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ANTIDEPRESSANT EFFECT OF *ZIZIPHUS MAURITANIA* AGAINST FORCED SWIM TEST INDUCED DEPRESSION IN WISTAR RATS

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Abstract:

Chronic stress increases disease susceptibility to depression and other neurodegenerative disorders. Depression is characterized by disturbances in emotions, cognition, autonomic and endocrine functions affecting 21% of the world's population. The present investigation aimed to explore the antidepressant effect of ethanolic extract of Ziziphus Mauritania (EEZM) against forced swim test induced depression in wistar rats. Rats were administered with EEZM in the doses of 200mg/kg and 400mg/kg daily orally and subjected to forced swim test. The study is carried out for 14 days. EEZM administration reduced the immobility duration in forced swim test. Neurobehavioural alterations were assessed using elevated plus maze and open field exploration and treatment with EEZM averted behavioural despair. Increased TBARS level and decreased antioxidant enzymes such as SOD, Gpx and Catalase levels due to stress were observed. Administration with EEZM reversed all these deleterious effects and raised SOD, Gpx and Catalase levels. Thus the present observation indicates the antidepressant activity of EEZM. Further neurochemical and molecular studies would unravel the possible mechanisms involved in antidepressant activity of EEZM.

Key Words: *Chronic stress, Cognition, Antidepressant, Antioxidant, Forced swim test.*

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INTRODUCTION:

Depression is one of the most prevalent chronic psychiatric disorder afflicts 21% of the world's population.[1] This depressive disorder is characterised by disturbances in emotions, cognition, autonomic and endocrine functions. World health organization reported that by the year 2030 this depressive disorder will be the leading cause of disability.[2] Despite its prevalence, the exact pathophysiology is not known. Although antidepressant medications are in use the mechanisms underlying their therapeutic effect remain elusive.[3] However preclinical and clinical studies data reveal that chronic stress is a triggering factor causing both neurochemical and neuroanatomical changes with deleterious effects on brain functioning.[4] The neurochemical mechanisms underlying the etiology and progression of depression include neurotransmitter dysregulation, oxidative stress and inflammation.

In response to chronic stress stimuli hippocampus controlling various brain functions is disturbed[5] and hippocampal function is controlled by serotonergic system. Serotonin, dopamine and noradrenaline levels are altered in depressive patient. Previous studies claimed that serotonin levels in brain of depressive patient are reduced.[6,7,8] Further noradrenaline and dopamine will act in tandem with serotonin.[9,10] Brain is susceptible to oxidative damage.[11] Chronic stress releases reactive oxygen species or free radicals in various brain areas which are involved in mood regulation[12] leading to depression. Hypothalamic – Pituitary adrenal (HPA) axis over activation and chronic inflammation leads to neuroplasticity reduction.[13] Studies reported that the levels of pro-inflammatory cytokines such as tumour necrosis factor alpha (TNF-α), Interleukins (IL-1& IL-6) are raised in depressive patient and the levels are proportional to the severity of the disease.[14,15] It is also evident from the past studies that genetic polymorphism in TNF-α, IL-1 confer susceptibility to develop depression.[16,17] Existing pharmacotherapy for depression is associated with low remission and several side effects.[18] So, alternative strategies such as phytomedicine could be valuable for the development of antidepressive drugs.

Ziziphus Mauritania is a spiny, green shrub belonging to the family Rhamnaceae. Leaves are reported for astringent and antityphoid activity.[19] Entire plant is reported for anti-cancer,[20] antidiabetic,[21] and antioxidant activity.[22] Thus, the aim of the present study was to investigate the antidepressant effect of ethanolic extract of *Ziziphus Mauritania* against behavioural deficits and biochemical modification on chronic stress induced depression in rats.

MATERIALS AND METHODS:

Collection, identification and authentication of plants

Plant materials of *Ziziphus Mauritania* were collected in the month of January- February from the region of Nalgonda. The plant materials were identified and authenticated by pharmacognosist- Karnati. Sushma, Swami Ramananda Tirtha Institute of Pharmaceutical Sciences, Nalgonda and a voucher specimen (No: SRTIPS/COG/2348) was deposited in Department of pharmacognosy. The collected plant material were shade dried thereafter reduced to powder form.

Preparation of extract

The powdered materials (100g) were extracted in a Soxhlet apparatus with ethanol (60 – 80ºC) for 18 hours. The extracts was filtered and dried.

Drugs and Reagents

Reduced glutathione, NADPH, pyrogallol and DPPH were procured from Sigma Aldrich, USA. All other chemicals were purchased from S. D Fine chemicals Pvt LTD, India.

Preliminary Phytochemical Screening

The ethanolic extract of *Ziziphus Mauritiana* was screened for the presence of various phytoconstituents like steroids, alkaloids, saponins, tannins, terpenoids, glycosides, flavonoids, carbohydrates, proteins and phenolic compounds.[23]

Experimental design

In vitro antioxidant assays

DPPH radical scavenging activity assay

The free radical scavenging activity of ethanolic extract of *Z. Mauritiana* on 2, 2-di-phenyl-2-picrylhydrazyl (DPPH•) radical was calculated by reduction of DPPH• to DPPHH (Di-phenyl picryl hydrazine).[24,25] IC50 value was determined and Vitamin C was used as positive reference.

Reducing power assay

Reducing power assay of the extract was calculated by spectrophotometric method.[26]

Assay of nitric oxide scavenging activity

Nitric oxide scavenging activity of the extract is estimated by using Griess Ilosvoy reaction.[27] IC50, which is an inhibitory concentration of the extract required to reduce 50% of the nitric oxide formation, was measured.

***In-vivo* Pharmacological studies**

Experimental animals

Male Wistar rats, weighing 200–250 g were used in the study and fed with standard laboratory pellet diet; Provimi limited (India), provided water ad libitum and were maintained at 23–25◦C, 35 to 60% humidity, and 12 h light/dark cycle. The rats were acclimatized to the laboratory conditions for a period of 7 days prior to experiment. The experimental protocol (SRTIPS/FM/1468/PO/a/11/CPCSEA/ 119/2017) was duly approved by institutional animal ethics committee (IAEC).

Acute Toxicity Studies

Acute toxicity studies are done by using OECD 423 annexure (D) acute toxic class method (OECD Guidelines).[28] This method is a stepwise procedure with three animals of a single sex per step. The starting dose of *Ziziphus Mauritiana* was 2000 mg/kg body weight p.o. using water as vehicle. Drug was administered to overnight fasted female rats. Food was withheld for 4 hours after administration of *Ziziphus Mauritiana* and observed for signs of toxicity.

Induction of Depression

The rats were divided into five groups (n=6). The EEZM extract was dissolved in distilled water and administered orally at doses of 200 and 400 mg/kg, 60 min before conduction of the behavioural tests and the study was carried out for 14 days.

Group I: control, administer saline 2 ml/kg orally.
Group II: FST
Group III: FST+ EEZM 200 mg/kg orally
Group IV: FST+ EEZM 400 mg/kg orally
Group V: FST+ Imipramine (10 mg/kg orally)

Forced swimming test (FST)

Rats were forced to swim individually in an open cylindrical tank (diameter10 cm, height 25 cm), filled with 19 cm of water at 25 ± 1◦C. The total time that each rat remained immobile during a 6 min section was recorded as immobility time.[12] Immobility is judged when the rat ceases struggling and remains floating motionless in the water, only making necessary movements to remain its head above water. A decrease in the immobility duration is an indicator for antidepressant effect.

Open field habituation

The exploratory behaviour of the rats was monitored by open field habituation task method. Rats was placed in a 40 cm × 60 cm × 50 cm open field and the floor was divided into 12 equal squares and left to explore it freely for 5 minutes. The number of line crossings and head dips were counted.[29]

Elevated Plus Maze

This test is used to measure anxiety behaviour in rodents. After administration of the EEZM extract, each rat was placed in the centre facing the open arm. The number of open arm entries, and the time spent in the open arm was calculated for 5minutes.[30,31,32]

Biochemical estimations

Rat brains were isolated and washed with phosphate buffer to remove blood, homogenized with 10% phosphate buffer saline solution and centrifuged. The resultant supernatant was utilized for further biochemical estimations, such as antioxidants.

Thiobarbituric acid reactive substances.

Lipid peroxidation was estimated by measuring the levels of thiobarbituric acid reactive substances (TBARS) in tissues.[33] The pink colour produced by the reaction of thiobarbituric acid with malondialdehyde was estimated at 532 nm.

Catalase

The rate of decomposition of hydrogen peroxide to water and molecular oxygen is proportional to the activity of catalase.[34]

Superoxide dismutase

Superoxide dismutase (SOD) activity was estimated by pyrogallol oxidation method.[35] One unit SOD activity is the amount of enzyme that inhibits the auto-oxidation of pyrogallol by 50%. The reaction is initiated by adding pyrogallol and the change in optical density was recorded at 420 nm.

Glutathione peroxidase (GPx)

The main principle involved in GPx determination is that the oxidation of glutathione to oxidized glutathione is catalyzed by an enzyme glutathione peroxidase, which is then coupled to their cycling of oxidized glutathione back to glutathione utilizing glutathione reductase and nicotinamide adenine dinucleotide phosphate (NADPH). Then, decrease in NADPH absorbance measured at 340 nm during the oxidation of NADPH to NADP+ is indicative of glutathione peroxidase activity.[36]

RESULTS:

Acute toxicity Studies

The drug EEZM was found to be non-toxic, and the LD50 of 2000 mg/kg and above is said to be unclassified according to OECD 423. Hence, (200 mg/kg) and (400 mg/kg) of the dose were selected for further investigation.

***In vitro* antioxidant assays**

DPPH radical scavenging activity assay

The EEZM exhibited potent free radical scavenging activity in a concentration-dependent manner. The IC50 value of the extract was 19.47 g/mL as opposed to that of standard ascorbic acid (6.82 g/mL). The results were depicted in the Table 1.

Table 1: In-vitro antioxidant assays

Extract	IC50 (µg of dried extract/ml) scavenging ability on DPPH radicals	Reducing Power	IC50 (µg of dried extract/ml) scavenging ability of nitric oxide
Ascorbic acid	6.82±0.09	18±2.15	7.22±0.31
Ethanolic extract of *Ziziphus Mauritiana*	19.47±2.17[a]	552.3±20.28[a]	36.62±3.53[a]

Values are expressed as mean ± SEM. Superscript letters represents the statistical significance done by unpaired t test.

[a] P < 0.001 indicates the significance on comparison of ethanolic extract of *Ziziphus Mauritania* with ascorbic acid.

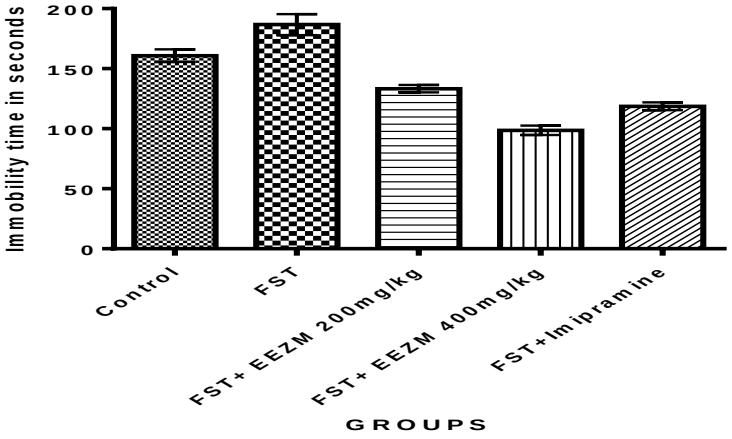

Fig 1: Effect of EEZM on Forced swim test

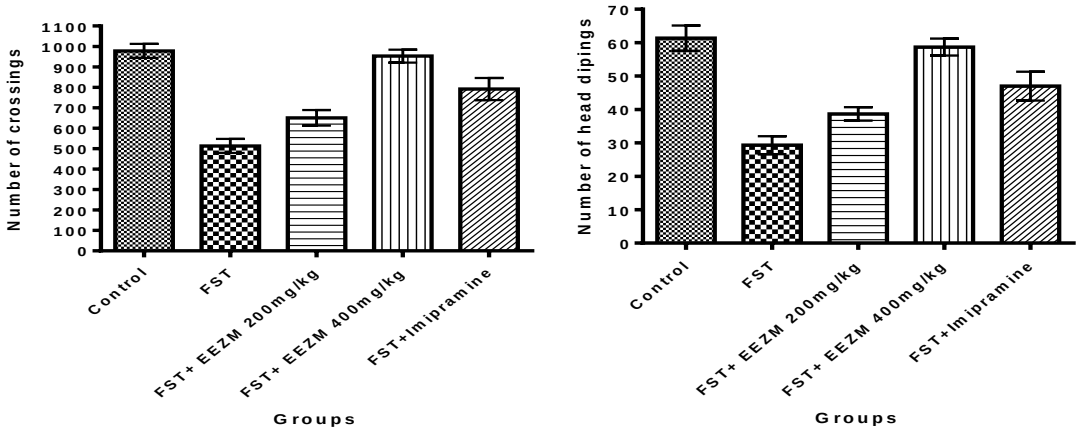

Fig 2: Effect of EEZM on Open Field Exploration

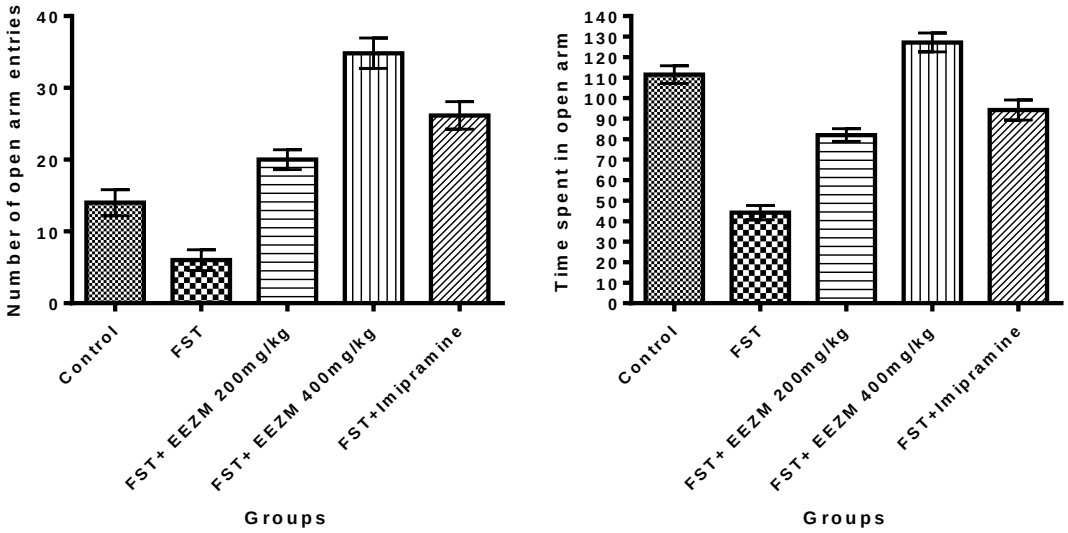

Fig 3: Effect of EEZM on Elevated Plus Maze

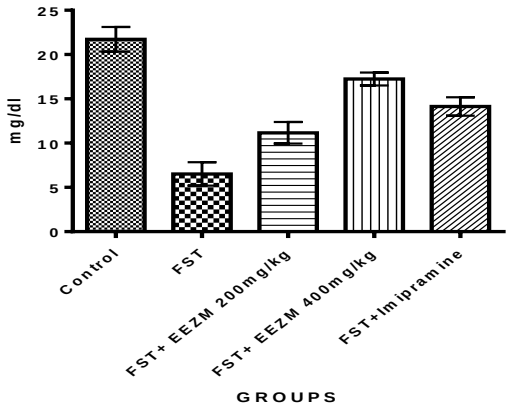

Fig 4: Effect of EEZM on Superoxide Dismutase Levels

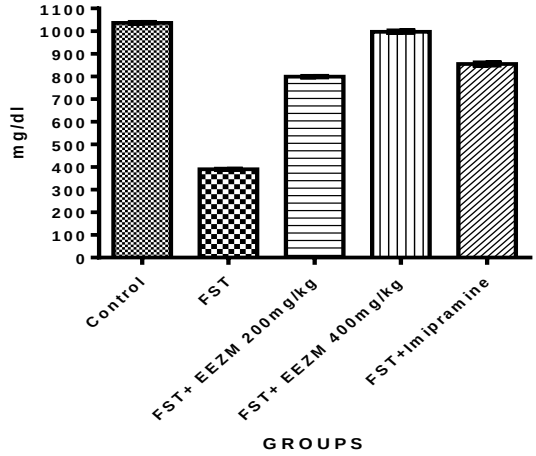

Fig 5: Effect of EEZM on Catalase Levels

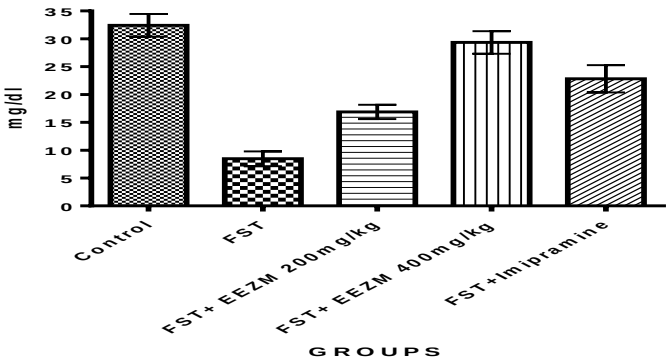

Fig 6: Effect of EEZM on Glutathione Peroxidase Levels

Reducing power

The reducing power is associated with its potential antioxidant activity. The EC50 value of the extract was 552.3 g/mL as opposed to that of standard ascorbic acid (18 g/mL). The results were shown in the Table 1.

Assay of nitric oxide scavenging activity

EEZM showed strong nitric oxide scavenging activity and it was compared with standard ascorbic acid. The IC50 value of the extract was 36.62 g/mL as opposed to that of standard ascorbic acid (7.22 g/mL). The results were shown in the Table 1.

Behavioural estimations

Effect of EEZM on Forced Swim test

Fig. 1 illustrates the effect of EEZM on the duration of immobility time in the FST model. One-way ANOVA revealed that there were significant differences between treatment groups when compared to control groups ($p< 0.01$; $p< 0.0001$). EEZM significantly decreased the duration of immobility time indicating antidepressant effect. There was significant dose-dependent effect of EEZM ($p< 0.001$; $p< 0.05$) on comparison with group III, V with Group IV respectively.

Effect of EEZM on open field habituation

The exploratory behaviour i.e., the number of line crossings and head dipping's decreased in FST group ($p < 0.0001$) in comparison with the control group. The number of line crossings and head dipping's increased significantly ($p < 0.05$; $p < 0.0001$, $p< 0.001$; $p< 0.01$) in 200 mg/kg EEZM, 400 mg/kg EEZM and imipramine treated groups respectively and indicates the improved open field habituation memory. There was significant dose-dependent effect of EEZM ($p< 0.0001$; $p< 0.001$; $p< 0.01$; $p< 0.05$) on comparison with group III, V with Group IV respectively. The results were shown in the Table 2; Figure 2.

Table: 2 Effect of EEZM on behavioural activity

Groups	Forced swim test	Open field exploration		Elevated plus maze	
	Time of immobility (seconds)	Line crossings	Head dips	Number of open arm entries	Time spent in open arm
Group I: Control	160.8±5.3	978.0±34	61.3±3.79	14±1.80	111.5±4.31
Group II: FST	186.7±8.8[a]	513.3±34.6[b]	29.33±2.67[b]	6±1.43[a]	44.1±3.51[b]
Group III: FST+EEZM 200 mg/kg	133.3±3.1[c]	650.7±38.2[d]	38.67±1.99[d]	20±1.39[c]	82±3.08[c]
Group IV: FST+ EEZM 400 mg/kg	98.67±3.8[c, h]	952.5±31.34[c, g]	58.67±2.52[c, h]	34.8±2.10[c, g]	127.2±4.62[c, g]
Group V: FST+ Imipramine	118.7±3.1[c, i]	791.7±53.8[e, j]	47±4.32[f, i]	26.1±1.90c, j	94.1±4.90[c, h]

Values are expressed as mean ± SEM of 6 animals. Superscript letters represents the statistical significance done by ANOVA followed by Newman-Keuls multiple comparisons test.

[a] $p < 0.01$; [b] $p < 0.0001$ indicates the significance on comparison of group II with group I

[c] $p < 0.0001$; [d] $p <0.05$; [e] $p <0.001$; [f] $p<0.01$ indicates the significance on comparison of group III, IV & V with group II.

[g] $p < 0.0001$; [h] $p < 0.001$, indicates the dose dependent significance on comparison of group III with group IV.

[i] $p< 0.05$; j $p<0.01$; h $p<0.001$, indicates the dose dependent significance on comparison of group IV with group V.

Effect of EEZM on elevated plus maze

FST group showed a significant reduction ($p < 0.0001$ and $p < 0.01$) in the time spent in the open arms and number of entries into the open arms when compared to control group. The group treated with 200, 400 mg/kg EEZM and imipramine exhibited significant increase in the number of entries into open arm and time spent in open arm ($p < 0.0001$) when compared with FST group. A dose-dependent activity of EEZM was found with significance of $p < 0.0001$; $p< 0.01$ and $p< 0.001$ on comparison with group III, V with Group IV respectively. The results were shown in the Table 2; Figure 3.

Antioxidant activities

Effect of EEZM on TBARS

FST group showed significantly ($p < 0.0001$) increased TBARS when compared with the control group. Treatment with EEZM 200, 400 mg/kg and imipramine significantly ($p < 0.05$; $p< 0.0001$; $p< 0.05$) attenuated the increase in TBARS. Results are tabulated in Table 3; Figure 7. A dose-dependent decrease in TBARS was found with significance of $p < 0.01$; $p< 0.05$ on comparison with group III, V with Group IV respectively.

Table: 3 Effect of EEZM on Antioxidant Parameters

Groups	Superoxide dismutase (mg/dl)	Catalase	Glutathione Peroxidase	TBARS
Group I: Control	21.72±1.39	1037±6.77	32.42±2.04	7.91±1.07
Group II: FST	6.52±1.32[a]	389±5.83[a]	8.46±1.32[a]	22.83±2.34[a]
Group III: FST+EEZM 200 mg/kg	11.1±1.21[b]	799±6.38[c]	16.87±1.28[b]	17.37±2.08[d]
Group IV: FST+ EEZM 400 mg/kg	17.2±0.73[c, f]	997±8.28[c, g]	29.35±2.02[c, h]	10.12±0.62[c, f]
Group V: FST+ Imipramine	14.4±1.04[e]	854±9.86[c, i]	22.82±2.44[c, i]	16.16±1.06[b, i]

Values are expressed as mean ± SEM of 6 animals. Superscript letters represents the statistical significance done by ANOVA followed by Newman-Keuls multiple comparisons test.

[a] $p < 0.0001$ indicates the significance on comparison of group II with group I

[b] $p < 0.01$; [c] $p <0.0001$; [d] $p <0.05$; [e] $p<0.01$ indicates the significance on comparison of group III, IV & V with group II.

[f] $p < 0.01$; [g] $p < 0.0001$; [h] $p<0.001$, indicates the dose dependent significance on comparison of group III with group IV.

[i] $p< 0.05$ indicates the dose dependent significance on comparison of group IV with group V.

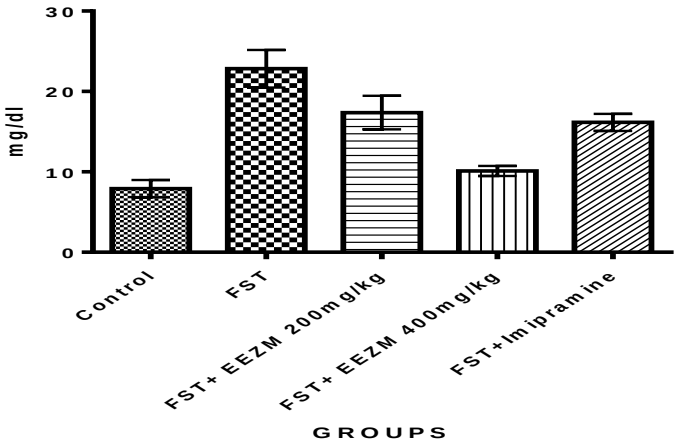

Fig 7: Effect of EEZM on TBARS Levels

Effect of EEZM on superoxide dismutase
FST group showed significantly ($p < 0.0001$) decreased levels of SOD when compared to the control group. Treatment with EEZM 200, 400 mg/kg doses and imipramine showed a significant increase ($p < 0.05$; $p < 0.0001$; $p < 0.001$) in activity of SOD, respectively compared to FST group. The results are shown in Table 3; Figure 4. A dose-dependent increase in SOD levels was found with significance of $p < 0.01$ on comparison with group III with Group IV respectively.

Effect of EEZM on glutathione peroxidase (GPx)
FST group showed significantly ($p < 0.0001$) decreased levels of GPx when compared to the control group. The groups treated with EEZM 200 mg/kg, 400 mg/kg and imipramine showed a significant increase ($p < 0.01$ and $p < 0.0001$) in activity of glutathione peroxidase, respectively compared to FST group. The results are shown in Table 3; Figure 6. A dose-dependent increase in GPx was found on administration of EEZM with significance of $p < 0.001$; $p < 0.01$ on comparison with group III, V with Group IV respectively.

Effect of EEZM on catalase
FST group showed significantly ($p < 0.0001$) decreased levels of catalase when compared to the control group. The groups treated with EEZM 200 mg/kg, 400 mg/kg and imipraine showed a significant increase ($p < 0.0001$) in activity of catalase compared to FST group. The results are shown in Table 3; Figure 5. A dose-dependent increase in catalase was found on administration of EEZM with significance of $p < 0.0001$; $p < 0.05$ on comparison with group III, V with Group IV respectively.

DISCUSSION:

The present study depicted that administration of EEZM produced antidepressant effect and prevented oxidative damage caused by forced swim test. Forced swim test predicted the efficacy of various antidepressants[37,38] and immobility behaviour of rats in forced swim test exactly resembles the state of depression in humans.[39] Treatment with EEZM significantly reduced immobility time in dose dependent manner indicating antidepressant activity.

Further to validate antidepressant activity of EEZM we subjected the rats for open field exploratory test. This test is used to examine the exploratory behaviour and the way of response to novel environment in experimental animals which mimic psychomotor retardation in humans.[40] Treatment with EEZM (400mg/kg) increased locomotor activity dose dependently and it may be due to modulation of hypothalamic- pituitary adrenal axis mediated by an increased function of glucocorticoid receptor.[41]
The elevated plus maze test is used widely to conduct behavioral assay for rodents and it has been validated to investigate anti-anxiety effects of various drugs.[42] In this task, compounds possessing anti-anxiety effects reduce the natural aversion of animals to the open arms. Hence, increase in the number of open arm entry or the time spent in the open arms; reflect the anxiolytic effect.[43] Our results showed that treatment with EEZM increased the open arm entries and augmented the time spent in the open arms in a dose dependent manner.

Oxidative stress is involved in pathogenesis of various neuropsychiatric disorders including depression.[11] A study also reported the involvement of free radical in pathogenesis of chronic depression.[44] Oxidative stress generates reactive oxygen species and this will exert deleterious effects on signal transduction and neural plasticity. Reactive oxygen species induce lipid peroxidation and cause damage to DNA and proteins.[45] Brain is vulnerable to peroxidative damage due to high oxygen tension, low antioxidant enzyme levels and rich oxidizable substrates.[46,47] In the present investigation, antioxidant activity of EEZM was assessed by both invitro and invivo assays. Invitro Dpph, reducing power, nitric oxide scavenging ability assay was carried out on EEZM and it revealed the free radical scavenging effect of EEZM.

In order to confirm the antioxidant activity of EEZM invivo antioxidant assays are carried out in brain homogenates. Forced swim test induced lipid peroxidation which was indicated by increase in TBARS levels and decrease in SOD, Catalase and Gpx. SOD converts super oxide anion to hydrogen peroxide and catalase removes hydrogen peroxide in the form of water. Gpx is one of the major enzymes involved in controlling the thiol content in brain.[48] Supplementation with EEZM increased the levels of SOD, Catalase and Gpx significantly in dose dependent manner which were compromised due to forced swim test.

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CONCULSION:

The present investigation explored the antidepressant effect of EEZM in forced swim test model of depression in rats. Results from behavioural

experiments indicate the antidepressant activity of EEZM and it may be due to facilitatory effect on both serotonergic and noradrenergic system. Further molecular studies have to be carried out on this line to confirm the protective effect of EEZM against depression.

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