

## EVALUATION OF ANTISTRESS ACTIVITY OF ETHANOLIC EXTRACT OF *CHROMOLAENA ODORATA* LEAVES IN ALBINO WISTAR RATS

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### ABSTRACT

**Objective:** The objective of this study was to study the effect of ethanolic extract of *Chromolaena odorata* (EECO) Linn. on acute restraint stress (ARS)-induced stress-like behavior and biochemical alterations in albino Wistar rats.

**Methods:** The ARS was induced by immobilizing the rats for a period of 12 h using rodent restraint device preventing them from any physical movement. Immediately, after 12 h rats were released and doses were given to each rat. 40 min post-release various behavioral parameters such as immobility time in force swim test and tail suspension test (TST), locomotor activity in open field test (OFT), and oxidative stress parameters and biochemical alterations in rat brain tissue were also performed.

**Statistical Analysis:** Expression of data was done as mean±standard error of mean. The normally distributed data were subjected to one-way ANOVA followed by Dunnett's test. p<0.05 was considered statistically significant.

**Results:** Experimental findings revealed that rats subjected to ARS exhibited significant increase in immobility time in forced swim test and TST models, decrease in locomotor activity in OFT model, and increase in malondialdehyde formation and impaired superoxide dismutase, and catalase activities in hippocampus and cerebral cortex as compared to non-stressed rats. EECO treatment (250 mg/kg and 500 mg/kg) significantly attenuated immobility time, locomotion, and restored the antioxidant enzymes after ARS.

**Conclusion:** EECO significantly alleviated ARS-induced stress-like behavior.

**Keywords:** Acute restraint stress, Diazepam, *Chromolaena odorata*, Ethanolic extract of *Chromolaena odorata*, Stress.

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### INTRODUCTION

Stress, which is a crucial determinant of health and disease, plays a significant role in the pathogenesis of neuropsychiatric disorders as these stressful events result in altered physiological, immunological, psychological, and neurobehavioral responses such as anxiety, depression, cognitive impairment, insomnia, anorexia, and activation of hypothalamic-pituitary-adrenal axis in animals and humans [1]. Detrimental effects on cellular functions, as a result of stressful conditions, arise due to oxidative damage produced by the release of free radicals or reactive oxygen species (ROS) which is implicated in neuropsychiatric disorders [2]. The central nervous system (CNS) is especially susceptible to free radical damage due to brain's high oxygen demand, abundant lipid content, and relative paucity of antioxidant enzymes [3]. Restraint stress exposure alters the free radical scavenging enzymes in discrete regions of brain [4]. Medicinal plants rich in phytochemicals such as phenolics and flavonoids, act as free radical scavengers and metal chelators, which are useful in preventing neurodegeneration [5].

*Chromolaena odorata* is commonly known as Siam weed belonging to sunflower family *Asteraceae*. It is an important medicinal plant which can be easily found in tropical Asia, West Africa, and parts of Australia. It is native to the America and found in Florida and Texas in the United States, and throughout Mexico and the Caribbean to South America. *C. odorata* contains a number of active chemical constituents, including flavonoids, phenolic acids, tannins, alkaloids, and vitamins which serve as useful antioxidants [6].

From literature review regarding the traditional uses and phytochemical properties of *C. odorata* are anti-bacterial, anticancer, anticonvulsant,

antidiabetic, anti-diarrheal, anti-fungal, anti-inflammatory, antioxidant, antiparasitic, hemostatic, wound healing, and hepatoprotective activities. These data indicate that this plant may certainly have some therapeutic effects on CNS. Nevertheless, scientific evidence about the potential effects of this plant on neurological disorders is lacking [6].

In this study, we sought to extend current literature dealing with the effect of ethanol extract of *C. odorata* (EECO) for the flavonoid and phenolic components on stress-like behavioral symptoms in albino Wistar rats and to analyze brain antioxidant elements such as superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and lipid peroxidation (LPO) in relation to the behavioral responses due to acute restraint stress (ARS)-induced stress-like behavior.

### METHODS

#### Collection and authentication of plant materials

The twigs (leaves and flowers) of *C. odorata* were collected from Sanjay Gandhi National Park, Borivali East, Mumbai, and were authenticated by Dr. Praveen Kale, Botanist at St. Xavier's College, Mumbai - 400 001.

#### Extraction and phytochemical evaluation

The authenticated leaves were washed and dried under shade and then powdered with a mechanical grinder to obtain a coarse powder. The resultant powder is extracted with ethanol (90% v/v) in Soxhlet apparatus at 60°C.

EECO was subjected to systematic chemical test analysis. The presence of different phytoconstituent such as flavonoids, carbohydrates, proteins and amino acids, alkaloids, glycosides, saponins, steroids, terpenoids, and tannins was investigated as described by the well-established methods [7,8].

### Experimental animals

Female albino Wistar Rats (200–300 g) were obtained from SA-FORD, Plot No.: V-10, MIDC, Taloja, Navi Mumbai, Dist. Raigad Maharashtra - 410 208 and maintained at constant room temperature (20–22°C) with free access to water and food, under a 12:12 h light: dark cycle. Animals were acclimatized to laboratory conditions before experiment. The use of these animals and the study protocols was approved by CPCSEA recognized Institutional Animal Ethics Committee (IAEC) of Oriental College of Pharmacy, Sanpada, Navi Mumbai - 400 705 under protocol no. OCP/IAEC/2018-19/05. All efforts were made to reduce animal suffering and the number of rats used in the experiments.

### Selection of doses

After thorough literature survey on available research data conducted on the EECO leaves, it was found that the extract was safe for animal use as LD<sub>50</sub> was considered as more than 5000 mg/kg [9].

Thus, for purpose of research, the doses of EECO were finalized as Intermediate dose – 250 mg/kg and high dose – 500 mg/kg.

### Experimental design

Thirty albino Wistar rats were randomly divided into five experimental groups containing six rats each as shown in Table 1. Group-I (normal control) rats received normal saline (2.0 mL/kg, p.o.) daily for 14 days; Group-II (stress control) rats received normal saline (2.0 mL/kg, p.o.) daily for 14 days and subjected to restraint stress on 13<sup>th</sup> day; Group-III (standard drug-treated) rats received diazepam (2 mg/kg, p.o.) daily for 14 days and subjected to restraint stress on 13<sup>th</sup> day; and Group-IV and V rats were treated with EECO (250 mg/kg and 500 mg/kg, p.o.) daily for 14 days and subjected to ARS on 13<sup>th</sup> day.

Stress-like behavior was assessed by subjecting the rats to behavioral paradigms such as tail-suspension test (TST) and open field test (OFT), 40 min post restraint stress procedure on 14<sup>th</sup> day. Pretest of 10 min for forced swim test (FST) was also given to each rat simultaneously. Then 23.5 h later, the relevant samples were administered and main test for FST performed 30 min later.

Oxidative stress parameters such as SOD, CAT, MDA, and extent of LPO were analyzed in restraint stress-induced animals and control groups, following FST on 15<sup>th</sup> day.

### Procedure for ARS

ARS was accomplished by placing rats in an individual plastic rodent restraint device for 12 h. This restrained all physical movements without subjecting the animal to pain. Animals were fasted and deprived of food and water during the entire period of exposure to stress. After 12 h, the animals were released from their enclosure and 40 min post-release and the animals were subjected to behavioral tests and then to biochemical estimations. In normal control group, the rats were kept in the animal cage in the experimental room [10,11].

### Behavioral tests

#### TST

The animals were suspended individually by end of tail with Micro pore adhesive tape (approximately 1 cm) with the head 50 cm from the bottom in a suspension box 40 min post restraint stress procedure. Rats were

suspended for a total of 6 min. During the final 4 min interval of the test, duration of immobility was recorded. Rats were considered immobile only when they will be hung passively and completely motionless. Antistress decreases the immobility of rats in these tests [12].

#### OFT

Open-field apparatus was made as reported. Each rat was placed in the center of the open field and its behavior observed for 5 min. The parameters evaluated were the total number of squares crossed, the number of outer squares (those adjacent to the walls) crossed, and the number of inner squares crossed; the three events referred to as total locomotion (TL), peripheral locomotion (PL), and central locomotion (CL), respectively. The numbers of leanings (one or two paws in contact with the wall), rearings (the mouse standing on its two hind paws without touching the walls), groomings (face cleaning, paw licking, fur licking, head scraping, and rubbing), and defecations were also recorded. At the end of each test, the whole area was cleaned with a wet sponge and a dry paper towel [13].

#### FST

On day 14, all the rats were allowed to swim individually for 10 min for adaptation. Then 23.5 h later, the relevant samples were administered and main test performed 30 min later, i.e., on day 15. Rats were forced to swim in a cylinder (diameter 40 cm and 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 to escape 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. Rats were considered to be immobile when they floated in an upright position, making only small movements to keep their head above the water level. Following swimming session, rats were dried using cotton towel and returned to home cages after experiment. A decrease in the duration of immobility is indicative of antistress-like effect, whereas an increase of immobility time, when compared with the control group, is associated with stress-like effects [14].

### Biochemical estimation

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.

#### CAT activity

The CAT activity in the supernatant was measured by the method of Aebi H. The supernatant (50 µl) was added to a cuvette containing 2.95 ml of 19 mM/L solution of H<sub>2</sub>O<sub>2</sub> prepared in potassium phosphate buffer. The change in absorbance was monitored at 240 nm wavelength at the 1 min interval for 3 min. The presence of CAT decomposes H<sub>2</sub>O<sub>2</sub> leading to a decrease in absorbance [15].

#### Sodium oxide dismutase activity

The SOD activity in the supernatant was measured by the method of Misra and Fridovich. The supernatant (500 µl) was added to 0.800 ml of carbonate buffer (100 mM, pH 10.2) and 100 µl of epinephrine (3 mM). The absorbance change of each sample was then recorded at 480 nm in spectrophotometer

Table 1: Grouping of animals

S. No.	Groups	Test substances	Animal required per group (albino Wistar rats)	Dose	Total number
1.	Normal control	Normal saline	6	2 ml/kg	6
2.	Stress control	Normal saline	6	2 ml/kg	6
3.	Standard control	Diazepam	6	2 mg/kg	6
4.	Test Group 1	EECO	6	250 mg/kg	6
5.	Test Group 2	EECO	6	500 mg/kg	6
Total animals required					30

\*EECO: Ethanolic extract of *Chromolaena odorata*

for 2 min at an interval of 15 s. A parallel run of blank and standard was done for determination of SOD activity. The reaction mixtures are diluted 1/10 just before taking the readings in a spectrophotometer [16].

**Determination of MDA formation**

From the 10% tissue homogenate, 1 ml of suspension medium was taken and 0.5 ml of 30% trichloroacetic acid (TCA) will be added to it followed by 0.5 ml of 0.8% thiobarbituric acid (TBA) reagent. The tubes were then be covered with aluminum foil and kept in shaking water bath for 30 min at 80°C. After 30 min tubes were taken out and kept in ice-cold water for 30 min. These were then be centrifuged at 3000 rpm for 15 min.

The absorbance of the supernatant was read at 540 nm at room temperature against an appropriate blank. Blank consists of 1 ml distilled water, 0.5 ml of 30% TCA, and 0.5 ml of 0.8% TBA [17].

**Determination of lipid peroxidation assay**

The level of lipid peroxides was estimated by TBA reaction method described by Ohkawa *et al.* To 0.2 ml of the test sample, 0.2 ml of Sodium Dodecyl Sulphate (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 min. After cooling, 1 ml of water and 5 ml of n-butanol/pyridine mixture were added and agitated smartly. After centrifugation at 4000 rpm for 10 min, the organic layer was taken and its absorbance was scan at 532 nm. The level of lipid peroxides was expressed as nmoles of MDA released/g wet tissue [17].

**Statistical analysis**

All values are expressed as mean±SD. Statistical significance was determined using one-way ANOVA followed by Dunnett’s test. p<0.05 was considered to be significant.

**RESULTS**

**Qualitative phytochemical screening**

The results of qualitative phytochemical analysis of the ethanolic extract of *C. odorata* leaves shown the presence of carbohydrates, alkaloids, flavonoids, steroids, terpenoids, and tannins.

**Antistress evaluation**

**TST**

Both the doses of the ethanolic extract of leaves of *C. odorata* showed dose dependent decrease in immobility time, when it was compared against stress control as well as against diazepam which was used as a standard. The results of TST are shown in Table 2 and Fig. 1.

**Table 2: Effect of ethanolic extract of *Chromolaena odorata* leaves on mobility and immobility time of tail suspension test in Albino Wistar rat**

S. No.	Treatment	Immobility	Mobility
1.	Normal control	112.17±12.59	127.83±12.59
2.	ARS	145.17±12.45	94.83±12.45
3.	Diazepam (2 mg/kg)+ARS	82.33±5.73	157.67±5.73
4.	EEO (250 mg/kg)+ARS	87.83±7.37	152.17±7.37
5.	EEO (500 mg/kg)+ARS	71±6.89	169±6.89

Values are the mean±SEM of n=6 rats/treatment. Significance \*\*p<0.01. EEO: Ethanolic extract of *Chromolaena odorata*, ARS: Acute restraint stress

**Table 3: Effect of ethanolic extract of *Chromolaena odorata* leaves in open field test in Albino wistar rats**

S. No.	Treatment	TL	PL	CL	Leaning	Rearing	Grooming	Defecation
1.	Normal control	50.33±3.37	46±3.51	4.33±1.49	9±2.59	0.83±0.4	3.17±0.60	2.83±1.4
2.	ARS	28.5±2.48	26.17±2.53	2.17±0.17	11.5±3.4	1.5±1.15	3±0.68	0.83±0.65
3.	Diazepam (2 mg/kg)+ARS	48.17±3.4	43.67±3.2	4.5±0.5	5.83±1.44	1±0.82	2±0.63	2.17±1.04
4.	EEO (250 mg/kg)+ARS	54.17±2.12	48.50±1.65	5.67±1.91	7.33±1.94	0.5±0.22	2.67±0.49	1.83±0.48
5.	EEO (500 mg/kg)+ARS	57.17±2.18	52.5±2.78	4.67±2.51	7.83±1.56	1.83±1.04	3.17±0.4	0.83±0.31

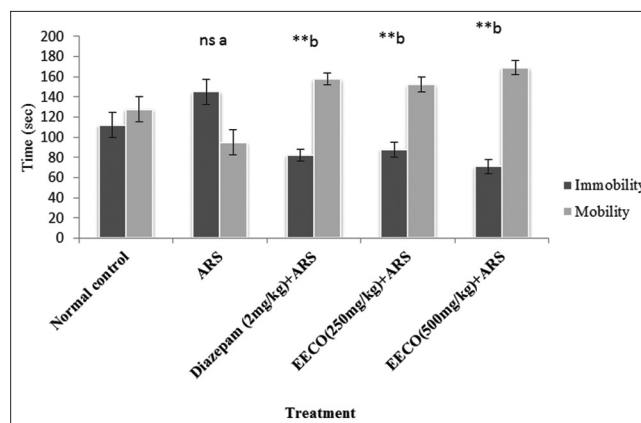
Values are the mean±SEM of n=6 rats/treatment. Significance \*\*p<0.01. TL: Total locomotion, PL: Peripheral locomotion, CL: Central locomotion. EEO: Ethanolic extract of *Chromolaena odorata*, ARS: Acute restraint stress

**OFT**

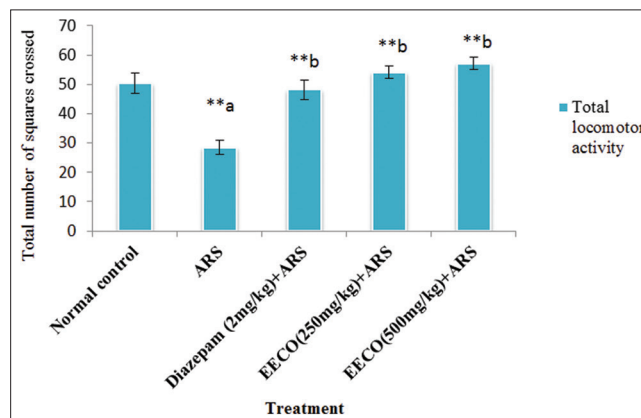
Both the doses of the ethanolic extract of leaves of *C. odorata* showed dose dependent increase in locomotor activity, when it was compared against stress control as well as against diazepam which was used as a standard. The results of OFT are shown in Table 3 and Fig. 2.

**FST**

Both the test groups of the ethanolic extract of leaves of *C. odorata* showed dose dependent decrease in immobility time when compared against stress control as well as against diazepam which was used as a standard. The results of FST are shown in Table 4 and Fig. 3.



**Fig. 1: Effect of EEO pretreatment on ARS-induced changes in duration of immobility in tail suspension test. NC: Normal control; ARS: Acute restraint stress; EEO: Ethanolic extract of *Chromolaena odorata*. Values are expressed as mean±standard error of mean (n=6). \*\*\*p<0.001, \*\*p<0.01, and \*p<0.05. a versus NC group and b versus ARS group**



**Fig. 2: Effect of EEO pretreatment on ARS-induced changes in total locomotor activity in open field test. NC: Normal control; ARS: Acute restraint stress; EEO: Ethanolic extract of *Chromolaena odorata*. Values are expressed as mean ± standard error of mean (n=6). \*\*\*p<0.001, \*\*p<0.01, and \*p<0.05. a versus NC group and b versus ARS group**

**Biochemical estimations**

The result of biochemical estimations for various oxidative parameters such as catalase, superoxide dismutase, malonaldehyde and lipid peroxidation are shown in Table 5.

**CAT activity**

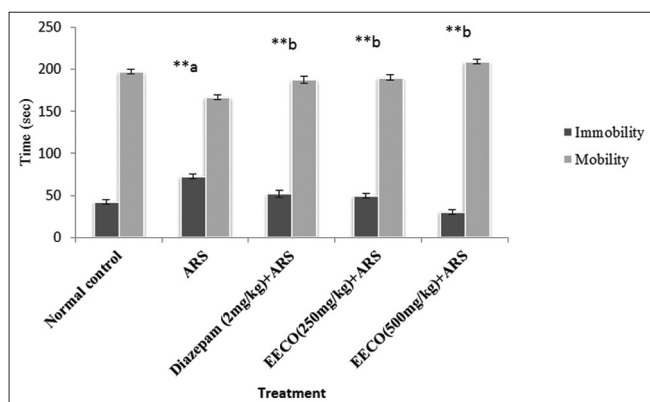
Evaluation of CAT activity revealed that stressed rats presented a significant decrease in CAT activity, which was significantly prevented by EEEO (250 mg/kg and 500 mg/kg) pretreatment, when compared to unstressed group as shown in Table 5 and Fig. 4.

**SOD activity**

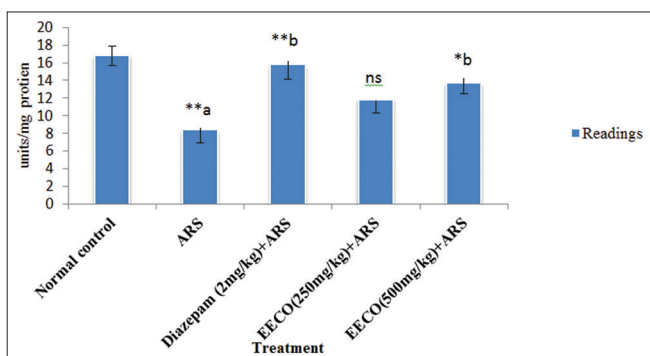
**Table 4: Effect of ethanolic extract of *Chromolaena odorata* leaves on mobility and immobility time of forced swim test in Albino Wistar rat**

S. 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.	Diazepam (2 mg/kg)+ARS	52.16±4.24	187.83±4.24
4.	EEEO (250 mg/kg)+ARS	49.66±3.21	190.33±3.21
5.	EEEO (500 mg/kg)+ARS	30.5±2.69	209.5±2.69

Values are the mean±SEM of n=6 rats/treatment. Significance \*\*p<0.01. EEEO: Ethanolic extract of *Chromolaena odorata*, ARS: Acute restraint stress



**Fig. 3: Effect of EEEO pretreatment on ARS-induced changes in duration of immobility in forced swim test. NC: Normal control; ARS: Acute restraint stress; EEEO: Ethanolic extract of *Chromolaena odorata*. Values are expressed as mean±standard error of mean (n=6). \*\*\*p<0.001, \*\*p<0.01, and \*p<0.05. a versus NC group and b versus ARS group**



**Fig. 4: Effect of EEEO pretreatment on ARS-induced changes on catalase activity. NC: Normal control; ARS: Acute restraint stress; EEEO: Ethanolic extract of *Chromolaena odorata*. Values are expressed as mean±standard error of mean (n=6). \*\*\*p<0.01 and \*p<0.05. a versus NC group and b versus ARS group**

In the rats pretreated with EEEO 250 mg/kg and 500 mg/kg p.o., the level of SOD was significantly increased (p<0.001) as compared to ARS rats. Table 5 and Fig. 5 show significant and dose-dependent recovery on ARS-induced reduced the level of SOD in animal due to EEEO.

**Determination of MDA formation**

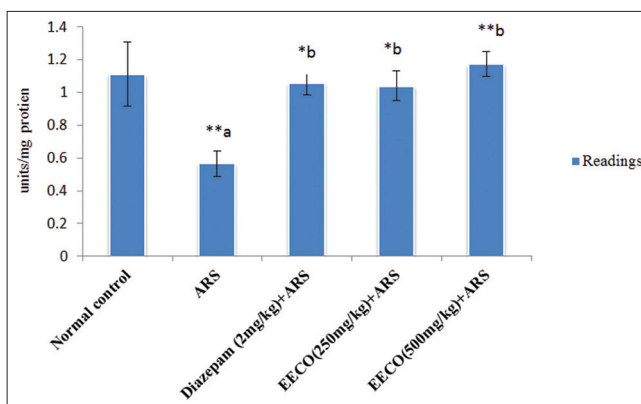
The results depicted in Table 5 and Fig. 6 illustrate that ARS significantly increased MDA level in rat brain as compared to unstressed rats. The results indicated that EEEO (250 mg/kg and 500 mg/kg) pretreatment and diazepam significantly abolished increase in MDA level caused by ARS.

**Lipid peroxidation assay**

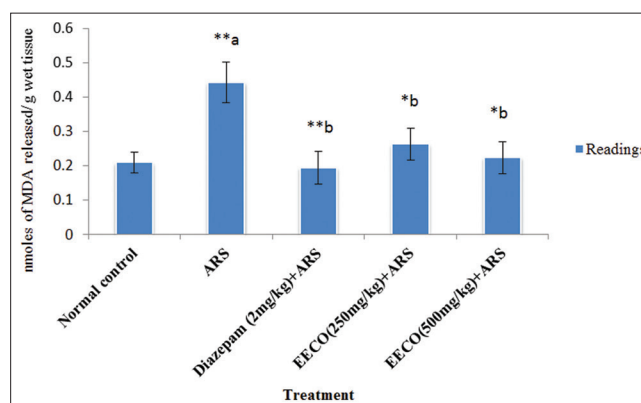
Quantitative measurement of LPO in the whole brain was assessed based on the amount of MDA formed; the statistical analysis revealed that ARS produced a significant increase in MDA level, whereas EEEO (250 mg/kg and 500 mg/kg) pretreatment significantly abolished increase in MDA level compared to stressed animals. The results are shown in Table 5 and Fig. 7.

**DISCUSSION**

Stress is the sum total of all the reaction of the body, which disturbs the normal physiological condition and results in a state of threatened homeostasis producing different physiological as well as pathological changes depending on severity, type, and extent of stress which



**Fig. 5: Effect of EEEO pretreatment on ARS-induced changes on superoxide dismutase activity. NC: Normal control; ARS: Acute restraint stress; EEEO: Ethanolic extract of *Chromolaena odorata*. Values are expressed as mean±standard error of mean (n=6). \*\*\*p<0.01 and \*p<0.05. a versus NC group and b versus ARS group**



**Fig. 6: Effect of EEEO pretreatment on ARS-induced changes on malondialdehyde activity. NC: Normal control; ARS: Acute restraint stress; EEEO: Ethanolic extract of *Chromolaena odorata*. Values are expressed as mean±standard error of mean (n=6). \*\*\*p<0.01 and \*p<0.05. a versus NC group and b versus ARS group**



Table 5: Effect of oxidative stress markers in brain homogenate of albino Wistar rats

S. 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.	Diazepam (2 mg/kg)+ARS	15.76±1.659	1.0577±0.07230	0.1944±0.0481	0.1326±0.0139
4.	EECO (250 mg/kg)+ARS	11.84±1.530	1.0396±0.09070	0.2627±0.0462	0.1446±0.0246
5.	EECO (500 mg/kg)+ARS	13.67±1.152	1.1742±0.07607	0.2243±0.0464	0.1233±0.0245

Values are the mean±SEM of n=6 rats/treatment. Significance \*\*p<0.01. CAT: Catalase, SOD: Superoxide dismutase, MDA: Malondialdehyde, LPO: Lipid peroxidase. EECO: Ethanolic extract of *Chromolaena odorata*, ARS: Acute restraint stress

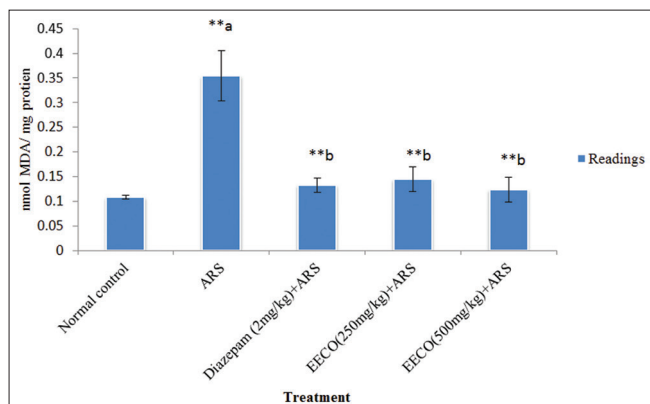


Fig. 7: Effect of EECO pretreatment on ARS-induced changes on LPO activity. NC: Normal control; ARS: Acute restraint stress; EECO: Ethanolic extract of *Chromolaena odorata*. Values are expressed as mean±standard error of mean (n=6). \*\*\*p<0.01 and \*p<0.05. a versus NC group and b versus ARS group

might cause changes in immunological and neurobehavioral profile during adaptational processes [18]. ARS, which is a type of stressful event, has been reported to induce anxiety and stress-like behavior in animals which can be monitored in various rodent behavioral models effectively [19]. ARS which is a reliable model of anxiety, stress, and depression induced by stress, produces an inescapable physical and mental stress, in addition to impairment in the *in vivo* antioxidant defense mechanism [20].

Stress-induced models, in rodents, used for evaluating antistress activity include FST and TST, which assess duration of immobility as an index of stress as immobility represents a state of behavioral despair and failure to adapt to stress. FST and TST provide rapid and reliable results for antistress activity and are also quite sensitive [20].

Conventional antistress agents reliably decrease duration of immobility in animals. In the present study, ARS significantly increased the duration of immobility in FST and TST, indicating stress-like behavior as demonstrated in earlier studies [4,19,20]. Pretreatment with EECO for 14 days provided significant protection against ARS-induced increased immobility time in FST and TST which supported the findings obtained in similar studies on evaluation of antistress-like effect.

Moreover, FST has some drawbacks as of obtaining false positive or negative responses because drugs that enhance motor activity may also give false positive responses in FST [21]. Similar to antidepressants and psychostimulants are also shown to decrease duration of immobility in FST and TST models, but at the same time, they cause a marked motor stimulation [22]. Thus, to exclude motor stimulation activity of EECO, locomotor activity test was also performed using OFT.

In OFT restraint stress caused a significant decrease in central and peripheral crossings in the indicating stress-like behavior. The dose of 250 mg/kg and 500 mg/kg of EECO increased the number of central and peripheral crossings. The observed results showed a significant dose-dependent antidepressant-like effect in OFT, as they increase in

locomotor activity when compared against stress control as well as against standard diazepam (2 mg/kg).

Various studies have shown that stressful events are associated with oxidative damage in the brain as a result of increase or decrease in the production of ROS which also plays an important role in the pathogenesis of stress. Huge decrease in SOD activity and CAT activity induced as a result of ARS is an index of pro-oxidative conditions [23]. In the present study, 12 h restraint stress-induced a significant oxidative damage, as indicated by decreased SOD and decreased CAT, which was significantly reversed by EECO pretreatment. Lipid peroxidation is considered as a critical mechanism in causing cell injury during oxidative stress. Several studies have demonstrated that restraint stress significantly elevated LPO level in the hippocampus of rats.

Our results are in line with these findings, showing significant LPO, evidenced by an increased amount of MDA, which was attenuated by EECO treatment. Thus, the antistress-like effect of EECO could be associated with its capacity to prevent the lipid peroxidative damage, induced by ARS.

The neuroprotection offered by EECO pretreatment might be attributed to the antioxidant constituents such as flavonoids and phenolics in the leaves of *C. odorata* [24].

## CONCLUSION

There is substantial evidence that flavonoids play an important and active role in providing anti-stress activity. This study is an attempt to find out the alternative medication for treating chronic stress with single medication which was shown a beneficial effect in animal models, may be useful for curing symptoms of stress.

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## AUTHORS' CONTRIBUTIONS

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Miss Sana Shaikh collected the data and analyzed the data. Dr. (Mrs.) Vanita Kanase proofread the whole manuscript and suggested the necessary changes and helps in designing manuscript.

## CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

## REFERENCES

1. Ansari I, Sorte RS. Evaluation of anti-stress activity of ethanolic extracts of *Terminalia catappa* L. in swiss albino mice. Asian J Pharm Clin Res 2018;11:253-7.
2. Kumar A, Garg R, Prakash AK. Effect of St. John's wort (*Hypericum*

- perforatum*) treatment on restraint stress-induced behavioral and biochemical alteration in mice. BMC Complement Altern Med 2010;10:18.
3. Halliwell B, Gutteridge JM. Oxygen radicals and the nervous system. Trends Neurosci 1985;8:22-6.
  4. Freitas AE, Bettio LE, Neis VB, Santos DB, Ribeiro CM, Rosa PB, et al. Agmatine abolishes restraint stress-induced depressive-like behavior and hippocampal antioxidant imbalance in mice. Prog Neuropsychopharmacol Biol Psychiatry 2014;50:143-50.
  5. Sundaram RS, Gowtham L. Microglia and regulation of inflammation-mediated neurodegeneration: Prevention and treatment by phytochemicals and metabolic nutrients. Int J Green Pharm 2012;6:81-92.
  6. Vanita K, Sana S. A pharmacognostic and pharmacological review on *Chromolaena odorata* (siam weed). Asian J Pharm Clin Res 2018;11:34-8.
  7. Kokate CK. Practical Pharmacognosy. 4<sup>th</sup> ed. New Delhi: Vallabh Prakashan; 2005.
  8. Khandelwal KR. Practical Pharmacognosy. Pune: Nirali Prakashan; 2000.
  9. Ogbonnia SO, Mbaka GO, Anyika EN, Osegbo OM, Igbokwe NH. Evaluation of acute toxicity in rats and subchronic toxicity of hydroethanolic extract of *Chromolaena odorata* (L.) King and Robinson (Fam. Asteraceae) in rats. Agric Biol J N Am 2010;1:859-65.
  10. Buynitsky T, Mostofsky DI. Restraint stress in biobehavioral research: Recent developments. Neurosci Biobehav Rev 2009;33:1089-98.
  11. Takada T, Yoshinari N, Sugiishi S, Kawase H, Yamane T, Noguchi T, et al. Effect of restraint stress on the progression of experimental periodontitis in rats. J Periodontol 2004;75:306-15.
  12. Hänell A, Marklund N. Structured evaluation of rodent behavioral tests used in drug discovery research. Front Behav Neurosci 2014;8:252.
  13. Moallem SA, Hosseinzadeh H, Ghoncheh F. Evaluation of antidepressant effects of aerial parts of *Echium vulgare* on mice. Iran J Basic Med Sci 2007;10:189-96.
  14. Yeap SK, Beh BK, Ali NM, Yusof HM, Ho WY, Koh SP, et al. Antistress and antioxidant effects of virgin coconut oil *in vivo*. Exp Ther Med 2015;9:39-42.
  15. Aebi H. Methods of Enzymatic Analysis. Vol. 2. New York: Academic Press; 1974. p. 674.
  16. Misra HP, Fridovich I. The oxidation of phenylhydrazine: Superoxide and mechanism. Biochemistry 1976;15:681.
  17. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
  18. Miller GE, Chen E, Parker KJ. Psychological stress in childhood and susceptibility to the chronic diseases of aging: Moving toward a model of behavioral and biological mechanisms. Psychol Bull 2011;137:959-97.
  19. Sulakhiya K, Patel VK, Saxena R, Dashore J, Srivastava AK, Rathore M, et al. Effect of *Beta vulgaris* Linn. Leaves extract on anxiety-and depressive-like behavior and oxidative stress in mice after acute restraint stress. Pharmacognosy Res 2016;8:1-7.
  20. Budni J, Zomkowski AD, Engel D, Santos DB, dos Santos AA, Moretti M, et al. Folic acid prevents depressive-like behavior and hippocampal antioxidant imbalance induced by restraint stress in mice. Exp Neurol 2013;240:112-21.
  21. Borsini F, Meli A. Is the forced swimming test a suitable model for revealing antidepressant activity? Psychopharmacology (Berl) 1988;94:147-60.
  22. Hsu LC, Ko YJ, Cheng HY, Chang CW, Lin YC, Cheng YH, et al. Antidepressant-like activity of the ethanolic extract from *Uncaria lanosa* wallich var. Appendiculata ridsd in the forced swimming test and in the tail suspension test in mice. Evid Based Complement Alternat Med 2012;2012:497302.
  23. Kolla N, Wei Z, Richardson JS, Li XM. Amitriptyline and fluoxetine protect PC12 cells from cell death induced by hydrogen peroxide. J Psychiatry Neurosci 2005;30:196-201.
  24. Sirinthipaporn A, Jiraungkoorskul W. Wound healing property review of siam weed, *Chromolaena odorata*. Pharmacogn Rev 2017;11:35-8.