Alteration in redox profile and behavioral effects following repeated administration of citral in rats

Sarwat Yousuf[1], Shaista Emad[1,2], Saara Ahmad[3], Sara Qadeer[1], Sana Sadaf[1], Sheeza Sheikh[1], Yousra Sarfaraz[1], Bushra Jabeen Mehdi[4] and Tahira Perveen[1]*

[1]Neurochemistry and Biochemical Neuropharmacology Research Unit, Department of Biochemistry, University of Karachi, Pakistan
[2]Department of Biochemistry, Jinnah Medical & Dental College, Karachi, Pakistan
[3]Department of Biochemistry, Agha Khan University Hospital, Karachi, Pakistan
[4]Department of Bio-Medical Engineering, Sir Syed University of Engineering and Technology, Karachi, Pakistan

Abstract: Essential oils are natural products having several important chemical constituents. Traditionally used worldwide as natural alternatives for treating various pathological conditions due to their antibacterial, anti-inflammatory, antifungal and antioxidants properties. Citral is one of the mono terpene present in lemon peel oil. The present study aimed to evaluate the effects of citral at low (0.1 mg/kg) and high (1 mg/kg) doses. In this study rats were subjected to different behavioral parameters such as tail suspension test (TST) to monitor depressive behavior, open field test (OFT) for locomotor activity, light/dark transition test (LDT) for the assessment of level of anxiety and the strength of muscles were monitored by Kondziela's inverted screen test. Plasma corticosterone and antioxidant enzymes activities were also estimated. The results from the present study showed that citral at 0.1mg/kg dose significantly increased the mobility time in TST, increased number of square crossed in OFT, increased time spent in LDT and showed muscles strengthen activity in Kondziela's inverted screen test. Lipid per oxidation (LPO) was decreased and antioxidant profile was improved along with the decrease in plasma corticosterone following the administration of 0.1mg/kg dose of citral in rats. However, at a high dose of 1 mg/kg of citral, behavioral alterations were observed along with the increased plasma corticosterone and decreased activities of antioxidant enzymes in rats. Therefore present findings suggested that citral at low dose has therapeutic potential as compared to high dose. It can be used as an alternative therapy for the treatment of various ailments in humans and animals.

Keywords: Anxiety, antioxidant, corticosterone, citral, depression.

INTRODUCTION

The therapeutic use of Essential oils (EO's) has a long tradition (Dobetsberger and Buchbauer, 2011). Previously EO's were mainly used for fragrance and flavoring drinks, foods and other products in industries (Ormancey *et al.,* 2001). Citral extracted from peel of citrus lemon has a fresh, strong, lemony and fruity odor (Odeh *et al.*, 2012). It has significant therapeutic properties such as antifungal, antibacterial, antiinflammatory and antioxidant (Vaio *et al.,* 2010). Terpenes are considered as the major components and organic properties of EO's are also determined by these components (Pichersky *et al.,* 2006). Monoterpenes that contribute 90% of EO's is one of the classes of terpenes comprising of two isoprene (C10) units (Bakkali *et al.,* 2008). Lemon oil is one of the commonly used essential oil with variety of culinary uses and pharmacological activity (Oboh *et al.,* 2014, Gonzalez *et al.,* 2010). Citral is one of monoterpene present in lemon peel oil. Numerous studies have been reported regarding the antioxidant properties of citral (Aazza *et al.,* 2011). Citral also acts on the central nervous system and helps in the treatment of fatigue, lethargy, insomnia, anxiety and depression (Yang *et al.*, 2009).

**Corresponding author:* e-mail: tahiraatiq@hotmail.com

Literature supports researches done on different doses of citral (Dobetsberger and Buchbauer, 2011, Oboh *et al.*, 2014). However, so far no comparative investigation has been carried out on different doses of citral. Therefore, the purpose of present work was to investigate and compare low and high doses of citral on psychological parameters and antioxidant profile in rats.

MATERIALS AND METHODS

Experimental protocol

Locally breed Sprague Dawley rats (180g to 200g) were purchased from HEJ Institute animal house (Voucher number: 7824/2016). Animals were caged separately in specifically designed cages in a quiet room to avoid any social interaction. Animals had free access to food and water for one week before commencement of experiment so that rats became habitual to the environment. Experiment was run in a controlled room temperature. Eighteen rats were divided into three groups (n=6): control group, test 1 (0.1mg/kg) and test 2 (1mg/kg) of citral groups. The control rats were given with 0.5 ml distilled water orally and test groups were administered with respective doses of citral intraperitoneally for 7 consecutive days. Citral was purchased from Sigma-Aldrich Company USA. All the animals of three groups

were then subjected to OFT to assess locomotor activity, light/dark transition test (LDT) to assess anxiolytic activity, tail suspension test (TST) to assess depressive behavior and Kondziela's inverted screen test to assessed the combine four paw and hind paw strength in rats. After behavioral analysis rats were decapitated using guillotine and their brain and plasma samples were collected and stored at -70^{o}C for biochemical investigations. Experiments were carried out between 10:00 am - 5:00 pm to avoid order and time influence. Formal approval of the study was taken from the Institutional Review Board (IRB) ref no: 02811/SC-2014. The guidelines made by National Institute of Health (NIH) Care and Use of laboratory animals were strictly followed (Publication No. 85-23, revised 1985).

Behavioral assessment

The locomotor activity in rats was monitored by open field test (Kennett *et al.,* 1985). The apparatus was same as used previously in our laboratory (Naqvi *et al.,* 2012).The levels of anxiety was determined by light/dark transition test described previously by Khaliq (Khaliq *et al.,* 2012). The muscular strength of all four limbs of rats was checked by Kondziela's inverted screen test (Kondziela, 1964). In the center of a square screen, rat is placed and immediately spin, too upside down with the rats head decline first; the time to fall was recorded. Scores were given as falling between 1 to 10 sec = 1, 11 to 25 sec =2, 26 to 60 sec =3 and over 60 sec =4 (Deacon, 2013).The depressive behaviors in rats were monitored by using tail suspension test. Rats were sling by the tail during which they tried to escape and showed agitation and immobility. The cut off time was six minutes and the duration of the immobility was noted as a measure of levels of depression in rats.

Biochemical estimations

Lipid peroxidation (LPO) was estimated as method described by Chow and Tappel (Chow and Tappel, 1972) and is represented as µmoles of MDA/g of brain. Activity of super oxide dismutase (SOD) in brain was estimated by the method of Chidambara (Chidambara *et al.,* 2002). The SOD activity was measured when nitro blue tetrazolium chloride (NBT) reduced to form blue formazan, a water-insoluble compound. SOD is expressed in U/g of brain. Catalase (CAT) was performed by the method described by Sinha (Sinha, 1972) and measured in terms of µmol/min/g of brain tissue. Glutathione peroxidase (GPx) activity was estimated by the method of Flohe (Flohe and Gunzler, 1984). GPx is represented as µmol/min/g of brain. Peterson and Pierce (Peterson and Pierce, 1960) described the procedure of extraction for plasma corticosterone. Plasma corticosterone concentration was measured by spectrofluorometric method as described earlier by Mattingly (Mattingly, 1962). 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 and measured in µg/ml.

STATISTICAL ANALYSIS

Results are represented as mean ± SD. Data of behavioral and biochemical analysis evaluated by one-way Anova of SPSS version 20. Post hoc analysis was done by Tukey's test. p >0.05 was considered to be non-significant.

RESULTS

Assessment of depression by tail suspension test

Fig. 1 shows the effect of citral at a dose of 0.1mg/kg and 1mg/kg on the mobility time by tail suspension test. Data analysis by one-way Anova (df 2, 15, F=45.57, p<0.01) showed significant effect of citral. Tukey's test showed significantly increased (p<0.01) mobility time in 0.1mg/kg and significantly decreased (p<0.5) mobility in 1mg/kg citral treated rats as compared to control.

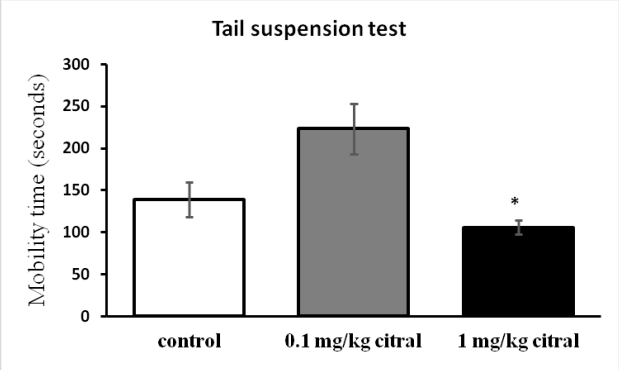

Fig. 1: Effect of citral on tail suspension test in rats. Values are mean ± SD (n=6) significant difference by Tukey's test *(p<0.05) from control.

Assessment of anxiety by light/dark transition test

Fig. 2 shows the effect of citral at a dose of 0.1mg/kg and 1mg/kg on anxiety by light/dark transition test of rats. Data analysis by one-way Anova (df 2, 15, F=47.89, p<0.01) showed significant effect of citral. Tukey's test showed significantly increased (p<0.01) time spent in light box in 0.1mg/kg and significantly decreased (p<0.01) time spent in light box in 1mg/kg citral treated rats as compared to control.

Assessment of locomotor activity by Open field test

Fig. 3 shows the effect of citral at a dose of 0.1mg/kg and 1mg/kg on the locomotor activity of rats. Data analysis by one-way Anova (df 2, 15, F=123.26, p<0.01) showed significant effect of citral. Tukey's test showed significantly increased (p<0.01) number of square crossed in 0.1mg/kg and 1mg/kg citral treated rats as compared to control.

Assessment of combine forepaw and hind paw strength by Kondziela's inverted screen test

Fig 4 shows the effect of citral at a dose of 0.1mg/kg and 1mg/kg on the forepaw and hind paw muscular strength

of rats. Data analysis by one-way Anova (df 2, 15, F=152.50, p<0.01) showed significant effect of citral. Tukey's test showed significantly increased (p<0.01) latency to fall in 0.1mg/kg and significantly decreased (p<0.01) latency to fall in 1mg/kg citral treated rats as compared to control.

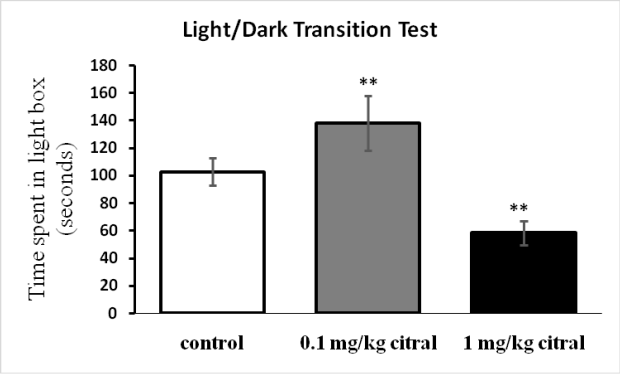

Fig. 2: Effect of citral on Light/Dark box activity in rats
Values are mean ± SD (n=6) significant difference by Tukey's test ** (p<0.01) from control.

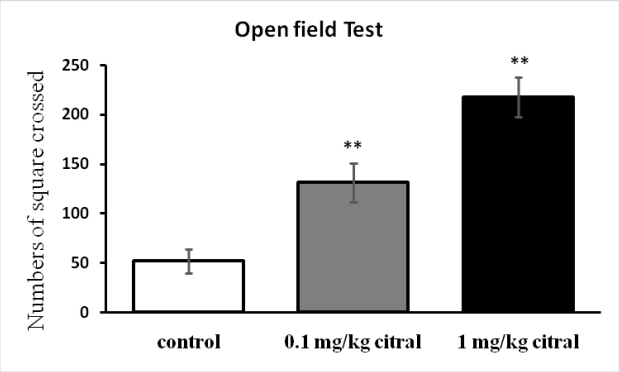

Fig. 3: Effect of citral on open field test in rats.
Values are mean ± SD (n=6) significant difference by Tukey's test ** (p<0.01) from control.

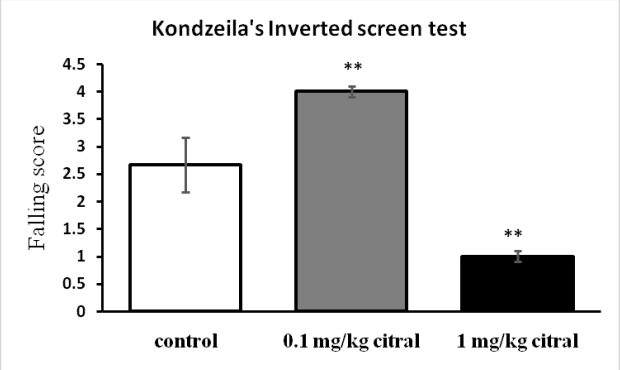

Fig. 4: Effect of citral on Kondzeila's inverted screen test in rats.
Values are mean ± SD (n=6) significant difference by Tukey's test ** (p<0.01) from control.

Effect of citral on lipid peroxidation

Table 1 shows the effect of citral at a dose of 0.1mg/kg and 1mg/kg on lipid peroxidation in rats. Data analysis by one-way Anova (df 2, 15, F=31.09, p<0.01) showed significant effect of citral. Tukey's test showed significantly decreased (p<0.01) MDA levels in 0.1mg/kg and significantly increased (p<0.01) MDA levels in 1mg/kg citral treated rats as compared to control.

Effect of citral on antioxidant enzymes (SOD, CAT and GPx) activities

Table 1 shows the effect of citral at a dose of 0.1mg/kg and 1mg/kg on the superoxide dismutase activity in rats. Data analysis by one-way Anova (df 2, 15, F=33.08, p<0.01) showed significant effect of citral. Tukey's test showed significantly increased (p<0.01) SOD activity in brain in 0.1mg/kg and significantly decreased (p<0.01) in 1mg/kg citral treated rats as compared to control. Table 1 shows the effect of citral at a dose of 0.1mg/kg and 1mg/kg on catalase activity in rats. Data analysis by one-way Anova (df 2, 15, F=5.85, p<0.01) showed significant effect of citral. Tukey's test showed significantly increased (p<0.05) CAT activity in 0.1mg/kg citral treated rats as compared to control. Table 1 shows the effect of citral at a dose of 0.1mg/kg and 1mg/kg on the glutathione peroxidase activity in rats. Data analysis by one-way Anova (df 2, 15, F=23.36, p<0.01) showed significant effect of citral. Tukey's test showed significantly increased (p<0.05) GPx activity in 0.1mg/kg and significantly decreased (p<0.01) in 1mg/kg in citral treated rats as compared to control.

Effect of citral on plasma corticosterone levels

Table 1 shows the effect of citral at a dose of 0.1mg/kg and 1mg/kg on the plasma corticosterone levels in rats. Data analysis by one-way Anova (df 2, 15, F=33.14, p<0.01) showed significant effect of citral. Tukey's test showed significantly decreased (p<0.01) in 0.1mg/kg and significantly increased (p<0.05) plasma corticosterone levels in 1mg/kg citral treated rats as compared to control.

DISCUSSION

The diversified properties of essential oils and their components are of great interest nowadays in the scientific world. The monoterpene is the third most abundant active component in the lemon peel oil. Citral has a safe profile for the human consumption as food additives (Gonzalez *et al.*, 2010). In this study we aimed to investigate the effects of low (0.1mg/kg) and high (1mg/kg) doses of citral on psychological parameters, antioxidant enzymes activities and corticosterone levels in rats. The results of present study showed improvement in behavioral activities in rats at low dose of citral. It is also observed that at low dose of citral, lipid peroxidation and corticosterone levels were decreased with subsequent increase in antioxidant enzyme activities in rats. In current study rats exposed to open field test showed increased locomotion as it is evident by increased number of square crossed at low dose citral. The marked increase in number of square crossed was also observed in rats at

high dose of citral. In open field test novel space sometime causes stress and anxiety, so rats remained to stay at corners and they do not prefer central area only wall to wall movement usually observed (Rebai and Djebi, 2008). Similar findings were observed in rats treated at high dose of citral as time spent in central area was decreased and wall to wall movement was observed. Here, a relation may be drawn between high dose of citral and stress like behavior in rats. The anxiolytic effects of low dose citral were monitored in LDT as there was increased time spent observed in light box. It has been reported previously that functional properties of various voltage and ligand gated ion channel can be modified by monoterpenes. The monoterpenes act via GABA gated chloride channels; tyramine, acetylcholine esterase and sodium channel on mammalian and rodent brain receptors (Lopez and Pascual, 2015). GABA receptors are mainly involved in behavioral changes (Tong and Coats, 2010). It is also reported that monoterpenes at high doses cause inhibition of GABA receptors (Czyzewska and Mozrzymas, 2013) and produce different effect at different doses. At low dose it produces anxiolytic effect however at high dose it produce anxiogenic effect. It is therefore suggested that anxiogenic and anxiolytic effects of high and low doses of citral respectively, observed in the study may be due to its effect on GABA receptors. Tail suspension test is a widely recognized test to monitor depressive behaviors in rats (Cherng *et al.,* 2013).The antidepressant effects of low dose citral was monitored as immobility time was significantly decreased in rats. However, at a high dose of 1mg/kg caused depressive behavior which is evident by increased immobility in rats. The plasma corticosterone levels were decreased in low dose citral as compared to high dose treated rats. High secretion of glucocorticoids (GCs) for prolonged duration has been linked with different pathological conditions and result in elevated oxidative stress by affecting the redox balance. The behavioral outcomes are also coupled with the physiological status and the GC mimic a condition of physiological stress (Costantini and Marosco, 2011). It could be suggested that high dose of citral produced a stress like condition in rat brain as a result of which corticosterone levels were increased and the behaviors were impaired in rats (Costantini and Marosco, 2011). However, low dose of 0.1mg/kg citral decreases the plasma GC levels and produces antidepressant effects. The Kondziela's inverted screen test used to monitor the muscular strength also reflects the brain functions. If brain functions are altered, it affects the coordination of the muscles. Citral at a dose of 0.1mg/kg improved the neuromuscular coordination in rats which is shown by the increased strength in four paws. Moreover, 1mg/kg of citral worsen the neuromuscular coordination in rats and it significantly decreased the four paw strength in rats in the present study. It is also reported previously that high corticosterone levels increase muscles proteolysis which ultimately decreased body weight (Bowes *et al.,* 1996). GCs also act on frontal lobes of the brain which is associated with the grip of muscles (Cahill and McGaugh, 1998). Therefore it may be suggested that high levels of corticosterone at 1mg/kg of citral causes muscle weakening which results in diminished muscular strength. Reactive oxygen species (ROS) are formed in our body by normal metabolic processes. If the levels of ROS increases and equilibrium are not maintained between ROS and antioxidants, it leads to a number of pathological ailments (Miguel, 2010). Because of the high consumption of oxygen; brain is more susceptible to oxidative stress (Floyd and Hensley, 2002). Severe neuronal damages can be caused by oxidative stress in humans and animals because the outer membrane of neurons is rich in polyunsaturated fatty acids (PUFA) and they are at risk of ROS attack (Haddadi *et al.,* 2014). Neuronal degeneration due to oxidative stress can provide difficulties in various behaviors (Fukui *et al.,* 2002). The endogenous antioxidants enzymes are also susceptible to ROS, therefore the dietary or exogenous antioxidants has usefulness against various pathological conditions caused by ROS (Gospodaryov and Lushchak, 2012). The antioxidants provide electron to the free radicals and make them stable and itself become oxidized. In the present study effect of citral on antioxidant enzymes and lipid peroxidation (LPO) was also monitored. Malondialdehyde (MDA) is formed by the oxidation of lipid as a marker of LPO (AK and Gulcin, 2008).Lipid peroxidation was decreased at a dose of 0.1mg/kg which shows its protective effects against free radicals. However, LPO was increased at dose of 1mg/kg of citral. The levels of SOD, CAT and GPx were also increased at 0.1mg/kg and decreased at 1mg/kg of citral. It is observed that dose of 0.1mg/kg improves the overall antioxidant profile in contrast to 1mg/kg that impaired them. Here, we may suggest that citral at low dose has ameliorating effects on behavior in rats. The activities of endogenous antioxidant enzymes were increased with decrease in plasma corticosterone levels at low dose of citral. On the other hand at 1mg/kg dose of citral the behavioral outcomes were hindered due to biochemical alterations in rat brain. This may be due to the fact that at high concentration, it acts as redox cycling prooxidant and produce toxic oxygen species particularly H_2O_2 (Asthana *et al.,* 1992). Citral is a nonpolar molecule previously it has been reported that toxicity limit of nonpolar antioxidant can exceed with the misuse of undiluted essential oils (Prashar *et al.,* 2006).The continuous generation of reactive oxygen species causes citral to react them and get oxidized (Misharina *et al.,* 2011). It is therefore suggested in the present study, as the dose of citral is higher it oxidized in large amount and start working as prooxidant molecule. Furthermore monoterpenes are volatile and lipophilic in nature and produce their effects by interacting with GABA synapses and modulate the physiological functioning (Lopez and Pascual, 2015). Present study reveals that at 0.1mg/kg

Table 1: Effect of citral on biochemical parameters

Biochemical estimations	Units	Control	0.1mg/kg Citral	1mg/kg Citral	One Way Anova (df 2, 15)
Lipid peroxidation (LPO) (MDA levels)	(µmol/g)	178.64±18.81	149.67±8.72**	205.46±4.51**	F=31.09,p<0.01
Superoxide dismutase (SOD)	(U/g)	1.73±0.05	1.85±0.03**	1.61±0.05**	F=33.08,p<0.01
Catalase (CAT)	(µmol/min/g)	431.49±7.0	442.32±5.73*	429.16±7.2	F=5.85, p<0.01
Glutathione peroxidase (GPx)	(µmol/min/g)	134.95±11.19	154.56±8.45*	109.10±14.27**	F=23.36,p<0.01
Corticosterone (CORT)	(µg/ml)	181.18±16.72	133.16±17.17**	208.84±14.86*	F=33.14,p<0.01

Values are mean ± SD (n=6) significant difference by Tukey's test ** ($p<0.01$) and *($p<0.05$) from control.

dose of citral exert antioxidant activity that may provide healthy environment for the neurons in brain and protect them from being damaged by ROS and ultimately improve the overall health. Prooxidants at a much higher concentration enter and can damage mitochondria due to permeability to cross the cell membrane (Bakkali *et al.*, 2008). In the present work it is suggested that low dose of citral prevented cellular damages whereas, at high concentration damages were accelerated in the cell. It resulted in the impairments of behaviors and antioxidant profile in the brain. It is also suggested that at low concentration cellular damages not occurred and antioxidant enzymes were not oxidized.

CONCLUSION

In conclusion "present study suggested that citral at low dose have beneficial effects on behaviors and improve the overall antioxidant profile as compare to the high dose at which it act as prooxidant, produced behavioral deficits and altered antioxidant enzymes".

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