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

Sarwat Yousuf¹, Shaista Emad², Mohammad Misbah ur Rehman¹, Zehra Batool¹, Sara Qadeer³, Yousra Sarfaraz¹, Sheeza Sheikh¹, Sana Sadaf⁶ and Tahira Perveen¹,*

¹Neurochemistry and Biochemical Neuropharmacology Research Unit, Department of Biochemistry, University of Karachi, Karachi-75270, Pakistan
²Department of Biochemistry, Jinnah Medical and Dental College/Sohail University, Karachi-74800 Pakistan.
³Hussain Lakhani Hospital, Iqra University, Karachi, 74600, Pakistan.
⁴Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan.
⁵Department of Biochemistry, Sir Syed College of Medical Sciences, Karachi, Pakistan.
⁶Department of Biochemistry, Faculty of Health and Medical Sciences, Hamdard University, Karachi, Pakistan.

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.

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 purposes 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, β-pinene, linalool, and citral, which are present in the form of stereoisomers (Oboh 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 (Oboh 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.
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 prooxidants 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^{2+} 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 β-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 (Baschieri 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°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 76×76 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 (Porstolt 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 central area in an open field following 14 days of administration.

**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 Na₂CO₃ (1 ml of 50 mM), NBT (0.4 ml of 24 μM), and EDTA (0.2 ml of 0.1 mM). H₂NO₃, 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 H₂O₂ (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 H₂O₂ 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 H₂O₂μmol/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 spent in the central area and the number of squares crossed.
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.

**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.

**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$_2$O$_2$ by SOD. H$_2$O$_2$ 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 peroxyl radicals may involve in the activation of the superoxide dismutase enzyme which converts free radicals into H$_2$O$_2$ (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$_2$O$_2$ 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 peroxyl radicals may define the pro-oxidant effects of lemon peel oil (Ayala et al., 2014). It has been reported that higher production of peroxyl radicals results in increased peroxidation of a lipid bilayer and other biomolecules (Ayala et al., 2014). The higher production of peroxyl 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 acts as a cofactor in different biochemical reactions tend to promote free radicals 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 starts autooxidation 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 degeneration 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 boots immunity as well as improves the biological activity of SOD resulting in the accumulation of free radicals.
various psychological ailments.

ACKNOWLEDGMENTS

Authors are thankful to The Higher Education Commission of Pakistan and University of Karachi, Pakistan for providing the funds and necessary facilities for the study.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Agatonovic-Kustrin, S., Kustrin, E., Gegechkori, V., and Morton, D.W., 2020. Anxiolytic terpenoids and aromatherapy for anxiety and depression. In: Reviews on new drug targets in age-related disorders (ed. P.C. Guest). Springer Cham. pp. 283-296. https://doi.org/10.1007/978-3-030-42667-5_11

Amorati, R., Foti, M.C., and Valgimigli, L., 2013. Antioxidant activity of essential oil. J. Agric. Fd. Chem., 61: 10835–10847. https://doi.org/10.1021/jf403496k

Ayala, A., Munoz, M.F., and Arguelles, S., 2014. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxynonenal. Oxid. Med. Cell. Longev., 2014: 360438. https://doi.org/10.1155/2014/360438

Bakkali, F., Averbeck, S., Averbeck, D., and Idaomar, M., 2008. Biological effects of essential oils. A review. Fd. Chem. Toxicol., 46: 446-475. https://doi.org/10.1016/j.fct.2007.09.006

Bascieri, A., Ajvazi, M.D., Tonfack, J.L.F., Valgimigli, L., and Amorati, R., 2017. Explaining the antioxidant activity of some common non-phenolic components of essential oils. Fd. Chem., 232: 656-663. https://doi.org/10.1016/j.foodchem.2017.04.036

Bouayed, J., and Bohn, T., 2010. Exogenous antioxidants double-edged swords in cellular redox state: Health beneficial effects at physiologic doses versus deleterious effects at high doses. Oxid. Med. Cell. Longev., 3: 228-237. https://doi.org/10.4161/oxim.3.4.12858

Cao, G., Sofic, E., and Prior, R.L., 1997. Antioxidant and prooxidant behavior of flavonoids: Structure activity relationships. Free Radic. Biol. Med., 22: 749–760. https://doi.org/10.1016/S0891-5849(96)00351-6

Eghbaliferiz, S., and Iranshahi, M., 2016. Prooxidant activity of polyphenols, flavonoids, anthocyanins and carotenoids. Phytother. Res., 30: 1379-1391. https://doi.org/10.1002/ptr.5643

Emad, S., Qadeer, S., Sadaf, S., Batoool, Z., Haider, S., and Perveen, T., 2017. Attenuation of stress induced memory deficits by nonsteroidal anti-inflammatory drugs (NSAIDs) in rats: Role of antioxidant enzymes. Pharmacol. Rep., 69: 300-305. https://doi.org/10.1016/j.pharep.2016.11.009

Fang, Y.Z., Yang, S., and Guoyao, W., 2002. Free radicals, antioxidants, and nutrition. Nutrition, 18: 872–879. https://doi.org/10.1016/S0899-9007(02)00916-4

Franke, S.I.R., Prá, D., da Silva, J., Erdtmann, B., and Henriquez, J.A.P., 2005. Possible repair action of Vitamin C on DNA damage induced by methyl methanesulfonate, cyclophosphamide, FeSO4 and CuSO4 in mouse blood cells in vivo. Mutat. Res. Genet. Toxicol. Environ. Mutagen., 583: 75-84. https://doi.org/10.1016/j.mrgentox.2005.03.001

Fung, T.K., Lau, B.W., Ngai, S.P., and Tsang, H.W., 2021. Therapeutic effect and mechanisms of essential oils in mood disorders: Interaction between the nervous and respiratory systems. Int. J. mol. Sci., 22: 4844. https://doi.org/10.3390/ijms22094844

Gilbert, D.L., 2000. Fifty years of radical ideas. Anals N. Y. Acad. Sci., 899: 1–14. https://doi.org/10.1111/j.1749-6632.2000.tb06172.x

Ginter, E., Simko, V., and Panakova, V., 2014. Antioxidants in health and disease. Bratisl. LekListy., 115: 603–606. https://doi.org/10.4149/BLL_2014_116

Gonzalez-Molina, E., Dominguez-Perles, R., Moreno, D.A., and Garcia-Viguera, C., 2010. Natural bioactive compounds of Citrus limon for food and health. J. Pharm. Biomed. Anal., 51: 327-345. https://doi.org/10.1016/j.jpba.2009.07.027

Gottesfeld, Z., Moore, A.N., and Dash, P.K., 2002. Acute ethanol intake attenuates inflammatory cytokines after brain injury in rats: a possible role for corticosterone. J. Neurotrauma, 19: 317-326. https://doi.org/10.1089/089771502753594882

Halliwell, B., 2008. Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and in vivo studies? Arch. Biochem. Biophys., 476: 107–112. https://doi.org/10.1016/j.abb.2008.01.028

Hao, C.W., Lai, W.S., Ho, C.T., and Sheen, L.Y., 2013. Antidepressant-like effect of lemon essential oil is through a modulation in the levels of norepinephrine, dopamine, and serotonin in mice: use of the tail suspension test. J. Funct. Fds., 5: 370–379. https://doi.org/10.1016/j.jff.2012.11.008
Kummer, R., Fachini-Queiroz, F.C., Estevao-Silva, C.F., Grespan, R., Silva, E.L., Bersani-Amado, C.A., and Cuman, R.K.N., 2013. Evaluation of anti-inflammatory activity of citrus latifolia tanaka essential oil and limonene in experimental mouse models. *Evid-Based Complement. Altern. Med.*, 2013. https://doi.org/10.1155/2013/859083

Mondelli, V., Cattaneo, A., Murri, M.B., Di, Forti, M., Handley, R., Hepgul, N., and Aitchison, K.J., 2011. Stress and inflammation reduce brain-derived neurotrophic factor expression in first-episode psychosis: A pathway to smaller hippocampal volume. *J. clin. Psychiat.*, 72: 1677-1684. https://doi.org/10.4088/JCP.10m06745

Nimse, S.B., and Pal, D., 2015. Free radicals, natural antioxidants, and their reaction mechanisms. *RSC Adv.*, 5: 27986–28006. https://doi.org/10.1039/C4RA13315C

Oboh, G., Olasehinde, T.A., and Ademosun, A.O., 2014. Essential oil from lemon peels inhibit key enzymes linked to neurodegenerative conditions and pro-oxidant induced lipid peroxidation. *J. Oleo. Sci.*, 63: 373–381. https://doi.org/10.5650/jos.ess13166

Porsolt, R.D., Bertin, A., and Jalfre, M., 1977. Behavioral despair in mice: a primary screening test for antidepressants. *Arch. Int. Pharmacodyn. Ther.*, 229: 327-336.

Procházková, D., Boušová, I., and Wilhelmová, N., 2011. Antioxidant and prooxidant properties of flavonoids. *Fitoterapia*, 82: 513-523. https://doi.org/10.1016/j.fitote.2011.01.018

Rahal, A., Kumar, A., Singh, V., Yadav, B., Tiwari, R., Chakraborty, S., and Dhama, K., 2014. Oxidative stress, prooxidants, and antioxidants: The interplay. *Biomed. Res. Int.*, 2014: 761264. https://doi.org/10.1155/2014/761264

Sacchetti, G., Maitetti, S., Muzzoli, M., Scaglioni, M., Manfredini, S., Radice, M., and Bruni, R., 2005. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Fd. Chem.*, 91: 621–632. https://doi.org/10.1016/j.foodchem.2004.06.031

Setzer, W.N., 2009. Essential oils and anxiolytic aromatherapy. *Nat. Prod. Commun.*, 4: 1305-1316. https://doi.org/10.1177/1934578X0900400928

Teixeira, B., Marques, A., Ramos, C., Serrano, C., Matos, O., Neng, N.R., Nogueira, J.M.F., Saraiva, J.A. and Nunes, M.L., 2013. Chemical composition and bioactivity of different oregano (*Origanum vulgare*) extracts and essential oil. *J. Sci. Fd. Agric.*, 93: 2707–2714. https://doi.org/10.1002/jsfa.6089

Wang, J., Zhai, Y., Ou, M., Bian, Y., Tang, C., Zhang, W., and Li, G., 2021. Protective effect of lemon peel extract on oxidative stress in H9c2 rat heart cell injury. *Drug Des. Dev. Ther.*, 15: 2047. https://doi.org/10.2147/DDDT.S304624

Yousuf, S., Emad, S., Ahmad, S., Qadeer, S., Sadaf, S., Sheikh, S., Sarfaraz, Y., Mehdi, B.J., and Perveen, T., 2018. Alteration in redox profile and behavioral effects following repeated administration of citral in rats. *Pak. J. Pharm. Sci.*, 31: 2639-2644.