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

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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* s, 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* s (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* s extract may possess an antidepressant like effect.

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

INTRODUCTION

Medicinal plants are various plants thought by some to have medicinal properties, but few plants or their phytochemical constituents have been proven by rigorous science or approved by regulatory agencies such as the United States Food and Drug Administration or European Food Safety Authority to have medicinal effects. World Health Organization (WHO) has provided a definition of medicinal plants, that is "A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for synthesis of useful drugs."¹

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 trees.² 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 defense 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.

1.1.1 History of plants in medicine⁷

The earliest known medical document is a 4000-year-old Sumerian clay tablet that recorded plant remedies for various illnesses. The ancient Egyptian Ebers papyrus from 3500 year ago lists hundreds of remedies. The Pun-tsao contains thousands of herbal cures attributed to Shennung, China's legendary emperor who lived 4500 years ago. In India, herbal medicine dates back several thousand years to the Rig-Veda, the collection of Hindu sacred verses. The Badianus Manuscript is an illustrated document that reports the traditional medical knowledge of the Aztecs. Western medicine can be traced back to the Greek physician Hippocrates, who believed that disease had natural causes and used various herbal remedies in his treatments. Early Roman writings also influenced the development of western medicine, especially the works of Dioscorides, who compiled information on more than 600 species of plants with medicinal value in *De Materia Medica*. Many of the herbal remedies used by the Greeks and Romans were effective treatments that have become incorporated into modern medicine (e.g., willow bark tea, the precursor to aspirin). Dioscorides' work remained the standard medical reference in most of Europe for the next 1500 years.

The beginning of the Renaissance saw a revival of herbalism, the identification of medicinally useful plants. This coupled with the invention of the printing press in 1450 ushered in the Age of Herbals. Many of the herbals were richly illustrated; all of them focused on the medicinal uses of plants, but also included much misinformation and superstition. The Doctrine of Signatures, for example, held that the medicinal use of plants could be ascertained by recognizing features of the plant that corresponded to human anatomy. For example, the red juice of bloodwort suggests that it should be used for blood disorders; the lobed appearance of liverworts suggests that it should be used to treat liver complaints; the "humanoid" form of mandrake root suggests that it should be used to promote male virility and ensure conception.

Many of the remedies employed by the herbalists provided effective treatments. Studies of foxglove for the treatment of dropsy (congestive heart failure) set the standard for pharmaceutical chemistry. In the 19th century, scientists began purifying the active extracts from medicinal plants (e.g. the isolation of morphine from the opium poppy). Advances in the field of pharmacology led to the formulation of the first purely synthetic drugs based on natural products in the middle of the 19th century. In 1839, for example, salicylic acid was identified as the active ingredient in a number of plants known for their pain-relieving qualities; salicylic acid was synthesized in 1853, eventually leading to the development of aspirin. It is estimated that 25% of prescriptions written in the U.S. contain plant derived ingredients (close to 50% if fungal products are included); an even greater percentage are based on semisynthetic or wholly synthetic ingredients originally isolated from plants.

While Western medicine strayed away from herbalism, 75% to 90% of the rural population of the rest world still relies on herbal medicine as their only health care. In many village marketplaces, medicinal herbs are sold alongside vegetables and other Wares. The People's Republic of China is the leading country for incorporating traditional herbal medicine into a modern health care system; the result is a blend of herbal medicine, acupuncture, and Western medicine. Plantations exist in China for the cultivation of medicinal plants, and thousands of species are thus available for the Chinese herbalist; prescriptions are filled with measured amounts of specific herbs rather than with pills or ointments. In India, traditional systems have remained quite separate from Western medicine. In addition to Ayurvedic medicine, which has a Hindu origin, Unani medicine, with its Muslim and Greek roots, is another widely practiced herbal

tradition in India. The renewed interest in medicinal plants has focused on herbal cures among indigenous populations around the world, especially those in the tropical rain forests. It is hoped that these investigations will add new medicinal plants to the world's pharmacopoeia before they are lost forever. In addition to the destruction of the forests, the erosion of tribal cultures is also a threat to herbal practices.

1.1.2 Traditional medicine

Traditional medicine is the synthesis of therapeutic experience of generations of practicing physicians of indigenous systems of medicine. Traditional preparation comprises medicinal plants, minerals and organic matters etc. Herbal drug constitutes only those traditional medicines that primarily use medicinal plant preparations for therapy. The ancient record is evidencing their use by Indian, Chinese, Egyptian, Greek, Roman and Syrian dates back to about 5000 years.

About 500 plants with medicinal use are mentioned in ancient texts and around 800 plants have been used in indigenous systems of medicine. Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments⁸, which also forms a rich source of knowledge. The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy use several plant species to treat different ailments⁹. In India around 20,000 medicinal plant species have been recorded recently, but more than 500 traditional communities use about 800 plant species for curing different diseases¹⁰. Currently 80 % of the world population depends on plant-derived medicine for the first line of primary health care for human alleviation because it has no side effects. Plants are important sources of medicines and presently about 25% of pharmaceutical prescriptions in the United States contain at least one plant-derived ingredient. In the last century, roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources.

The use of traditional medicine has increased in developed countries also, mainly due to the failure of modern medicine to provide effective treatment for chronic diseases and emergence of multi-drug resistant bacteria and parasites. The adverse effects of chemical drugs, questioning of the approaches and assumptions of allopathic medicine, their increasing costs and greater public access to information on traditional medicine has also led to an increase in interest in alternative treatments (WHO 2002). Plant extracts have become a source of hope as a wide group of medicinal plant preparations are available that have been used over the centuries almost exclusively on the basis of empirical evidence. Hence, it has become necessary to revisit the importance of these herbal medicines.

Traditional medicine is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illnesses. Traditional medicine that has been adopted by other populations (outside its indigenous culture) is often termed alternative or complementary medicine. Herbal medicines include herbs, herbal materials, herbal preparations, and finished herbal products that contain parts of plants or other plant materials as active ingredients.

1.1.3 Trends of using traditional medicine

In some Asian and African countries, 80% of the population depend on traditional medicine for primary health care. In many developed countries, 70% to 80% of the population has used some form of alternative or complementary medicine (e.g. acupuncture). Herbal treatments are the most popular form of traditional medicine, and are highly lucrative in the international marketplace. Annual revenues in Western Europe reached US\$ 5 billion in 2003-2004. In China sales of products totaled US\$ 14 billion in 2005. Herbal medicine revenue in Brazil was US\$ 160 million in 2007¹¹.

1.1.4 Modern medicine from medicinal plants

Medicinal plants play a vital role for the development of new drugs. During 1950-1970 approximately 100 plants based new drugs were introduced in the USA drug market including deserpidine, reseinnamine, reserpine, vinblastine and vincristine which are derived from higher plants. From 1971 to 1990 new drugs such as ectoposide, Eguggulsterone, teniposide, nabilone, plaunotol, Z-guggulsterone, lectinan, artemisinin and ginkgolides appeared all over the world. 2% of drugs were introduced from 1991 to 1995 including pacitaxel, topotecan, gomishin, irinotecan etc.

Plant based drugs provide outstanding contribution to modern therapeutics; for example: serpentine isolated from the root of Indian plant *Rauwolfia serpentina* in 1953, was a revolutionary event in the treatment of hypertension and lowering of blood pressure. Vinblastine isolated from the *Catharanthus roseus* is used for the treatment of Hodgkins, choriocarcinoma, nonhodgkins lymphomas, leukemia in children, testicular and neck cancer. Vincristine is recommended for acute lymphocytic

leukemia in childhood advanced stages of hodgkins, lymphosarcoma, small cell lung, cervical and breast cancer¹². Phophyllotoxin is a constituent of Phodophyllum emodi currently used against testicular, small cell lung cancer and lymphomas. Indian indigenous tree of Nothapodytes nimmoniana (Mappia foetida) are mostly used in Japan for the treatment of cervical cancer. Plant derived drugs are used to cure mental illness, skin diseases, tuberculosis, diabetes, jaundice, hypertension and cancer.

Medicinal plants play an important role in the development of potent therapeutic agents. Plant derived drugs came into use in the modern medicine through the uses of plant material as indigenous cure in folklore or traditional systems of medicine. More than 64 plants have been found to possess significant antibacterial properties; and more than 24 plants have been found to possess antidiabetic properties, antimicrobial activity.

The present investigation explores the isolation and purification of another active compound from the aqueous and alcoholic leaves extract of *Allium cepa*, which was responsible for snake venom neutralization. Antagonism of both viper and cobra venom and antiserum action potentiation, antioxidant property of the active compound was studied in experimental animals. Recently, from this laboratory reported that an active compound from the Strychnus nux vomica seed extract, inhibited viper venom induced lipid peroxidation in experimental animals. The mechanism of action of the plant derived micromolecules induced venom neutralization need further attention, for the development of plant-derived therapeutic antagonist against snakebite for the community in need. However, the toxicity of plants has known for a long period of time, and the history of these toxic plants side by side with medicinal ones are very old and popular worldwide, they considered the major natural source of folk medication and toxication even after arising of recent chemical synthesis of the active constituents contained by these plants. Before the introduction of modern medicines, disease treatment was entirely managed by herbal remedies. It is estimated that about 80% of the world population residing in the vast rural areas of the developing and under developed countries still rely mainly on medicinal plants. Medicinal plants are the only affordable and accessible source of primary health care for them, especially in the absence of access to modern medical facilities. Studies reveal that there are more traditional medicine providers than the allopathic providers especially in the rural areas (WHO 2002). Increasing interest by multinational pharmaceutical companies and domestic manufacturers of herbal-based medicines is contributing to a significant economic growth of the global medicinal plants sector.

However, a large proportion of medicinal plant research is focused on nutraceuticals, chronic and metabolic disorders (diabetes, cardiovascular, etc.) and other diseases like HIV/AIDS, malaria, etc. Whereas, the common diseases of resource poor communities such as diarrhoeal diseases and acute respiratory tract infections (ARI) are often not addressed. Moreover, unlike the rural communities who use fresh/dried plant material or their crude extracts, the industry lays importance on isolation of active principles or standardized fractions since crude extracts are not patentable. However, it is often seen that a crude extract is more active compared to the isolated active fractions.

Among the main armaments in defense of the medicinal plants we have to mention the followings future medicine bank to disease: There are approximately half million plants with flowers, most of which have not been investigated & which principles could be decisive in the treatment of present or future. Novel structures of biologically active compounds, isolated from plant sources, often prompt the chemists to synthesize similar or biologically more potent semi-synthetic compounds.

Synergic medicine: Many application of isolated component has not had the wished effect due to turn out to be toxic. Thus the uses complement or damage or negative effects. Synthetic drugs with similar or more potent therapeutic activity are often prepared by structural modification of the plant derived compounds with known biological activity.

Support of official medicine: The treatment of vary complex disease can require in some cases the support of the medicinal properties of the plants or the derivatives that they provide. Various analogues and derivatives of plant constituents with similar or better pharmacological actions and therapeutic properties are often prepared by the chemists for use as potent drugs.

Preventive medicine: Finally we do not have to forget the preventive character that the plants have regarding the appearance of disease. In this sense the plants are better than the chemical remedies that are applied essentially when the disease has already appeared. Homatropine (a synthetic tropane alkaloid similar to atropine), syrosingopine (a synthetic derivative of reserpinre), chloroquine (a synthetic derivative of quinine), dihydromorphinone, methyl dihydromorphinone, oxy morphine, ethyl morphine, and nallyl-normorphine (synthetic derivative of morphine) are some of the examples of such synthetic drugs which plants have contributed indirectly. Even in this age of synthetic drugs, there are some naturally occurring drugs, such as Digitalis glycosides used in cardiac complications and the Catharanthus alkaloids

used in cancers, which have no synthetic alternatives. In such cases, plants continue to remain as their principal and only sources.

Artuso (1997) has outlined the entire process which includes formulating an appropriate strategy and he estimates that the entire process would take more than 10–20 years. This approach is very demanding since there is an estimated 250,000 species of higher plants present on this earth (Ayensu et al, 1978). However, this scenario would change with use of the high throughput advanced screening methods that are available today. Another approach than can prove to be a highly productive and cost effective in development of safe, effective and acceptable therapeutic agents is reverse pharmacology which is based on the documented therapeutic effects of plants in ancient texts.

It focuses on aspects of the medicinal plant research: from collection of plant material, to efficacy and safety evaluation through preclinical studies and phytochemical standardization. Billions of dollars are spent for developing a new drug every year, but little is spent to know their exact pattern of use, and how much devastation it is causing at the u

6. 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.

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 Flouxetine capsules were from Cadila Pharmaceuticals. Norepinephrine ampoules were purchased from IGMCI 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 Sulfanilamide 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 color 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 Ethanolic 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

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

Hager's test: The extract was treated with few ml of Hager's reagen.

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, fi

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 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,

B compared with Imipramine.

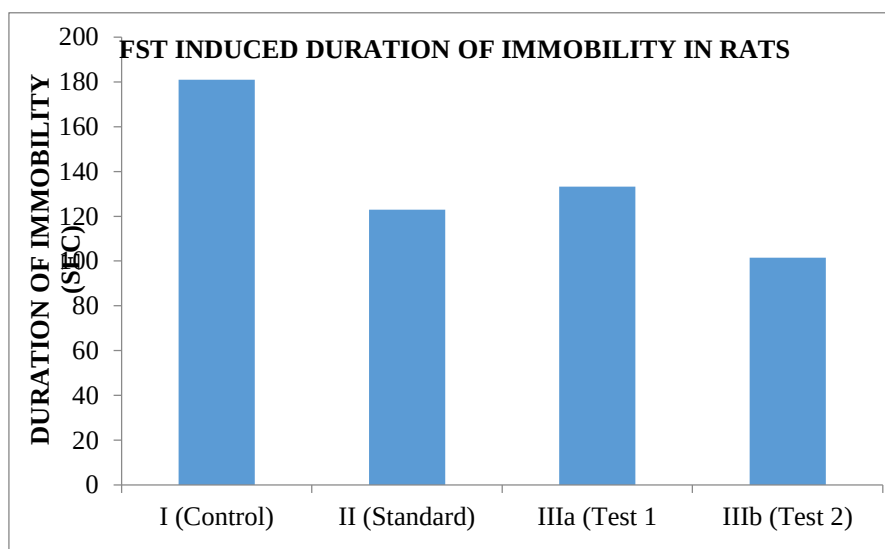
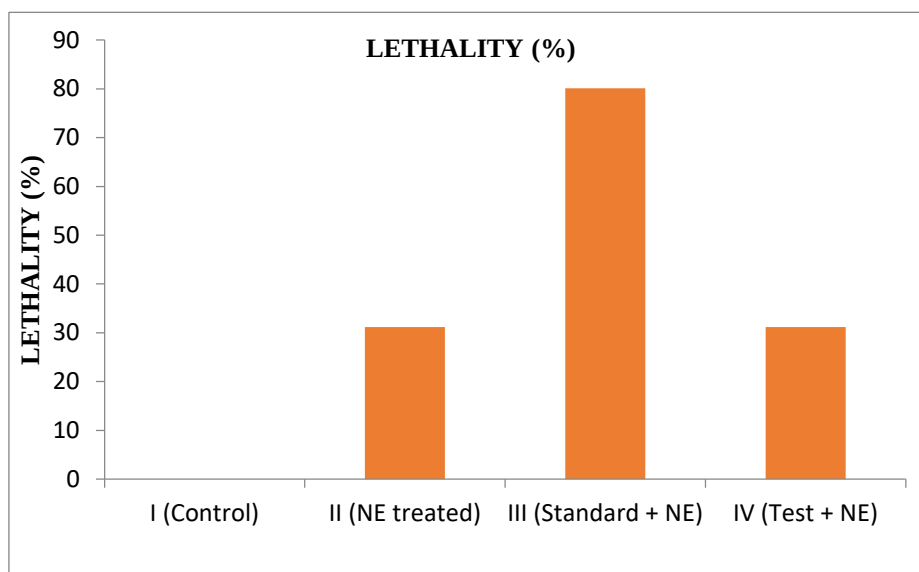
**Fig: Effect of METP on FST induced duration of immobility in rats**

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) + Haloperidol (1mg/kg, i.p)	79.415± 1.369b***	82.000± 3.256b***	87.000± 1.564b***
IV (Test drug)	METP (500mg/kg, p.o) + Haloperidol (1mg/kg, i.p)	115.256± 1.696b***	120.147± 1.249b***	124.647± 1.597b***

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.

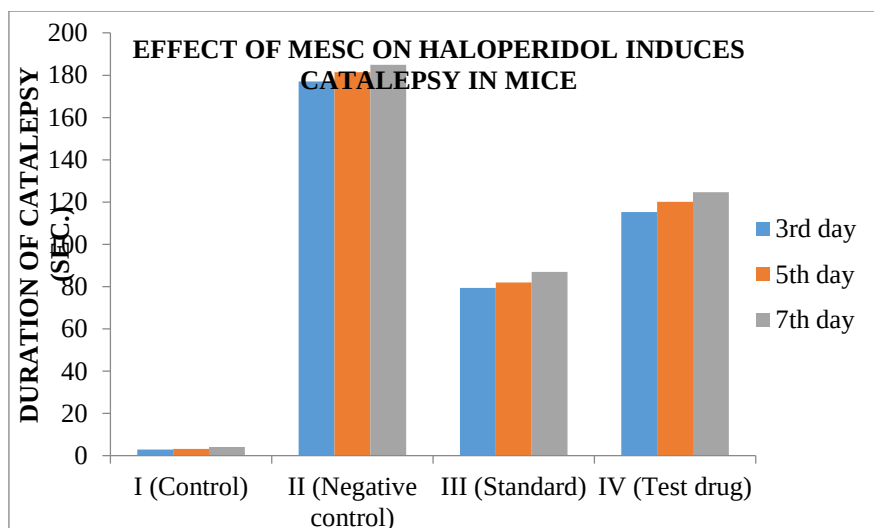


Fig: Effect of METP on Haloperidol induces catalepsy in mice

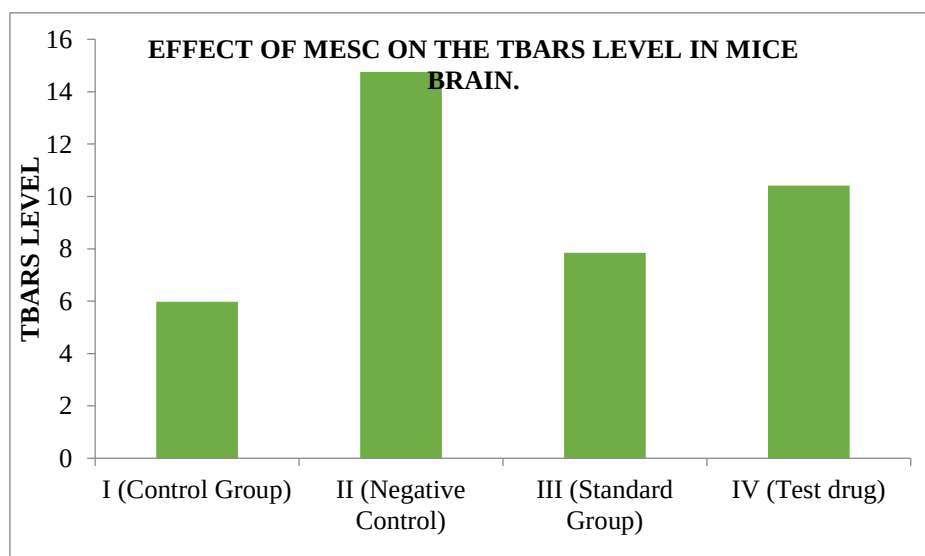
Table. 4: Effect of METP on the TBARS level in mice brain

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

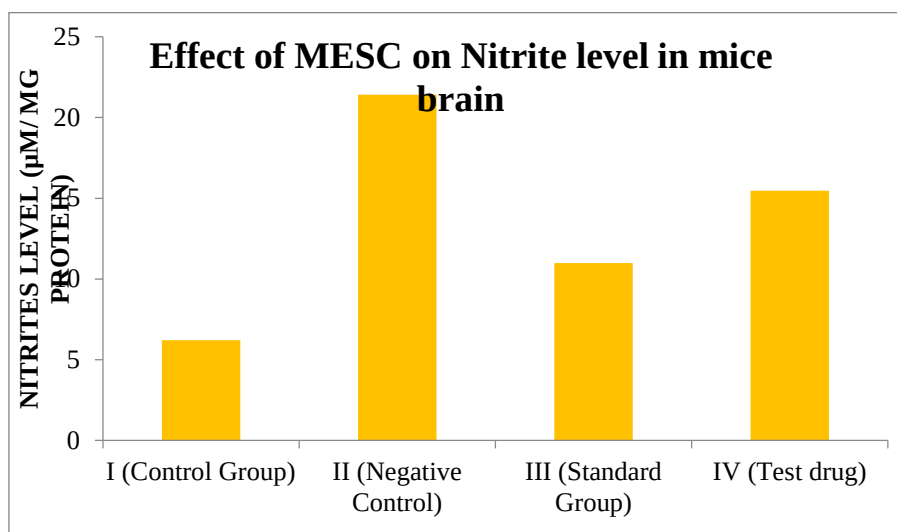
Table. 5: Effect of METP on Nitrite level in mice brain

Group	Treatment for 7 days	Nitrites level ($\mu\text{M}/\text{mg}$ 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 ($\mu\text{M}/\text{mg}$ protein) [Mean $\pm$ SEM]
I (Control Group)	Normal Saline (5 ml/kg, i.p)	31.128 $\pm$ 2.369
II (Negative Control)	Haloperidol (1 mg/kg, i.p)	9.187 $\pm$ 1.354a***
III (Standard Group)	Flouxetine(5mg/kg,i.p)+ Haloperidol (1 mg/kg, i.p)	21.548 $\pm$ 0.123b***
IV (Test drug)	METP (500mg/kg,p.o)+Haloperidol (1 mg/kg, i.p)	18.421 $\pm$ 0.320b**

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

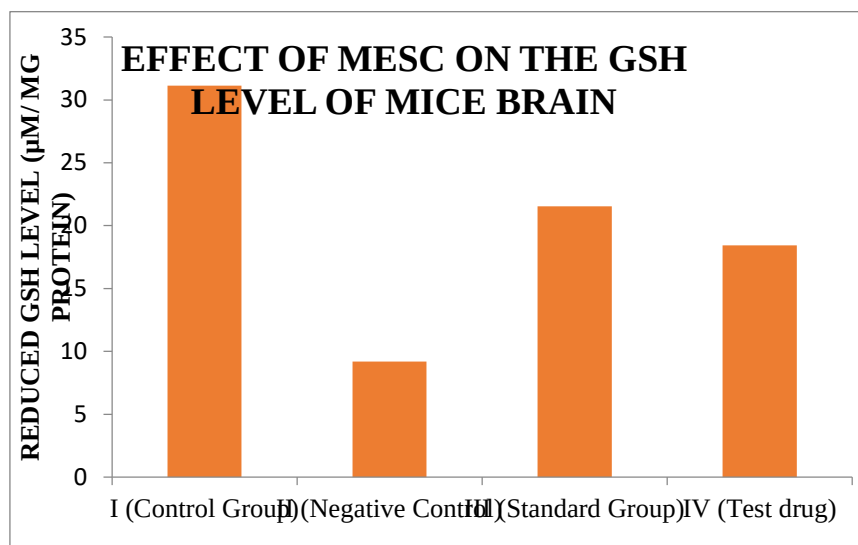


Fig: Effect of METP on the GSH level of mice brain

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.)

DISCUSSION

1. 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.
2. 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.
3. 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.
4. 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

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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