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Dr. Vanita Kanase
Department of Pharmacology, Oriental College of Pharmacy, Sanpada, Navi, Mumbai, Maharashtra, India

Aman Gupta
Department of Pharmacology, Oriental College of Pharmacy, Sanpada, Navi, Mumbai, Maharashtra, India

Anjali Gupta
Department of Pharmacology, Oriental College of Pharmacy, Sanpada, Navi, Mumbai, Maharashtra, India

Pratik Mishra
Department of Pharmacology, Oriental College of Pharmacy, Sanpada, Navi, Mumbai, Maharashtra, India

Corresponding Author:
Dr. Vanita Kanase
Department of Pharmacology, Oriental College of Pharmacy, Sanpada, Navi, Mumbai, Maharashtra, India

Antidepressant activity of ethanolic extract of *Trapa natans L.* fruits in Swiss albino mice (Pharmacological research)

Dr. Vanita Kanase, Aman Gupta, Anjali Gupta and Pratik Mishra

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Abstract
Trapa natans L. commonly known as Water Chestnut, is a member of the Trapaceae family and has gained recognition as a nutritional powerhouse and a potential source of bioactive compounds with diverse psychopharmacological properties. Nevertheless, the available scientific information about this species is scarce and there are no reports related to its possible effect on the CNS. In this work, the effects of ethanolic extract of fruits of *Trapa natans L.* (EETNL) were evaluated in Swiss Albino Mice using behavioral tests sensitive to clinically effective antidepressant compounds. The ethanolic extract (200 and 400mg/kg), administered orally was able to decrease the immobility time of mice dose-dependently when subjected to both tail suspension and forced swim tests and the effects are compared to that of standard drug i.e., imipramine (15mg/kg) and control group. In addition to behavioural tests, EETNL also normalized oxidative stress markers such as Catalase, SOD, MDA, and LPO in a dose dependant manner. It was observed that the plant extracts possess Antidepressant activity at higher dose levels. It is suggested that more studies in this regard should be pursued to obtain more knowledge about the role of *Trapa natans L.* in depression.

Keywords: Okra, genetic combining ability, specific combining ability, variance, growth, yield and quality

Introduction
According to the World Health Organization (WHO) report, globally approximately 450 million people suffer from a mental or behavioural disorder. This contributes to 12.3% of the global burden of disease, and will increase to 15% by year 2026 [1]. Psychological illness is also often associated with attempting suicide and there are between 10 to 20 million suicide attempts every year [2]. Depression is the most prevalent mental disorder and depression is recognized to be symptomatically, emotionally, intellectually, psychologically and biologically heterogeneous [3]. The psychiatric depression disorder is characterized by, hallucination, insomnia, apathy, loss of energy, retardation of thinking and activity, as well as profound feelings of gloominess, loss of appetite, despair and suicidal ideation. In spite of the availability of various antidepressant drugs like tricyclic antidepressants (TCA), selective reversible inhibitors of monoamine oxidase-A (MAO-A), selective serotonin reuptake inhibitors (SSRIs) and selective noradrenaline reuptake inhibitors (SNRIs), depression Continues to be a serious and major medical health problem [4]. Basic neurological science offers the promise of improving our understanding of disease pathophysiology, balancing of neurotransmitters in brain and identifying novel mechanisms of action that can be targeted by more effective pharmacotherapies and screening of herbal sources of drugs. To combat the noxious stressful situation of life and stress response of the body, we need efficient anti-depressant agent. Since the herbal medicines are potential and efficient source for treatment of diseases, its exploration as anti-depressant agent is prerequisite to overcome depression [5].

Literature Survey
According to Literature survey, *Trapa natans L* is commonly known as Water Chestnut which belongs to family Trapaceae. Water chestnut is a plant with promising pharmacological action, owing to the presence of its constituents, phenols and flavonoids, which have been shown to have hepatoprotective, antioxidant, anti-inflammatory, anti-diabetic, antifungal, and antimicrobial activity. The fruits of Water Chestnut plant contains phenolic compounds such as gallic acid, Caffeic acid, ellagic acid, ferulic acid and flavonoids such as Pinobanksin, quercetin, naringenin, etc all this have antidepressant effect.

Antioxidant potential are the best supplements for the diseases associated with oxidative stress. The fruits of *Trapa natans L.* contains high amount of quercetin. Quercetin is a flavonoid which is responsible to show antidepressant activity. Hence, an attempt has been made to investigate antidepressant activity of fruit extract of *Trapa natans L* [6,7].

Fig 1: Fruit of Water Chestnut plant

Fig 2: Whole plant of water chestnut

Materials and Methods

Plant Material

The fruits of *Trapa natans L.* were purchased from Agricultural Produce Market Committee (APMC), Vashi, Navi Mumbai in October 2023. Sample specimen were send for authentication to Mahesh R. Atale, Botanist at Alarsin Ayurvedic Research Centre, Andheri, Mumbai. The fruits were washed with tap water and shade dried at normal room temperature with the aid of circulating air flow using fan. After drying they were powdered coarsely and extraction was done using soxhlet extraction. The resultant EETNL was kept in a refrigerator for a further use.

Preparation of Extract

The dried plant fruit material was crushed into fine particles (powder) using an electronic mixer. The powdered plant material (500 g) was packed in a soxhlet apparatus and subjected to continuous hot percolation for 8-9 hour using ethanol (1:4) as solvent. Extract obtained was passed through the Whatman filter paper No.1 and the ethanol was evaporated (at 400 °C) with the help of heating mantle and dried in a desiccator.

Animals

Male Swiss Albino Mice (20-35 g) were used for the study. The animals were obtained from Jay Agro, Posari, District-Raigad 410201. All the mice were kept in standard polypropylene mice cages with stainless steel coverlids and wheat husk was used as bedding material. The animals were maintained under standard environmental conditions (25 + 2° C and relative humidity of 45 to 55%) and were fed with standard pellet diet and water ad libitum. The use of these animals and the study protocols were approved by CPCSEA recognized Institutional Animal Ethics Committee (IAEC) of Oriental College of Pharmacy, Sanpada, Navi Mumbai-400705 under Protocol No. OCP/IAEC/2023-24/07 titled "Evaluation of antidepressant activity of ethanolic extract *Trapa natans L.* fruit in Swiss Albino Mice" of the thesis entitled "Evaluation of Pharmacological Activity of Selected Medicinal Plant". CPCSEA guidelines were adhered during the maintenance and experiment. All experimental work were carried out between 10:00- 17:00 hours.

Drugs and chemicals

Hydrogen peroxide, Ethanol, Saline, n-butanol, epinephrine, Thiobarbituric acid, etc and imipramine hydrochloride (Sigma-Aldrich, St. Louis, USA) were used reference standards for antidepressant activity.

Screening of antidepressant activity

Thirty mice were randomly divided into five experimental groups. Group-I (Normal control) mice received normal saline (1.0 mL/kg, P.O.) daily for 14 days; Group-II (stress control) mice received normal saline (1.0 mL/kg, P.O.) daily for 14 days and subjected to restraint stress on 13th day. Group-III (standard drug-treated) rats received Imipramine (15 mg/kg, I.P.) daily for 14 days and subjected to restraint stress on 13th day. Group-IV and V mice were treated with EETNL (200 mg/kg and 400 mg/kg, P.O.) daily for 14 days and subjected to ARS on 15th day.

Stress-like behavior was assessed by subjecting the mice to behavioural paradigms such as tail-suspension test (TST), 40 min post restraint stress procedure. Pretest of 10 minute for forced swim test was also given to each mice simultaneously. Then 23.5 hours later, the relevant samples were administered and main test of forced swim test performed 30 minutes later.

Oxidative stress parameters such as Superoxide Dismutase (SOD), Catalse test (CAT), Malondialdehyde formation (MDA), and extent of Lipid peroxidase (LPO) were also analyzed in restraint stress-induced animals and control group, following forced swim test on 15th day [8].

Behavioral Tests

Tail suspension test (TST)

Tail suspension test (TST) commonly employed behavioral model for screening of antidepressant-like activity in mice, was first given by Steru, *et al*. Animals were suspended individually by end of tail with Micro pore adhesive tape (approximately 1cm) with the head 50 cm from the bottom in a suspension box 40 min post restraint stress procedure. Mice were suspended for a total of 6 min. During the final 4 min interval of the test, duration of immobility was recorded. Mice were considered immobile only when they will be hung

passively and completely motionless. Each animal under test was both acoustically and visually isolated from other animals during the test. The total period of immobility was recorded manually for 6 min. Animal was considered to be immobile when it didn't show any body movement, hung passively and completely motionless. The TST test was conducted in a dim lighted room and each mice were used only once in the test [9].

Forced Swim Test (FST)

Forced swim test, the most frequently used behavioral model for screening of antidepressant-like activity in rodents, was first proposed by Porsolt, *et al*. The FST procedure is same as followed previously. Mice were forced to swim in a cylinder (diameter 40 cm, height 60 cm) containing 30 cm of fresh water maintained at 25 °C±1 °C. Water in the cylinder was changed after each animal to prevent the behavioral alteration among animals due to used water. Each animal showed vigorous movement during initial 2 min period of the test. Duration of immobility will be manually recorded during the next 4 min of total 6 min testing period by the observer. Mice were considered to be immobile when they floated in an upright position, making only small movements to keep their head above the water level. Mice were considered to be immobile when they ceased struggling and remained floating motionless in water, making only those movements necessary to keep their head above water. Following swimming session, mice were towel dried and returned to their housing conditions [10].

Biochemical estimation tests

All the animals were sacrificed by euthanasia, after behavioral observations. The brains were quickly removed, washed in ice-cold sterile isotonic saline, and weighed. A 10% (w/v) tissue homogenates were prepared with phosphate buffer solution (PBS), (pH 7.4). The supernatant was obtained by centrifugation of the homogenate at 1000 rpm for 20 min at 5°C and used for further biochemical estimation [11, 12].

Catalase activity (CAT)

Procedure: Supernatant (50 µl) was added to cuvette containing 2.95 ml of 19 mM/L solution of H2O2 prepared in potassium phosphate buffer. The change in absorbance was monitored at 240 nm wavelength at 1-minute interval for 3 minutes. Presence of catalase decomposes H2O2 leading to a decrease in absorbance.

Superoxide Dismutase activity (SOD)

Procedure: The SOD activity in supernatant was measured by the method of Misra and Fridovich. The supernatant (500 µl) was added to 0.800ml of carbonate buffer (100mM, pH 10.2) and 100 µl of epinephrine (3mM). The change in absorbance of each sample was then recorded at 480 nm in spectrophotometer for 2 min at an interval of 15 sec. Parallel blank and standard were run for determination SOD activity. The reaction mixtures are diluted 1/10 just before taking the readings in spectrophotometer.

Determination of malondialdehyde (MDA) formation

Procedure

1 ml of suspension medium was taken from the 10% tissue homogenate. 0.5 ml of 30% TCA will be added to it, followed by 0.5 ml of 0.8% TBA reagent. The tubes were then be covered with aluminium foil and kept in shaking water bath for 30 minutes at 80 degree celsius. After 30 minutes tubes were taken out and kept in ice-cold water for 30 minutes. These were then be centrifuged at 3000 rpm for 15 minutes. The absorbance of the supernatant was read at 540 nm at room temperature against appropriate blank. Blank consist of 1 ml distilled water, 0.5 ml of 30% TCA and and 0.5 ml of 0.8% TBA.

Lipid Peroxidation Assay (LPO)

Procedure

To 0.2 ml of test sample, 0.2 ml of SDS, 1.5 ml of acetic acid and 1.5 ml of TBA were added. The mixture was made up to 4 ml with water and then heated in a water bath at 95°C for 60 minutes. After cooling, 1 ml of water and 5 ml of n-butanol/pyridine mixture were added and shaken vigourously. After centrifugation at 4000 rpm for 10 minutes, the organic layer was taken and its absorbance was read at 532 nm. The level of lipid peroxides was expressed as nmoles of MDA released/ g wet tissue.

Statistical Analysis

The data were analysed with InStat Software by GraphPad (version 3.10). The results are expressed as the mean±SEM for each group. Statistical differences were evaluated using a One-way analysis of variance (ANOVA) followed by Dunnett's t-test. Results were considered to be statistically significant at $p \leq 0.05$.

* indicates $p \leq 0.5$ as significant;
** indicates $p \leq 0.01$ as highly significant;
*** indicated $p \leq 0.001$ as very significant

Results

Tail Suspension Test (TST)

Both the doses of the Ethanolic extract of fruits of *Trapa natans L.* showed dose dependent decrease in immobility time when it was compared against stress control as well as against imipramine which was used as a standard.

Table 1: Effect of ethanolic extract of *Trapa natans L.* Fruits on mobility and immobility time of tail suspension test in swiss albino mice

1.	Normal control	112.17±12.59	127.83±12.59
2.	ARS	145.17±12.45	94.833±12.45
3.	Imipramine (15mg/kg)+ARS	82.33±5.73	157.67±5.73
4.	EETNL (200mg/kg)+ARS	87.83±7.37	152.17±7.37
5.	EETNL (400mg/kg)+ARS	71±6.89	169±6.89

Table 1: Effect of ethanolic extract of *Trapa natans L.* Fruits on mobility and immobility time of Tail suspension test in Swiss Albino Mice. Values are the mean ± SEM of N=6 mice/treatment. Significance $^{**}p \leq 0.01$.

Forced swim test

Both the test groups of the Ethanolic extract of fruits of *Trapa natans L.* showed dose dependent decrease in immobility time when compared against stress control as well as against imipramine which was used as a standard.

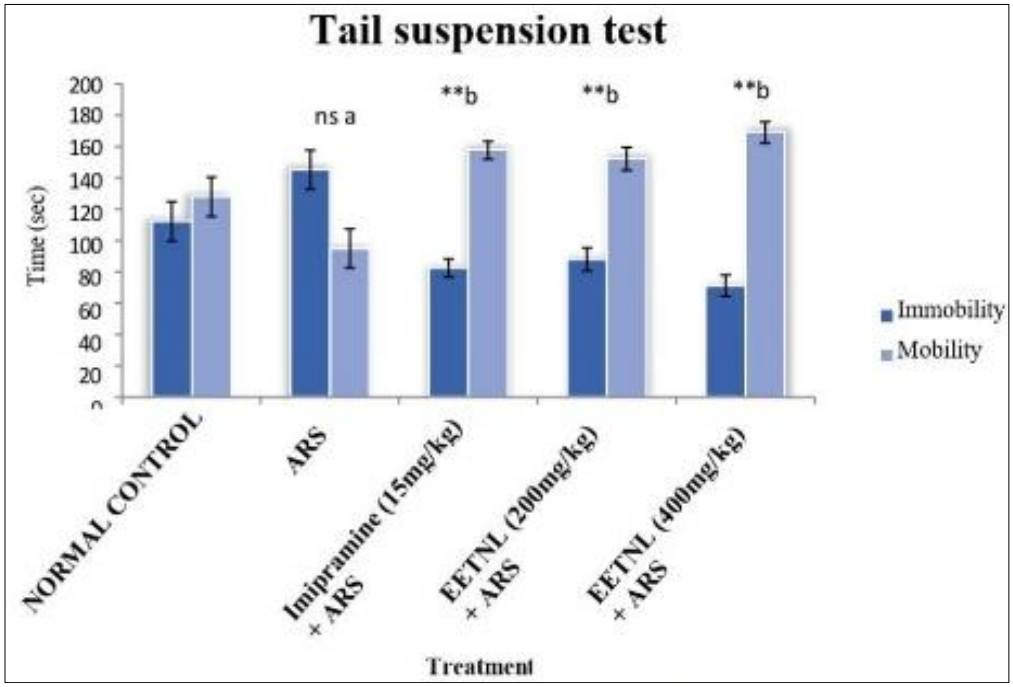

Fig 3: Effect of EETNL pretreatment on ARS induced changes in duration of immobility in Tail suspension test. NC: Normal control; ARS: Acute restraint stress; EETNL: Ethanolic extract of *Trapa natans L.* Values are expressed as mean±standard error of mean (N=6). ***p<0.001, **p<0.01, *p<0.05. a versus NC group and b versus ARS

Table 2: Show treatment, immobility and mobility

Sr. No.	Treatment	Immobility	Mobility
1.	Normal control	42.5±2.63	197.5±2.63
2.	ARS	72.83±2.62	167.16±2.62
3.	Imipramine (15mg/kg)+ARS	52.16±4.24	187.83±4.24
4.	EETNL (200mg/kg)+ARS	49.66±3.21	190.33±3.21
5.	EETNL (400mg/kg)+ARS	30.5±2.69	209.5±2.69

Figure 2: Effect of ethanolic extract of *Trapa natans L.* Fruits on mobility and immobility time of Forced swim test in Swiss Albino Mice. Values are the Mean ± SEM of N=6 mice/treatment. Significance $^{**}p \leq 0.01$

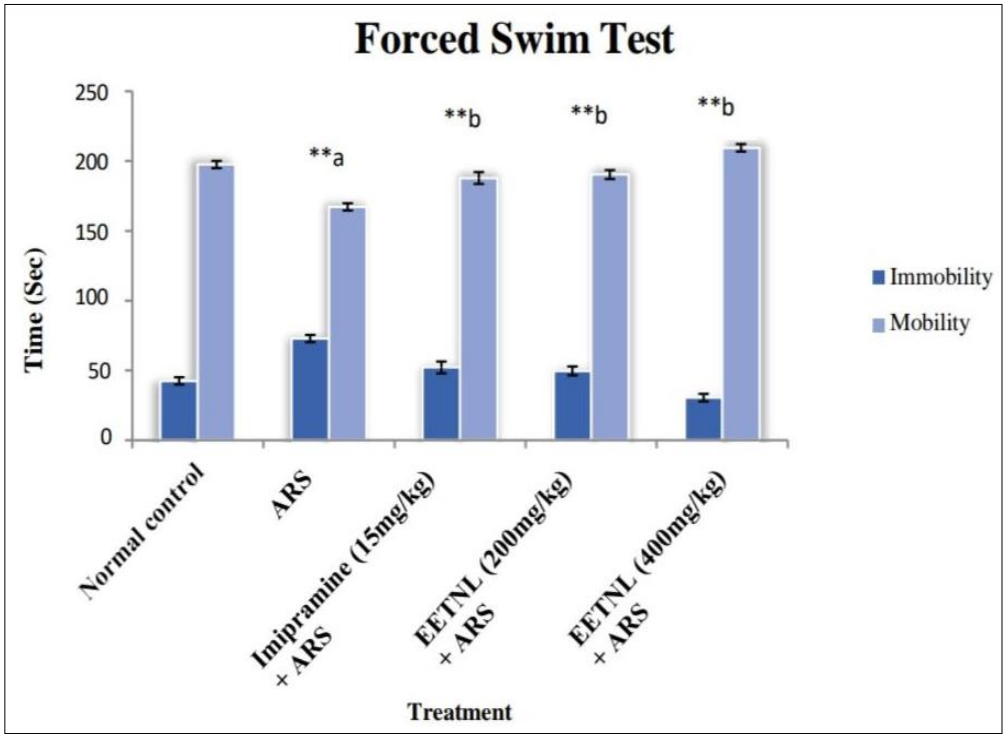

Fig 4: Effect of EETNL pretreatment on ARS induced changes in duration of immobility in Forced swim test. NC: Normal control; ARS: Acute restraint stress; EETNL: Ethanolic extract of *Trapa natans L.* Values are expressed as mean±standard error of mean (N = 6). ***P<0.001, **P<0.01, *P<0.05. a versus NC group and b versus ARS group

Biochemical test

Table 3: Effect of oxidative stress markers in brain homogenate of Swiss Albino Mice. Values are the Mean ± SEM of N=6 mice/treatment. Significance **$p \leq 0.01$ CAT-Catalase, SOD-Superoxide Dismutase, MDA-Malonaldehyde, LPO-Lipid peroxidase.

SR. No	Treatment	CAT	SOD	MDA	LPO
1.	Normal control	16.8±1.082	1.1107±0.1956	0.2093±0.0308	0.1082±0.0037
2.	ARS	8.41±1.502	0.5662±0.07900	0.4423±0.0584	0.3543±0.0508
3.	Imipramine (15mg/kg)+ARS	15.76±1.659	1.0577±0.07230	0.1944±0.0481	0.1326±0.0139
4.	EETNL (200mg/kg)+ARS	11.84±1.530	1.0396±0.09070	0.2627±0.0462	0.1446±0.0246
5.	EETNL (400mg/kg)+ARS	13.67±1.152	1.1742±0.07607	0.2243±0.0464	0.1233±0.0245

Catalase activity

Evaluation of CAT activity revealed that stressed mice presented a significant decrease in CAT activity, which was significantly prevented by EETNL (200 mg/kg and 400 mg/kg) pretreatment, when compared to unstressed group as shown in table 3.

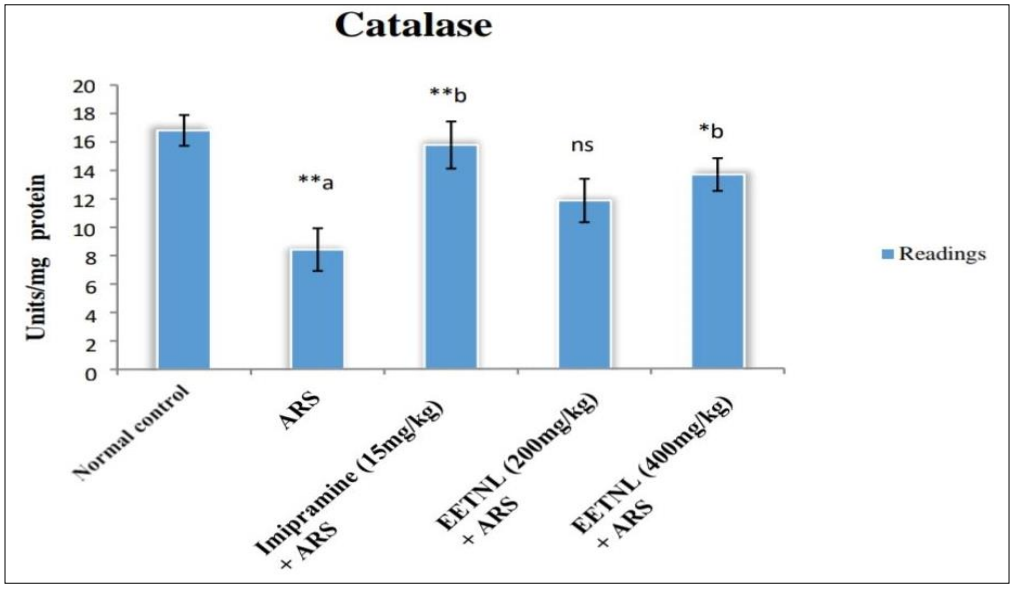

Fig 5: Effect of EETNL pretreatment on ARS induced changes on catalase activity. NC: Normal control; ARS: Acute restraint stress; EETNL: Ethanol extract of *Trapa natans L.* Values are expressed as mean ± standard error of mean (N=6). ***p<0.01, *p<0.05. a verus NC group and b versus ARS group.

Superoxide Dismutase activity

In the mice pretreated with EETNL 200 mg/kg and 400 mg/kg P.O. the level of SOD was significantly increased (p<.001) as compared to ARS mice. Table-3 shows significant and dose dependent recovery on ARS induced reduced level of SOD in animal due to EETNL.

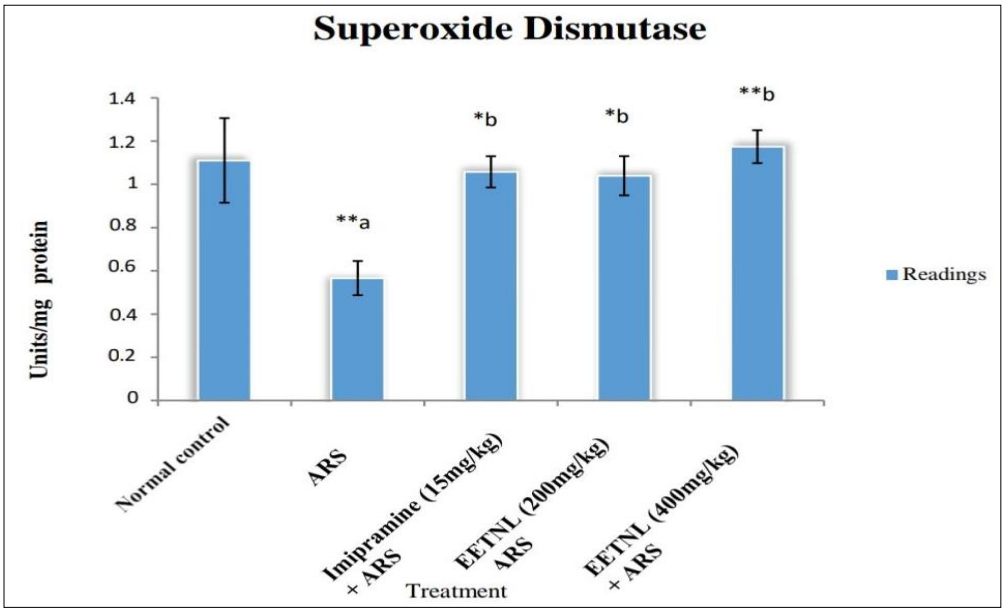

Fig 6: Effect of EETNL pretreatment on ARS induced changes on SOD activity. NC: Normal control; ARS: Acute restraint stress; EETNL: Ethanol extract of *Trapa natans L.* Values are expressed as mean ± standard error of mean (N=6). ***p<0.01, *p<0.05. a verus NC group and b versus ARS group.

Determination of Malondialdehyde (MDA) formation

The results depicted in Figure 7 illustrate that ARS significantly increased MDA level in mice brain as compared to unstressed mice. The results indicated that EETNL (200 mg/kg and 400 mg/kg) pretreatment and Imipramine significantly abolished increase in MDA level caused by ARS.

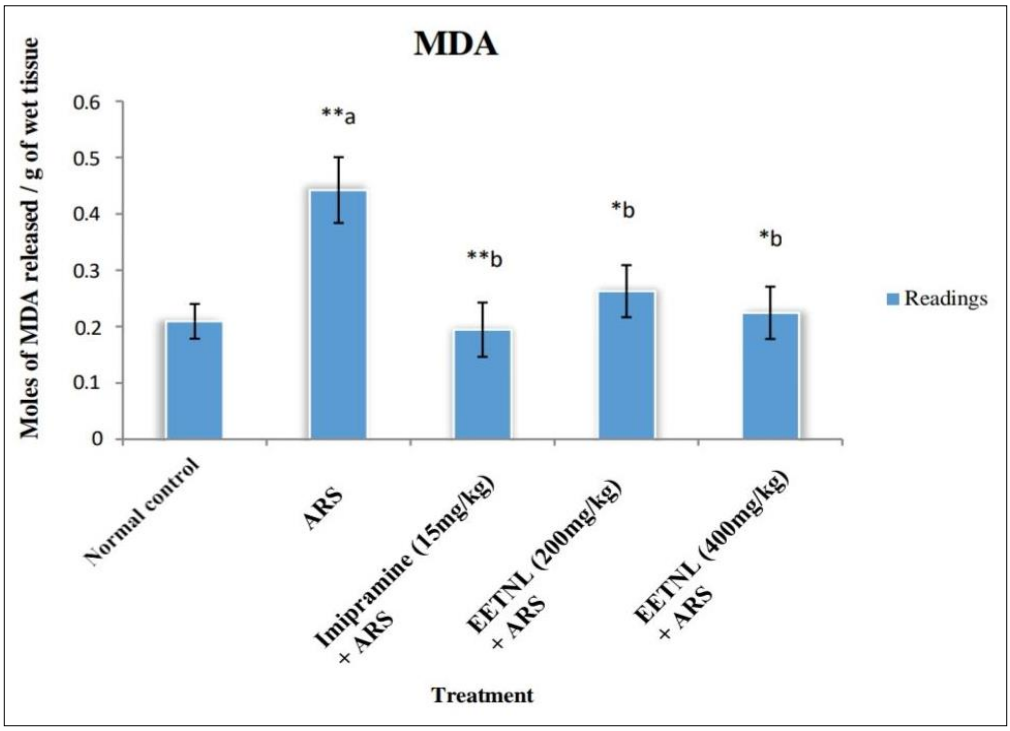

Fig 7: Effect of EETL pretreatment on ARS induced changes on MDA activity. NC: Normal control; ARS: Acute restraint stress; EETNL: Ethanol extract of *Trapa natans L.* Values are expressed as mean ± standard error of mean (N=6). ***p<0.01, *p<0.05. a verus NC group and b versus ARS group.

Lipid peroxidation assay

Quantitative measurement of LPO in the whole brain was assessed based on the amount of malondialdehyde (MDA) formed, the statistical analysis revealed that ARS produced a significant increase in MDA level whereas EETNL (200 mg/kg and 400 mg/kg) pretreatment significantly abolished increase in MDA level compared to stressed animals.

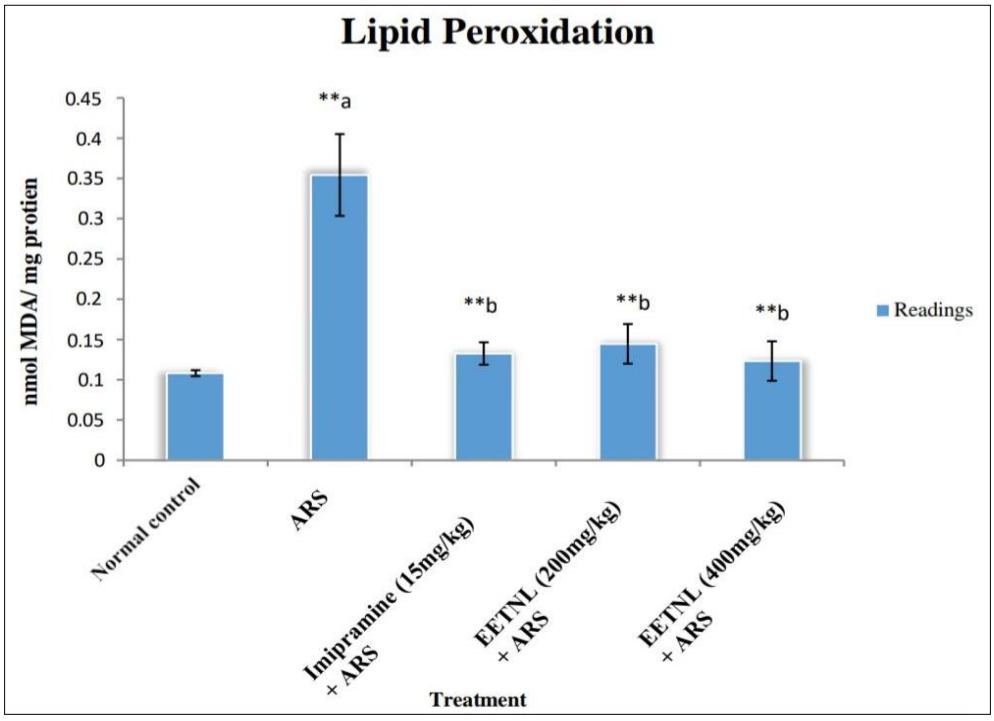

Fig 8: Effect of EETNL pretreatment on ARS induced changes on LPO activity. NC: Normal control; ARS: Acute restraint stress; EETNL: Ethanol extract of *Trapa natans L.* Values are expressed as mean ± standard error of mean (N=6). ***p<0.01, *p<0.05. a verus NC group and b versus ARS group.

Discussion

It was observed from the results that *Trapa natans L*. showed a significant dose-dependent decrease in duration of immobility time in TST and FST when compared with the control group in a dose dependent manner. In addition to behavioral tests, EETNL also normalized oxidative stress markers such as Catalase, SOD, MDA, and LPO in a dose-dependent manner.

Conclusion

It can be concluded from the study that the ethanolic extract of *Trapa natans L* fruit possess significant antidepressant property, which is probably due to flavonoids which play an active role in providing Antidepressant-like effect. Although the results of crude drug extract are promising yet more studies need to be performed on isolated flavonoids and then we can proceed for clinical research studies. *Trapa natans L*. plant can be used for the treatment of neurological disorders and may be recommended as a supplement for the antidepressant activity.

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