochemical analysis

The aqueous extract of Tridax Procumbens linn were screened for the presence of various phytochemical constituents using conventional protocols .[5]

Experimental Animals

All animals were conducted after approval from the Institution of ethical committee, Madurai Medical College, Madurai. Albino mice (25-30g) were used for the study were housed in care facility institute of Pharmacology, MMC, Madurai. The animal were used in polypropylene cage (room temperature(25± 1°C) with 12 hrs light and 12 hrs dark cycle relative humidity approximately 60°C. With free standard pellets and tap water mice were Accelimatized for 10 days before experimentations.

Test methods

Animals were divided into various groups such that 6 animals were in each group. Animals treated with 0.1 ml normal saline served as control, Diazepam (0.4mg/kg i.p.)11 served as standards and animals in test group were treated with AETP (200 mg/ kg i.p.) respectively. Each animal was treated with respective drug 30 min before experimentation. Following are the details of experiments performed.

1. Rota-rod performance

Dunham and Miya (1957) suggested that neurological depression in mice/rats could be evaluated by testing their ability to remain on a Rota-rod. Rota-rod apparatus (Dolphin make) is a four panel techno device with timer. Animals (4 at a time) were placed on rod rotating at 20-25 rpm speed. Only the mice, which demonstrated their ability to remain on the revolving rod (20-25 rpm) for 5 min after training sessions during pretest screening, were selected for studies. The fall off time was recorded in all the groups before and 30 min after drug administration. Decrease in fall off time is suggestive of CNS depression[6,7.]

2. Actophotometer test

The animal locomotor behavior was monitored using Actophotometer, described by Dews P.B. (1953). Actophotometer (Dolphin make) provided with a digital counter, photocell and a light source were used to measure locomotor activity (horizontal movement) of animals.

Each animal was placed in Actophotometer for 5 minutes and basal activity score was recorded for all animals. Each animal was treated with respective drug and activity

score was recorded after 30 min and 1hr. Deceased activity score was taken as index of CNS depression[8,9,10 .]

3. Open field test (OFT):

Open field apparatus was designed as described by Gray and Lalji (1971) with few modifications. Dimensions were 50cm x 50cm x 40cm made up of plywood open from top and bottom kept on white table top; surface was divided into 25 equal squares i.e. 9 central and 16 peripheral. The animals were pretreated with samples (0.1 ml normal saline, AETP and Diazepam) 1hr before the trial. During 5 min session of observation, each animal was placed in the corner of open field apparatus and behavior of animal as determined by ambulation (number of

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squares entered with both forelimbs), rearing, preening and defecation was recorded [11]

III. STATISTICAL ANALYSIS

Data was analyzed by ANOVA test followed by Dunnett's post hoc test. All the results were expressed as Mean (±SEM). *P <0.001 was considered significant. Percent reduction in activity score and fall off time calculated with reference to respective basal recordings.

IV. RESULTS

1) Phytochemical analysis

Phytochemical screening of aqueous extract of leaves of Tridax Procumbens Linn reveals that the presence of various phytochemical constituents like alkaloids, flavonoids, tannins, saponins, terpenoids and phytosterols (Table-1)

2) Rota-rod method

Diazepam (0.4mg/kg) and AETP (200mg/kg) treated groups showed significant CNS depressant activity when compared to control however this depression was less with AETP treated group than diazepam treated group. (Table-2)

3) Actophotometer test

Diazepam (0.4mg/kg) and AETP (200mg/kg) treated groups showed significant CNS depressant activity when compared to control however this depression was less with AETP treated group than diazepam treated group. (Table-3)

4) Open field Test

Diazepam (0.4mg/kg) and AETP (200 mg/kg i.p.) significantly (p<0.001) exhibited anxiolysis evidenced by increased ambulation, rearing and preening at the same time decreased defecations compared to control. (Table-4)

Table 1: Phytochemical screening of extract (AETP)

S. No.	Chemical Constituents	AETP
1	Test for alkaloids	+
2.	Test for carbohydrates	+
3.	Test for saponins	+
4.	Test for triterpenoids	+
5	Test for flavonoids	+
6	Test for tannins	+
7	Test for phytosterols	+

NEUROPHARMACOLOGICAL ACTIVITY
ROTA-ROD-METHOD

Table 2: Fall of Rota Rod Method

S. NO	TREATMENT	DOSE	FALL OF TIME (Seconds)	
			BEFORE DRUGS	AFTER DRUGS
1	Normal-Control	0.1 ml/ mouse	223±18.9	226.7±22.3

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2	**Standard-Diazepam**	**0.4 mg/kg**	**229.7±22.9**	**86.3±2.4***
3	**Aqueous extract of Tridax Procumbens Linn**	**200 mg/kg**	**225±18.5**	**139.3±20.1**

Values indicate mean±SEM. (n=6). (Anova followed by Dennetts post-hoc test). Value are significantly different at *p<0.001 compared with normal control.

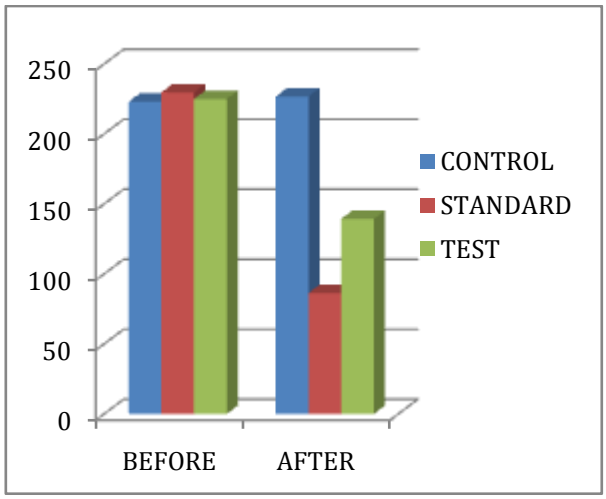

Table: 3

S.NO	**TREATMENT**	**DOSE**	**LOCOMOTOR ACTIVITY IN 5 MIN(COUNTS)**	
			BEFORE DRUGS	**AFTER DRUGS**
1	**Normal-Control**	**0.1 ml/mouse**	**350±12.5**	**344.2±12.1**
2	**Standard-Diazepam**	**0.4 mg/kg**	**362±57.7**	**144.2±36.8***

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3	**Aqueous extract of Tridax Procumbens Linn**	**200 mg/kg**	**344.3±9.8**	**258.8±9.9***

ACTIVITY SCORE IN ACTOPHOTOMETER METHOD

Values indicate mean±SEM. (n=6). (Anova followed by Dennetts post-hoc test). Value are significantly different at *p<0.001 compared with normal control.

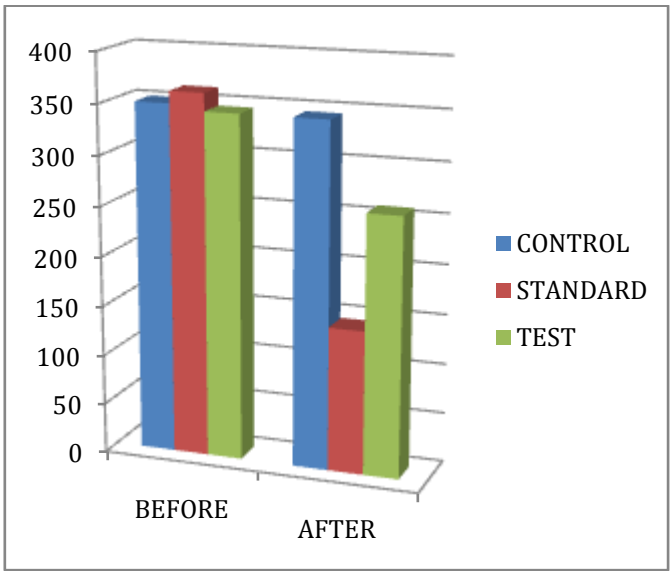

Table : 4

MEAN SCORE IN OPEN FIELD METHOD

S. NO	TREATMENT	DOSE	NO.OF CROSSINGS IN 5 MIN (SQUARES CROSSED WITH ALL FOUR LIMBS)	
			BEFORE DRUGS	AFTER DRUGS
1	**Normal-Control**	**0.1 ml/ mouse**	**101.7±31**	**100.6±5.6**
2	**Standard-Diazepam**	**0.4 mg/kg**	**99.4±1.4**	**25.2±3.2***
3	**Aqueous**	**200**	**108.2±4.**	**45.8±5.6**

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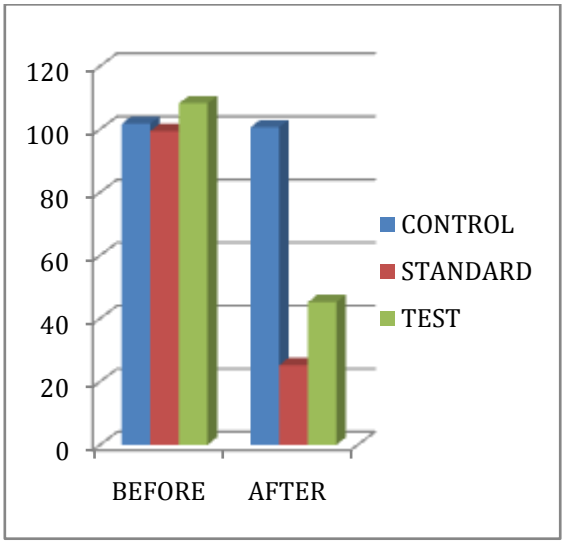
	extract of Tridax Procumbens Linn	**mg/kg**	**3**	*****

Values indicate mean±SEM. (n=6). (Anova followed by Dennetts post-hoc test). Value are significantly different at *p<0.001 compared with normal control.



V. DISCUSSION

Anxiety and hypnosedation are principally medicated in the CNS by the GABA receptor complex, which is also involved in other physiological functions related to behavior and in various psychological and neurological disorders such as epilepsy, anxiety, depression, Parkinson syndrome and Alzhimer's disease.[12]

Diverse drugs that are used in various psychological and neurological disorders might modify the GABA- system at the level of the synthesis of GABA , induce anxiolysis or hypnosis in animals by potentiating the GABA –medicated postsynaptic inhibition through an allosteric modification of GABA receptors[13] Thirdly by direct increase in chloride conductance or indirectly by potentiating GABA induced chloride conductance with simultaneous depression of voltage activated Ca- Currents like barbiturates.[14]

In this study, CNS depressant activity of TP was evaluated by rota rod test, which has clearly demonstrated the CNS depressant activity evidenced by decreased fall off time. Another important step in evaluating CNSdrug action is to observe its effect on locomotor activity of the animal. The activity is a measure of the level of excitability of the CNS , and decreased activity results from CNS depression.[15] The extract significantly decreased the locomotor activity as observed in the results of the actophotometer test.

Moreover , anxiolysis was studied by measuring external signs like ambulation, rearing, preening and defection in open field test. It is used for evaluating the effect of drug on gross general behavior and is used to measure the level of nervous excitability when the animals are exposed to a novel environment. Exploration in a new environment is an essential part of of normal behavior in animals, lower ambulation , exploration and reduction in normal rearing and preening behavior with increased defection in new environment are due to anxiety and fear. However, disinhibitory actions of anxiolytics increase these behavioral avtivites in new environment by releasing novelty-induced suppression of behavior.

As mentioned in results,TP possesses various phytochemical substance such as triterpenoid, saponins, alkaloids, flavonoids, CNS depressant and anxiolytic activity TP was supposed to be attributed to these phytochemicals found in extract. Several plants have been reported to have CNS depresent and anxiolytic activity due to the presence of Triterpenoids, saponins and flavanoids. Triterpenoids , saponin flavonoids are reported to

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have agonistic/faciliatory activites at GABA receptor complex.[16]Which led to the hypothesis that they act as benzodiazepine – like molecules. This is supported by their behavioral effects in animal models of CNS depression and anxiety.

VI. CONCLUSION

From the results we can concluded that TP possess considerable CNS depressant and anxiolytic activity which is comparable with the standard. The presence of triterpenoids, saponins and flavonoids may be the phytochemicals possible for this activity.

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