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Research Article
Anti-depressant Activity of Standardized *Macrotyloma uniflorum* Extract in Experimental Models of Depression in Rats

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Abstract

Background and Objective: *Macrotyloma uniflorum* (MU) is claimed to be used in treatment of depression traditionally, in ayurveda, siddha and unani medicine. Also, the role of MU has potential source in curing depression is still unexplored. The present study was designed to evaluate the ethanolic extract of *Macrotyloma uniflorum* (EEMU) in experimental models of depression in rats. **Materials and Methods:** In the present study, phytochemical screening, including Thin-Layer Chromatography (TLC) and High-Performance Thin-Layer Chromatography (HPTLC) was performed to confirm the presence of isoflavone, daidzein, genisteins in standardized EEMU. The EEMU was screened at two doses (200 and 400 mg kg^{-1}/p.o), based on acute toxicity study in rats. Each dose of EEMU was given to rats twice a day. Antidepressant activity was accessed by force swim test, tail suspension test and potentiation of nor-epinephrine toxicity. The duration of study was 1 week for all four groups. Antioxidants parameters like Thiobarbituric Acid Reactive Substances (TBARs), Reduced Glutathione (GSH) and nitrite/nitrate level were also accessed in brain homogenate to check the oxidative defence of EEMU extract. **Results:** The extract showed the presence of daidzein, genistein in EEMU. Further, study indicates that EEMU at doses (200 mg kg^{-1}/p.o.) and (400 mg kg^{-1}/p.o.) increase the mobility levels in rats as compared to control group. The treatment of rat with EEMU at a dose of (400 mg kg^{-1}/p.o.) produced significant decrease in the levels of TBARs, nitrite/nitrate contents and increases the level of GSH that revealed the antioxidant nature of the extract. **Conclusion:** The antidepressant activity of EEMU due to its antioxidant and tyrosine kinase inhibiting nature.

Key words: *Macrotyloma uniflorum*, anti-depressant, antioxidants, tyrosine kinase, nor-epinephrine

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Depression is a brain disorder, characterized by different symptoms like change in cognition, psychomotor and emotional disturbance[1-3]. It is one of the most common diseases and according to WHO it may become a primary cause of disability in the future. Depression is due the alteration in neurotransmitters signalling or release of biogenic amines[4,5]. These neurotransmitters include adrenaline, dopamine, serotonin and nor-adrenaline in which nor-adrenaline and serotonin increase susceptibility in control of mood and emotional behaviour[6,7].

One of the therapeutic approaches to treat depression is by increasing levels of serotonin and decreasing its uptake, which leads to increase in overall function of serotonin[8]. Selective Serotonin Reuptake Inhibitors (SSRIs), Tricyclic Antidepressants (TCAs) and irreversible Monoamine Oxidase Inhibitors (MAOIs) are three existing classes of antidepressants. The synthetic drugs are frequently prescribed especially tricyclic antidepressants for the treatment of depression[9]; but have common side effects like dry mouth, mydriasis, constipation, temporary fatigue, restlessness and headache[10-14]. Thus, researchers are exploring natural resources for newer, safer and effective antidepressant drugs.

Plants like soy beans, *Pueraria lobata* have been used traditionally in depression due to the presence of isoflavones having polyphenolic nature. Natural compound genistein, a soy isoflavone, has been proven as antidepressant at dose of 10 mg kg^{-1} for 14 days in ovariectomized rats[15,16].

Macrotyloma uniflorum has been used traditionally in various ailments like fever, dysmenorrhoea, increase biliary secretion, in dysentery as astringent, dyspepsia, vomiting, indigestion, constipation, diarrhoea, snake bite, cancer, depression and infertility[17,18].

The seeds of the plant *Macrotyloma uniflorum* belonging to family Fabaceae has variety of chemical compounds in it, like flavonoids, phenols, tannins, carbohydrates, saponins, sterols, alkaloids and coumarins[6,19]. This plant was traditionally used to treat various diseases including the neurodegenerative disease[5] and it's the main rational to evaluate it against depression. The present study aimed to phytochemically screen and to evaluate antidepressant activity of the ethanol extract of *Macrotyloma uniflorum* seeds in various models predictive of antidepressant action.

MATERIALS AND METHODS

Plant material: Standardized ethanolic extract of *Macrotyloma uniflorum* seeds was procured from Herbo Nutra, New Delhi, India. The extract dose was prepared using 2% CMC as suspending agent prior to oral administration. The study was conducted from October, 2015-April, 2016.

Drugs and chemicals: All the chemicals like imipramine, dihydrogen orthophosphate, nor-epinephrine etc. used in the study were of analytical grade, procured from Himedia Laboratories (Mumbai).

Experimental animals: Experiments were carried out on Wistar rats of either sex weighing 150-180 g. Rats were procured from authorized dealer. The animals were housed in polypropylene cages. Paddy husk was provided as bedding material and was changed every day. The cages were maintained clean and hygienic. They were fed with standard pellet diet and water. The nutrient composition of the rat diet was: Casein 210; corn starch 440; sucrose 100; maltose dextrin 100; cellulose 50; soya bean oil 50; vitamin mix 10 and minerals 35 g kg^{-1}. They were kept in a well-aerated room and a 12 h light and dark cycle was maintained. The room temperature was maintained at 22-24°C. The experiments were conducted in noise free environment between 9:00 AM to 2:00 PM. All procedures were approved and carried out as per the guidelines of Committee for the purpose of Control and Supervision of Experimental Animals (CPCSEA). Prior approval from Institutional Animal Ethics Committee (IAEC) was obtained for conduction of experiments (IAEC/2013-15/02).

Phytochemical screening: Phytochemical screening of ethanolic extract of *Macrotyloma uniflorum* was done by using various methods listed in literature[6].

TLC methodology: Ethanol extract of *Macrotyloma uniflorum* sample was applied on merck aluminium plate pre-coated with silica gell $60F_{254}$ of 0.2 mm thickness. The plates were developed in toluene:ethyl acetate 5:1, ethyl acetate: methanol 9:1, n-butanol:acetic acid:water 67:23:10, solvent system respectively. The plates were dried and visualized under radiations 254 and 366 nm, then, plates were dipped in vanillin sulphuric acid and heated in oven at 105°C till colour spots appeared[7,20].

HPTLC methodology: *Macrotyloma uniflorum* ethanolic extract (0.3 g) was dissolved in alcohol filtered and made upto 10 mL in standard flask and the solution was applied on "Merck" aluminium plate coated with silica gel $60F_{254}$ using linomat IV applicator. The plate was developed in butanol: acetic acid:water 67:23:10, ethyl acetate:methanol 9:1, v/v. The

plate was scanned at 254 nm using deuterium lamp in "Camag" HPTLC instrument provided with cats 4.05 version software. From the standard stock solution, 2.5-12.5 µL solutions were applied on coated plate of Silica Gel G, to produce the range of 1.25-6.25 µg of RT-F2 per spot, respectively[21].

Pharmacological studies

Forced swim test (FST) and tail suspension model: In both the models, all the rats of either sex were randomly divided in 4 groups of 6 animals each. The drug administration in each model was done as follow:

Group I : Control group received normal saline (0.9% sodium chloride 5 mL kg^{-1}/p.o.)
Group II : Positive control group received standard drug, imipramine (10 mg kg^{-1}/p.o.)
Group III : Test drug (200 mg kg^{-1}/p.o.) EEMU treated group
Group IV : Test drug (400 mg kg^{-1}/p.o.) EEMU treated group

The behavioural parameters were estimated 1 h after the administration in all four groups for both the models.

Potentiation of nor-epinephrine toxicity: In this model, rats were divided into 4 groups containing 6 rats in each group:

Group I : Received normal saline (0.9% sodium chloride 5 mL kg^{-1}/p.o.)
Group II : Received nor-epinephrine (4 mg kg^{-1}/i.p.)
Group III : Received standard drug, imipramine (10 mg kg^{-1}/p.o.) twice a day, 30 min before administration of nor-epinephrine
Group IV : Received EEMU (400 mg kg^{-1}/p.o.) twice daily 30 min before nor-epinephrine (NE) administration

Evaluation

Behavioural tests

Force swim test: The animals were acclimatized for 7 days before the study. The method described in literature was used in this study[9]. Each animal was placed individually in vertical Plexiglass cylinder filled with water up to 15 cm and was observed for duration of 6 min. The duration of immobility was recorded during the last 4 min of the observation period[1]. The animal was considered immobile when it floated motionlessly or made only those moments necessary to keep its head above the water surface. The water was changed after each test[9,22].

Tail suspension test (TST): The animal was hanged by the tail on a rod 75 cm above the surface with the help of an adhesive tape and was observed for duration of 8 min. The duration of immobility was recorded during the last 6 min of the observation period. Animal was considered immobile only when it was completely motionless[10,23].

Potentiating nor-epinephrine toxicity: This model described that antidepressants blocks the reuptake of biogenic amines into nervous tissue. By this way, the toxic effects of NE were potentiated. The test drug was given orally and standard drug was given by i.p. route 30 min prior to the i.p injection of the sub lethal dose of 4 mg kg^{-1} nor-epinephrine. Rats were placed in plastic cages with free access to food and water. The mortality was assessed 48 h post-dosing ED_{50} or dose which cause death of 50% of the treated subjects[11].

Statistical analysis: The data was statistically analyzed by using graph pad prism version-6.0 software. The p-value of <0.01 was taken as significant. Results were expressed as Mean±SEM followed by one way ANOVA and Dunnett's multiple comparison tests[12,24].

RESULTS

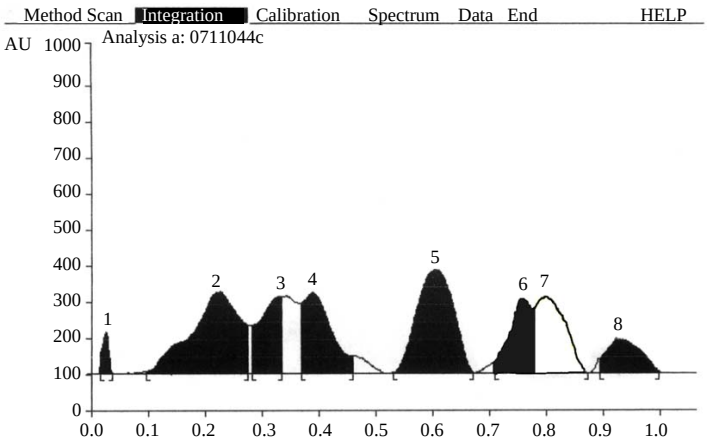
Phytochemical screening of EEMU: Specific standard reagents were used for screening various extracts of *Macrotyloma uniflorum* for different classes of phyto-constituents and are shown in Table 1. The percentage yield of ethanolic extract was 4.25 g/100 g of seeds.

TLC finger printing: The plate was scanned at 254 nm and then the spots were visualized by spraying with vanillin/sulphuric acid reagent (heated at 105°C for 5 min). All the results were confirmed using n- butanol: acetic acid: water (6.7:2.3:1.0) as solvent system and reported R_f values (0.05,

Table 1: Qualitative chemical evaluations of EEMU extract

Test constituents	Results
Flavonoids	+
Phenols	+
Tannins	+
Carbohydrates	+
Saponins	+
Sterols	+
Alkaloids	+
Coumarins	+
Triterpenoids	-
Glycosides	-

+: Present and -: Absent



Peak	start		max			end		area	
#	Rf	H	Rf	H	[%]	Rf	H	F	[%]
1	0.02	56.7	0.03	113.8	8.44	0.04	1.8	1067.8	1.56
2	0.10	5.6	0.23	222.1	16.47	0.28	131.4	16393.4	24.02
3	0.28	131.7	0.33	209.5	15.54	0.34	208.6	6895.0	10.10
4	0.37	191.4	0.39	219.5	16.28	0.46	47.8	9505.3	13.93
5	0.53	1.9	0.61	282.0	20.91	0.67	2.9	16019.5	23.47
6	0.71	27.0	0.80	207.6	15.39	0.88	0.4	13507.1	19.79
7	0.90	39.1	0.93	94.1	6.97	1.00	7.0	4869.8	7.13
		Total height =		1348.7			Total area =	68257.9	

Fig. 1: HPTLC chromatogram of ethanolic extract of MU seeds. (1) Daidzein, (2) Genistein

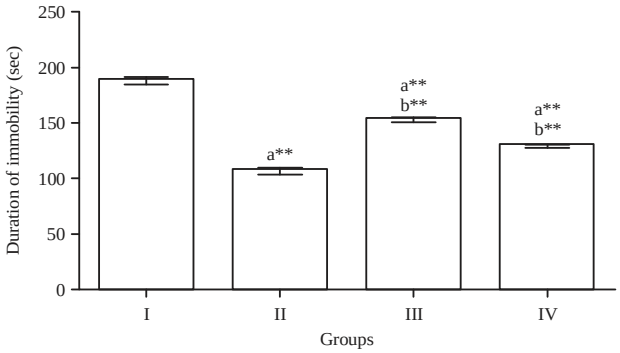


Fig. 2: Effect of EEMU on FST induced duration of immobility in rats. Data are Mean±SEM values, n = 6. Group I: Control group received normal saline (0.9% sodium chloride 5 mL kg^{-1}/p.o.), group II: Positive control group received standard drug, imipramine (10 mg kg^{-1}/p.o.), group III: Test drug (200 mg kg^{-1}/p.o.) EEMU treated group and group IV: Test drug (400 mg kg^{-1}/p.o.) EEMU treated group

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

0.23, 0.74, 0.95) were matched with presence of isoflavones (daidzein, 0.95) and further used for identification using HPTLC.

HPTLC finger printing: The HPTLC chromatogram scanned at 254 nm showed seven spots and are marked in Fig. 1 and confirmed by previous literature published by Wojciak-Kosior *et al.*[15] and the different standard compounds chromatogram (Fig. 2 and 3). Major constituents that were found are daidzein and genistein (main components of isoflavones).

Acute toxicity: The EECA did not produce any toxic symptoms or mortality upto dose level of 2000 mg kg^{-1} b.wt., orally in rats and hence the drug was considered safe for further pharmacological screening. The body weight of rats before and after administration was noted. No changes in skin, fur, eyes, mucus membrane, respiratory, circulatory, autonomic central nervous system, motor activity and behaviour pattern were observed. Animal were kept under observation for any signs of tremor, convulsion, salivation and diarrhoea. Throughout, study there was no toxicity/death was observed at these levels.

Effect of EEMU on forced swim test induced duration of immobility in rats: There was significant (p<0.01) decrease in duration of immobility in animals of group II as compared to group I. There was a significant (p<0.01) dose dependent decrease in duration of immobility in animals treated with test

Table 2: Effect of EEMU on nor-epinephrine toxicity in rats

Groups	Treatment	Number of mortalities	Lethality (%)
I (Control)	Normal saline (10 mL kg^{-1}/p.o.)	0	0.0
II (NE treated)	NE (4.0 mg kg^{-1}/i.p.)	2	40.0
III (Standard+NE)	Imipramine (10 mg kg^{-1}/i.p.)+NE (4.0 mg kg^{-1}/i.p.)	5	100.0
IV (Test+NE)	EEMU (400 mg kg^{-1}/p.o.)+NE (4.0 mg kg^{-1}/i.p.)	2	40.0

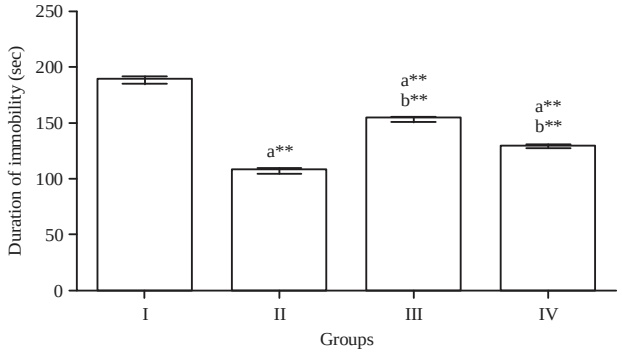


Fig. 3: Effect of EEMU on TST induced duration of immobility in rats. Data are Mean±SEM values, n = 6. Group I: Control group received normal saline (0.9% sodium chloride 5 mL kg^{-1}/p.o.), group II: Positive control group received standard drug, imipramine (10 mg kg^{-1}/p.o.), group III: Test drug (200 mg kg^{-1}/p.o.) EEMU treated group and group IV: Test drug (400 mg kg^{-1}/p.o.) EEMU treated group

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

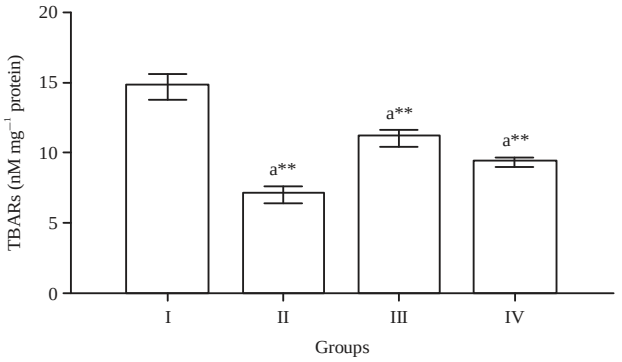


Fig. 4: Effect of EEMU on the TBARs level in rat brain. Data are Mean±SEM values, n = 6. Group I: Control group received normal saline (0.9% sodium chloride 5 mL kg^{-1}/p.o.), group II: Positive control group received standard drug, imipramine (10 mg kg^{-1}/p.o.), group III: Test drug (200 mg kg^{-1}/p.o.) EEMU treated group and group IV: Test drug (400 mg kg^{-1}/p.o.) EEMU treated group

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

drug EEMU in group III and group IV. Group IV showed a significant (p<0.01) effect when compared to group I and II (Fig. 2).

Effect of EEMU on induced duration of immobility in rats: Group II showed significant (p<0.01) decrease in duration of immobility as compared to group I. There was a significant (p<0.01) dose dependent decrease in duration of immobility in animals treated with test drug EEMU in group III and group IV. Group IV showed a significant (p<0.01) potent effect when compared to group II (Fig. 3).

Effect of EEMU on nor-epinephrine potentiation toxicity in rats: Animals of group I showed zero mortality rates. Group II animals showed mortality rate of 2, group III showed significant mortality rate. Group IV showed mortality of 2. Imipramine showed marked NE toxicity in rats, there was no such effect seen with EEMU administration (Table 2).

Biochemical estimations

Effect of EEMU on TBARs level in rat brain: The TBARS level in standard treated group II was found to be decreased when compared with controlled rats (14.67±0.88 nM mg^{-1}) protein which was significantly (p<0.01) decreased in group III and group IV in a dose dependent manner when compared to group I (Fig. 4).

Effect of EEMU on GSH levels in rat brain: The GSH level in group I animals was found to be (8.2±0.66 μM mg^{-1}) of protein. Group II showed significant (p<0.01) increase in the levels of GSH in brain tissues when compared to group I. EEMU treated rats, group IV showed more significant (p<0.01) increase in the levels of GSH in comparison to group III and found significant when compared with control group (Fig. 5).

Effect of EEMU on nitrites level in rat brain: The level of nitrite in group I was found to be (22.016±0.728 μM mg^{-1}) of protein. Standard treated rats, group II showed significant (p<0.001) low nitrite levels in the brain when compared to group I. EEMU treated rats also showed significant (p<0.01) decrease nitrite levels in the brain with respective dose (group-III-17.534±0.3910 and group-IV-14.29±0.4247 μM mg^{-1}) of protein when compared with group I (Fig. 6).

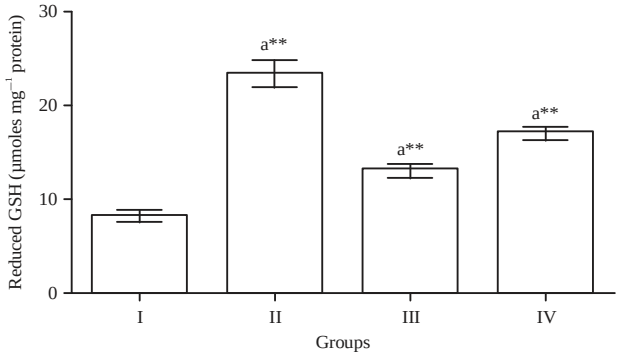


Fig. 5: Effect of EEMU on the GSH level in rat brain. Data are Mean±SEM values, n = 6. Group I: Control group received normal saline (0.9% sodium chloride 5 mL kg^{-1}/p.o.), group II: Positive control group received standard drug, imipramine (10 mg kg^{-1}/p.o.), group III: Test drug (200 mg kg^{-1}/p.o.) EEMU treated group and group IV: Test drug (400 mg kg^{-1}/p.o.) EEMU treated group

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

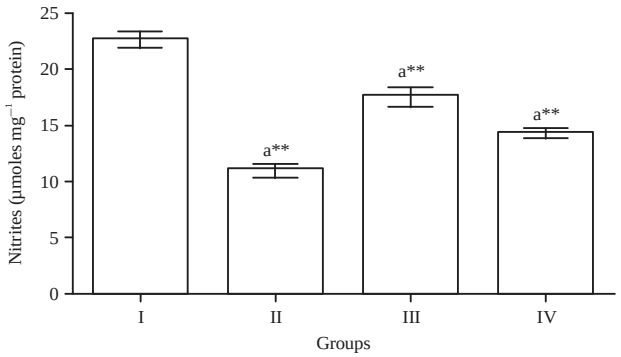


Fig. 6: Effect of EEMU on nitrite level in rat brain. Data are Mean±SEM values, n = 6. Group I: Control group received normal saline (0.9% sodium chloride 5 mL kg^{-1}/p.o.), group II: Positive control group received standard drug, imipramine (10 mg kg^{-1}/p.o.), group III: Test drug (200 mg kg^{-1}/p.o.) EEMU treated group and group IV: Test drug (400 mg kg^{-1}/p.o.) EEMU treated group

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

DISCUSSION

In this study, it has found that EEMU extract significantly decreased (p<0.01) the immobility and increased swimming times compared to the saline group. Forced swimming test is the well-known model for evaluation of antidepressant activity in rodents. Where, animal forced to swim in a restricted area it initially shows vigorous activity followed by immobile posture and its movement are only restricted and those movements that keep its head above water. Immobile behaviour is considered to be proportional to lower mood and depression. Present study also reported the change in immobility[22] and decrease in immobility by EEMU extract can be considered because of its antidepressant effects. Also, tail suspension test and potentiation of nor-epinephrine toxicity are excellent preclinical animal models that correctly emulate the important clinical features of depression.

As per previous hypothesis of depression, monoamines, serotonin and nor-adrenaline play important role in development of depression and impairment of monoaminergic neurotransmission and decreased level of serotonin and nor-adrenaline are most common causes of depression. The treatment therapy involves the classical antidepressant drugs such as imipramine which act by increasing synaptic level of monoamines and show anti-depressant effect[23]. These synthetic drugs involve the long term complications and number of side effects which includes drowsiness, fatigue, cardiac arrhythmias gastro intestinal dysfunction etc. Thus, it is very important to address the problem and find effective alternate remedies. Herbal plants play very important role for the treatment of various diseases and disorders including depression. Various animal models have been developed in both rodents and non-human primates that have been used successfully in the evaluation for their anti depressive activities. The development of new antidepressant drugs from medicinal plant is an attractive preposition because diverse chemical compounds have been isolated from medicinal plants with antidepressant activity which have been shown to produce promising results in the treatment of depression some of which are *Hypericum perforatum* Linn*, Areca catechu, Bacopa monnieri*[24] and present study also support the presence of various chemical compounds in MU which is responsible for antidepressant effect, which were earlier reported as antidepressants[25].

The forced swim test showed a strong sensitivity to monoamine alterations and is sensitive to monoaminergic manipulations[16]. The result of current study indicates that the EEMU reduced the immobility and involve in monoaminergic manipulations in rats.

Another model used was potentiation of norepinephrine toxicity. This model reveals an adrenergic component of pharmacological activity of antidepressants. Several

antidepressants block not only uptake noradrenaline, but also of dopamine and of serotonin[17,25-26]. In the present study, imipramine potentiated markedly NE toxicity in mice but EEMU did not potentiate markedly NE toxicity. The oxidative stress was measured through determination of levels of TBARs (or MDA), reduced glutathione and nitrate/nitrite contents. Also generation of oxidative stress is indicated by decrease in levels of endogenous antioxidant marker (GSH), increase in the extent of lipid peroxidation (TBARs), increase in nitrate/nitrite content in brain was found to be the cause of depression[26] and the data reported in this study also showed the significant antioxidant effect of MU and act as symptomatic relief with progression of depression.

It was also observed in the study that with increase in doses of EEMU, leads to more significant effect ($p<0.01$) on immobility and improved anti-depressive action. Further studies can be done to understand this dose dependent relation.

CONCLUSION AND FUTURE RECOMMENDATIONS

The present study proves the presence of isoflavones in ethanolic extract of *Macrotyloma uniflorum* and its therapeutic role as antidepressive agent. It also brings forward the dose dependent relation when it comes to its pharmacological effects. Apart from its preclinical uses as antidepressant activity further activities need to be done on human to test its efficacy with fewer side effects.

The study will help researcher to explore the beneficial effects of MU in neurological disorder and it will also help society to use its constituents or raw seed as nutraceuticals or protective effects against various stress or neurodegenerative disease. This study is applicable to prepare the commercial extract of MU and better alternative to adjuant therapy in depression.

SIGNIFICANCE STATEMENT

The present study addresses the role of *Macrotyloma uniflorum* seeds extract in a search of newer drugs in the treatment of depression. The drugs available in the market have numerous side effects associated with their long-term use. This study confirms the presence of active constituents like isoflavone, daidzein, genisteins in *Macrotyloma uniflorum* seeds extract and found as better cure in plant related drugs to treat depression. This study will help researchers to identify the active constituents, with significant interaction to serotonin receptors and help in various therapy related to depression.

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