



ISSN: 2347-6567

International Journal of Allied Medical Sciences and Clinical Research (IJAMSCR)

IJAMSCR |Vol. 14 | Issue 3| Jul - Sep 2026

www.ijamscr.com

DOI : <https://doi.org/10.61096/ijamscr.v14.iss3.2026.1543.1553>

Phytochemical Analysis and Antidepressant Activity of *Tridax Procumbens* Leaves Extracts in Experimental Models of Depression in Wister Albino Rats and Mice

*MD MINHAZ US SIRAZ, Md. Rasheed

*Gurram Balanarsaiah Institute of Pharmacy (GBN), Edulabad, Hyderabad, Telangana.
Affiliated to Jawaharlal Nehru Technological University, Hyderabad, Telangana.

*Corresponding author: MD MINHAZ US SIRAZ

Email: minhazetc@gmail.com



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16.09.2026
Published by:
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Abstract: The present study was undertaken to investigate the effects of methanolic extract of *Tridax procumbens* Leaves (family: Asteraceae), popularly known as *Tridax procumbens* on depression using force swim test in rats, potentiation of norepinephrine toxicity in mice and haloperidol induce catalepsy in mice. The extract of *Tridax procumbens* (250 and 500 mg/kg) was administered orally to rats used in FST and 500mg/kg was administered in HIC and same dose administered in NE toxicity in mice. The dose of 250mg/kg and 500mg/kg of extract significantly ($p < 0.001$) reduced the immobility times in rats but dose of 500 mg/kg showed more potent effect than Imipramine (30mg/kg). So this dose was used in HIC and NE toxicity in mice. But in NE toxicity model it was observed that METP is not good adrenergic component. A significant ($P < 0.001$) reduction in the duration of catalepsy was observed in the METP treated group and Fluoxetine group as compared to the haloperidol treated group. In HIC, mice were sacrificed on the seventh day and TBARS, glutathione, nitrite activities were estimated. Monoamine oxidase inhibiting effect and anti-oxidant effect of *Tridax procumbens* s may be contributing favorably to the antidepressant-like activity. Thus, it is concluded that *Tridax procumbens* extract may possess an antidepressant like effect.

Keywords: *Tridax procumbens*, Antidepressant activity, and forced swim test.

Introduction:

World Health Organization (WHO) reported that 80% of the world's population depends on medicinal plants for their primary health care. In the Plant Kingdom, Medicinal plants form the largest single grouping of plants. It is estimated that 30,000 species worldwide fall in this group, of which around 33% are trees² Plants are known to be the source of many chemical compounds. Medicinal plants were used by people of ancient cultures without knowledge of their active ingredients. The common practice of taking crude extract orally is laden with hazards as the extracts may contain some toxic constituents. There is an ever increasing need to limit toxic clinical drugs. In modern times, the active ingredients and curative actions of medicinal plants were first investigated through the use of European Scientific methods³. The most important ingredients present in plant communities turn out to be alkaloids, terpenoids, steroids, phenols glycosides and tannins².

The information obtained from extracts of medicinal plants makes pharmacological studies possible. The mode of action of plants producing therapeutic effects can also be better investigated if the active ingredients are characterized. Infectious diseases are the leading cause of death worldwide. The clinical efficiency of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens. Bacterial pathogens have evolved numerous defence mechanisms against antimicrobial agents and resistance to old and newly produced drug is on the rise. The increasing failure of chemotherapeutics

and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity⁴.

There are several reports in the literature regarding the antimicrobial activity of crude extracts prepared from plants⁵. Plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines. Higher plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health care since ancient times. Over 50% of all modern clinical drugs are of natural product origin and natural products play a vital role in modern drug development in the pharmaceutical industry⁶.

PLANT PROFILE: SCIENTIFIC CLASSIFICATION

BOTONICAL NAME: *Tridax procumbens*

Kingdom : Plantae
Order : Asterales
Family : Asteraceae
Genus : Tridax
Species : T. procumbens



Fig: *Tridax procumbens*

MATERIALS AND METHODS: The designing of methodology involves a series of steps taken in a systematic way in order to achieve the set goal(s) under the prescribed guidelines and recommendations. It includes in it all the steps from field trip to the observation including selection and collection of the medicinal plant, selection of dose value, standardization of protocol, usage of instruments, preparation of reagents, selection of specific solvents for extraction, formation of protocols and final execution of the standardized protocol. All this requires good build of mind and a good and soft technical hand to handle the materials and procedure in a true scientific manner.

Instruments: Following instruments were required for the study:

Table No: List of Instruments: Centrifuge, Digital weighing balance, Glucometer, Heating mantle, Refrigerator, Actophotometer, Elevated Plus maze apparatus, Glass Adhesive tape, cylinder, Thread, Stop watch, Syringes, Needles, Soxhlet extractor, Condenser, Burette stand, Round bottom flask, Oven Water bath, Stirrer/glass rod, Watch glass, Whatmann, filter paper, Butter paper, Spatula, Rubber pipes. Etc.

EXPERIMENTAL ANIMALS

Animals: Wistar albino rats and Swiss albino mice of either sex, weighed 150-180g procured from CPCSEA registered source. They were housed in polypropylene cages under standard light/dark cycle, with food and water provided ad libitum.

The experimental protocols were approved by the Institutional Animal Ethics Committee and conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Chemicals: Imipramine tablets Procured from Intas Pharmaceutical, Haloperidol ampoules Procured from Crescent Pharma and Fluoxetine capsules were from Cadila Pharmaceuticals. Norepinephrine ampoules were purchased from IGMSC Shimla (H.P). 5,5'-Dithio-bis (2-nitrobenzoic acid) (DTNB) Reduced

Glutathione standard was purchased from Sanjay biological Amritsar, India. NEDA and TCA was purchased from (SDFCL) S D Fine-Chem Ltd, Mumbai. TBA was purchased from Loba Chem Pvt Ltd., Mumbai and Sulphanilamide was purchased from Titan Biotech Limited. All the reagents and chemicals used in the study were of analytical grade and were procured from Spruce Enterprises (Ambala, Haryana).

Plant material: The fresh leaves of *Tridax procumbens* were collected from local market. The plant material was cleaned, reduced to small fragments, air dried under shade at room temperature.

Preparation of extract: The collected plant Leaves were made dried by fan aeration in shade. The air-dried plant material was then grinded to reduce them into coarse powder with the help of a suitable grinder. The powder was then subjected to extraction with methanol in Soxhlet extractor. After the complete extraction, the solvent was distilled off and concentrated on a water bath to a dry residue. The extract was concentrated by distilling of the solvent and then evaporated to dryness on water bath. A gummy concentrate of dark chocolate black colour obtained was designated as methanolic extract of *Tridax procumbens* (METP). The yield of extract was found to be 7.2%.

PHYTOCHEMICAL EVALUATION

Preliminary phytochemical screening of Methanolic leaves extract of *Tridax procumbens*

The Methanolic leaves extract of *Tridax procumbens* was used for testing preliminary phytochemical screening in order to detect major chemical groups.

Test for carbohydrates: Molisch's test: Dissolved small quantity of 300mg alcoholic and dried leaves extract powder of *Pimenta dioica* separately in 4ml distilled water and filtered. The filtrate was subjected to Molisch's test. **Fehling's test:** Dissolve a small portion of extract in water and treat with Fehling's solution. **Phenols test:** The extract was spotted on a filter paper. A drop of phosphomolybdic acid reagent was added to the spot and was exposed to ammonia vapours.

Test for flavanoids: Shinoda test: To 2 to 3ml of extract, a piece of magnesium ribbon and 1ml of concentrated HCl was added. **Lead acetate test:** To 5ml of extract 1ml of lead acetate solution was added.

Test for tannins: Braemer's test: To a 2 to 3ml of extract, 10% alcoholic ferric chloride solution was added.

Test for steroid/terpenoid: Liebermann-Burchardt test: To 1ml of extract, 1ml of chloroform, 2 to 3ml of acetic anhydride and 1 to 2 drops of concentrated Sulphuric acid are added.

Test for alkaloids: Dragendorff's test: A drop of extract was spotted on a small piece of precoated TLC plate and the plate was sprayed with modified Dragendorff's reagent.

Hager's test: The extract was treated with few ml of Hager's reagent. **Wagner's test:** The extract was treated with few ml of Wagner's reagent. **Tests for Glycosides: Legal's test:** Dissolved the extract [0.1g] in pyridine [2ml], added sodium nitroprusside solution [2ml] and made alkaline with Sodium hydroxide solution.

Test for Saponins: Foam test: 1ml of extract was dilute with 20ml of distilled water and shaken with a graduated cylinder for 15 minutes.

Test for Anthraquinones: Borntrager's test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml of concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia.

Test for Amino acids: Ninhydrin test: Dissolved a small quantity of the extract in few ml of water and added 1ml of ninhydrin reagent.

Test for fixed oils and fats: Press small quantity of the petroleum ether extract between two filter paper.

METHODOLOGY: Overnight fasted animals were selected randomly on the day of experiment for administration of vehicle, standard drug and study drug.

Force swim test (FST): Method of behavior despair or force swim test use as a model was proposed by (Porsolt et al., 1977, 1978) to evaluate antidepressant activity. Rats were forced to move in open cylindrical container (diameter of 15cm, height 25 cm), containing fresh water of 15cm height and maintained at 25°C.

All the rats were divided in to three groups of six animals in each.

Group I represented as control group that received normal saline, Nacl (5ml/kg, i.p). Group II is standard group that received Imipramine (30mg/kg, i.p). Group III was represented as drug treated group which was further divided in to two groups IIIa, IIIb that received two different doses (250, 500mg/kg, p.o) of METP.

In total time period of 10 min, the duration of immobility was recorded during the last 6 min (Thamarai et al., 2012). After an initial 4 min period of vigorous activity, each animal assumed a typical immobile posture. A rat was considered to be immobile when it remained floating motionless in the water, ceased for struggling and making only minimum movements of its limbs necessary to keep its head above water. Changes in duration of immobility of each group were studied in this model.

Potentiation of norepinephrine toxicity in mice: This model is proposed by (Sigg, 1959) to evaluate antidepressant activity based on mechanism of action (Vogel, 2002). Mice were divided into four groups of six animals each.

Group I animals received Normal saline (5ml/kg, i.p) and this group used as control group. Group II animals received norepinephrine (4mg/kg, i.p) and this group used as NE group. Group III animals received Imipramine (40mg/kg x 2) and norepinephrine (4mg/kg, i.p) this group used as standard group. Group IV animals received dose of METP (500mg/kg x 2) and norepinephrine (4mg/kg, i.p) this group used as drug treated group.

In this model, Imipramine and METP administered p.o twice 24 hr and 30 min prior to norepinephrine. Within 48 hrs after norepinephrine injection, number of lethality's or mortalities were recorded and calculated.

Haloperidol induce catalepsy (HIC) in mice: In this method catalepsy was induced by haloperidol. Mice were divided into four groups of six animals each. Group I served as normal control that received normal saline (5ml/kg, i.p). Group II served as negative control that received haloperidol (1mg/kg, i.p). Group III served as standard group that received 5mg/kg fluoxetine, i.p (Pires at al., 2005) and haloperidol (1mg/kg, i.p). Group IV served as drug treated group that received METP (500mg/kg, p.o) and haloperidol (1mg/kg, i.p). All these doses were given to mice for seven days.

The duration of catalepsy was measured at 30, 60, 90, 120, 150, 180 min. Catalepsy was assessed by means of standard bar test on every 3rd, 5th and 7th day of the drug treatment (MD et al., 2012). The catalepsy was measured in time for which the mouse maintained an imposed posture with both the front limbs extended and resting on a 4 cm high wooden bar (1.0cm diameter). The end point of the catalepsy was considered to occur when both the front paws were removed from the bar or if the animal moved its head in an exploratory manner. A cut-off time of 200 seconds was applied. The effects of the test drug METP (500 mg/kg, p.o) and the standard drug fluoxetine (5mg/kg, i.p) were assessed after their repeated dose administration in mice for seven days, 30 minutes prior to the administration of haloperidol. The mice were sacrificed on the 7th day and the TBARS, glutathione and nitrite estimations were estimated.

Biochemical parameters: Preparation of Brain homogenate on 7th day of dosing of all groups, for the biochemical analysis, animals were scarified by anesthetized with diethyl ether immediately after behavioural assessment. The brains were removed and rinsed with 0.9%Nacl solution and weighed. Tissue was homogenized with phosphate buffer solution (ph-8) for 1 minute and then centrifuged the homogenized mixture for 10 min at 2000 rpm. An aliquot supernatant phase was collected and stored at 2-8° C.

Estimation of TBARS: Lipid peroxidation was estimated spectrophotometrically in brain tissue by quantifying TBARS. In brief, for the estimation of TBARS the supernatant of the tissue homogenate was treated with 10 % TCA reagent and kept the solution in ice bath for 15 minutes. Then centrifuged the solution for 5minutes at 2000 rpm. Then 2 ml of supernatant was taken out and mixed with freshly prepared 2 ml of 0.67% TBA solution. The mixture was kept in boiling water bath for 15 minutes. After cooling, the tubes were centrifuged for 10 minutes and the supernatant taken for measurement. The developed color was read at 532 nm using a UV spectrophotometer (Shimadzu UV-1700, UV-VIS double-beam Spectrophotometer) against a reagent blank and expressed as nm /mg of protein.

Estimation of Reduced glutathione: The supernatant of brain homogenate was mixed with trichloroacetic acid (10% w/v) in 1:1 ratio. The tubes were centrifuged at 10000 rpm for 10 min at 4oC. The supernatant obtained (0.5 ml) was mixed with 2 ml of 0.3 M disodium hydrogen phosphate. Then 0.25

ml of 0.001 M freshly prepared DTNB [5, 5-dithiobis (2-nitrobenzoic acid) dissolved in 1% w/v sodium citrate] was added and absorbance was noted spectrophotometrically (Shimadzu UV-1700, UV-VIS doublebeam Spectrophotometer) at 412 nm. A standard curve was plotted using 10-100 μ M of reduced form of glutathione and results were expressed as micromoles of reduced glutathione per mg of protein.

Estimation of nitrite: The accumulation of nitrite in the supernatant is an indicator of production of nitric oxide (NO), which has produced due to oxidative stress occurring in the brain. Production of NO was determined by spectrophotometric assay with Griess reagent (0.1% N-1-naphthyl ethylene amine dihydrochloride, 1% sulphanilamide and 2.5% phosphoric acid). Equal volumes of brain homogenate and Griess reagent were mixed; the mixture was incubated for 10 min at room temperature and the absorbance was measured at 546 nm. The concentration of nitrite in the supernatant was determined from the standard curve and expressed in nm/mg of protein.

RESULTS:

Phytochemical screening test: The freshly prepared extract of the leaves of *Tridax procumbens* was subjected to phytochemical screening tests for the detection of various active constituents. The extract showed the presence of alkaloids, tannins, steroids, phenolic and flavonoids, carbohydrates, and glycosides in crude extract of *Tridax procumbens* leaves as depicted in Table 1.

Table 1: Result of chemical group tests of the Methanolic extract of *Tridax procumbens* leaves.

Class of compounds	Results
Carbohydrates	+
Tannins	++
Flavonoid	++
Saponin	++
Phenols	+
Steroids	+++
Alkaloids	+
Glycosides	+

ANTIDEPRESSANT ACTIVITY OF *TRIDAX PROCUMBENS*

Effect of METP on FST induce immobility in rats: Animals administered with Imipramine 30mg/kg showed the decreased duration of immobility. Thus, showing the significant ($p < 0.001$) difference as compared to control. There was a significant ($p < 0.001$) dose dependent decrease in duration of immobility in animals treated with 250 and 500 mg/kg doses of METP. METP showing a greater effect at 500 mg/kg dose when compared to vehicle control group. Whereas, METP at 500mg/kg showed a significant ($p < 0.01$) potent effect when compared to Imipramine treated group. Animals treated with METP 250 mg/kg and 500mg/kg showed average duration of immobility respectively for a period of 6 min after 1 hr of METP treatment (Table 2).

Table. 2: Effect of METP on FST induced duration of immobility in rats

Group	Treatment	Duration of immobility (Sec) Mean $\pm$ SEM
I (Control)	Normal saline (5ml/kg, i.p)	181.0 $\pm$ 1.687
II (Standard)	Imipramine (30 mg/kg, i.p)	122.9 $\pm$ 3.364a***
IIIa (Test 1)	METP (250 mg/kg, p.o)	133.3 $\pm$ 0.541 a***, b*
IIIb (Test 2)	METP (500 mg/kg, p.o)	101.5 $\pm$ 2.974 a***, b**

Data are mean $\pm$ SEM values, n=6. Data were analysed by One way ANOVA followed by Dunnett's Multiple Comparison Test. *P < 0.05, **P < 0.01, ***P<0.001, compared with control, compared with Imipramine.

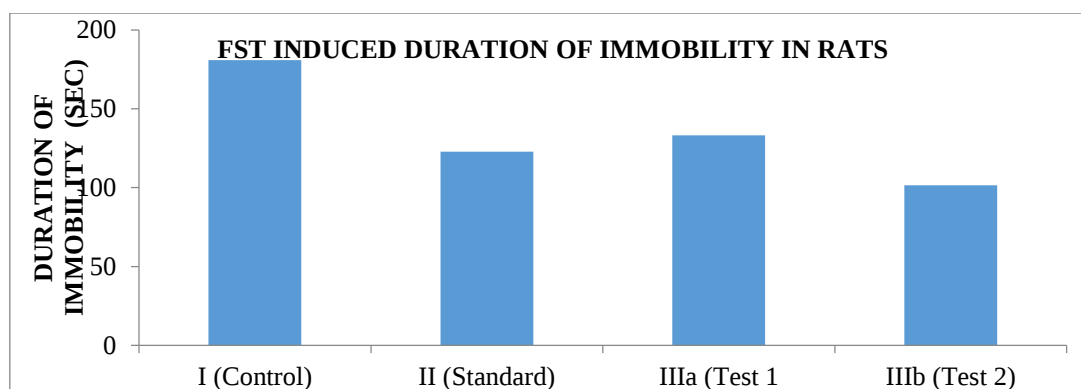


Fig: Effect of METP on FST induced duration of immobility in rats

Table. 3: Effect of METP on norepinephrine Potentiation toxicity in mice

Group	Treatment	Death number of animals	Lethality (%)
I (Control)	-	0	0.0
II (NE treated)	NE (4.0 mg/kg, i.p)	2	31.2
III (Standard + NE)	Imipramine (40 x 2 mg/kg, i.p) + NE (4.0 mg/kg, i.p.)	5	80.1
IV (Test + NE)	METP (500 x 2 mg/kg, p.o) + NE (4.0 mg/kg, i.p)	2	31.2

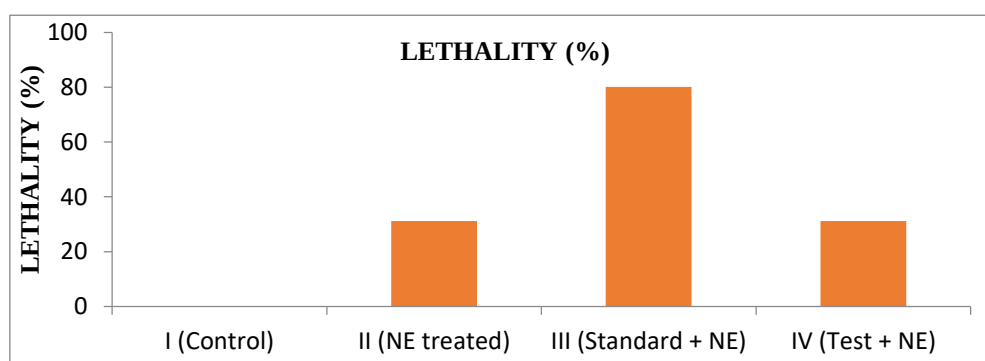


Fig: Effect of METP on norepinephrine Potentiation toxicity in mice

Table 4: Effect of METP on Haloperidol induces catalepsy in mice

Group	Treatment	Duration of Catalepsy (Sec.)		
		3rd day	5th day	7th day
I (Control)	Normal saline (5ml/kg, i.p)	2.987± 1.14	3.249± 2.964	4.189± 0.247
II (Negative control)	Haloperidol (1mg/kg, i.p)	177.12± 1.254a***	181.445± 3.256a***	184.981± 1.647a***
III (Standard)	Flouxetine (5mg/kg, i.p) +	79.415±	82.000±	87.000±
	Haloperidol (1mg/kg, i.p)	1.369b***	3.256b***	1.564b***
IV (Test drug)	METP (500mg/kg, p.o) +	115.256±	120.147±	124.647±
	Haloperidol (1mg/kg, i.p)	1.696b***	1.249b***	1.597b***

Data are mean ± SEM values, n=6. Data were analysed by One way ANOVA followed by Dunnett's Multiple Comparison Test. *P < 0.05, **P < 0.01, ***P<0.001.

A compared with control group-b compared with haloperidol treated group.

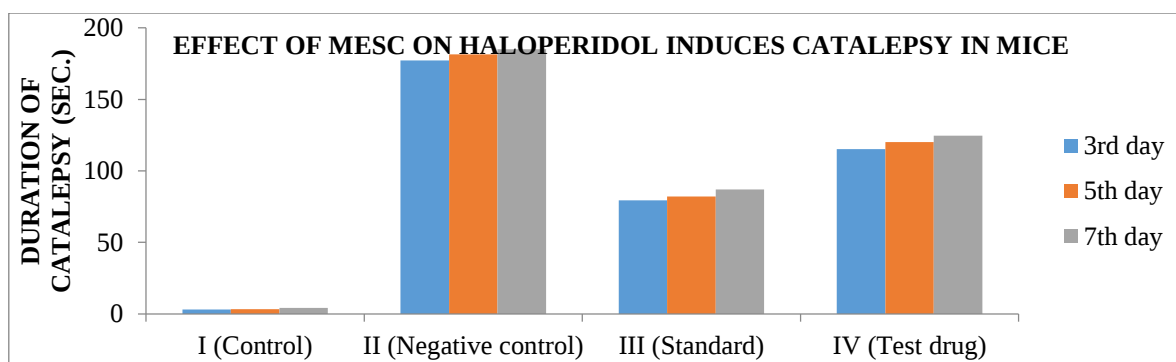
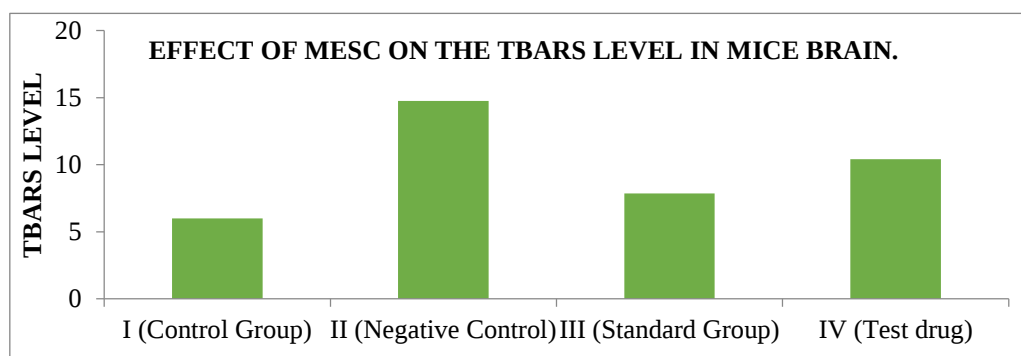


Fig: Effect of METP on Haloperidol induces catalepsy in mice

Table. 4: Effect of METP on the TBARS level in mice brain

Group	Treatment for 7 days	TBARS level (nM/ mg protein) [Mean $\pm$ SEM]
I (Control Group)	Normal Saline (5 ml/kg, i.p)	5.98 $\pm$ 0.14
II (Negative Control)	Haloperidol (1 mg/kg, i.p)	14.76 $\pm$ 0.10a***
III (Standard Group)	Flouxetine(5mg/kg,i.p)+ Haloperidol (1 mg/kg, i.p)	7.85 $\pm$ 0.415b***
IV (Test drug)	METP (500mg/kg,p.o)+Haloperidol (1 mg/kg, i.p)	10.421 $\pm$ 0.315b***

Data are mean $\pm$ SEM values, n=6. Data were analyzed by One way ANOVA followed by Dunnett's Multiple Comparison Test. *P < 0.05, **P < 0.01, ***P<0.001. a compared with control group b compared with haloperidol treated group.



Effect of METP on the TBARS level in mice brain

Table. 5: Effect of METP on Nitrite level in mice brain

Group	Treatment for 7 days	Nitrites level (μ M/ mg protein) [Mean $\pm$ SEM]
I (Control Group)	Normal Saline (5 ml/kg, i.p)	6.198 $\pm$ 1.352
II (Negative Control)	Haloperidol (1 mg/kg, i.p)	21.415 $\pm$ 2.824a***
III (Standard Group)	Flouxetine(5mg/kg,i.p)+ Haloperidol (1 mg/kg, i.p)	10.981 $\pm$ 1.325b***
IV (Test drug)	METP (500mg/kg,p.o)+Haloperidol (1 mg/kg, i.p)	15.475 $\pm$ 2.469b***

Data are mean $\pm$ SEM values, n=6. Data were analyzed by One way ANOVA followed by Dunnett's Multiple Comparison Test. *P < 0.05, **P < 0.01, ***P<0.001. a compared with control group, b compared with haloperidol treated group.

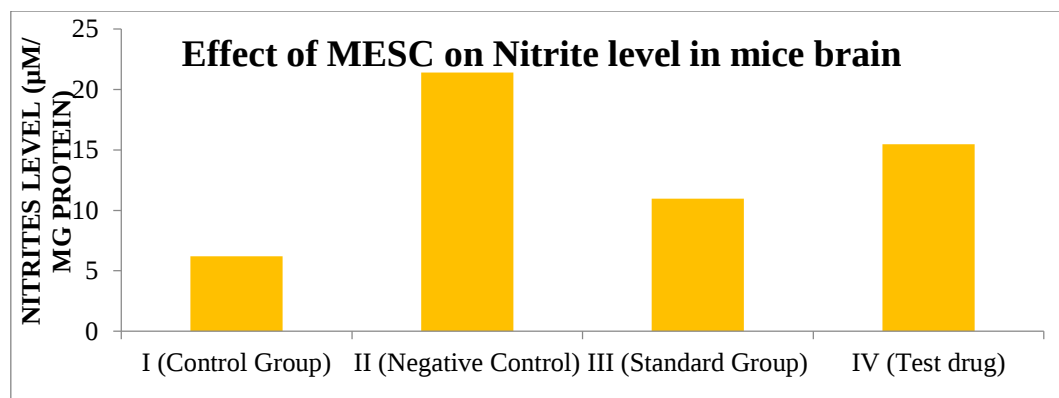


Fig: Effect of METP on Nitrite level in mice brain

Table. 6: Effect of METP on the GSH level of mice brain

Group	Treatment for 7 days	Reduced GSH level (µM/ mg protein) [Mean ± SEM]
I (Control Group)	Normal Saline (5 ml/kg, i.p)	31.128 ± 2.369
II (Negative Control)	Haloperidol (1 mg/kg, i.p)	9.187 ± 1.354a***
III (Standard Group)	Flouxetine(5mg/kg,i.p)+ Haloperidol (1 mg/kg, i.p)	21.548 ± 0.123b***
IV (Test drug)	METP (500mg/kg,p.o)+Haloperidol (1 mg/kg, i.p)	18.421 ± 0.320b**

Data are mean ± SEM values, n=6. Data were analyzed by One way ANOVA followed by Dunnett's Multiple Comparison Test. *P < 0.05, **P < 0.01, ***P<0.001. a compared with control group b, compared with haloperidol treated group

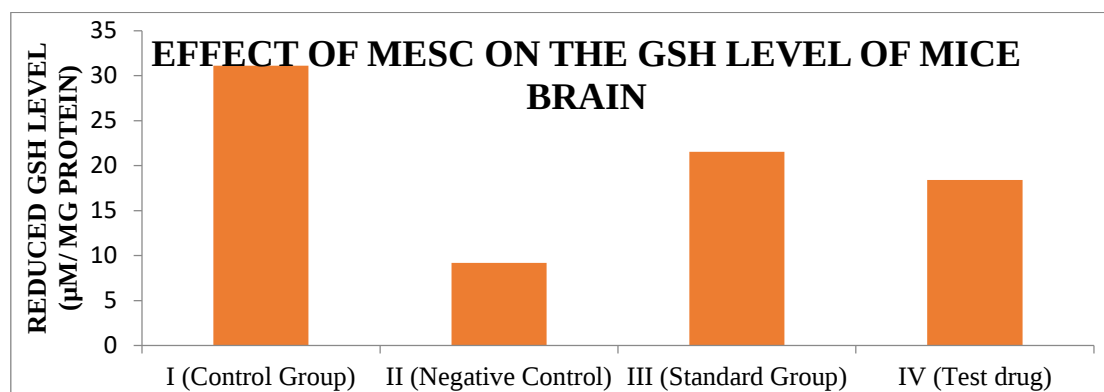


Fig: Effect of METP on the GSH level of mice brain

Effect of METP on norepinephrine potentiation toxicity in Mice

Animals of Group I received normal saline showed no mortality in mice. Group II animals received NE (4 mg/kg i.p) showed 2 mortality in mice, Group III animals receive Imipramine (40 x 2 mg/kg i.p) twice daily 30 min before NE administration showed significant increase in mortalities of mice. And in IV group animals receive MESC (500 x 2 mg/kg p.o) twice daily 30 min before NE administration showed only 2 mortalities in mice. Imipramine potentiated markedly NE toxicity in mice but METP did not show potentiated markedly NE toxicity in mice (Table 3.)

Effect of METP on Haloperidol induce catalepsy in mice

The cataleptic behavior (inability to correct abnormal posture) of haloperidol (1mg/kg, i.p) treated animal was found to increase significantly on 3rd or 5th day and also on the 7th day of treatment when compared to control group animals. Administration of Flouxetine (5mg/kg, i.p) and METP (500 mg/kg, p.o) to haloperidol (1mg/kg, i.p) treated animals significantly (p < 0.001) prevented the increase in catalepsy when compared to haloperidol treated group on 7th day (Table 4).

Biochemical parameters: Effect of METP on TBARS level in mice brain Haloperidol treatment to mice for 7 days induced lipid peroxidation as indicated by a significant ($P<0.001$) rise in brain MDA levels compared with the vehicle treated mice. Administration of Fluoxetine (5mg/kg, i.p) and METP (500mg/kg, p.o) to haloperidol treated animals' animals significantly ($P<0.001$) respectively reversed the extent of lipid peroxidation compared with haloperidol treated mice (Table 5).

Effect of METP on Nitrites levels in mice brain: As shown in table 5 the brain nitrite levels in haloperidol treated group significantly ($p<0.001$) increase as compared to control group. Administration of Fluoxetine (5mg/kg, i.p) and METP (500mg/kg, p.o) to haloperidol treated animals significantly ($P<0.001$) decrease the nitrite level as compared to haloperidol treated group (Table 6).

Effect of METP on GSH levels in mice brain: Statistical analysis of brain GSH levels showed a significant difference ($P<0.001$) between the vehicle treated and haloperidol treated mice. Administration of Fluoxetine (5mg/kg, i.p) and METP (500mg/kg, p.o) to haloperidol treated animals significantly ($P<0.001$) and ($p<0.01$) increase GSH levels as compared to haloperidol treated group (Table 6).

DISCUSSION: Depression is a mood disorder which is the most common illness, which affects the mood, lack of interest in surroundings, decreased energy level, and lack of confidence, poor concentration, disturbed sleep and the arousal of negative thoughts. It is mainly associated with anxiety. Ayurveda provides lot of medicinal plants to counteract these side effects. *Tridax procumbens* is one of the important medicinal plants knowing for its various medicinal properties. The most widely used model for antidepressant screening is FST. And the other models which are used in this study are Potentiation of norepinephrine toxicity in mice and haloperidol induce catalepsy in mice. FST is quite sensitive and relatively specific to all major classes of antidepressants. In FST, Results showed that the administration of *Tridax procumbens* produce antidepressant activity at a dose of 250 and 500 mg/kg body weight in a dose dependent manner as compared to control group and Imipramine group. *Tridax procumbens* at a dose of 500mg/kg showed potent effect to decrease the immobility period as compared to Imipramine (30 mg/kg). Numerous studies have demonstrated that antidepressant drugs such as Imipramine stimulated the action of serotonin and act by inhibiting the reuptake of biogenic amines in CNS. There is another model Potentiation of norepinephrine toxicity in mice is used. This model reveals an adrenergic component of pharmacological activity of antidepressants. In the present study Imipramine potentiated markedly NE toxicity in mice but METP did not potentiated markedly NE toxicity in mice. So, results showed that Imipramine is a good adrenergic component but METP may not be good adrenergic component.

In Haloperidol induce catalepsy, administration of *Tridax procumbens* at dose 500 mg/Kg p.o. for 7 days and the duration of catalepsy were observed on 3rd 5th and 7th day showed to decrease the duration of catalepsy as compared to haloperidol treated group. The standard drug Fluoxetine (5mg/kg) also showed significant reduction in duration of catalepsy as compared to haloperidol treated animals. From various previous researches showed that the treatment with haloperidol induces the production of free radical but the exact mechanisms by which haloperidol increased free radical production were not clear. With chronic dosing in mice for 7 days, haloperidol is associated with the greatest level of oxidative stress. The oxidative stress was measured through determination of levels of TBARs (or MDA), reduced glutathione and nitrite. Also, generation of oxidative stress, indicated by decrease in levels of endogenous antioxidant marker (GSH) and increase in the extent of lipid peroxidation (TBARs) and increase in nitrite levels was found to be the cause of depression and catalepsy. In the present study treatment of mice for seven days with Fluoxetine at the dose of 5 mg/kg (i.p) and METP (500 mg/kg) significantly ($p<0.001$) decreased the levels of TBARs and nitrites whereas increase the level of GSH revealed the antioxidant nature of the extract and also an indication of effective herbal antidepressant. In the present study, preliminary phytochemical studies of the methanolic extract of *Tridax procumbens* showed the presence of flavonoids, saponins, tannins and steroids. It has been reported that flavonoids may be responsible for antidepressant activity in experimental animal models.

CONCLUSION

A large number of traditional herbal formulations are being used successfully for the treatment of a number of ailments as suggested by the growing body of literature related to scientific justification of traditional herbal medicine. With the modernization, a huge number of folklore traditional knowledge tends to be lost in near future unless properly document based upon scientific revisiting. That is why; it is becoming imperative to take upon researches and implementing the process of documentation.

The present study was designed to undertake pharmacognostic studies, phytochemical screening and antidepressant evaluation of *Tridax procumbens* leaves. The leaves parts of *Tridax procumbens* were

selected for antidepressant evaluation based on the ethno pharmacological importance of this plant in treatment of CNS related problems.

The present study thus proves that the methanolic extract of *Tridax procumbens* possesses significant antidepressant activity due to its reduction in the immobility period in FST and reduction in the duration of catalepsy in haloperidol induces catalepsy. But METP does not potentiate markedly NE toxicity in mice. The study though supports the traditional claim; further studies are needed to identify the chemical constituents that are responsible for the antidepressant effect.

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