

# Dose Dependent Effects of Lemon Peel Oil on Oxidative Stress and Psychological Behaviors in Rats

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## ABSTRACT

The therapeutic potency of plants has been known for ages and the ability to divulge their biological activity is an area of great interest. *Citrus lemon* is traditionally used as an antioxidant, antibacterial and anti-inflammatory agent. This study intended to determine the potential role of lemon peel oil in neurobehavioral function and oxidative stress. Rats were treated with lemon peel oil at a dose of 0.7, 1.4, 2.1, 2.7, 3.5 g/kg for 14 days. Results showed that lemon peel oil at low doses has antidepressant and anxiolytic activity. Muscular strength was also improved at low doses. The brain antioxidant defense enzymes were also enhanced whereas plasma corticosterone levels were significantly decreased following the administration of a low dose of lemon peel oil. However, rats administered with higher doses of lemon peel oil that act as prooxidants showed depression and anxiety-like effects, and impaired muscular strength. Altered brain antioxidant enzyme activity and elevated corticosterone in plasma were also observed in rats treated with a high dose of lemon peel oil. The present study demonstrates that a low dose of lemon peel oil has a potential therapeutic effect on psychological functions following 14 days of oral administration in rats. Lemon peel oil could be considered for therapeutic use against deleterious effects of oxidative stress, which a low dose of lemon peel oil dramatically reduced in rats.

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## Authors' Contribution

SY, SE designed the experiment and wrote the manuscript. MM analyzed the data. ZB provided technical support. SQ, YS and S Sheikh conducted the experiment. S Sadaf designed graphical representation. TP was principal investigator and evaluated the article.

## Key words

Lemon peel oil, Antioxidants, Prooxidants, Depression, Anxiety

## INTRODUCTION

The effectiveness and use of essential oil in various inflammatory conditions, as well as oxidant-related diseases, are increasing nowadays. Essential oils extracted from different parts of the plant are used for various therapeutic purpose and food industries (Teixeira *et al.*, 2013). Lemon (*Citrus limon*) oil has been reported to have many medicinal uses. It has antioxidant characteristics and has neuroprotective effects. Essential oil from the peel of lemon

has many important nutritive and active components like limonene,  $\beta$ -pinene, linalool, and citral, which are present in the form of stereoisomers (Obob *et al.*, 2014). The active components have calming, anxiolytic, antidepressant, and antispasmodic effects as reported earlier (Agatonovic-Kustrin, 2020). It is a rich source of antioxidants due to the presence of chemical components such as flavonoids, minerals, ascorbic acid, and phenols (Obob *et al.*, 2014). The oxidative stress that is brought by free radicals is responsible for different ailments and it is therefore treated by the antioxidants (Ginter *et al.*, 2014). Moreover, there is an expanding concern to identify the protective agents particularly from food plants having scavenging activity

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## Abbreviations

OFT, open field test; LDT, light/Dark transition test; FST, forced swim test; MDA, malondialdehyde; LPO, lipid peroxidation; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; ROS, reactive oxygen species; H<sub>2</sub>O<sub>2</sub>, hydrogen peroxide; GCs, glucocorticoids.

against reactive species generated as a result of oxidative stress (Bouayed and Bohn, 2010). Lemon oil possesses anti-inflammatory properties by inhibiting the release of proinflammatory cytokines and lipid peroxidation (Kummer *et al.*, 2013). Both oxidative stress and inflammation have an important role in the development of neurodegeneration. Reactive oxygen species triggers cellular apoptosis and pro-inflammatory signaling, which in turn produce undesirable repercussions that are supposed to be a possible cause of depression (Fung *et al.*, 2021). Essential oils may be beneficial in depression due to their antioxidant and anti-inflammatory characteristics, based on the notion that ROS and inflammatory signaling play role in depression (Fung *et al.*, 2021). Inflammation is associated with glucocorticoid resistance and reduced negative feedback of the HPA axis in response to high cortisol and cytokine levels (Mondelli *et al.*, 2011). Corticosterone is the end product of the activation of the HPA axis which mimics the physiological stress response. It has been reported in previous studies that lemon oil effectively reduced the plasma corticosterone levels in rodents and concomitant levels of depression (Fung *et al.*, 2021). Though the treatment with exogenous antioxidants causes the elimination of reactive species, however, a high dose of these compounds is reported to be toxic and exhibit prooxidant effects (Rahal *et al.*, 2014). Therefore, the potential dose of exogenous dietary antioxidants should be investigated for optimal cellular functioning. In vitro studies showed that the treatment of isolated mitochondria and cultured cells with a high dose of flavonoids potentiated the formation of superoxide radicals, decreased cell survival, and viability (Bouayed and Bohn, 2010). Reduced total antioxidant capacity, decreased glutathione and diminished activity of superoxide dismutase (SOD) and catalase (CAT) were also reported following flavonoids treatment at high concentration (Eghbaliferiz and Iranshahi, 2016). Besides the concentration of exogenous antioxidants, the presence of transition metal in a cellular environment may also provide a possible mechanism for the induction of prooxidant effects (Halliwell, 2008). The antioxidants scavenge free radicals from non-reactive species because of having the strong reducing ability, it can also affect transition metals such as  $Fe^{+3}$  and  $Cu^{+2}$ , due to this their tendency to produce more reactive species from peroxides anions become raised (Cao *et al.*, 1997). Supplements of various antioxidants such as  $\beta$ -carotene, epigallocatechin gallate, lycopene, lutein, zeaxanthin have been shown to induce prooxidant effects (Prochazkova *et al.*, 2011). Terpenes including limonene, citral, and linalool have also shown toxic effects at high concentrations (Bascieri *et al.*, 2017). It has been suggested that limonene, linalool, and citral have a narrow concentration range to produce antioxidant effects. Although lemon peel oil has been reported to have beneficial effects

at lower doses, however, higher doses of lemon peel have not been investigated. Previously, a dose up to 1600 mg/kg was tested to monitor the antidepressant effects of lemon oil (Hao *et al.*, 2013). Therefore, this study was conducted to determine the dose dependent effects of lemon peel oil on anxiety, depression, and general motor coordination.

## MATERIALS AND METHODS

### *Animals*

Sprague Dawley rats were purchased from the Animal Facility of International Center for Chemical and Biological Sciences, University of Karachi, weighing about 180-200 g. Animals were kept individually in their cages in a quiet room for at least a week for acclimation. The experiment was approved by the institutional Board of Advance Studies and Research (BASR no: 02811/Sc) and performed as per under National Institute of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1995). All experimental procedures were made in a balanced design to avoid the effect of time and order. The chemicals and reagents used in the experiment were purchased from Sigma Aldrich Company.

### *Experimental protocol*

Thirty-six rats were divided into six (n=6) groups. The first group was treated with saline and designated as control whereas groups 2, 3, 4, 5, and 6 were designated as tests and administered with 0.7, 1.4, 2.1, 2.7, and 3.5 g/kg doses of lemon peel oil respectively for 14 days. Open field test (OFT), forced swim test (FST), light/dark transition (LDT) test, and Kondziela's test were performed between 0900-1400 h. Rats were decapitated after the completion of behavioral tests and a brain sample was collected within 30-60 sec of decapitation. Plasma samples were collected from blood. All samples were stored at  $-20^{\circ}C$  until analyzed. Lipid peroxidation (LPO) and activity of SOD and CAT were determined in collected brain samples and corticosterone levels were measured in plasma.

### *Behavioral analysis*

#### *Open field test*

In a novel environment locomotor activity of all groups i.e., control and tests rats were observed in an open field arena with the dimensions of  $76 \times 76$  cm with walls that were opaque with a height of 42 cm. The floor of the open field was divided into 25 equal squares. To avoid any noise effect test was performed in a quiet room under white light as described earlier. Animals were placed in the center square of the open field (one at a time) and different observations such as time spent in the central area and number of squares crossed were monitored during the cutoff of 5 min (Yousuf *et al.*, 2018).

*Forced swim test (FST)*

As a measure of depression-like behavior, FST was performed. The test was conducted according to the method of (Porsolt *et al.*, 1977). FST is performed in a container of size (53 x 19 x 28) of a glass tank in which up to 18 cm of water is filled. The water height is such that animals were able to dive in under an inescapable condition. The animal is placed in the container and allowed to swim for 5 min, struggling time is then monitored by using a stopwatch. The rat was considered to be immobile when the observer judged that the rat was exhibiting no overt behaviors other than small postural movements. The immobility indicates the animal's helpless behavior. Rats were dried with a towel after each test and placed in their home cage.

*Light/dark transition (LDT)*

LDT is employed for examining anxiety and it consists of two boxes. The dimensions of both boxes were measured as 26×26×26 cm and were separated by having a door with the measurements as 12×12 cm. The two plastic boxes were different i.e. one had a black coating on its walls and the other plastic wall was kept transparent. Initially, the compartment with light was selected for the rat to be put inside having a cutoff time of 5 min, and the degree of anxiety-like behavior was discerned by the number of entries and time, which was spent by the rat in the lightbox. A bulb, 60 W, exhibiting white light is used to determine activity in the box (Yousuf *et al.*, 2018).

*Kondziela's test*

Kondziela's test was performed to check the strength, resistance, and exercise ability of the rat. In this test rat was placed in the middle of the screen, turn to an inverted position with the rat's head declining first, and falling time was recorded (Yousuf *et al.*, 2018).

*Biochemical estimations**Determination of MDA content*

Estimation of lipid peroxidation was performed with slight modifications (Yousuf *et al.*, 2018). In a mixture of TCA (15%)–TBA (0.375%) (2 ml) brain homogenate (100–500 µl) was added. For 20 min, the mixture was boiled in a water bath and forthwith it was cooled down to centrifuge at 2000×g for 10 minutes. The supernatant collected had a color of light pink, and absorbance was noted at 532 nm. The representation of LPO data is done in µmoles of MDA/g of the brain.

*Determination of SOD activity*

SOD activity, gauged by the mitigation of NBT, in the brain, to form blue formazan, a water-insoluble compound (Yousuf *et al.*, 2018). Brain homogenate (10%, 0.5 ml) was mixed with Na<sub>2</sub>CO<sub>3</sub> (1 ml of 50 mM), NBT

(0.4 ml of 24 µM), and EDTA (0.2 ml of 0.1 mM). H<sub>3</sub>NO. HCl (0.4 ml of 1 mM) was poured to initiate the reaction. At time 0 min, and at 5 min, measurements were noted for the difference in absorbance at 560 nm. For each batch of samples, control was set without having a brain homogenate. Representation of SOD activity was done as U/g of the brain. Enzyme quantity, leading to inhibition of up to 50% reduction in NBT, equals 1 unit.

*Determination of catalase activity*

CAT activity was evaluated by taking 0.1 ml of brain homogenate was added into the reaction mixture containing 0.01 M phosphate buffer at pH 7.4 (1 ml) and 0.2 M H<sub>2</sub>O<sub>2</sub> (0.4 ml). For 90 seconds, at 37 °C, the tubes were put for incubation. The reaction came to halt upon the introduction of a dichromate reagent (2 ml of 5%). To determine the consumption of H<sub>2</sub>O<sub>2</sub> throughout the reaction, the sample, at 100 °C, was incubated for 15 minutes and was read at 570 nm. Representation of the CAT activity was noted as the consumption of H<sub>2</sub>O<sub>2</sub> µmol/min/g of the brain (Yousuf *et al.*, 2018).

*Determination of plasma corticosterone activity*

Plasma corticosterone concentration was performed by fluorometry method. The lower layer of acid alcohol reagent was transferred to a small cuvette for fluorescence to be read at 460 nm excitation and 570 nm emission wavelengths. Results were reported as µg/dl of plasma (Yousuf *et al.*, 2018).

*Statistical analysis*

The representation of the results is taken as mean ± SD. Data of behavioral analysis and biochemical estimations were evaluated by one-way ANOVA. Post hoc analysis was done by Tukey's test. p<0.05 was measured to be significant.

**RESULTS***Locomotor activity by OFT*

Effects of different doses of lemon peel oil on the number of squares crossed and time spent in the central area in an open field following 14 days of administration are shown in Figure 1a and b. Statistical analysis of data by one-way ANOVA revealed significant effects of lemon peel oil on the number of squares crossed (F= 133.95, df 5, 30, p<0.01) and time spent in the central area (F= 49.72, df 5, 30, p<0.01). Post hoc analysis by Tukey's test showed that there was a gradual increase in time spent in the central area and the number of squares crossed with an increasing dose of lemon peel oil. Optimum effects were observed at the dose of 1.4 g/kg but further increases in the dose (2.1, 2.7, and 3.5 g/kg) reversely affected the time

duration spent in the central area and also in the number of squares crossed.

*Anxiety by light/dark transition test and depression by forced swim test*

Effect of different doses of lemon peel oil on LDT and FST activity following 14 days of administration is shown in Figure 2a and 2b. Statistical analysis of data by one-way ANOVA revealed significant effects of lemon peel oil in LDT ( $F=168.84$ ,  $df\ 5, 30$ ,  $p<0.01$ ) and FST ( $F=75.00$ ,  $df\ 5, 30$ ,  $p<0.01$ ). Post hoc analysis by Tukey's test showed that there was a gradual increase in time spent in the lightbox and struggling time in FST with an increasing dose of lemon peel oil. Optimum effects were observed at the dose of 1.4 g/kg further increase in dose reversely affected the time spent in the lightbox and struggling time. Rats administered with 2.7 g/kg and 3.5 g/kg doses of lemon peel oil resulted in decreased time spent in the lightbox. 2.1, 2.7, and 3.5 g/kg doses of lemon peel oil also reduced struggling time in forced swim test significantly.

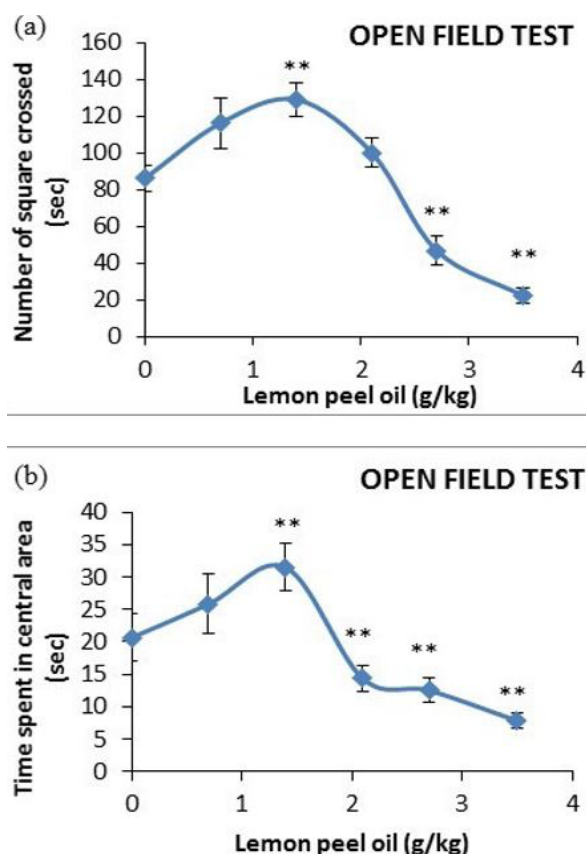


Fig. 1. Dose dependent effects of lemon peel oil on open field. (a) Number of square crossed and (b) Time spent in central area. Values are mean $\pm$ SD (n=6). Significant difference was obtained by Tukey's post hoc test, \*\* $p<0.01$  as compared to control animals.

*Combine forepaw and hind paw strength by kondziela's inverted screen*

Effect of different doses of lemon peel oil on combine forepaw strength following 14 days of administration is shown in Figure 3. Statistical analysis of data by one-way ANOVA revealed a significant effect of lemon peel oil ( $F=21.45$ ,  $df\ 5, 30$ ,  $p<0.01$ ). Post hoc analysis by Tukey's test showed that there was a gradual increase in the strength of forepaws with an increasing dose of lemon peel oil. Optimum effects were observed at the dose of 1.4 g/kg but further increase in dose reversely affected forepaw strength.

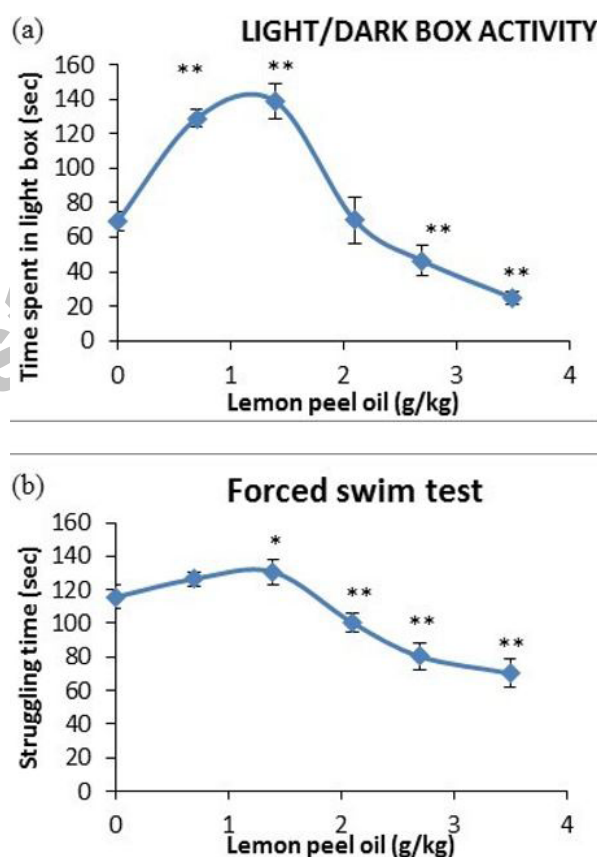


Fig. 2. Dose dependent effects of lemon peel oil on (a) light/dark transition box and (b) forced swim test. Values are mean $\pm$ SD (n=6). Significant difference was obtained by Tukey's post hoc test, \* $p<0.05$ , \*\* $p<0.01$  as compared to control animals.

*Lipid peroxidation*

The effect of different doses of lemon peel oil on MDA levels following 14 days of administration is shown in Figure 4. Statistical analysis of data by one-way ANOVA revealed significant effects of lemon peel oil ( $F=51.42$ ,  $df\ 5, 30$ ,  $p<0.01$ ). Post hoc analysis by Tukey's test showed

that there was a gradual decrease in MDA levels with an increasing dose of lemon peel oil. Optimum effects were observed at the dose of 1.4 g/kg but further increase in dose reversely affected the MDA levels. Rats administered with 2.1, 2.7, and 3.5 g/kg doses of lemon peel oil resulted in increased MDA levels.

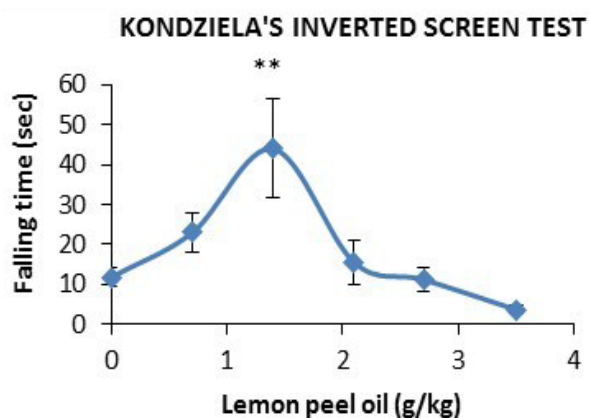


Fig. 3. Dose dependent effects of lemon peel oil on combine forepaw and hindpaw strength by Kondziela's inverted screen test. Values are mean±SD (n=6). Significant difference was obtained by Tukey's post hoc test, \*\*p<0.01 as compared to control animals.

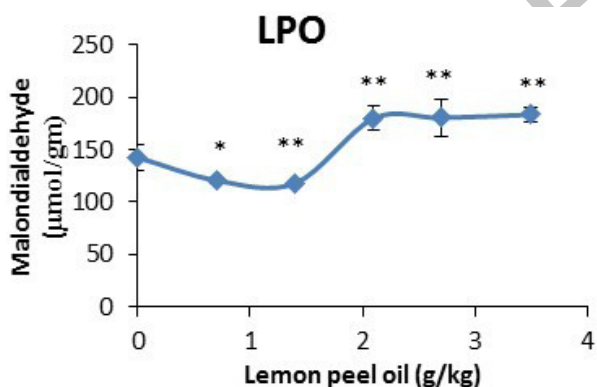


Fig. 4. Dose dependent effects of lemon peel oil on lipid peroxidation. Values are mean±SD (n=6). Significant difference was obtained by Tukey's post hoc test, \*p<0.05, \*\*p<0.01 as compared to control animals.

#### SOD and CA

Effect of different doses of lemon peel oil on SOD and CAT levels following 14 days of administration are shown in Figure 5. Statistical analysis of data by one-way ANOVA revealed significant effects of lemon peel oil on (a) SOD (F= 115.90, df 5, 30, p<0.01) and (b) CAT (F= 81.79, df 5, 30, p<0.01). Post hoc analysis by Tukey's test showed that there was a gradual increase in SOD and CAT

levels with an increasing dose of lemon peel oil. A dose of 1.4 g/kg shows the maximum effect but a further increase in dose reversely affected the SOD and CAT levels.

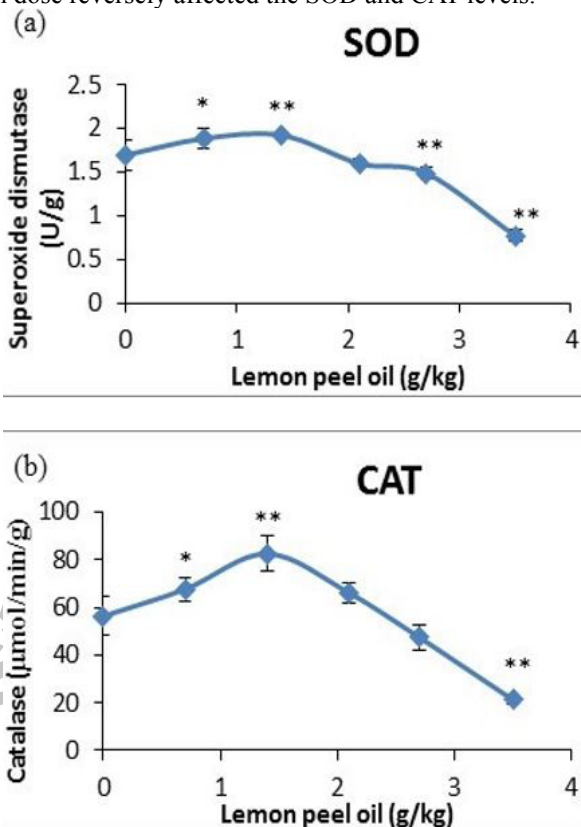


Fig. 5. Dose dependent effects of lemon peel oil on antioxidant enzymes (a) superoxide dismutase and (b) catalase activity. Values are mean±SD (n=6). Significant difference was obtained by Tukey's post hoc test, \*p<0.05, \*\*p<0.01 as compared to control animals.

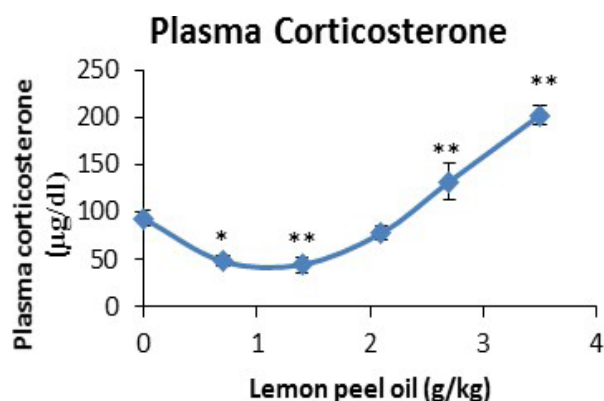


Fig. 6. Dose dependent effects of lemon peel oil on plasma corticosterone level. Values are mean±SD (n=6). Significant difference was obtained by Tukey's post hoc test, \*p<0.05, \*\*p<0.01 as compared to control animals.

### *Plasma corticosterone*

The effect of different doses of lemon peel oil on corticosterone levels following 14 days of administration is shown in [Figure 6](#). Statistical analysis by one-way ANOVA revealed significant effects of lemon peel oil ( $F= 185.45$ ,  $df 5, 30$ ,  $p<0.01$ ). Post hoc analysis by Tukey's test showed that there was a gradual decrease in corticosterone levels with an increasing dose of lemon peel oil. Optimum effects were observed at the dose of 1.4 g/kg but further increase in dose reversely affected the corticosterone levels. Rats administered with 2.7 g/kg and 3.5 g/kg doses of lemon peel oil resulted in increased corticosterone levels.

## DISCUSSION

Essential oils obtained from the citrus plants including lemon oil, have long been used in alternative medicine as well as in aromatherapy. A large number of psychological ailments such as anxiety, depression, and stress have long-term adverse effects in humans ([Setzer, 2009](#)). The use of traditional antipsychotics has its adverse effects, therefore herbal medicine has gained eminence for treating various diseases. Lemon peel oil contains terpenes, polyphenols and carotenoids that are known to have antioxidant effects and are able to remove the reactive oxygen species (ROS) ([Bouayed and Bohn, 2010](#)). ROS generation is a normal mechanism of the aerobic metabolic process in organisms. Increased ROS generation can lead to different pathological conditions as well as psychological and behavioral alterations and have a crucial role in the pathogenesis of neurodegenerative diseases ([Fung \*et al.\*, 2021](#)). To avoid the deleterious health effects of ROS, it is imperative to keep the equity between the formation and removal of ROS ([Bouayed and Bohn, 2010](#)). The human body has its defense system to remove ROS that is endogenous antioxidant enzymes and it is called a primary defense system. These endogenous antioxidant enzymes are also prone to oxidative damage which lead to the increasing requirement of exogenous antioxidant to restore against the oxidative response ([Yousuf \*et al.\*, 2018](#)). The present study investigated the effects of lemon peel oil at different doses and interestingly it has been found for the very first time the biphasic nature of lemon peel oil. It is observed that at a low dose (0.7 g/kg) lemon peel oil acts as an anxiolytic, antidepressant and antioxidant. Motor coordination was also improved following the administration of 0.7 g/kg of lemon peel oil. These effects were optimum at 1.4 g/kg however at high doses (2.1, 2.7, and 3.5 g/kg) it exhibited prooxidant effects and produced neuropsychiatric alterations and altered antioxidant enzyme activity. Essential oils have the ability to quench free radicals and this is why they play a crucial role in

brain-related dysfunctions and other diseases ([Hao \*et al.\*, 2013](#)). OFT is used to assess the exploratory activity. Lemon peel oil at a dose of 0.7 and 1.4 g/kg increased the number of squares crossed and spent more time in the central area and showed improved locomotor activity. However, results showed that a dose of 0.7 g/kg and 1.4 g/kg also has antidepressant effects as it increased the struggling time in FST. Repeated administration of 0.7 g/kg and 1.4 g/kg showed anxiolytic effect as it is observed by increased time spent in the lightbox. Malondialdehyde (MDA) is a biomarker of LPO which is formed during oxidation of lipids ([Yousuf \*et al.\*, 2018](#)). In the current study, significantly decreased levels of MDA were observed in rats treated with 0.7 g/kg and 1.4 g/kg lemon peel oil which shows that lemon peel oil at a low dose inhibited LPO in the brain. SOD and CAT provide a primary defense system to counteract oxidative stress ([Fang \*et al.\*, 2002](#)). Superoxide radicals are converted to  $H_2O_2$  by SOD.  $H_2O_2$  itself is a highly reactive species which are further destroyed by mutual scavenging activities of glutathione peroxidase (GPx) and CAT and form water ([Emad \*et al.\*, 2017](#)). The non-phenolic content of lemon peel oil including limonene, linalool, and citral has shown antioxidant effects ([Baschieri \*et al.\*, 2017](#)). Moreover, the citral-rich essential oil obtained from lemon grass showed antioxidant capacity and reduced autoxidation of unsaturated fatty acids ([Sacchetti \*et al.\*, 2005](#)). The generation of peroxy radicals may involve in the activation of the superoxide dismutase enzyme which converts free radicals into  $H_2O_2$  ([Amorati \*et al.\*, 2013](#); [Emad \*et al.\*, 2017](#)). In the present study rats treated with 0.7 g/kg and 1.4 g/kg showed increased activity of SOD which could suggest the generation of  $H_2O_2$  which is further catalyzed by CAT. It is positively related to the increased activity of CAT as observed in the present results. Under normal and stressful situations, nuclear factor-erythroid factor 2-related factor 2 (Nrf2) is a key transcription factor that regulates the expression of several genes in the cell and it is resistant to oxidative stress ([Wang \*et al.\*, 2021](#)). It could be suggested from the findings of the present study that active components limonene, linalool, and citral present in lemon peel oil may regulate the signaling pathway of Nrf2 to increase the activity of antioxidant enzymes SOD, CAT, and GPx. In that way shielding the cells against damaging effects of reactive oxygen species and lipid peroxidation and hence improved behaviors in rats. Lemon peel oil at a very high doses showed pro-oxidant effects as evidenced by increased lipid peroxidation and reduced activity of antioxidant enzymes at 2.1 g/kg, 2.7 g/kg, and 3.5 g/kg doses. These results were associated with depressogenic and anxiogenic-like responses in rats. The findings of the present study showed

that at higher doses of lemon peel oil, there is decreased number of squares crossed and reduced time spent in the central area. It also showed significantly reduced struggling time in FST exhibiting depressant effects of lemon peel oil. Moreover, lemon peel oil at higher doses also significantly decreased the time spent in the light compartment and produced anxiogenic effects. The non-phenolic components of lemon peel oil have been reported to increase oxygen consumption (Baschieri *et al.*, 2017). The ability of limonene, linalool and citral to produce peroxy radicals may define the pro-oxidant effects of lemon peel oil (Ayala *et al.*, 2014). It has been reported that higher production of peroxy radicals results in increased peroxidation of a lipid bilayer and other biomolecules (Ayala *et al.*, 2014). The higher production of peroxy radical may also exceed the biological activity of SOD resulting in the accumulation of free radicals which may further exacerbate the oxidation of vital cellular molecules (Nimse and Pal, 2015). In the present study reduced activity of antioxidant enzymes and increased lipid peroxidation represent the accumulation of reactive species and increased oxidative burden in rat brain. Corticosterone regulates the synthesis of cytokines. It has previously been reported that patient neurologic impairment is related to plasma cortisol concentration, implying that excessive cortisol levels may have neurotoxic effects. Pro-inflammatory cytokines have been shown to increase the activity of the HPA axis, causing corticosterone to be released. Corticosterone levels in the blood may influence the production of pro-inflammatory cytokines (Emad *et al.*, 2017). A modest increase in pro-inflammatory cytokines has been suggested to have neuroprotective properties, whereas large quantities may be neurotoxic. The balance between pro and anti-inflammatory cytokines is disturbed when corticosterone levels are too high (Gottesfeld *et al.*, 2002). Administration of higher doses of lemon peel oil increases plasma corticosterone levels in rats. GCs can easily and quickly cross the blood brain barrier. Prolonged elevated levels of glucocorticoids (GCs) are associated with different pathological conditions which result in oxidative stress and the elevated levels of GCs imitate the stress-like effects that cause dysfunction and cause biochemical and behavioral changes (Yousuf *et al.*, 2018). Previous findings also reported that increased levels of GCs are associated with inflammatory responses together with neuronal toxicity (Gilbert, 2000). In the present study, MDA levels in rats treated with 2.1 g/kg, 2.7 g/kg, and 3.5 g/kg lemon peel oil were increased as compared to controls. Whereas, these doses significantly decreased SOD and CAT activity. Various transition metals that act as a cofactor in different biochemical reactions tend to promote free radical generation. Lemon peel oil

contains selenium, iron, copper, manganese, zinc, and sodium and these ions require as co-factor in various reactions (González *et al.*, 2010). Auto oxidation of flavins and monoamines can be produced by transition metals (Franke *et al.*, 2005). It is therefore suggested that the possible mechanism behind alteration in behaviors is due to the cofactor-dependent enzyme activation enhanced by antioxidant enzymes activity which is provided by lemon peel oil (Halliwell, 2008). In the present study administration of higher doses of lemon peel oil showed impairment in behaviors with significantly decreased activity of antioxidant enzymes. It may be suggested that the alterations in behaviors may be due to the auto oxidative effects of lemon peel oil at a high dose. Our study is in accordance with the previous findings that at higher concentrations essential oils may act as pro-oxidants and start autoxidation in the cellular machinery (Halliwell, 2008). Earlier it is also narrated that due to the lipophilic nature essential oils can easily penetrate the neuronal cell membrane and cause alteration in the lipid bilayer structure and make them permeable and ultimately leads to brain alterations (Bakkali *et al.*, 2008). It could be suggested that the neurodegeneration which could be induced by oxidative stress can be controlled by a low dose of lemon peel oil as it inhibits LPO and has antioxidant activity. Therefore, it is suggested that at high concentration antioxidants could also disrupt the redox balance due to their ability to interact with reactive species present at a physiological concentration resulting in cellular dysfunction.

## CONCLUSION

The current study provided a better understanding of the chemical mechanisms of lemon peel oil and its targeted impact on anxiety and depression. It is suggested that supplementation at lower doses has health-benefiting effects by reducing oxidative stress and GCs levels. Whereas, at higher doses, lemon peel oil may exert deleterious effects by inducing oxidative stress and increasing GCs levels. Present findings, therefore propose that the balance between antioxidant and pro-oxidant are crucial for maintaining a healthy life. To the best of our knowledge, no literature has been reported earlier on the biphasic effect of lemon peel oil as an antioxidant and prooxidant compound. In the current COVID-19 pandemic where the major global public health crisis is evident and has generated worldwide anxiety in people due to its relatively high infections, rapid progression as well as relatively high death rate, it is highly suggested to take natural antioxidants such as lemon and its related compounds that boost immunity as well as improves

various psychological ailments.

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### Statement of conflict of interest

The authors have declared no conflict of interest.

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