

CNS Activity of various leaf Extracts of '*Tridax procumbens*' In Mice

ABSTRACT

The present study was designed to evaluate the anti-depressant activity of Successive extraction of Petroleum ether (PEETP), Chloroform (CHETP), ethylacetate (EAETP), Ethanolic (EETP) & Aqueous (AETP) extracts of *Tridax procumbens* (TP) by Forced swim test (FST) and Tail suspension test (TST). The mice 25-30g were divided into six groups. Dose was fixed as per OECD 425 Guidelines acute toxicity studies. Drug and extracts was administered 1 hr prior to study. Duration and percentage reduction of immobility was noted. The CETP & EAETP 200 mg/kg extracts shows significant ($p < 0.01$) decreased immobility time compared to control. The EETP 200 mg/kg effect was equal to escitalopram (10mg/kg), as compared to control more significant ($P < 0.001$) then other extracts. AETP was shows $p < 0.05$ significant whereas PEETP no significant to negative control, the studies result recommends that ethanol extract good antidepressant effect.

Keywords: Anti-depressant, *Tridax procumbens*, Tail suspension test, Mice, Immobility

INTRODUCTION

Depression is a state of low mood and aversion to activity that can affect a person's thoughts, behaviour, feelings and sense of well-being. [1] Mental depression is one of the common chronic illnesses that affect the mood, thought, physical health and behaviour of an individual. According to the WHO, approximately 450 million people suffer from a mental or behavioural disorder [2]. This amounts to 12.3% of the global burden of disease, and will rise to 15% by 2020. [3] Psychiatric illness is also often associated with suicide and there are between 10 and 20 million suicide attempts every year. It has been estimated that 5.8% of men and 9.5% of women experience a depressive episode in their lifetime and suicide is one of the most common outcomes of depression. [4,5]

There are many effective antidepressants available today the current armamentarium of therapy is often inadequate with unsatisfactory results and mainly side effect like weight gain or loss etc. To avoid such problem and tried to achieve better efficacy with less side effect the present study was carried with Ayurveda plant of *Tridax procumbens*.

MATERIALS AND METHODS

Collections of plant material

The Leaves of *Tridax Procumbens* L were collected in Namakkal District, Tamilnadu and authenticated by Dr. Raju, M.Sc, M.Phil, Ph.D, Associate Professor, Kandar Arts College, Paramathi velur.

Preparation of extracts and Preliminary phytochemical screening

The collected leaves of *c Tridax Procumbens* L were shade dried completely. The dried leaf was then coarsely powdered and was sieved (sieve # 60) to get uniform powdered. The 500 g powdered material was successively extracted with Petroleum ether, Chloroform, Ethyl acetate, ethanol and aqueous by maceration Process. Final compound was concentrated by vacuum drying. The traces of the solvents were removed by keeping the dried extracts in to desiccators. The Leaf extracts of *Tridax Procumbens* L were subjected to chemical test for identification. [6]

DOSE DETERMINATION

Acute oral toxicity studies [7]

Acute oral toxicity studies was performed as per OECD-425 guidelines with Leaf extracts of *Tridax Procumbens* L using albino mice of either sex, selected by random sampling for acute toxicity study. Animals are fasted prior to dosing (e.g. with the rat, food but not water should be withheld overnight with the mouse, food but not water should be withheld for 3-4 hr). Following the period of fasting, the animals should be weighed and the test substance administered. After the substance has been administered, food may be withheld for a further 3-4 hr in mice. Three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 500 and 2000 mg/kg body weight. Animals are observed individually after dosing at least once during the first 30 min, periodically during the first 24 hr, with special attention given during the first 4 hr, except where they need to be removed from the study and humanely killed for animal welfare reasons are found dead. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in animal, then the same dose was repeated again to confirm the toxic dose, if mortality was not observed, the procedure was repeated for higher doses.

Table 1: Study period and observation parameters of acute toxicity studies

Initial once observation	First 30 min and periodically 24 hr
Special attention	First 1-4 hr after drug administration

Long term observation	Up to 14 days
Direct observation parameters	Tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma.
Additional observation parameters	Skin and fur, eyes and mucous membrane, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern etc.

Experimental Design for anti-depressant activity

The mice were divided into control and experimental groups (n=6). Drugs/ vehicle were administered to the animals 30min prior to study.

- Group I : Negative control received 1% DMSO.
Group II : Positive control and receive standard drug Escitalopram (10 mg/kg) orally.
Group III : Receive *Tridax procumbens* Petroleum Ether Extract (200 mg/kg) orally.
Group IV : Receive *Tridax procumbens* Chloroform Extract (200 mg/kg) orally.
Group V : Receive *Tridax procumbens* Ethyl Acetate Extract (200 mg/kg) orally.
Group VI : Receive *Tridax procumbens* Ethanol Extract (200 mg/kg) orally.
Group VII : Receive *Tridax procumbens* Aqueous Extract (200 mg/kg) orally.

Experimental models of Anti-depression.

Tail suspension test (TST)

All the animals were fasted for 12 hrs prior to the administration of vehicle/standard/test compounds. The vehicle/standard/test compounds were administered p.o. 30 minutes prior to testing. The mice were suspended on the edge of a shelf 58cm above the table top by adhesive tape placed approx.1cm from the tip of tail. The duration of immobility was recorded for the period of 6 minutes by using stop watch. After the initial period of vigorous motor activity, the mice would become still. Mice were considered immobile when they hang passively and completely motionless. [8,9]

Statistical analysis

The data were analysed using one way ANOVA followed by Dunnet's multiple comparison test. P<0.001 was considered significant.

RESULT

Preliminary phytochemical screening

The Various leaf extract of *Tridax procumbens L* was subjected to Preliminary Phytochemical results showed the presence of alkaloids, carbohydrates, flavonoids or polyphenols etc.

Table 2: Preliminary phytochemical screening of various extracts of *Tridax procumbens L*

S. No	Constituents	Tests	PEETP	CETP	EAETP	EETP	AETP
1	Alkaloids	Mayer's test Dragendroff's test Hager's test Wagner's test	- - + -	+ + + +	+ + + +	+ + + +	+ + - +
2	Sterols	Burchard test Salkowski	+ +	+ -	+ -	- -	- -
3	Carbohydrates	Molisch's test Fehling's test Benedict's test Barfoed's test	- - - -	- - - -	+ + + +	+ + + +	+ - + +
4	Glycosides	Legal test Keller kiallani test Borntrager's test	+ - -	+ - -	+ + +	+ + +	+ + +
5	Fixed oils & Fats	Spot test Saponification test	- -	- -	- -	- -	- -
6	Phenolic Compounds	Ferric chloride	+	+	+	+	+
7	Proteins & amino acids	Biuret test Ninhydrin test Millon's test	+ + +	+ + +	+ + +	+ + +	+ + +
8	Terpenoids & Saponins	Foam test Hemolysis test	- -	- -	- -	- -	- -
9	Tannins	Gelatin test FeCl ₃ test	+ +	+ +	+ +	+ +	+ +
10	Gums & mucilage	Precipitation to 90% alcohol	-	-	-	+	+
11	Flavonoids	Shinoda test Lead acetate test Ferric chloride test Zinc HCL test	- - - +	+ - + +	+ - + +	+ + + +	- - + +

+ve: Present, -ve: Absent

Acute Oral Toxicity Study

The various extracts of *Tridax procumbens L* was administered orally to different groups of mice at different dose levels and was found to be safe up to the dose level of 2000 mg/kg, p.o, and did not

produce any toxic symptoms. The survived animals were sacrificed and complete absorption of drug through GIT was observed. Hence 1/10th Maximum Therapeutic Dose (2000 mg/kg) of 200 mg/kg was selected for the pharmacological models.

Functional Observational Battery (FOB)

The various extracts of *Tridax procumbens* L was subjected to FOB, which is a non-invasive procedure to detect gross functional deficits basing upon various behavioral parameters. The scoring of various parameters was shown in Table. The results showed a normal in stereotypic behaviors. The various parameters observed in Functional observational battery for extract at doses of 200 mg/kg, p.o were tabulated as follows.

Table 3: Functional Observation Battery of extracts of *Tridax procumbens* L

S. NO	BEHAVIORAL PARAMETERS	NORMAL SCORE	30 MIN	60 MIN	120 MIN	240 MIN
1	Spontaneous Motor Activity	4	4	4	4	4
2	Respiration	4	4	4	4	4
3	Ataxia	0	0	0	0	0
4	Inclined plane	0	0	0	0	0
5	Body tremor	0	0	0	0	0
6	Convulsions	0	0	0	0	0
7	Reactivity to Sound & Touch	0	0	0	0	0
8	Pinna reflex, Corneal reflex, Righting reflex	4	4	4	4	4
9	Analgesia	4	4	4	4	4
10	Writhing	0	0	0	0	0
11	Stereotype behavior	0	0	0	0	0
12	Body tone	4	4	4	4	4
13	Limb tone	4	4	4	4	4
14	Urination	0	0	0	0	0
15	Lacrimation	0	0	0	0	0
16	Salivation	0	0	0	0	0

17	Diarrhoea	0	0	0	0	0
18	Piloerection	0	0	0	0	0
19	Pupil size	4	4	4	4	4
20	Ptosis	0	0	0	0	0
21	Struab tail	0	0	0	0	0
22	Catalepsy	0	0	0	0	0
23	Hypothermia	0	0	0	0	0
24	Stratle response	0	0	0	0	0
25	Cyanosis	0	0	0	0	0
26	Exophthalmus	0	0	0	0	0

Tail suspension test

The standard (Escitalopram 10mg/kg) and ethanol extract (200 mg/kg) treated groups, the peak values of immobility time significantly decreased to 72.22 ± 6.42 , 91.25 ± 3.94 when compare to control 192.80 ± 2.30 . There is no significant variation between ethanol extract and escitalopram treated group immobility time. But it is reveals significant variation ($p < 0.001$) compare to other extracts and negative control. Thus, the Chloroform and ethyl acetate 200mg/kg extracts shows less significant ($p < 0.01$) then the Ethanol when compared to negative control in immobility time 112.31 ± 2.80 & 100.80 ± 5.66 , but no significant variation between the groups. Aqueous extracts result revealed less significant ($p < 0.05$) comparatively with other extracts. Petroleum extract treated group decreases the immobility time but it shows no significant to negative control.

Table 4: Effect of *Tridax Procumbens* L on Immobility time in tail suspension test model of mice

Group	Treatment	Immobility time in seconds	% Inhibition
I	Negative control	192.80 ± 2.30	0
II	Positive control	$72.22 \pm 6.42^{***a}$	62.54
III	PEETP 200mg/kg	186.26 ± 5.7^{nsd}	3.3
IV	CETP 200mg/kg	$112.31 \pm 2.8^{**b}$	41.74
V	EAETP 200mg/kg	$100.80 \pm 5.66^{**b}$	47.71
VI	EETP 200mg/kg	$91.25 \pm 3.94^{***a}$	52.67
VII	AETP 200mg/kg	$148.72 \pm 5.51^{*c}$	22.86

Results were analyzed by one-way ANOVA using Dunnett's multiple comparison test; N=6 in each group; Significance at ***p<0.001, **p < 0.01, *p<0.05, Mean Bearing same superscript do not differ significantly, mean bearing different superscript differ significantly. Non Significance (ns) at p > 0.05 Vs negative.

DISCUSSION

The incidence of anxiety and depression in the community is very high and is associated with lot of morbidity. Hence, it is very important to address these problems and find effective remedies. Though several drugs are available, all are associated with some limitations and there is an urgent need for alternative medications for these disorders. Despite the widely popular use of *Tridax procumbens L* for treating nervous disorders, there is an absence of scientific reports about the evaluation of its pharmacological effects. In this work, it was demonstrated that the administration of different extracts of *Tridax procumbens L* in mice was able to induce antidepressant effects. On the basis of the clinical association of depressive episodes and stressful life events, many of the animal models for the evaluation of antidepressant drug activity assess stress-precipitated behaviors. The two most widely used animal models for antidepressant screening are the forced swimming and tail suspension tests. These tests are quite sensitive and relatively specific to all major classes of antidepressants [10]. In TST, immobility reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. This induces a state of behavioral despair in animals, which is claimed to reproduce a condition similar to human depression [11, 12]. It has been seen that the TST is less stressful and has higher pharmacological sensitivity than FST [13].

Results showed that the administration of the *Tridax procumbens L* produced a diminution of immobility time of mice exposed to tail suspension tests. In the present study, ethanol extract of *Tridax procumbens L* (200 mg/kg, po) administered to mice, produced significant antidepressant-like effect in TST and both CETP & EAETP 200mg/kg immobility time increasing compare to ethanol extract it might be less effective in release of 5-Hydroxy Tryptamine or Nor adrenaline.

Data in the literature demonstrated that 5HT₃ and α adrenergic receptors on the nerve terminals and inhibit the release/ block post neuron binding site/ inhibit the reuptake of 5HT and NA, which indicates that the plant extract exerts antidepressant effects by significantly modifying release of 5HT & NA in the terminals. It has been established that the shortening of immobility time in tail suspension tests depends mainly on the enhancement of central 5-HT and NA. [14,15] There is a little effect was found due to the increase of dopamine also. Exact mechanisms underlying the antidepressant action might be due to the extract act through the serotonergic and adrenergic neuron and increase the same. The action of the extract may due to presence of large number of

phytochemicals in the EETP. However, the antidepressant activity may be attributed to the presence of flavonoids, tannin, phenolic compounds, alkaloids and glycoside in the extract.

CONCLUSION

As per our results obtained from experiments performed on the mice were succeeded and showing very positive results. We believe that Ethanol leaf extract of *Tridax procumbens L* has the marked efficacy in the treatment of depression and other mood disorders. From all the above findings, the present investigation suggests that the ethanol extract of *Tridax procumbens L* may possess antidepressant activity by inhibiting reuptake of Serotonin and noradrenaline or increase the release of the neurotransmitter none selectively. Therefore lend pharmacological credence to the unclaimed use of this plant in the treatment of depression. However, an extensive Pharmacological study of this plant is required for complete understanding of the antidepressant activity of ethanol extract of *Tridax procumbens L*. Further investigation should be carried out to isolate, identify the chemical constituent and elaborate mechanism of action which is responsible for its antidepressant activity.

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