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Article

Antidepressant-like activity of ethanol extract of leaves of *Caesalpinia pulcherrima* in unstressed and stressed mice

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In the present study, antidepressant-like activity of ethanol extract of leaves of *Caesalpinia pulcherrima* was evaluated in Swiss young male albino mice. Stress was induced in mice by subjecting them to unpredictable mild stress for 21 successive days. Ethanol extract of the leaves (100, 200 and 400 mg/kg, p.o.) and fluoxetine (20 mg/kg, p.o.) were administered for 21 consecutive days to separate groups of unstressed and stressed mice. Ethanol extract (200 and 400 mg/kg) and fluoxetine significantly decreased immobility period of unstressed as well as stressed mice in tail suspension test (TST). However, the lowest dose (100 mg/kg) of the extract also significantly decreased immobility period of stressed mice in TST. The extract significantly restored reduced sucrose preference in stressed mice. There was no significant effect on locomotor activity of mice. Ethanol extract of the leaves significantly decreased plasma nitrite and corticosterone levels; brain MAO-A activity and MDA level; and increased brain reduced glutathione and catalase activity in unstressed as well as stressed mice as compared to their respective vehicle treated controls. Thus, ethanol extract of leaves of *Caesalpinia pulcherrima* showed significant antidepressant-like activity in unstressed and stressed mice probably through inhibition of brain MAO-Aactivity, reduction of oxidative stress and plasma corticosterone levels.

Keywords: Chronic unpredictable mild stress. Depression. Sucrose preference test. Tail suspension test. *Caesalpinia pulcherrima.*

INTRODUCTION

Depression is characterized by feelings of guilt, sadness, disturbed sleep or appetite, loss of interest, feeling of tiredness and poor concentration. World Health Organization (WHO) estimated that globally, over 322 million people have suffered from depression which is equivalent to 4.4% of the world's population. Prevalence rates of depression vary by age. It is more common in the elderly people (above 7.5% among females and above 5.5% among males). It also occurs in children and adolescents below the age of 15 years, but at a lower level than older age groups. Prevalence and burden of depression disorder in India in the year 2015 was equivalent to 4.5% of its total population (WHO, 2017). The depression may be caused by functional deficits of monoamines (norepinephrine, serotonin and dopamine) at certain sites in brain such as limbic system, frontal cortex and hippocampus (Gold, Goodwin, Chrousus, 1998; Tanabe, Nomura, Rinsho, 2007). Monoamine oxidase (MAO) is an enzyme responsible for metabolizing monoamines such as nor-epinephrine, dopamine and serotonin. In case of depression, the levels of MAO in brain increase which leads to decrease in the levels of monoamines. Selective MAO-A inhibitors such as brofaromine and moclobemide are as effective as the tricyclic antidepressants (Lotufo-Neto, Tridevi, Thase, 1999).

In addition to the role of monoamines in depression, there is co-existence of increased oxidative stress with depression-like behaviour in patients, as evidenced by defective plasma antioxidant defence in association with

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enhanced susceptibility to lipid peroxidation (Maes*et al*., 2000; Bilici*et al*., 2001). Levels of malondialdehyde were raised in patients with affective disorders (Ozcan*et al*., 2004). So exogenous antioxidants may be effective in treating depression.

Nitric oxide (NO) is an important neurotransmitter in the nervous system and regulates many behavioural, cognitive and emotional processes, including depression (Harvey, 1996). Nitrite is the stable end product of nitric oxide in living system. Stressful situations in mice have been reported to significantly increase plasma nitrite levels. Imipramine and venlafaxine showed protective effect against acute immobilization-induced behavioral and biochemical alterations in mice through involvement of nitric oxide mechanism (Kumar *et al*., 2009). Thus, nitrosative stress seems to be an important contributor of stress-induced depression.

There is hyper secretion of corticotrophin-releasing hormone in depressed patients (Pariante, Lightman 2008). Chronic hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis may also be responsible for depression (Schutter, 2012). When chronic stress is given to animals, hyperactivity of HPA axis has been observed (Young *et al*., 1991). Mice subjected to CUMS (chronic unpredictable mild stress) develop depressive symptoms similar to human depression (Willner*et al*., 1987; Willner, 1997). CUMS-induced increase in brain oxidative stress has been considered as a major factor for neurotoxicty and neuronal death, which may be lead to development of chronic stress-induced depression (Madrigal *et al*., 2001).

Caesalpinia pulcherrima(L.) Sw., (Family: Fabacaeae; Sub-family: Caesalpinioideae) is a leguminous, perennial large shrub native of South America. In India, it is cultivated as an ornamental plant. Commonly, it is known as Guletura, Gulutura (Hindi), Peacock flower, Barbados pride (English), Ratnagandhi(Sanskrit) (Kumar *et al*., 2009). *Caesalpinia pulcherrima* leaves extract have been reported to possess anticonvulsant (Kumar *et al*., 2009), weight lowering (Christina *et al*., 2011), anti-ulcerogenic (Ayaz*et al*., 2015), wound healing (Kavith, Naira, 2012), anti-fertility activities (Kumar *et al*., 2013). Traditionally, leaves of *Caesalpinia pulchirrima* have been reported to possess purgative, tonic, antipyretic, emmenagogue activities, while roots have folkfore use in convulsions, intermittent fever, lungs and skin diseases (Chatterjee, Prakashi, 2006).

Since *Caesalpinia pulcherrima* leaves have been reported to possess anticonvulsant property (Kumar *et al*., 2009), so leaves of this plant might possess neuroprotective property. Further, leaves of this plant have not been evaluated for antidepressant activity. So the present study was designed to evaluate the effect of ethanol extract of *Caesalpinia pulcherrima* leaves on chronic unpredictable mild stress-induced depression in mice by employing behavioural models such as tail suspension test and sucrose preference test. Further, the mechanisms of action of the extract on CUMS-induced depression were also explored by carrying out estimations of plasma corticosterone and nitrite levels; brain MAO-A and catalase activities; malondialdehyde and reduced glutathione levels.

MATERIAL AND METHODS

Experimental animals

Swiss male albino mice (3 months old, weighing around 25–30 g) were purchased from Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar (Haryana, India). The female mice were excluded from the present study because estrogen (female sex hormone) has been reported to have antidepressant effect (Kandi, Hayslett 2011). Animals were housed separately in groups of 08 per cage (Polypropylene cage size: 29×22×14 cm) under laboratory conditions with alternating light and dark cycle of 12 h each. The animals had free access to food and water. The animals were kept fasted 2 h before and 2 h after drug administration. The animals were acclimatized for at least five days before behavioural experiments which were carried out between 09:00 and 17:00 h. The experimental protocol was approved by Institutional Animals Ethics Committee (IAEC) in its meeting held on 18th May, 2017 (letter number of minutes of IAEC meeting - IAEC/2016/26-34, dated 5th December, 2017). Animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Fisheries, Animal Husbandry and Dairying, Government of India (Registration No. CPCSEA/436/PO/ReBi/S/2001).

Drugs and chemicals

Fluoxetine (Psychotropic India Limited, Haridwar, India), *N*-(1-Naphthyl) ethylenediaminedihydrochloride, p-nitroso-*N*,*N*-dimethylaniline, 5-hydroxytryptamine creatinine sulphate monohydrate, thiobarbituric acid (HiMedia Laboratories Pvt. Ltd., Mumbai), sulphanilamide, meta-phosphoric acid, potassium ferricyanide, hydrogen peroxide, trichloro acetic acid

(CDH), 5,5-Dithio*bis*-2-(nitro benzoic acid) (SRL), sulfosalicylic acid (Spectrochem), total protein estimation kit, (Siemens, Siemens Ltd., Vadodara, Gujrat); were used in the present study. Fluoxetine was dissolved in normal saline (0.9% w/v sodium chloride). Ethanol extract of leaves of Caesalpinia pulcherrima was dissolved in 1% w/v carboxy methyl cellulose.

Plant material

The leaves of *Caesalpinia pulcherrima* were collected in December, 2016 from campus of Guru Jambheshwar University of Science &Technology, Hisar (Haryana), India. The plant species (Reference no. NHCP/NBPGR/2016-19) was identified by Dr. Anjula Pandey, Principal Scientist, in the Institute of Division of Plant Exploration and Germplasm Collection, National Herbarium of Cultivated Plants (NHCP), ICAR- National Bureau of Plant Genetic Research (NBPGR), New Delhi, as *Caesalpinia pulcherrima* (L.) Sw. (Family: Caesalpiniaceae). Collected plant material was shade dried, coarsely powdered and used for further studies.

Preparation of extract

Shade dried powdered leaves were defatted with petroleum ether and extraction was done by using 70% v/v ethanol as per the reported method (Zhu *et al*., 2010). Defatting of the powdered leaves was done using petroleum ether for 24 hours. This was followed by extraction of the defatted leaves using 70% v/v ethanol on soxhlet apparatus at 100 ºC for 24 hours. Solvent was then evaporated under vacuum and the dried extract was stored below 10 ºC in a desiccator.

Gas chromatography-mass spectroscopy (GC-MS) analysis of extract

The extract of leaves of *Caesalpinia pulcherrima* (50 mg/mL) was injected (1 µL) into gas chromatogram GCMS-QP2010 Plus computerized system (Shimadzu Corporation, Kyoto, Japan) by using an auto injector (AOC-20i) connected with it. For separation of components, Rtx-5MS (crossbond, 5% diphenyl/95% dimethyl polysiloxane) capillary column (Restek Corporation, Bellefonte, USA) with dimensions 30 m (length) × 0.25 mm (diameter) × 0.25 µm (film thickness) was used. GC-MS spectra was obtained using the following conditions: interface temperature 260 ºC, ion source temperature 230 ºC, solvent cut off time 2.50 minute, ionization mode - electronic impact at 70 eV and m/z range 40 to 990. Carrier gas used was helium (>99.999%) with flow rate 1.21 mL/min in split mode (10:1). Injection temperature was 250 ºC and sample injection volume was 1.0 µL. Programmed oven temperature was 100 ºC for 3 minutes and then increased to 280 ºC at a rate of 10 ºC/min and held at 280 ºC for next 19 minutes. Constituents in ethanol extract of leaves of *Caesalpinia pulcherrima* were identified by their retention index which was determined relative to the alkane homologous series injected with the sample. The GC solution software post run analysis option and compound responsible for each peak were confirmed by matching their mass fragmentation patterns to the National Institute of Standard Technology (NIST) Library and Wiley Library.

Selection of doses

The doses - 100, 200 and 400 mg/kg of ethanol extract of leaves of *Caesalpinia pulcherrima* (Kumar *et al*., 2009) and fluoxetine (20 mg/kg) were employed.

Chronic unpredictable mild stress procedure

Animals were subjected to chronic unpredictable mild stress (CUMS) as described by Kumar *et al*. (2011). The mice were subjected to stress paradigm between 9.00 AM to 5.00 PM once a day over a period of 3 weeks. The order of stressors was as follows:

Weeks	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6	Day-7
Week-1	I	F	E	O	T1	X	T2
Week-2	O	X	I	T2	F	T1	E
Week-3	O	F	T1	X	T2	I	E

I—Immobilization for 2 h; F—Exposure to foreign object for 24 h (e.g. piece of plastic); E—Exposure to empty water bottles for 1 h; O— overnight illumination; T1—tail pinch (30 s); X—Tilted cage at 45 degree for 7 h; T2—tail pinch (60 s);

Tail suspension test

Tail suspension test (TST) is a widely used behavioural model for evaluating effect of drugs on depressant-like behaviour in mice (Steru *et al*., 1985). In this test, the mice were individually suspended 50

cm above the surface of a floor, using an adhesive tape placed 1 cm away from the tip of the tail. 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.

Sucrose preference test

Sucrose preference test (Willner *et al*., 1987) was employed herein to determine anhedonia, one of the core symptoms of major depression in humans. After 1 week of acclimatization, mice were trained to consume 1% (w/v) sucrose solution before the start of the CUMS protocol. In training course, mice were deprived of food and water for 48 h and only exposed to 1% w/v sucrose solution. Three days later, after 23-h food and water deprivation, 1-h baseline test was performed, in which mice could select between two pre-weighed bottles, one with 1% w/v sucrose solution and the other with tap water. Then, the sucrose preference was calculated according to the following formula:

Sucrose preference (%)= A/A+B × 100

Where A indicates sucrose solution intake in gram; B indicates water intake in gram.

The test was again performed on 22nd day to evaluate the effect of drug treatments in unstressed and stressed mice.

Measurement of Locomotor activity

Horizontal locomotor activities of control and test animals were recorded for a period of 5 min using photoactometer (INCO, Ambala, India). This was done to rule out the effects of various drug treatments on locomotor activity. The photoactometer operates on photoelectric cells which are connected in circuit with a counter. When the beam of light falling on the photocell is cut off by the movement of an animal, a count is recorded (Kumar, Kuhad, Chopra, 2011).

Experimental protocol

The animals were divided into following 20 groups (n = 8 mice in each group):

Groups for tail suspension test (TST)

Groups 1 to 5: Vehicle (1% w/v CMC), ethanol extract of leaves of *Caesalpinia pulcherrima* (100, 200 and 400 mg/kg) and fluoxetine (20 mg/kg) respectively were administered orally to mice for 21 successive days. TST was performed on the mice 60 min after vehicle/drug administration. On 22nd day, locomotor activities of these animals were recorded.

Groups 6 to 10: Vehicle (1% w/v CMC), ethanol extract of leaves of *Caesalpinia pulcherrima* (100, 200 and 400 mg/kg) and fluoxetine (20 mg/kg) respectively were administered orally 30 min before induction of stress to mice for 21 successive days. TST was performed on the mice 60 min after vehicle/drug administration. On 22nd day, locomotor activities of these animals were recorded.

Groups for sucrose preference test

Groups 11 to 20: Separate mice were employed for sucrose preference test, but their treatments were same as mentioned under groups 1 to 10.

Biochemical estimations: Collection of blood samples

After subjecting unstressed and stressed mice to testing of locomotor activity on 22nd day and one hour after drug administration on 23rd day, blood (1.0–1.5 mL) was withdrawn from retro-orbital plexus of mice. Plasma was separated using refrigerated centrifuge (Remi, Mumbai, India) at 350 *g* for 10 min and employed for estimation of corticosterone and nitrite levels.

Estimation of plasma nitrite levels

Plasma nitrite was measured by using the method of Green *et al*., 1982. A mixture of 1% w/v sulphanilamide in 5% w/v aqueous solution of m-phosphoric acid (1 part) and 0.1% (w/v) *N*-(1-Naphthyl) ethylene diamine dihydrochloride (1 part) was prepared and kept at 0 ºC for 60 min. 0.5 mL plasma was mixed with 0.5 mL of the above mixture and kept in dark for 10 min at room temperature. The absorbance was read at 546 nm using UV–visible spectrophotometer (Varian Cary 5000 UV–VIS–NIR Spectrophotometer, The Netherlands).

Estimation of plasma corticosterone levels

Plasma corticosterone levels were measured by the method of Bartos, Pesez, 1979. To 1.0 mL of sample in ethanol, 0.50 mL of 0.10% w/v solution of *p*-nitroso-*N*,*N*-dimethylaniline in ethanol was added and the tubes were

immersed in ice water for 5 min; and then 0.50 mL of 0.10 M sodium hydroxide was added. The tubes were plugged with cotton-wool, and were let to stand at 0 ºC for 5 h, protected against light. To the above solution, 2.0 mL of sodium carbonate/biocarbonate buffer (pH 9.8), 5.0 mL of 0.10% solution of phenol in ethanol and 0.50 mL of 1.0% w/v aqueous solution of potassium ferricyanide were added. The tubes were kept in a water bath at 20 ± 2 ºC for 10 min. The solution was read at 650 nm using UV–visible spectrophotometer (Varian Cary 5000 UV–VIS–NIR Spectrophotometer, The Netherlands).

Biochemical estimations in brain homogenate

After withdrawing blood samples on 23rd day, the mice were sacrificed by decapitation and their brains were isolated. Isolated brain samples were washed with cold 0.25 M sucrose–0.1 M Tris–0.02 M ethylenediamine tetraacetic acid buffer (pH 7.4) and weighed. The buffer washed brain sample was homogenized using a potter teflon glass homogenizer in 9 volumes of cold 0.25 M sucrose–0.1 M Tris–0.02 M ethylenediamine tetraacetic acid buffer (pH 7.4) buffer and centrifuged twice at 350 *g* for 10 min at 4 ºC in a cooling centrifuge (Remi Instruments, Mumbai, India). The pellet was discarded and the supernatant was collected, which was then centrifuged at 8064 *g* for 20 min at 4 ºC in a cooling centrifuge. This centrifuged supernatant was separated into two parts:
Part I: The precipitates (mitochondrial fraction) were used for estimation of MAO-A activity.
Part II: The remaining supernatant was used to assay lipid peroxidation, reduced glutathione and catalase activity.

Measurement of MAO-A activity

MAO-A activity was assessed spectrophotometrically (Charles, McEwan, 1977; Schurr, Livne, 1976). The mitochondrial fraction of brain was washed twice with about 100 mL of sucrose–Tris– EDTA buffer and suspended in 9 volumes of cold sodium phosphate buffer (10 mM, pH 7.4, containing 320 mM sucrose) and mingled well at 4 ºC for 20 min. The mixture was then centrifuged at 12600 *g* for 30 min at 0 ºC and the pellets were again suspended in cold sodium phosphate buffer. Then, 2.75 mL sodium phosphate buffer (100 mM, pH 7.4) and 100 µL of 4 mM 5-hydroxytryptamine were mixed in a quartz cuvette which was placed in UV–visible spectrophotometer (Varian Cary 5000 UV–VIS–NIR Spectrophotometer, The Netherlands). This was followed by the addition of 150 µL solution of mitochondrial fraction to initiate the enzymatic reaction and the change in absorbance was recorded at 280 nm for 5 min against the blank containing sodium phosphate buffer and 5-hydroxytryptamine.

Estimation of protein concentration

Estimation of total protein concentration in brain homogenate was done by using a total protein kit (Siemens Ltd., Vadodara, Gujrat), using semi-automatic autoanalyzer (Chem 5 plus-V2 semi-autoanalyzer; Erba Mannheim, Germany). Total protein concentration was estimated by Biuret method at 546 nm wavelength. The protein standard used was albumin which was supplied along with the kit (Henry, Winkelman, 1974).

Estimation of lipid peroxidation

The thiobarbituric acid-reactive substances (TBARS), a measure of lipid peroxidation, were assayed by the method of Wills (1965). Briefly, 0.5 mL of post mitochondrial supernatant and 0.5 mL of Tris– HCl were incubated at 37 ºC for 2 h. After incubation, 1 mL of 10% w/v trichloroacetic acid was added and centrifuged at 5 *g* for 10 min. To 1 mL of supernatant, 1 mL of 0.67% w/v thiobarbituric acid was added, and the tubes were kept in boiling water for 10 min. After cooling, 1 mL of double distilled water was added, and absorbance was measured at 532 nm. Thiobarbituric acid-reactive substances were quantified using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} and expressed as nanomole of malondialdehyde equivalents per milligram protein.

Estimation of reduced glutathione

Reduced glutathione was assayed by the method of Jollow *et al*. (1974). Briefly, 1.0 mL of post mitochondrial supernatant (10% v/v) was precipitated with 1.0 mL of sulfosalicylic acid (4% w/v). The samples were kept at 4 ºC for at least 1 h and then subjected to centrifugation at 81*g* for 15 min at 4 ºC. The assay mixture contained 0.1 mL supernatant, 2.7 mL phosphate buffer (0.1 M, pH 7.4), and 0.2 mL 5,5-dithio*bis*-(2-nitrobenzoic acid) (Ellman's reagent, 0.1 mM, pH 8.0) in a total volume of 3.0 mL. The yellow colour developed was read immediately at 412 nm. GSH levels were calculated by using molar extinction coefficient as 1.36×104 M^{-1} cm^{-1} and expressed as micromole per milligram protein.

Estimation of catalase activity.

Catalase activity was assayed by the method of Claiborne (1985). Briefly, the assay mixture consisted of 1.95 mL phosphate buffer (0.05 M, pH 7.0), 1.0 mL hydrogen peroxide (0.019 M), and 0.05 mL post mitochondrial supernatant (10% v/v) in a final volume of 3.0 mL. Changes in absorbance were recorded at 240 nm. Catalase activity was quantified using the extinction coefficient of H_2O_2 (43.6 M^{-1} cm^{-1}) and expressed as micromoles of H_2O_2 decomposed per minute per milligram protein.

Statistical analysis

All the results are expressed as Mean ± S.E.M. Data were analyzed by one - way ANOVA (analysis of variance) followed by Tukey–Kramer multiple comparison test using Graph Pad Instat (GPIS) package, version 3.05. $p < 0.05$ was considered as statistically significant.

RESULTS

Gas chromatography-mass spectroscopy (GC-MS) analysis of extract

In GC-MS of ethanol extract of *Caesalpinia pulcherrima* leaves, 66 components were detected (Table I, Figure 1). Some of the important constituents detected include vitamin E, beta-sitosterol, inositol, squalene, ethyl palmitate and benzoic acid. Among these constituents, beta-sitosterol (0.96%) (Zhao *et al*., 2016), inositol (61.28%) (Einat *et al*., 2001) have been reported to possess antidepressant activity.

TABLE I – Constituents detected in ethanol extract of leaves of *Caesalpinia pulcherrima*

S. No.	Chemical constituent	% area	Molecular formula
	4-Oxopentanethioic acid	0.28	$C_5H_8O_2S$
	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furanone	0.10	$C_6H_8O_4$
	1,2,3-propanetriol	0.25	$C_3H_8O_3$
	4-Methoxy-2,6-dipropyl-1,3-dioxane	0.19	$C_{11}H_{22}O_3$
	2-Methyl pyromeconic acid	0.39	$C_6H_6O_3$
	Furaneol	0.07	$C_6H_8O_3$
	Isopentyl acetate	15.06	$C_7H_{14}O_2$
	4H-1,3,2-dioxazine-2-acetic acid	0.87	$C_9H_{17}NO_4$
	2-Methyldecahydronaphthalene	0.04	$C_{11}H_{20}$
	Benzoic acid	0.83	$C_7H_6O_2$
	1-Dodecanol	0.07	$C_{12}H_{26}O$
	1,1,3,3-Tetramethyl-1,3-disiletane	0.23	$C_6H1_6Si_2$
	2,3-Dihydro-benzofuran	0.32	C_8H_8O
	2-butyl-3-methoxy-2-cyclopenten-1-one	0.22	$C_{10}H_{16}O_2$

(continuing)

TABLE I – Constituents detected in ethanol extract of leaves of *Caesalpinia pulcherrima*

S. No.	Chemical constituent	% area	Molecular formula
	Glycerol 1-acetate	0.60	$C_5H_{10}O_4$
	3,9-dimethylundecane	0.04	$C_{13}H_{28}$
	Ethyl 3-hydroxy-4-methylpentanoate	0.09	$C_8H_{16}O_3$
	Cis-2-vinyl-2,4-dimethyl-1,3-dioxolane	0.30	$C_7H_{12}O_2$
	Tridecane	0.18	$C_{13}H_{28}$
	2-methoxy-4-vinylphenol	0.04	$C_9H_{10}O_2$
	Nerolic acid	0.20	$C_{10}H_{16}O_2$
	1,2,3-benzenetriol	1.86	$C_6H_6O_3$
	1-tetradecene	0.09	$C_{14}H_{28}$
	Tetradecane	0.58	$C_{14}H_{30}$
	Cinnamic acid	0.14	$C_9H_8O_2$
	3,4,5-trimethylphenol	0.05	$C_9H_{12}O$
	1-chlorooctadecane	0.03	$C_{18}H_{37}Cl$
	3,7,11-trimethyldodeca-1,6,10-trien-3-ol	0.06	$C_{15}H_{26}O$
	Megastigmatrienone	0.52	$C_{13}H_{18}O$
	1-octadecanol	0.11	$C_{18}H_{38}O$
	Hexadecane	0.19	$C_{16}H_{34}$
	(-)-t-muurolol	0.04	$C_{15}H_{26}O$
	Myristic acid	1.08	$C_{14}H_{28}O_2$
	Inositol	61.28	$C_7H_{14}O_6$
	Morpholino 2-benzothiazolyl disulfide	0.06	$C_{11}H_{12}N_2OS_3$
	3,5,11,15-tetramethyl-1-hexadecen-3-ol	0.04	$C_{20}H_{40}O$
	L-(+)-ascorbic acid 2,6-dihexadecanoate	2.71	$C_{38}H_{68}O_8$
	Ethyl palmitate	0.29	$C_{18}H_{36}O_2$
	Pentacosane	0.13	$C_{25}H_{52}$

(continuing)

TABLE I – Constituents detected in ethanol extract of leaves of *Caesalpinia pulcherrima*

S. No.	Chemical constituent	% area	Molecular formula
	3,7,11,15-tetramethylhexadec-2-en-1-ol	3.98	$C_{20}H_{40}O$
	Linoleic acid	1.47	$C_{18}H_{32}O_2$
	2-(2-heptadecynyloxy)tetrahydro-2*H*-pyran	0.02	$C_{22}H_{40}O_2$
	14-methyl-8-hexadecyn-1-ol	0.03	$C_{17}H_{32}O$
	Stearic acid	0.69	$C_{18}H_{36}O_2$
	Ethyl (9z,12z)-9,12-octadecadienoate	0.12	$C_{20}H_{36}O_2$
	N-tetracosanol-1	0.22	$C_{24}H_{50}O$
	3-cyclopentylpropionic acid, 2-dimethylaminoethyl ester	0.05	$C_{12}H_{23}NO_2$
	1-bromodocosane	0.03	$C_{22}H_{45}Br$
	Palmidrol	0.05	$C_{18}H_{37}NO_2$
	2-dodecylcyclobutanone	0.04	$C_{16}H_{30}O$
	2,6,10,14-tetramethylhexadecane	0.03	$C_{20}H_{42}$
	3-cyclopentylpropionic acid, 2-dimethylaminoethyl ester	0.05	$C_{12}H_{23}NO_2$
	2-monopalmitin	0.48	$C_{19}H_{38}O_4$
	Longifolenaldehyde	0.06	$C_{15}H_{24}O$
	2,3-*bis*(acetyloxy)propyl palmitate	0.05	$C_{23}H_{42}O_6$
	3-(4-methoxyphenyl)propan-1-ol	0.14	$C_{10}H_{14}O_2$
	Squalene	0.38	$C_{30}H_{50}$
	Tetrapentacontane	0.06	$C_{54}H_{110}$
	2,2-dimethyl-3-(3,7,16,20-tetramethyl-heneicosa-3,7,11,15,19-pentaenyl)-oxirane	0.03	$C_{29}H_{48}O$
	2,2-dimethyl-3-[(3e,7e,11e,15e)-3,7,12,16,20-pentamethyl-3,7,11,15,19-henicosapentaenyl]oxirane	0.10	$C_{30}H_{50}O$
	Gamma-tocopherol	0.06	$C_{28}H_{48}O_2$
	Stigmast-5-en-3-yl 9-octadecenoate	0.07	$C_{47}H_{82}O_2$
	Vitamin-E	0.56	$C_{29}H_{50}O_2$

(continuing)

TABLE I – Constituents detected in ethanol extract of leaves of *Caesalpinia pulcherrima*

S. No.	Chemical constituent	% area	Molecular formula
	(2E)-7-ethoxy-3,7-dimethyl-2-octen-1-ol	0.18	$C_{12}H_{24}O_2$
	(22E)-stigmasta-5,22-dien-3-ol	0.11	$C_{29}H_{48}O$
	Beta-sitosterol	0.96	$C_{29}H_{50}O$

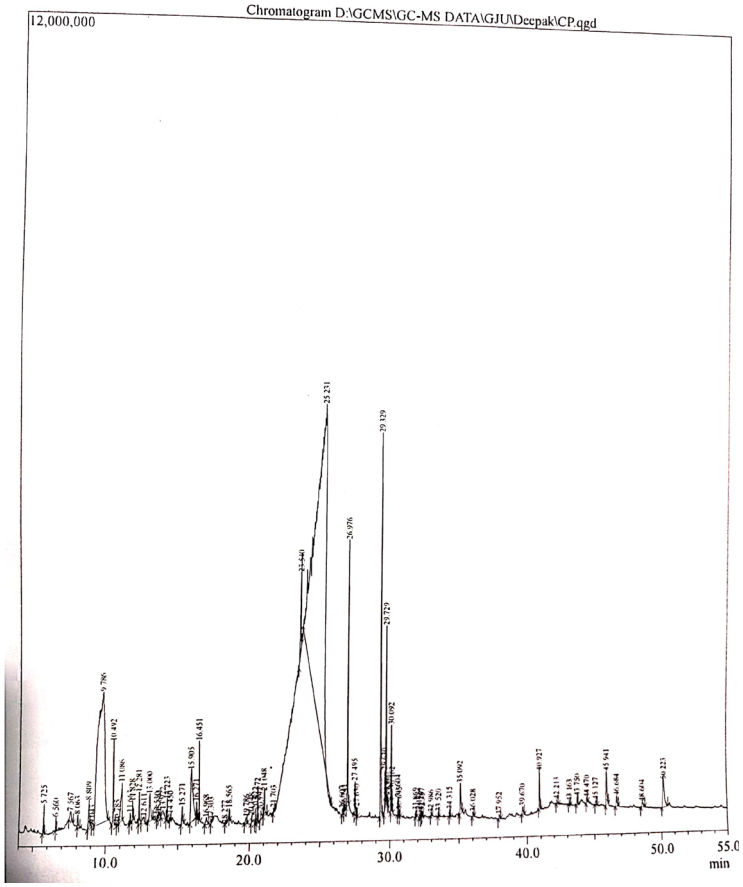

FIGURE 1 – GCMS of ethanol extract of leaves of *Caesalpinia pulcherrima.*

Effect of ethanol extract of leaves of *Caesalpinia pulcherrima* and fluoxetine on immobility time of mice in TST

Exposure of the mice to unpredictable mild stress for 21 successive days significantly ($p < 0.05$) increased their immobility time as compared to the unstressed mice. Ethanol extract of leaves of *Caesalpinia pulcherrima* (200 and 400 mg/kg, p.o.) and fluoxetine (20 mg/kg, p.o.) administered for 21 successive days significantly decreased immobility time of the unstressed mice ($p <0.05$) as compared to its vehicle treated control. Ethanol extract of leaves of *Caesalpinia pulcherrima* (100, 200 and 400 mg/kg, p.o.) and fluoxetine (20 mg/kg, p.o.) administered for 21 successive days also significantly decreased immobility time of the stressed mice ($p < 0.05$,$p < 0.001$, $p < 0.001$ and $p < 0.001$ respectively) as compared to vehicle treated stressed mice (Figure 2)

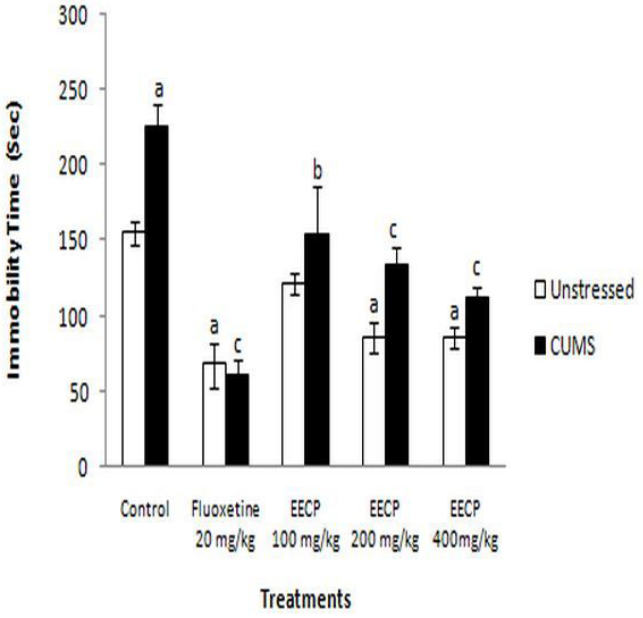

FIGURE 2 – Effect of ethanol extract of *Caesalpinia pulcherrima* and fluoxetine on immobility time of unstressed and stressed mice in tail suspension test.
U = unstressed mice; CUMS = chronic unpredictable mild stress.
Values are expressed as Mean ± S.E.M. The data were analyzed by one-way ANOVA followed by Tukey–Kramer multiple comparison test.
F (9, 70) =12.433; $p < 0.05$.
a = $p < 0.05$, as compared to vehicle treated unstressed mice.
b and c = $p < 0.05$ and $p < 0.001$, respectively as compared to vehicle treated stressed mice.
EECP stands for ethanol extract of *Caesalpinia pulcherrima*.

Effect of ethanol extract of leaves of *Caesalpinia pulcherrima* and fluoxetine on sucrose preference test

Exposure of the mice to unpredictable mild stress for 21 successive days significantly ($p < 0.01$) decreased their sucrose preference (%) as compared to the unstressed mice. There was no significant difference in sucrose preference (%) among all the groups in the baseline test. Ethanol extract of leaves of *Caesalpinia pulcherrima* (100, 200 and 400 mg/kg) and fluoxetine (20 mg/kg) administered for 21 successive days significantly restored the reduced sucrose preference (%) in the unstressed mice ($p < 0.05$, $p < 0.001$, $p < 0.001$ and $p < 0.001$) as compared to vehicle treated unstressed mice. Ethanol extract of leaves of *Caesalpinia pulcherrima* (200 and 400 mg/kg) and fluoxetine (20 mg/kg) significantly restored the reduced sucrose preference (%) in the stressed mice ($p < 0.001$, $p < 0.001$ and $p < 0.001$) as compared to vehicle treated stressed mice. There was no significant effect of the lowest dose (100 mg/kg) of the extract on sucrose preference of stressed mice as compared to its vehicle treated control (Table II).

TABLE II – Effect of ethanol extract of *Caesalpinia pulcherrima* (EECP) and fluoxetine on sucrose preference (%) of unstressed and stressed mice.

S. No.	Drug treatments for 21 days	Dose (kg^{-1})	Sucrose preference (%)- baseline test	Sucrose preference (%)- after 21 days
1	Vehicle (U)	10 mL	59.58±1.75	42.71±1.13
2	Vehicle(CUMS)	10 mL	56.71±1.25	37.06±1.18b
3	Fluoxetine (U)	20 mg	59.02±1.07	52.89±0.74c
4	EECP (U)	100 mg	57.64±1.76	48.06±1.83a
5	EECP(U)	200 mg	58.81±2.06	51.40±0.96c
6	EECP (U)	400 mg	56.94±0.99	51.98±0.73c

(continuing)

TABLE II – Effect of ethanol extract of *Caesalpinia pulcherrima* (EECP) and fluoxetine on sucrose preference (%) of unstressed and stressed mice.

S. No.	Drug treatments for 21 days	Dose (kg^{-1})	Sucrose preference (%)- baseline test	Sucrose preference (%)- after 21 days
7	Fluoxetine (CUMS)	20 mg	54.51±1.52	46.23±0.42d
8	EECP (CUMS)	100 mg	54.18±0.89	38.61±0.81
9	EECP (CUMS)	200 mg	55.23±1.24	45.90±0.56d
10	EECP (CUMS)	400 mg	54.92±0.80	45.91±0.82d

n = 8 each group. U = unstressed mice; CUMS = chronic unpredictable mild stress.
Values are expressed as Mean ± S.E.M. The data were analyzed by one-way ANOVA followed by Tukey–Kramer multiple comparison test.
For Sucrose preference (%)- baseline test; $F(9, 70) = 2.136$; $p < 0.05$.
For Sucrose preference (%)- after 21 days; $F(9, 70) = 29.397$; $p < 0.05$.
a, b and c = $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively as compared vehicle treated unstressed mice.
d = $p < 0.001$, as compared to vehicle treated stressed mice.
EECP stands for Ethanol extract of *Caesalpinia pulchirrhima*

Effect of ethanol extract of leaves of *Caesalpinia pulcherrima* and fluoxetine on locomotor activity of mice

Various treatments did not significantly affect the spontaneous loco motor activities of unstressed and stressed mice as compared to their respective vehicle treated controls (Table III).

TABLE III – Effect of ethanol extract of leaves of Caesalpinia pulcherrima and fluoxetine on locomotor activity

S. No.	Drug treatments for 21 days	Dose (kg-1)	Locomotor activity
1	Vehicle (U)	10 mL	230.25±11.10
2	Vehicle(CUMS)	10 mL	314.88±17.60
3	Fluoxetine (U)	20 mg	278.00±35.59
4	EECP (U)	100 mg	362.88±12.74
5	EECP (U)	200 mg	361.75±55.81
6	EECP (U)	400 mg	309.38±25.63
7	Fluoxetine (CUMS)	20 mg	415.38±35.76
8	EECP (CUMS)	100 mg	319.88±21.32
9	EECP (CUMS)	200 mg	293.00±20.05
10	EECP (CUMS)	400 mg	292.50±28.25

(continuing)

TABLE III – Effect of ethanol extract of leaves of Caesalpinia pulcherrima and fluoxetine on locomotor activity

n = 8 each group. U = unstressed mice; CUMS = chronic unpredictable mild stress.
Values are expressed as Mean ± S.E.M. The data were analyzed by one-way ANOVA followed by Tukey–Kramer multiple comparison test.
For Locomotor activity; $F(9, 70) = 3.122$; $p < 0.05$.
EECP stands for Ethanol extract of *Caesalpinia pulchirrhima*

Effect of ethanol extract of leaves of *Caesalpinia pulcherrima* and fluoxetine on plasma nitrite levels

Plasma nitrite levels were significantly ($p < 0.001$) increased in the mice subjected to chronic unpredictable mild stress. Ethanol extract of leaves of *Caesalpinia pulcherrima* (200 and 400 mg/kg) and fluoxetine (20 mg/kg) administered for 21 successive days significantly ($p < 0.01$, $p < 0.01$ and $p < 0.001$ respectively) decreased plasma nitrite levels in the unstressed mice. But lowest dose (100 mg/kg) of the extract did not significantly decrease plasma nitrite levels in both unstressed and stressed mice as compared to their respective vehicle treated controls. The leaves extract (200 and 400 mg/kg) and fluoxetine (20 mg/kg) administered for 21 successive days significantly ($p < 0.05$, $p < 0.001$ and $p < 0.001$ respectively) decreased plasma nitrite levels in the stressed mice as compared to their vehicle treated control (Figure 3).

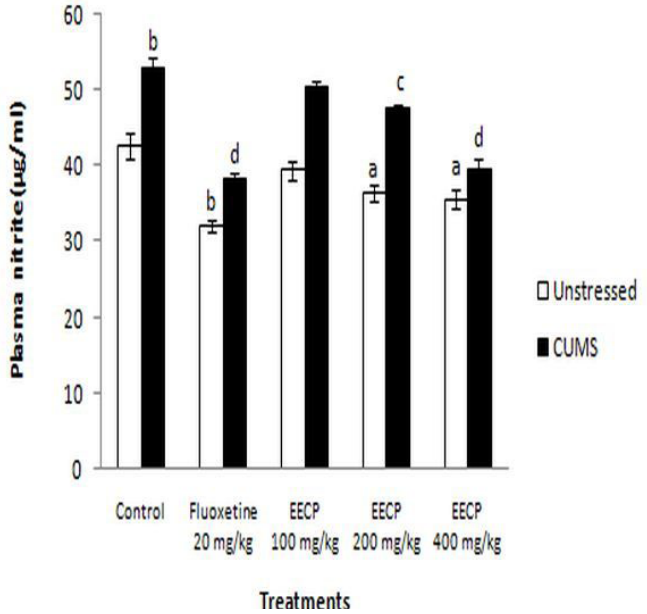

FIGURE 3– Effect of ethanol extract of *Caesalpinia pulcherrima* and fluoxetine on plasma nitrite level of unstressed and stressed mice.
U = unstressed mice; CUMS = chronic unpredictable mild stress.
Values are expressed as Mean ± S.E.M. The data were analyzed by one-way ANOVA followed by Tukey–Kramer multiple comparison test.
F (9, 70) = 35.759; $p < 0.05$.
a and b= $p < 0.01$ and $p < 0.001$, respectively as compared to vehicle treated unstressed mice.
c and d = $p < 0.05$ and $p < 0.001$, respectively as compared to vehicle treated stressed mice.
EECP stands for ethanol extract of *Caesalpinia pulcherrima*.

Effect of ethanol extract of leaves of *Caesalpinia pulcherrima* and fluoxetine on plasma corticosterone levels

Chronic unpredictable mild stress significantly ($p < 0.001$) increased plasma corticosterone levels as compared to vehicle treated unstressed mice. Ethanol extract of leaves of *Caesalpinia pulcherrima* (100, 200 and 400 mg/kg) and fluoxetine (20 mg/kg) administered for 21 successive days significantly ($p < 0.05$, $p < 0.001$, $p < 0.001$ and $p < 0.001$ respectively) decreased corticosterone levels of both unstressed and stressed mice as compared to their respective vehicle treated control (Figure 4).

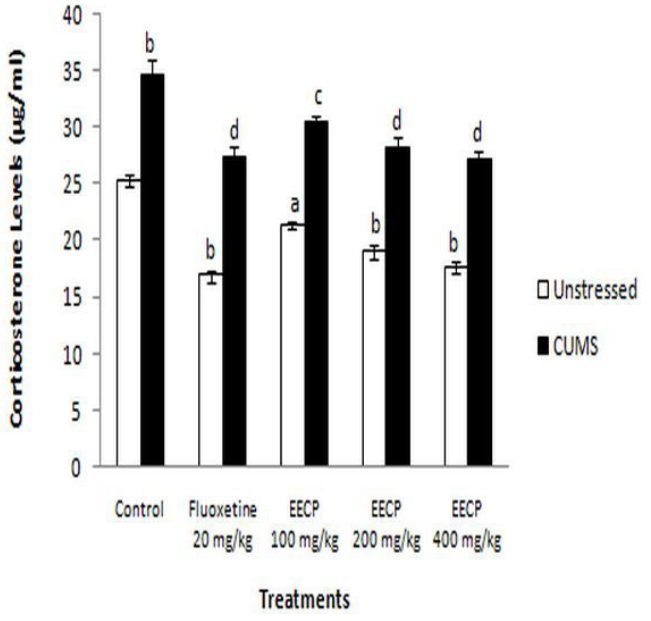

FIGURE 4 – Effect of ethanol extract of *Caesalpinia pulcherrima* and fluoxetine on plasma corticosterone level of unstressed and stressed mice.
U = unstressed mice; CUMS = chronic unpredictable mild stress.
Values are expressed as Mean ± S.E.M. The data were analyzed by one-way ANOVA followed by Tukey–Kramer multiple comparison test.
F (9, 70) = $p < 0.05$.
a and b = $p < 0.05$ and $p < 0.001$, respectively as compared to vehicle treated unstressed mice.
c and d= $p < 0.05$ and $p < 0.001$, respectively as compared to vehicle treated stressed mice.
EECP stands for ethanol extract of *Caesalpinia pulcherrima*.

Effect of ethanol extract of leaves of *Caesalpinia pulcherrima* and fluoxetine on brain MAO-A activity

Chronic unpredictable mild stress significantly ($p < 0.001$) increased brain MAO-A activity as compared to vehicle treated unstressed mice. Ethanol extract of leaves of *Caesalpinia pulcherrima* (100, 200 and 400 mg/kg) and fluoxetine (20 mg/kg) administered for 21 successive days significantly ($p < 0.05$) decreased MAO-A activity in unstressed mice as compared to vehicle treated unstressed mice. The extract (100, 200 and 400 mg/kg) and fluoxetine (20 mg/kg) also significantly ($p < 0.05$, $p < 0.001$, $p < 0.001$ and $p < 0.001$) decreased brain MAO-A activity in stressed mice as compared to vehicle treated stressed mice (Figure 5).

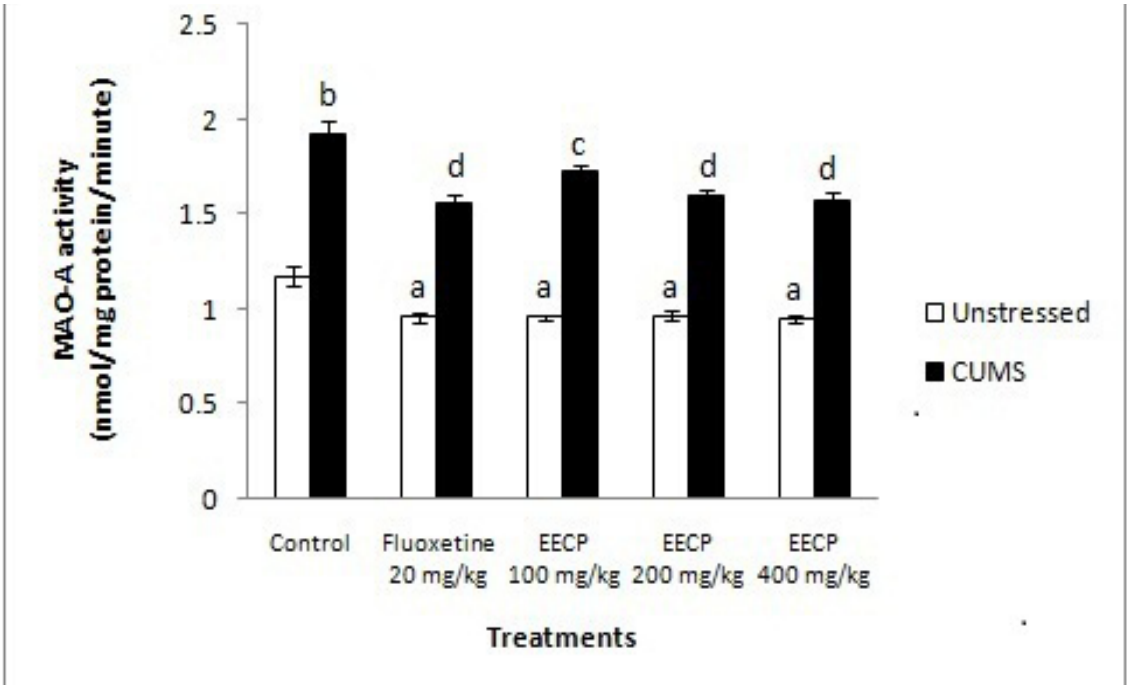

FIGURE 5 – Effect of ethanol extract of *Caesalpinia pulcherrima* and fluoxetine on MAO- A activity of unstressed and stressed mice.
U = unstressed mice; CUMS = chronic unpredictable mild stress.
Values are expressed as Mean ± S.E.M. The data were analyzed by one-way ANOVA followed by Tukey–Kramer multiple comparison test.
$F(9, 70) = 75.318$; $p < 0.05$.
a and b = $p < 0.05$ and $p < 0.001$, respectively as compared to vehicle treated unstressed mice.
c and d= $p < 0.05$ and $p < 0.001$, respectively as compared to vehicle treated stressed mice.
EECP stands for ethanol extract of *Caesalpinia pulcherrima*.

Effect of ethanol extract of leaves of *Caesalpinia pulcherrima* and fluoxetine on brain malondialdehyde equivalents

Levels of malondialdehyde equivalents were increased significantly ($p < 0.001$) in the mice subjected to CUMS as compared to the vehicle treated unstressed mice. Ethanol extract (100, 200 and 400 mg/kg) and fluoxetine (20 mg/kg) administered for 21 days significantly ($p < 0.05$, $p < 0.05$, $p < 0.01$ and $p < 0.05$, respectively) decreased malondialdehyde equivalents levels in the unstressed mice as compared to vehicle treated unstressed mice. The extract (100, 200 and 400 mg/kg) and fluoxetine (20 mg/kg) also significantly ($p < 0.001$ respectively) decreased malondialdehyde equivalents levels in the stressed mice as compared to vehicle treated stressed mice (Figure 6).

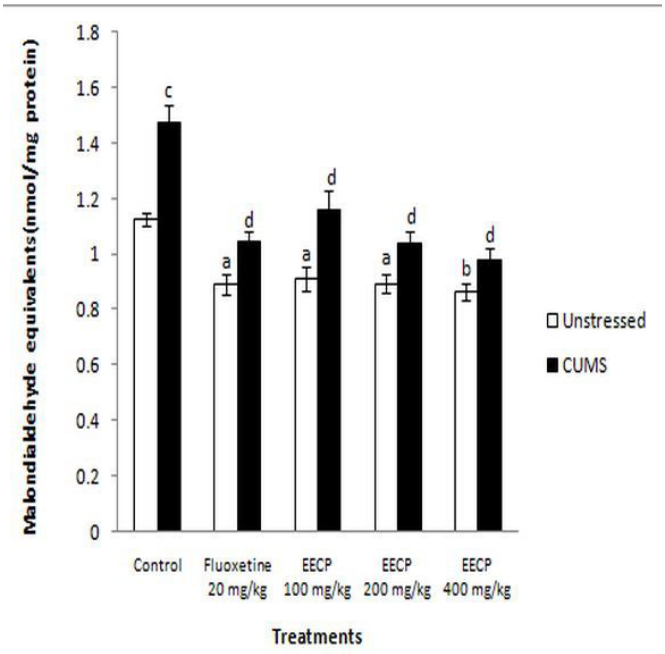

FIGURE 6 – Effect of ethanol extract of *Caesalpinia pulcherrima* and fluoxetine on brain malondialdehyde equivalents of unstressed and stressed mice.
U = unstressed mice; CUMS = chronic unpredictable mild stress.
Values are expressed as Mean ± S.E.M. The data were analyzed by one-way ANOVA followed by Tukey–Kramer multiple comparison test.
F (9, 70) = 15.942; $p < 0.05$.
a, b and c = $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively as compared to vehicle treated unstressed mice.
d = $p < 0.001$, as compared to vehicle treated stressed mice.
EECP stands for ethanol extract of *Caesalpinia pulcherrima*.

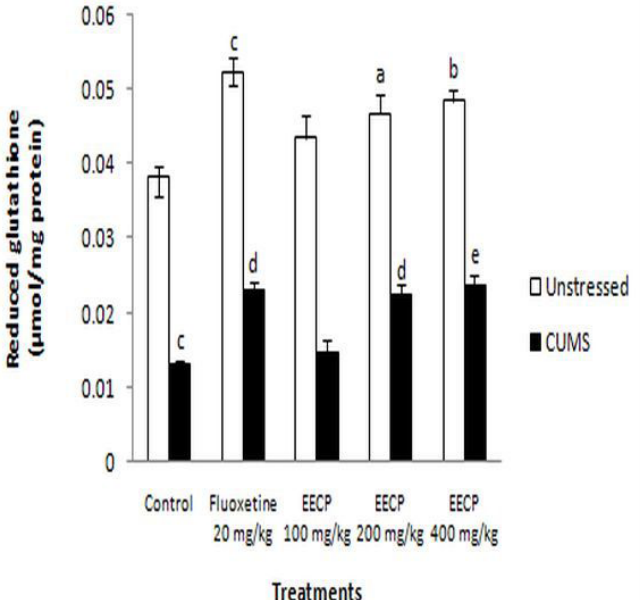

FIGURE 7 – Effect of ethanol extract of *Caesalpinia pulcherrima* and fluoxetine on brain reduced glutathione of unstressed and stressed mice.
U = unstressed mice; CUMS = chronic unpredictable mild stress.
Values are expressed as Mean ± S.E.M. The data were analyzed by one-way ANOVA followed by Tukey–Kramer multiple comparison test.
F (9, 70) = 62.521; $p < 0.05$.
a, b and c = $p < 0.05$ $p < 0.01$ and $p < 0.001$ respectively as compared to vehicle treated unstressed mice.
d and e = $p < 0.05$ and $p < 0.01$ respectively as compared to vehicle treated stressed mice.
EECP stands for ethanol extract of *Caesalpinia pulcherrima*.

Effect of ethanol extract of leaves of *Caesalpinia pulcherrima* and fluoxetine on brain reduced glutathione levels

Reduced glutathione levels were significantly ($p < 0.001$) decreased in the stressed mice as compared to vehicle treated unstressed mice. Lowest dose (100 mg/kg) of the extract administered for 21 successive days did not significantly increase the reduced glutathione levels in both unstressed and stressed mice as compared to their respective vehicle treated controls. But higher doses of the extract (200 and 400 mg/kg) and fluoxetine (20 mg/kg) administered for 21 successive days significantly increased reduced glutathione levels in both unstressed mice ($p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively) and stressed mice ($p < 0.05$, $p < 0.01$ and $p < 0.05$ respectively) as compared to their respective vehicle treated controls (Figure 7).

Effect of ethanol extract of leaves of *Caesalpinia pulcherrima* and fluoxetine on brain catalase activity

Catalase activity significantly ($p < 0.05$) decreased in brain of the stressed mice as compared to respective vehicle treated unstressed mice. Lowest dose of the extract (100 mg/kg) administered for 21 successive days did not significantly increase catalase activity in the unstressed mice as compared to its vehicle treated control. But the higher doses of the extract (200 and 400 mg/kg) and fluoxetine (20 mg/kg) administered for 21 successive days significantly ($p < 0.05$, $p < 0.05$ and $p < 0.01$ respectively) increased catalase activity in the unstressed mice as compared to their vehicle treated control. All the three doses (100, 200 and 400 mg/kg) of the extract and fluoxetine (20 mg/kg) administered for 21 successive days significantly ($p < 0.05$, $p < 0.01$, $p < 0.01$ and $p < 0.001$ respectively) increased catalase activity in

the stressed mice as compared to vehicle treated stressed mice (Figure 8).

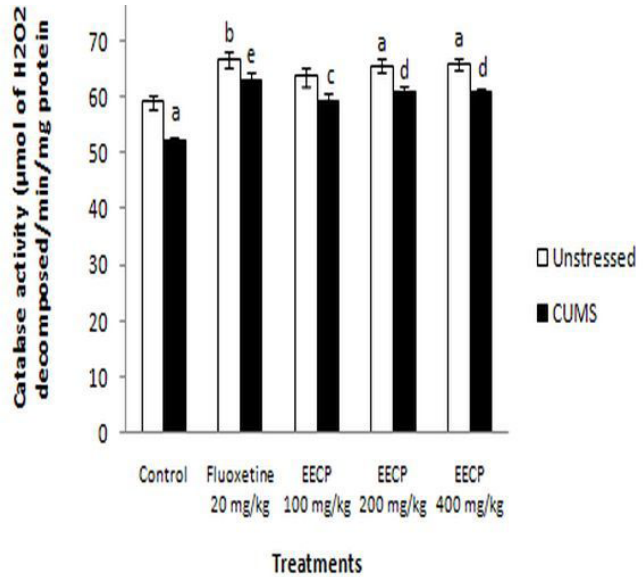

FIGURE 8 – Effect of ethanol extract of *Caesalpinia pulcherrima* and fluoxetine on brain catalase of unstressed and stressed mice.
U = unstressed mice; CUMS = chronic unpredictable mild stress.
Values are expressed as Mean ± S.E.M. The data were analyzed by one-way ANOVA followed by Tukey–Kramer multiple comparison test.
F (9, 70) = 10.717; $p < 0.05$.
a and b = $p < 0.05$ and $p < 0.01$, respectively as compared to vehicle treated unstressed mice.
c, d and e = $p < 0.05$ and $p < 0.01$ and $p < 0.001$, respectively as compared to vehicle treated stressed mice.
EECP stands for ethanol extract of *Caesalpinia pulcherrima*.

DISCUSSION

In the present investigation, ethanol extract of leaves of *Caesalpinia pulcherrima* administered for 21 successive days showed significant antidepressant-like activity in unstressed mice as well as in stressed mice. Induction of depression using CUMS is considered as the most valid animal model of depressive behaviour as observed in humans (Willner, 1991, 2005). TST (Steru*et al*., 1985) and sucrose preference test (Willner*et al*., 1987) were used to evaluate the effect of the drugs on depression-like behaviour in the mice. CUMS resulted in increase in immobility periods of mice in TST as compared to control unstressed animals, thus indicating induction of depression-like behaviour. Chronic treatment with fluoxetine (20 mg/kg, p.o.) and ethanol extract (100, 200 and 400 mg/kg, p.o.) per se produced significant decrease in immobility period of stressed mice in TST, indicating their significant antidepressant-like effects. Fluoxetine and ethanol extract (200 and 400 mg/kg, p.o.) also significantly decreased immobility period of unstressed mice in TST, indicating their antidepressant-like effect in unstressed mice also. Fluoxetine and ethanol extract did not affect the locomotor activities of both unstressed and stressed mice as compared to their respective controls, thus ruling out their CNS stimulant activities.

We also employed another model, sucrose preference test for evaluation of antidepressant-like activity of the extract in mice. This test is an indicator of anhedonia-like behaviour, such as loss of interest or pleasure observed in patients of depression. Anhedonia was modelled in mice by inducing a decrease in responsiveness to rewards reflected by reduced consumption and/or preference of sweetened solutions (Willner 1997, 2005). In our study, stressed mice showed a significant decrease in sucrose preference as compared to the unstressed mice. Sucrose preference was significantly restored in both unstressed and stressed mice after administration of fluoxetine (20 mg/kg, p.o.) and ethanol extract for 21 successive days, which suggested their antidepressant-like actions. Thus, the results obtained from behavioural studies indicated that ethanol extract of leaves of *Caesalpinia pulcherrima* produced significant antidepressant-like action in both unstressed and stressed mice.

Hypothalamic–pituitary–adrenal (HPA) axis is activated in response to stress, with resultant increase in circulating glucocorticoids such as corticosterone in rodents or cortisol in primates. Hyper-activation of HPA axis is associated with abnormally high blood glucocorticoid levels, which may eventually lead to depression (Pan *et al*., 2006). Cortisol is known to regulate neuronal survival, neuronal excitability, neurogenesis and memory acquisition. High levels of cortisol may contribute to the manifestation of depressive symptoms by impairing these brain functions (Sousa,Cerqueira, Almeida,2008). It has been reported that chronic antidepressant treatment in rodents reduced HPA activity (Mason, Pariante2006). Thus, restoration of a normal functional status of HPA axis may be involved in the treatment of clinical depression (Pan *et al*., 2006). In the present study, CUMS resulted in significant increase in serum corticosterone levels, which is also supported by observations from other studies (Swaab,Bao, Lucassen, 2005). Fluoxetine and ethanol extract significantly

reduced plasma corticosterone levels in both unstressed and stressed mice.

Reactive oxygen species (ROS) play a role in major depression. Activation of immune-inflammatory process, increased monoamine catabolism, and abnormalities in lipids may cause overproduction of ROS, lipid peroxidation, and reduced antioxidant enzyme activities, and these processes may lead to depression (Bajpai *et al*., 2014). In the present study, 21 days of exposure to different stressors resulted in increase of brain malondialdehyde and plasma nitrite levels; and decrease in brain reduced glutathione levels and catalase activity. This is supported by an earlier study where CUMS impaired the antioxidant status (increased lipid peroxidation and nitrite levels, decreased glutathione levels and catalase activity) of brain tissue, presumably through production of excessive reactive oxygen species (Kumar,Kuhad, Chopra, 2011; Dhingra, Bansal, 2015). Chronic administration of ethanol extract and fluoxetine per se showed significant decrease in brain malondialdehyde in both unstressed and stressed mice. Ethanol extract (200 and 400 mg/kg, p.o.) and fluoxetine also showed significant increase in brain reduced glutathione levels and catalase activity in both unstressed and stressed mice. Thus, ethanol extract and fluoxetine showed significant antioxidant activity in both unstressed and stressed mice. Stress has been reported to significantly increase plasma nitrite levels in rats (Lee, Cheng, Sim, 2007). Ethanol extract and fluoxetine significantly reduced nitrosative stress in mice, as indicated by reduction of plasma nitrite levels. Thus, ethanol extract of leaves of *Caesalpinia pulcherrima* showed strong protective effect against oxidative and nitrosative stress in mice.

Chronic exposure to different stressors led to increased activity of brain MAO-A. Chronic treatment with the ethanol extract significantly inhibited brain MAO-A activity in both unstressed and stressed mice. Further, antidepressant activity of the extract might be due to presence of inositol and beta-sitosterol which are present in the extract to the extent of 61.28% and 0.96%. Antidepressant activity of inositol and beta-sitosterol has been reported in the literature (Einat*et al*., 2001; Zhao *et al*., 2016).

In conclusion, ethanol extract of leaves of *Caesalpinia pulcherrima* showed significant antidepressant-like activity in both unstressed and stressed mice, probably through inhibition of brain MAO-A activity; decrease in plasma corticosterone levels and alleviation of oxidative and nitrosative stress. Thus, ethanol extract of leaves of *Caesalpinia pulcherrima* may be explored further for treatment of depression in humans.

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