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# ANTIDEPRESSANT-LIKE EFFECT OF ESSENTIAL OIL OF *CYMBOPOGON FLEXUOSUS* IN A CHRONIC UNPREDICTABLE MILD STRESS-INDUCED DEPRESSION MODEL IN MICE

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## Abstract

*Cymbopogon flexuosus*, a famous Indian perennial grass containing citral, is traditionally used to alleviate stress, anxiety and depression. *Cymbopogon flexuosus*, commonly known as lemongrass-are cultivated mainly for essential oils and are native to India. Due to lack of experimental studies, it is prerequisite to evaluate the antidepressant potential of *C. flexuosus* in experimental animals.

The present study was employed to explore the antidepressant potential of *C. flexuosus* in chronic unpredictable mild stress (CUMS) model induced depression-like behaviour in mice and to identify probable mechanism of action of *C. flexuosus* in amelioration of oxidative stress and depression.

Mice were subjected to 28 days of chronic unpredictable mild stress (CUMS) procedure, and treated with Cymbopogon flexuosus (50 and 100 mg/kg, po) or imipramine (15 mg/kg, po). CUMS induced depression like behavior was assessed by forced swimming test (FST) and tail suspension test (TST) and followed by treatment with *Cymbopogon flexuosus.* Furthermore, oxidative stress parameters were determined by measuring the levels of reduced glutathione (GSH), malondialdehyde (MDA) formation, nitrite, superoxide dismutase (SOD), catalase (CAT).

Lemongrass oil or imipramine significantly decreased immobility in CUMS mice as evaluated by FST and TST. In addition to this, lemongrass oil decreased malondialdehyde formation and nitrite levels, while glutathione, superoxide dismutase and catalase levels were found to be increased in the whole brain.

The results of the present study demonstrated that lemongrass oil attenuated the CUMS-induced depression in mice, by reduction of oxidative–nitrosative stress in the mice brain.

***Keywords:*** *Cymbopogon flexuosus*, depression, chronic unpredictable mild stress, oxidative stress, immobility.

## Introduction

Depression is a mental health disorder characterized primarily by a sustained low mood, hopelessness and lack of daily activities (Chen *et al.*, 2008) with a chance of 10 to 20% lifetime prevalence (Wong & Licinio, 2001). Based on World Health Organization (WHO) reports, depression is expected to rank second in causing disease-related disability by the year 2020.

The monoamine hypothesis delineates the cause of depression that suggests monoaminergic function impairment and the decrease of neurotransmitters such as serotonin (5-HT), norepinephrine (NE) and dopamine (DA) levels in the brain (Delgado, 2000; Chuang *et al.*, 2011). Several antidepressant drugs have been developed on the basis of monoamine hypothesis including tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), selective norepinephrine reuptake inhibitors (SNRIs), selective norepinephrine-dopamine reuptake inhibitors (SNDRI) and monoamine oxidase inhibitors (MAOIs), that function by inhibiting the transport of 5-HT, DA and NE to increase the concentration of these neurotransmitters in synaptic cleft by blocking their reuptake to relieve the symptoms of depression (Fava, 2003).

The classical antidepressants present limited efficacy and produce severe side effects including nausea, diarrhoea, sexual dysfunction, sedation, and sleep disorders that adversely affect health (Seol *et al.*, 2010). Also, these drugs are ineffective on a certain population of patients suffering from depression (Liu *et al.*, 2012). Therefore, there is a need to develop new antidepressants with lesser adverse effects; that can prove to be advantageous in treatment of majority of patients suffering from depression.

Recent studies suggest that herbal medicines may serve as prospective alternatives to treat depression due to their wider safety profile and enhanced tolerability in clinical patients. (Sarris, 2007; Iovieno *et al.*, 2011; Yeung *et al.*, 2018). Lemon grass (*Cymbopogon flexuosus* (Steud.) Wats) is one of the most important essential oil bearing crops of India. Due to high citral content in lemongrass essential oil (LEO), it has a strong lemon-like odour and possesses germicidal, medicinal and flavouring properties. Traditionally, lemongrass oil has analgesic, antiseptic, carminative, astringent, febrifuge, fungicidal, bactericidal and antidepressant properties (Ganjewala & Gupta, 2013).

The chronic unpredictable mild stress (CUMS) model of depression is a widely accepted model used in rodents that produces physiological effects, similar to clinical patients with major depression and is, therefore, used to study the symptoms of depression and mechanism of action of the new antidepressant drugs (Song & Leonard, 2005). The preclinical studies to evaluate the antidepressant properties of *C. flexuosus* have not been conducted till date. Hence, the objective of this study was to evaluate the behavioural and biochemical effects of the essential oil of *C. flexuosus* by employing the chronic unpredictable mild stress (CUMS) model of depression in mice.

## Materials and Methods

### Chemical and reagents

Imipramine (Torrent Pharmaceuticals, India) was used in the study. All other reagents used were of analytical grade.

### Plant collection

Leaves of *Cymbopogon flexuosus* (lemongrass) were collected from medicinal garden of Birla Institute of Technology, Ranchi, India. The leaves were dried at room temperature under shed for 2 days and later stored in moisture free environment. The plant material was taxonomically identified at National Institute of Pharmaceutical Education and Research, Mohali, India and preserved against herbarium no. NIP-H-225.

### Isolation of essential oil

Fresh herb (leaves) of *C. flexuosus* were hydrodistilled in a clevenger apparatus for 6 hrs (Chowdhury *et al.*, 2010). The volume of the essential oil was measured and stored in small vials. To the vials, anhydrous sodium sulphate was added to remove the moisture content from essential oils. To prevent the degradation of the essential oil, the vials were stored at 4°C in refrigerator.

### Experimental animals

Swiss albino mice (20-30g) were procured from the Institute Animal House of Birla Institute of Technology, Mesra, Ranchi. The animals were acclimatized under standard laboratory conditions for a week with a 12-h light/dark cycle (lights turned on at 7 AM). The mice were kept in polyacrylic cages and fed with standard rodent diet and provided with purified water and food *ad libitum*. The behavioral assessments were carried out between 09:00 and 15:00 h. The experimental protocol was approved (PROV/BIT/PH/IAEC/33/2014) by the Institutional Animal Ethics committee (IAEC) of Birla Institute of Technology, Mesra, Ranchi.

### Chronic unpredictable mild stress (CUMS) procedures

Mild unpredictable stress parameters practised for 4-6 weeks in mice produce depression-like traits in mice. This makes CUMS protocols as valid depression models in rodents (Nestler & Hyman, 2010). The CUMS procedure was performed as described by Zhong (Zhong *et al.*, 2006) with some modifications. The method of CUMS procedure including the following stimulations was performed in the experimental animals for 28 days: (1) physical restraint for 2 hours, (2) exposure to an empty water bottle for 1 hour, (3) exposure to a foreign object for 24 hours, (4) overnight illumination, (5) tail pinch (60 sec), (6) tilted cage at 45 for 7 hours (7) tail pinch (30 sec) (Jindal *et al.*, 2013; Liu *et al.*, 2012).

### Drug administration and experimental groups

The animals were divided into 8 groups (n=8). Group 1: nonstressed mice administered with vehicle (Tween 80, 0.1%, po); Group 2: nonstressed mice received imipramine (15 mg/kg, po); Groups 3 and 4: nonstressed mice with LEO treatment (50 and 100 mg/kg, po); Group 5: CUMS stressors with vehicle; Group 6: CUMS stressors administered with imipramine (15 mg/kg, po); Groups 7 and 8: CUMS stressors with LEO treatment (50 and 100 mg/kg, po). The essential oil and imipramine were given daily 1 hour before the CUMS stressors for 28 days. LEO was dissolved in vehicle before use and all drugs were administered orally (via intragastric gavage) between 9:00 am to 10:00 am for 4 weeks.

## Behavioral procedures

### Tail suspension test (TST)

TST was used to measure the total duration of immobility in mice according to the method of Steru (Steru *et al.*, 1985). Animals were suspended by an adhesive tape placed approximately 1 cm from the tip of the tail and at a height of 50 cm above the floor surface. The test lasted for 6 minutes and immobility duration was recorded. Immobility was defined as the state of freezing or absolute loss of movement in mice.

### Forced swimming test (FST):

The FST was performed according to a previously described method with minor modifications (Yankelevitch-Yahav *et al.*, 2015). Briefly, each mouse was individually placed in a glass cylinder filled with water (height: 50 cm; diameter: 20 cm; water depth: 35 cm; temperature: 23–25 °C) and was forced to swim for a period of 6 min. The duration of immobility was recorded for the last 4 min of the test. The water in the cylinder was replaced after each trial.

## Biochemical parameters

### Tissue preparation

On 29$^{th}$ day following behavioral analysis, the animals were sacrificed under anaesthesia. The brains were removed and weighed. A 10% (w/v) brain homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 g for 15 min, aliquots of supernatant were separated for biochemical analysis (Gawali *et al.*, 2017).

### Reduced Glutathione estimation

Reduced glutathione was estimated according to the method described by Ellman, 1959. 1 ml of supernatant was precipitated with equal volume of 4% sulfosalicylic acid and cold digested for 1 h at 4°C. Samples were centrifuged at 1200 g for 15 min at 4°C. To 1 ml of the supernatant, added 2.7 ml of phosphate buffer (0.1 mmol/L, pH 8) and 0.2 ml of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB). The absorbance was read at 412 nm using a UV spectrophotometer (Shimadzu, Japan). The results were expressed as µmol per mg protein.

### Lipid peroxidation assay

The extent of lipid peroxidation in brain homogenate was measured by the method described by Wills, 1966. The amount of MDA (an end product of lipid peroxidation), was measured after its reaction with thiobarbituric acid at 532 nm using a UV spectrophotometer (Shimadzu, Japan). The concentration of MDA was determined from a standard curve and expressed as nmol per mg protein.

### Nitrite estimation

The accumulation of nitrite that indicates the production of nitrite oxide, was determined by Griess reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1%

sulfanilamide and 5% phosphoric acid (Green *et al.*, 1982). The supernatant and Griess reagent were mixed in equal volumes and this mixture was incubated for 10 min at room temperature in the dark. The absorbance was measured at 546 nm using a UV spectrophotometer (Shimadzu, Japan). The concentration of nitrite was determined from standard curve of sodium nitrite and expressed as µmol/mg protein.

**Catalase activity**

Brain catalase activity was measured in accordance to Sinha, 1972. To 1.0 ml of phosphate buffer (0.01 mol/l, pH 7), added 0.1 ml of brain homogenate supernatant and 0.4 ml of hydrogen peroxide (2 mol/l). To this, added 2 ml of dichromate–acetic acid reagent (5% potassium dichromate and glacial acetic acid were dissolved to prepare a 1:3 ratio). The absorbance was measured at 620 nm and expressed as U/mg protein.

**Superoxide dismutase activity**

Superoxide dismutase (SOD) activity was measured by the method described by Misra & Fridovich, 1972. The reaction mixture was prepared by adding 1ml of sodium carbonate (50 mM), 0.4 ml of nitroblue tetrazolium (25 µM), and 0.2 ml hydroxylamine hydrochloride (0.1 mM). Later, 0.1 ml of brain homogenate supernatant was added to the previously made reaction mixture. The absorbance was measured at 560 nm and expressed as U/mg protein.

**Statistical analysis**

All the results were expressed as mean ± SEM. Data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test using Graphpad Prism 7 software. $p < 0.05$ was considered as statistically significant.

## Results

**Effect of LEO treatment on immobility times in the FST and TST:** In Fig 1&2, there was no significant effect of imipramine (15 mg/kg) and LEO (50 mg/kg and 100 mg/kg) in unstressed mice on immobility time in FST and TST. In stressed mice, the CUMS treatment significantly increased the immobility time in both the tests in comparison to the vehicle treated group ($p<0.05$). Treatment with imipramine significantly reduced immobility time in stressed mice than vehicle group that received CUMS treatment ($p<0.05$). LEO (50 and 100 mg/kg) dose dependently decreased the immobility time than CUMS treated vehicle group ($p<0.05$).

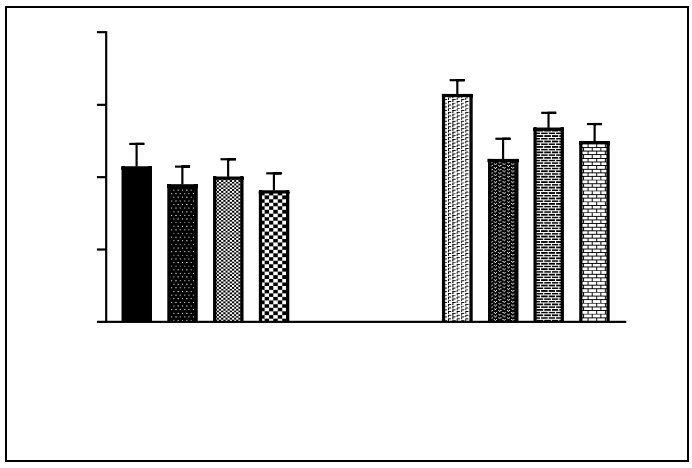

**Fig. 1:** Effect of LEO on immobility time in FST in seconds. $^{**}P<0.05$ vs vehicle; $^{\#\#}P<0.05$ vs CUMS

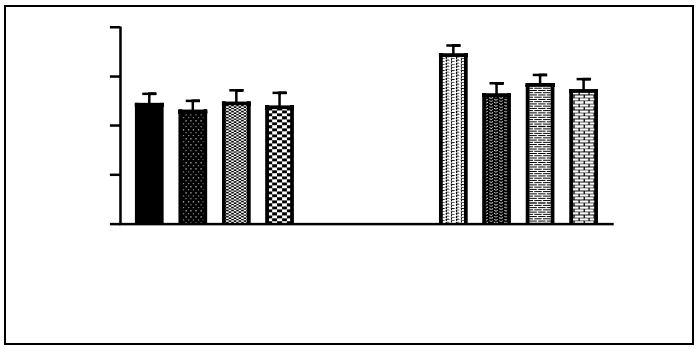

**Fig. 2:** Effect of LEO on immobility time in TST in seconds. $^{**}P<0.05$ vs vehicle; $^{\#\#}P<0.05$ vs CUMS

**Effect of LEO treatment on oxidative stress markers**

**Effect of LEO treatment on lipid peroxidation levels in the brain:** In Fig. 3, there was no significant effect of imipramine (15 mg/kg) and LEO (50 and 100 mg/kg) in unstressed mice on lipid peroxidation levels. Lipid peroxidation levels were significantly elevated in mice subjected to CUMS treatment than the vehicle treated group ($p<0.05$). Imipramine significantly reduced lipid peroxidation levels in stressed mice than vehicle group that received CUMS treatment ($p<0.05$). LEO (50 and 100 mg/kg) dose dependently reduced brain lipid peroxidation levels in CUMS subjected mice ($p<0.05$).

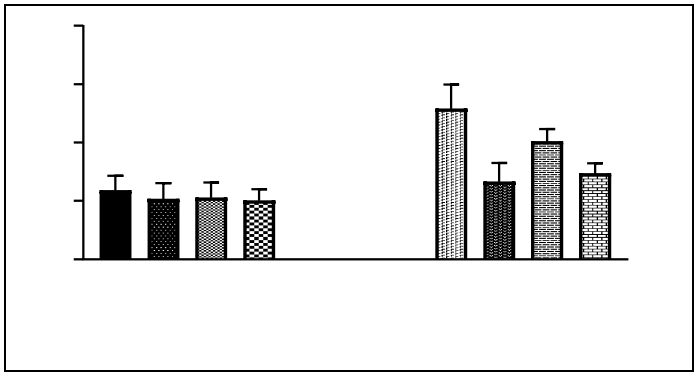

**Fig. 3:** Effect of LEO on lipid peroxidation levels in the brain. $^{**}P<0.05$ vs vehicle; $^{\#\#}P<0.05$ vs CUMS

**Effect of LEO treatment on plasma nitrite levels:** In Fig 4, there was no significant effect of imipramine (15 mg/kg) and LEO (50 and 100 mg/kg) in unstressed mice on plasma nitrite levels. Plasma nitrite levels were significantly elevated in mice subjected to CUMS treatment than the vehicle treated group ($p<0.05$). Imipramine significantly reduced plasma nitrite levels in stressed mice than vehicle group that received CUMS treatment ($p<0.05$). LEO treatment dose dependently reduced brain plasma nitrite levels in CUMS subjected mice ($p<0.05$).

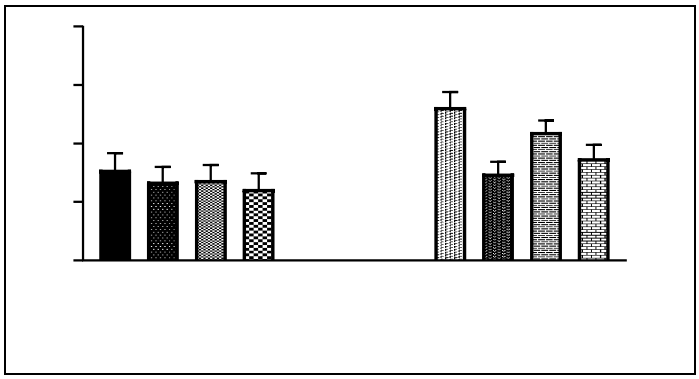

**Fig. 4:** Effect of LEO on plasma nitrite levels in the brain. $^{**}P<0.05$ vs vehicle; $^{\#\#}P<0.05$ vs CUMS

**Effect of LEO treatment on glutathione levels in the brain:** In Fig 5, there was no significant effect of imipramine (15 mg/kg) and LEO (50 and 100 mg/kg) in unstressed mice on glutathione levels in the brain. CUMS treatment resulted in significant reduction of GSH levels in the mice brain in comparison to vehicle control group ($p<0.05$). Imipramine (15 mg/kg) treatment significantly increased glutathione levels in stressed mice than vehicle group that received CUMS treatment ($p<0.05$). Treatment with LEO dose dependently restored the glutathione levels in the mice brain ($p<0.05$).

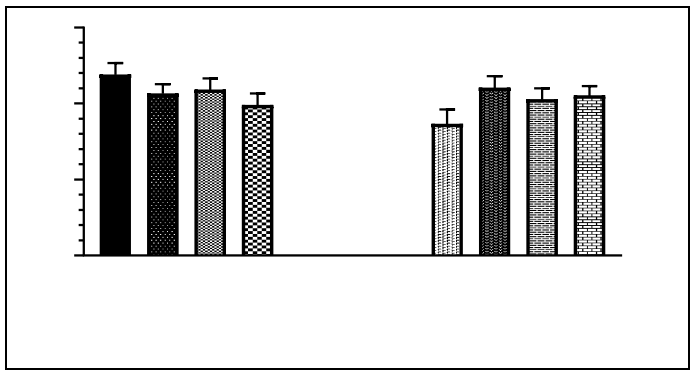

**Fig. 5:** Effect of LEO on glutathione levels in the brain.
$^{**}P<0.05$ vs vehicle; $^{\#\#}P<0.05$ vs CUMS

**Effect of LEO treatment on catalase levels in the brain:** In Fig 6, there was no significant effect of imipramine (15 mg/kg) and LEO (50 and 100 mg/kg) in unstressed mice on catalase levels in the brain. In CUMS treated mice, there was a significant decrease in the catalase levels in the mice brain than the vehicle treated group ($p<0.05$). Imipramine (15 mg/kg) treatment significantly increased glutathione levels in stressed mice than vehicle group that received CUMS treatment ($p<0.05$). LEO (50 and 100 mg/kg) dose dependently restored the catalase levels in the mice brain ($p<0.05$).

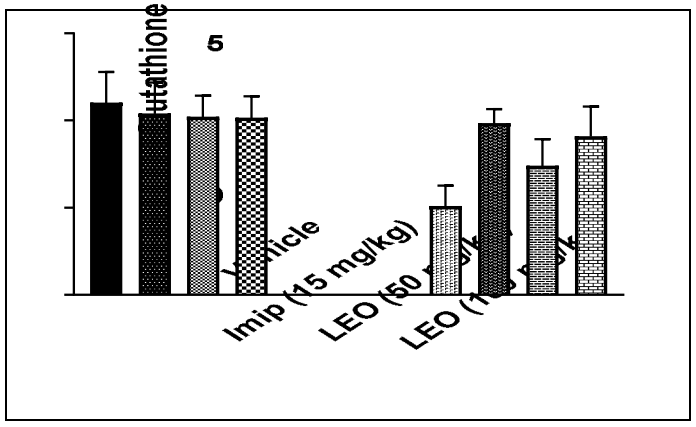


**Fig. 6:** Effect of LEO on catalase levels in the brain.
$^{**}P<0.05$ vs vehicle; $^{\#\#}P<0.05$ vs CUMS

**Effect of LEO treatment on SOD levels in the brain:** In Fig 7, there was no significant effect of imipramine (15 mg/kg) and LEO (50 and 100 mg/kg) in unstressed mice on catalase levels in the brain. SOD levels were significantly decreased in the mice brain on CUMS exposure than the vehicle treated group ($p<0.05$). Imipramine (15 mg/kg) treatment significantly increased SOD levels in stressed mice than vehicle group that received CUMS treatment ($p<0.05$). LEO (50 and 100 mg/kg) treatment produced restoration of the SOD levels in the CUMS treated mice ($p \leq 0.05$).

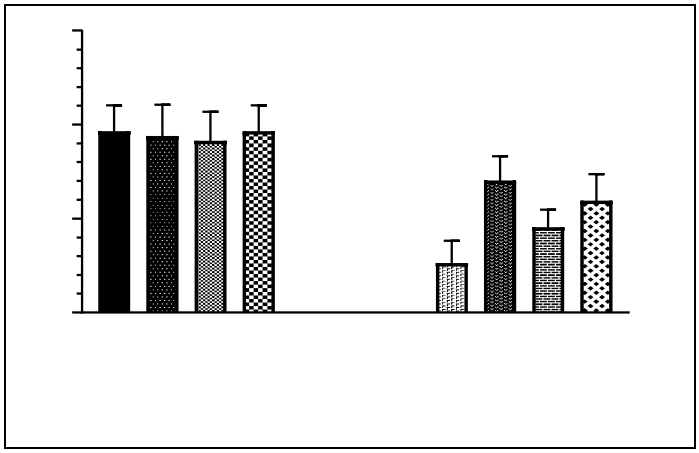

**Fig. 7:** Effect of LEO on SOD levels in the brain.
$^{**}P<0.05$ vs vehicle; $^{\#\#}P<0.05$ vs CUMS

## Discussion

In the present experiment, we have demonstrated the potential antidepressant-like activity of LEO in the CUMS mice model. The CUMS model is commonly used to induce depression in mice and also to understand the pathophysiology of depression, as it simulates human depression like state in mice (Willner, 1997, 2005). The behavioral tests employed in present study are FST and TST suggest that CUMS stressors applied for 28 days produced the depressive-like behavior in mice (Nirmal *et al.*, 2008; Kumar *et al.*, 2011). FST and TST are widely used behavioural despair tests of depression. In the FST, mice swam vigorously in a beaker from which they are unable to escape (aan het Rot *et al.*, 2010; Murrough *et al.*, 2013). The duration of immobility time in mice that are confined and forced to swim is reflective of helplessness (Chen *et al.*, 2012). It was observed that exposure to CUMS protocol significantly increased immobility time in FST. While, administration of LEO (50 and 100 mg/kg, po) in the CUMS treated mice for 28 successive days demonstrated dose dependent reduced levels of despair as immobility period was reduced in comparison to CUMS+vehicle group. In a similar manner, in the TST test, mice were subjected to inescapable stressful situation. Unlike the forced swim test, in the TST there is no risk of hypothermia induced by submersion in water (Thierry *et al.*, 1986). A significant treatment effect of LEO was observed in stressed mice as immobility time was reduced dose-dependently than in CUMS+vehicle group.

Recent studies indicate that oxidative stress in rodent brain contributes in the pathogenesis of depression. In the CNS, decreased antioxidant levels result in increased reactive oxygen species (ROS) production and thus cause damage to endogenous structural molecules such as protein, fat, and DNA (Bilici *et al.*, 2001; Khanzode *et al.*, 2003). In the present study, recurrent exposure to different stressors for 4 weeks produced increased lipid peroxidation, nitrite levels, and lowered endogenous antioxidant activity in the brain of the mice. However, the chronic lemongrass oil treatment significantly decreased the MDA, nitrite levels and increased the glutathione, SOD and CAT activity in the brain of CUMS mice.

In conclusion, exposure to CUMS protocol resulted in depression like behaviour, increased the oxidative stress markers and decreased the antioxidant enzymes activity. In CUMS mice, the chronic treatment of imipramine and the

high dose of lemongrass oil produced a similar beneficial effect on depressant-like behavior and improved oxidative stress. The direct pharmacological targets of imipramine and LEO are different. Imipramine, a tricyclic antidepressant, inhibits reuptake of NA and 5-HT from pre-synaptic membrane, while LEO acts as an antioxidant and reduced oxidative stress in the CNS. Therefore, LEO has potential antidepressant activity and can be used in the treatment of depression disorders.

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