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Full Length Article

Carvacrol ameliorates behavioral disturbances and DNA damage in the brain of rats exposed to propiconazole

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ABSTRACT

Propiconazole (PCZ) is an ergosterol biosynthesis inhibiting fungicide. Carvacrol (CAR) is a monoterpenoid phenol that has various beneficial health effects. The current research was designed to study the impact of PCZ on the behavior of rats and its ability to induce DNA damage in neurons as well as to clarify the ameliorative effect of CAR against these toxic impacts. Sixty Sprague-Dawley rats were randomly and equally divided into 4 experimental groups and treated daily by oral gavage for 2 months as follows: Group 1 (control); group 2 treated with PCZ (75 mg/kg); group 3 treated with CAR (50 mg/kg) and group 4 treated with both PCZ and CAR. Behavioral tests demonstrated that exposure to PCZ had a deleterious effect on psychological, motor and cognitive neural functions. Additionally, antioxidant enzyme activities, SOD and GSH-Px, were declined in brain tissue following exposure to PCZ. Moreover, comet assay revealed a high percent of DNA damage in the brain of rats exposed to PCZ. On the other hand, CAR administration ameliorated the harmful effects induced by PCZ through a protective mechanism that involved the improvement of neural functions and attenuation of oxidative stress and DNA damage.

1. Introduction

Fungicides are a class of pesticides used for the protection of human and animals against various diseases caused by fungi (Tabassum et al., 2016). Conazoles are fungicides containing an azole ring and are divided into two groups, imidazoles, and triazoles, according to the number of nitrogen atoms in their azole ring (Kjærstad et al., 2010). Triazole fungicides have been reported to induce various neurobehavioral toxic effects in rats and mice. Neurobehavioral abnormalities and pathological lesions were produced in rats following perinatal exposure to tebuconazole (Moser et al., 2001). Additionally, triadimefon is known to block the reuptake of the neurotransmitter dopamine (DA), and increase ambulatory and vertical activity (Reeves et al., 2003). Moreover, intra-striatal administration of flutriafol produced a significant increase in DA levels (Santana et al., 2009). Furthermore, Xi et al. (2012) found that the decrease in hippocampal retinoic acid concentration after exposure to triadimefon might be responsible for its suppressive effect on spatial learning and reference memory in rats. Recently, penconazole was found to induce oxidative stress and lipid peroxidation as well as various pathological injuries in the brain of rats (Chaâbane et al., 2017).

Propiconazole (*cis-trans*-1-[2-(2,4-dichlorophenyl)4-propyl-1,3-dioxolan-2-ylmethyl]-1*H*-1,2,4-triazole) is a triazole fungicide used for protecting a wide variety of crops against invasion of fungi (Sun et al., 2005). PCZ exerts its fungicidal activity via inhibition of lanosterol 14α-demethylase, a cytochrome P450-dependent enzyme, which is responsible for the transformation of lanosterol into ergosterol. Since ergosterol is a primary component of fungal cell membrane, inhibition of its synthesis brings about the death of fungi (Debeljak et al., 2003). Few studies in fish have explored the neurobehavioral toxic effects of PCZ (Li et al., 2010b, 2011; Srivastava and Singh, 2014; Tabassum et al., 2016). PCZ exposure had resulted in increased levels of reactive oxygen species and inhibition of enzymatic and non-enzymatic antioxidants, in addition to decreased activities of acetylcholinesterase (AChE), Na^+/K^+ ATPase and monoamine oxidase (MAO) enzymes in the brain of fish. Moreover, behavioral disturbances and pathological injuries were observed in the nervous system of PCZ-exposed fish.

Carvacrol (2-methyl-5-[1-methylethyl]phenol) is one of the volatile constituents of essential oils produced by various aromatic plants, such as oregano and thyme (Pezzani et al., 2017; Sharifi-Rad et al., 2018). Several studies have clarified the neuroprotective effect of CAR in rats and mice. Savelev et al. (2004) mentioned that the small molecular size of CAR and its lipophilicity makes it easy to cross the blood-brain barrier and produce its therapeutic impacts in an effective way.

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Additionally, Jukic et al. (2007) stated that the AChE inhibitory activity of CAR is 10 times more potent than its isomer thymol and appears to play an essential role in improving cognitive function. Melo et al. (2010) and Melo et al. (2011) also reported that CAR has anxiolytic and antidepressant effects in mice. Later on, Azizi et al. (2012) demonstrated that the ability of CAR, to ameliorate amyloid β or scopolamine-induced cognitive impairments, depends on its antioxidant and anti-inflammatory properties. In addition, Yu et al. (2012) found that CAR had decreased the level of cleaved caspase-3, a marker of apoptosis, suggesting the anti-apoptotic activity of CAR. Moreover, Deng et al. (2013) showed that CAR had prevented behavioral, biochemical and molecular abnormalities associated with diabetes, depending on its hypoglycemic, anti-inflammatory and antioxidant effects. Furthermore, Baluchnejadmojarad et al. (2014) reported that CAR has an ameliorative effect against oxidative stress and lipid peroxidation induced by 6-hydroxydopamine where it decreased the levels of MDA and nitrite and increased CAT enzyme activity. Recently, Li et al. (2016) stated that the levels of inflammatory cytokines and myeloperoxidase enzyme activity were decreased in ischemic cortical tissues in addition to an increase in SOD activity and a decrease in MDA level after treatment with CAR. Finally, Wang et al. (2017) mentioned that CAR ameliorated the cognitive impairment, free radicals synthesis, and apoptosis in mice treated with ethanol and also decreased ethanol-induced apoptosis in hippocampal nerve cells in vitro.

Our previous study (Noshy et al., 2018) has indicated that PCZ had an adverse effect on the behavior of rats where exposure to PCZ caused fear, depression, and dysfunction in spatial memory. In addition, various biochemical and pathological changes were reported in the brain of rats after exposure to PCZ. Furthermore, Concurrent administration of CAR with PCZ ameliorated these harmful effects. The present study aims to investigate the effects of PCZ on another group of neural functions such as anxiety, motor functions and cognitive memory by conducting a new set of behavioral tests, as well as to clarify whether DNA damage in the nerve cells plays a role in the emergence of these effects, and also to emphasize the ameliorative role of CAR against such toxic impacts.

2. Materials and methods

2.1. Chemicals

The source of PCZ is Zhejiang Rayfull Chemicals Co., Ltd., China. While the source of CAR is Sigma-Aldrich Chemicals Co., St. Louis, MO, USA. The other chemicals are of analytical grade and were purchased from accredited local companies.

2.2. Animals

Sixty Sprague-Dawley rats (240–260 g) were utilized as laboratory animals in this experiment. They were bought from the animal house in the department of veterinary hygiene and management, faculty of veterinary medicine, Cairo University. Rats were reared in cages made of plastic and fed with pelleted feed and water was supplied ad libitum. Their health status was regularly examined and they were adapted to the laboratory environment for 10 days before use. The experimental design was approved by the institutional animal care and use committee (IACUC) of Cairo University (IACUC protocol number: CU-II-S-50-17). Our experiment follows the rules of the National Institutes of Health (NIH).

2.3. Experimental protocol and animal grouping

Rats were indiscriminately and equally divided into 4 groups, 15 rats each, and were given PCZ and/or CAR daily by oral gavage for 2 months as follows:

The 1st group: It was used as a control group.

The 2nd group: It was exposed to 1/20 of the LD_{50} of PCZ (75 mg/kg) according to Sun et al. (2005).

The 3rd group: It was treated with CAR (50 mg/kg) according to previous studies (Nafees et al., 2013; Deng et al., 2013; Wang et al., 2017).

The 4th group: It was given both PCZ and CAR by the aforementioned doses.

2.4. Behavioral evaluation

At the end of the exposure period, neural functions of rats were assessed using the following behavioral tests:

2.4.1. Open field test

The open field is a square arena (90 × 90 × 25 cm) made of wood and covered with Formica laminate to prevent absorption of fluids (urine of rats). The floor is divided by cross lines into 36 small squares (15 × 15 cm). The rat was placed randomly into one of the four corners of the open field, facing the center, and permitted to move freely for 5 min. The number of peripheral squares crossed, rearing against the wall, fecal boli, urination, and entries into the central area were recorded (Gould et al., 2009).

2.4.2. Rod walking test

The rat was placed at the middle of a horizontal elevated rod (100 cm in length and 2.6 cm in diameter) for 60 s. The time of falling of the rat from the rod onto a cushion placed below was recorded (Shukitt-Hale et al., 1998).

2.4.3. Object recognition test

Two identical objects were placed in the back left and right corners of an empty cage. The rat was placed at the middle of the wall opposite to the objects. The rat was permitted to explore these objects for 10 min and then the rat was removed from the cage and returned to its colony for 1 h. To test for object recognition, one of the familiar objects was placed in one back corner of the cage; and a novel object was placed in the other back corner and the rat was placed as described before for 4 min. The time spent by the rat exploring each object was recorded (Bevins and Besheer, 2006).

2.5. Collection and preparation of samples

After behavioral evaluation, rats were weighed, anesthetized by diethyl ether and sacrificed. The whole brain of each rat was collected and weighed; half of the brain was placed in a plastic bag and stored at −20 °C for determination of SOD and GSH-Px activities, while the other half was placed into ice cold phosphate buffered saline for comet assay.

2.6. Evaluation of enzymatic antioxidants

2.6.1. Homogenization of brain tissue

Cold buffer, i.e. 50 mM potassium phosphate buffer containing 1 mM EDTA, was used for homogenization of brain tissue using a tissue homogenizer followed by centrifugation. The supernatant was then collected for determining the activity of antioxidant enzymes.

2.6.2. Superoxide dismutase (SOD) enzyme activity

SOD enzyme activity in brain tissue was determined by a colorimetric method according to Nishikimi et al. (1972). This method depends on the ability of SOD enzyme to inhibit reduction of nitroblue tetrazolium dye by phenazine methosulphate. SOD kit was purchased from Biodiagnostic Company, Egypt.

2.6.3. Glutathione peroxidase (GSH-Px) enzyme activity

GSH-Px enzyme activity in brain tissue was determined by a colorimetric method according to Paglia and Valentine (1967). Oxidized glutathione, resulting from the reduction of organic peroxide by GSH-Px is recycled to its reduced state by glutathione reductase enzyme with concurrent oxidation of NADPH to NADP+ resulting in a decrease in absorbance at 340 nm providing a spectrophotometric mean for measuring GSH-Px enzyme activity. GSH-Px kit was purchased from Bio-diagnostic Company, Egypt.

2.7. Comet assay

The percentage of DNA damage in the brain tissue was determined using the comet assay according to the method described by Olive and Banáth (2006). Nerve cells were embedded in agarose and cast on a microscope slide. Embedded cells were then lysed with detergent and concentrated salt to form nucleoids consisting of supercoiled loops of DNA attached to the nuclear matrix. Electrophoresis at alkaline pH led to comet-like structures, visualized by staining with propidium iodide; the ratio of the comet tail to the head indicates the number of DNA breaks. The possible basis for this is that loops containing a break lose their supercoiling and become free to spread toward the positive electrode. CometScore analysis software was used for measurement of different comet assay parameters.

2.8. Statistical analysis

The results are presented as mean ± SE. Statistical analysis was done by using SPSS 16.0. One-way ANOVA was used for analysis of data followed by LSD post-hoc test for comparison between different groups. p values < 0.05 were considered statistically significant.

3. Results

3.1. Weight gain and relative brain weight

Exposure to PCZ resulted in a significant decrease in weight gain and a non-significant change in relative brain weight compared to the control group. While concurrent administration of CAR with PCZ resulted in a non-significant change in weight gain and relative brain weight compared to PCZ group (Table 1).

3.2. Behavioral evaluation

3.2.1. Open field test

Exposure to PCZ resulted in a significant increase in the number of peripheral squares crossed, rearing against the wall, fecal boli, and urine streaks as well as a significant decrease in the number of entries into the central area compared to control group. On the other hand, concurrent administration of CAR with PCZ ameliorated most of the effects of PCZ as there was a non-significant change in all recorded parameters compared to control group except the number of rearing against the wall which was significantly different from control and PCZ groups (Table 2).

3.2.2. Rod walking test

Exposure to PCZ resulted in a significant decrease in falling time compared to the control group. While concurrent administration of CAR with PCZ ameliorated the effect of PCZ as there was a non-significant change in falling time compared to the control group (Fig. 1).

3.2.3. Object recognition test

Exposure to PCZ resulted in a non-significant change in exploration time of new object and a significant increase in exploration time of familiar object compared to the control group. On the other hand, concurrent administration of CAR with PCZ ameliorated the effects of PCZ as there was a non-significant change in exploration time of new and familiar objects compared to the control group (Fig. 2).

3.3. Evaluation of enzymatic antioxidants

Exposure to PCZ resulted in a significant decrease in activities of SOD and GSH-Px enzymes in brain tissue compared to the control group. While concurrent administration of CAR with PCZ ameliorated the effects of PCZ as there was a significant increase in activities of SOD and GSH-Px enzymes in brain tissue compared to PCZ group (Figs. 3 and 4).

3.4. Comet assay

Exposure to PCZ resulted in a significant increase in all comet assay parameters measured in brain tissue compared to the control group. While concurrent administration of CAR with PCZ ameliorated the effects of PCZ as there was a significant decrease in all comet assay parameters measured in brain tissue compared to PCZ group (Table 3) and (Fig. 5).

4. Discussion

A significant decrease in weight gain was observed after exposure to PCZ. This may result from PCZ-induced general toxicity bringing about anorexia and reduced food intake or due to reduced food efficiency following exposure to PCZ. Schmidt et al. (2016) reported that exposure to PCZ at the highest dose level (2400 ppm) led to a reduction in food efficiency during the second half of treatment. Moreover, Goetz et al. (2007) observed a decrease in body weight with a higher dose of PCZ (250 mg/kg). Contrary to our results, Sun et al. (2005) stated that PCZ had no effect on weight gain after 14 days of gavage treatment of rats or mice; this may be due to the short duration of exposure. Yet again, Costa et al. (2015) also mentioned that exposure to PCZ (4 mg/kg and 20 mg/kg) did not affect the body weight gain; this may be resulting from administration of small doses. Nonetheless, no significant change in weight gain was noticed after administration of CAR with PCZ. Furthermore, relative brain weight showed no difference between various groups of rats after exposure to PCZ and/or CAR. In line with our results, Sellers et al. (2007) agreed that changes in brain weight are rarely associated with neurotoxicity. Additionally, Piao et al. (2013) stated that the weight of the brain showed low variability compared with other organs and is not considered to be influenced by nutritional factors.

Behavioral evaluation is a very important assay of neural functions because the behavior of human and animals is affected by changes occurring within the nervous system (Kalueff and Tuohimaa, 2004).

Table 1
Effects of PCZ and/or CAR on weight gain and relative brain weight.

Group	Control	PCZ	CAR	PCZ + CAR
Weight gain (g)	26.93 ± 1.426	19.33 ± 1.214[a]	24.07 ± 0.636[b]	22.47 ± 1.434[a]
Relative brain weight (%)	0.673 ± 0.0121	0.697 ± 0.0134	0.683 ± 0.0075	0.676 ± 0.0102

Values are presented as mean ± SE. (n = 15 rats/group).
[a] Indicates statistically significant difference from the control group.
[b] Indicates statistically significant difference from PCZ group at p < 0.05.

Table 2
Effects of PCZ and/or CAR on different parameters measured during the open field test.

Group	Control	PCZ	CAR	PCZ + CAR
No. of peripheral squares crossed	47.46 ± 4.21	73.31 ± 5.42[a]	49.13 ± 4.56[b]	60.85 ± 4.20
No. of rearing against the wall	10.23 ± 0.802	18.92 ± 1.389[a]	9.93 ± 0.881[b]	14.62 ± 1.071[a,b]
No. of fecal boli	1.92 ± 0.431	4.23 ± 0.778[a]	1.67 ± 0.333[b]	2.85 ± 0.564
No. of urine streaks	0.23 ± 0.122	0.62 ± 0.140[a]	0.20 ± 0.107[b]	0.38 ± 0.140
No. of entries into the central area	1.31 ± 0.308	0.31 ± 0.133[a]	1.13 ± 0.307[b]	0.85 ± 0.274

Values are presented as mean ± SE. (n = 15 rats/group).
[a] Indicates statistically significant difference from control group.
[b] Indicates statistically significant difference from PCZ group at $p < 0.05$.

Fig. 1. Effects of PCZ and/or CAR on falling time (sec) during the rod walking test. Values are presented as mean ± SE. (n = 15 rats/group). * indicates statistically significant difference from the control group and + indicates statistically significant difference from PCZ group at $p < 0.05$.

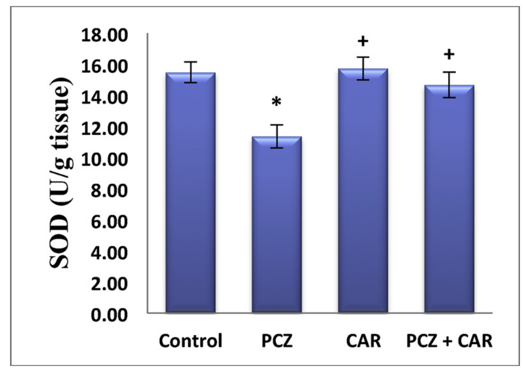

Fig. 3. Effects of PCZ and/or CAR on SOD enzyme activity (U/g tissue) in the brain. Values are presented as mean ± SE. (n = 15 rats/group). * indicates statistically significant difference from the control group and + indicates statistically significant difference from PCZ group at $p < 0.05$.

Three behavioral tests were conducted in our study for behavioral assessment of rats following exposure to PCZ and/or CAR, namely, the open field test (OFT), the rod walking test and the object recognition test. The OFT is a simple and popular test used to assess anxiety-related conflict emerging between exploration tendency of rats by wandering into the center of the arena and safety by remaining in a corner or along the periphery (Weisstaub et al., 2006). Increased ambulation and rearing in the periphery of the open field noticed in PCZ treated rats had been proven to reflect higher levels of anxiety and emotionality (fearfulness). In addition, enhancement of both horizontal and vertical activities in the corners and against the wall of the open field at first exposure might be on account of novelty-provoked more fear-related behavior as well as the tendency to escape in rats (Choleris et al., 2001; Anderson and Hughes, 2008). Moreover, frequent entries of central squares had been reported to indicate curious animal with lower levels of anxiety (Frye et al., 2000) and here again, PCZ was found to decrease entries of central squares in the open field. Finally, increased defecation and urination scores observed in PCZ treated rats are also satisfactory and sensitive measures for anxiety and emotionality (Homberg et al., 2002; Fromm et al., 2004). In conclusion, exposure to PCZ had adversely affected all the parameters measured during the OFT which is an indication of PCZ induced anxiety and emotionality in rats. However, rats concomitantly exposed to PCZ and CAR were non-significantly different from corresponding control rats in the majority of the recorded parameters which indicates that CAR had ameliorated most of the effects induced by PCZ due to its anxiolytic effect (Melo et al., 2010). What's more, the anxiogenic and anxiolytic effects of PCZ and CAR respectively were also demonstrated in our previous study in rats using the elevated plus maze test (Noshy et al., 2018).

Motor performance deficits include slow movement, decreased balance, and muscle strength as well as coordination difficulty (Seidler et al., 2002). Missions requiring control and coordination of motor and reflexive responses, for instance, the period of time an animal can cross/balance on a wooden rod or plank, are among the tests which are utilized to evaluate motor incapacities (Joseph and Lippa, 1986). PCZ

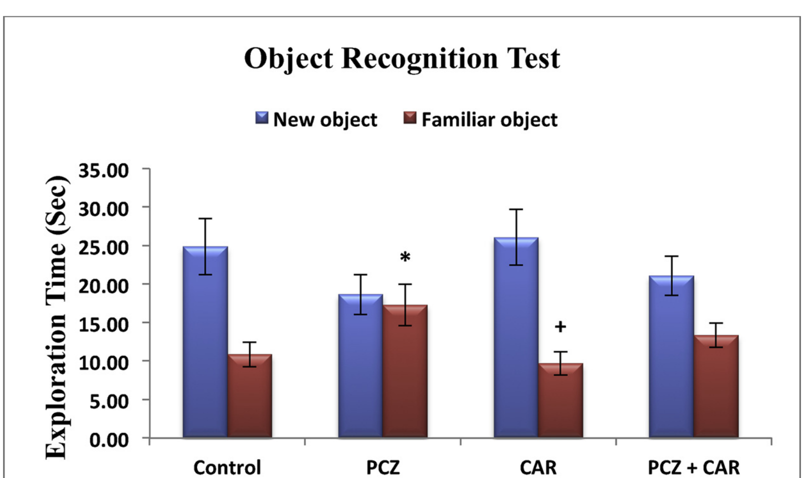

Fig. 2. Effects of PCZ and/or CAR on exploration time (sec) of new and familiar objects during the object recognition test. Values are presented as mean ± SE. (n = 15 rats/group). * indicates statistically significant difference from the control group and + indicates statistically significant difference from PCZ group at $p < 0.05$.

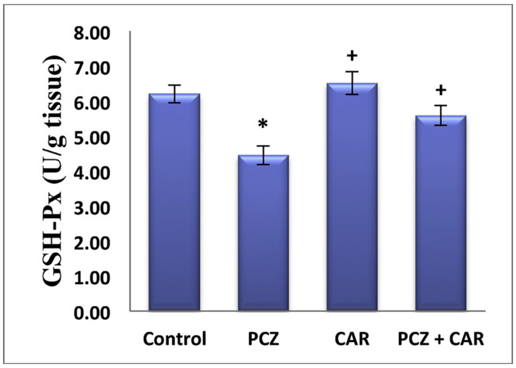

Fig. 4. Effects of PCZ and/or CAR on GSH-Px enzyme activity (U/g tissue) in the brain. Values are presented as mean ± SE. (n = 15 rats/group). * indicates statistically significant difference from the control group and + indicates statistically significant difference from PCZ group at $p < 0.05$.

treated rats showed a poor performance in rod walking test that relies on balance and coordination. Deficits in motor performance may result from cerebellum disorders, where the cerebellum is known to be crucial for functions related to movement, gait, posture, and balance (Konarski et al., 2005). As such, walking ataxia is considered one of the most important signs of cerebellar damage (Morton and Bastian, 2004). In line with our results, PCZ had been reported to induce behavioral abnormalities in fish like hypomovement and loss of equilibrium (Srivastava and Singh, 2014). Yet again, behavioral disorders such as hypoactivity, muscle weakness and imbalance were observed in rats following exposure to penconazole (Chaâbane et al., 2017). On the other hand, improvement in motor functions of rats was observed following concurrent administration of CAR with PCZ as there was no significant difference between the group exposed to PCZ and CAR and control group.

Rats and mice have a tendency to interact more with a novel object than a familiar object. This inclination has been utilized by behavioral pharmacologists and neuroscientists to study learning and memory (Bevins and Besheer, 2006). Although, there was no significant difference between all groups in the time spent in exploration of the novel object; the time spent in exploration of the familiar object was significantly higher in PCZ treated rats which is considered as an indicator for cognitive impairment (Wolf et al., 2016). On the other hand, CAR had improved recognition memory as there was no significant difference between rats concomitantly exposed to PCZ and CAR and control rats. In agreement with our results, Azizi et al. (2012) reported that CAR had ameliorative effect against amyloid β-induced intellectual weaknesses in rats. In addition, our previous study had revealed the adverse effect of PCZ as well as the ameliorative effect of CAR on spatial memory of rats using the Y-maze test (Noshy et al., 2018).

The toxicity of various xenobiotics depends on oxidative stress resulting from the extraordinary production of reactive oxygen species (ROS). The increased amount of ROS in brain tissue is supposed to be the cause for poor performance in motor and cognitive tasks in both mammals and invertebrates (Hu et al., 2006; Farooqui, 2008; Harrison et al., 2009). To prevent the possible adverse effects of ROS, an enzymatic antioxidant defense system made out of SOD, CAT, GSH-Px and other enzymes in addition to non-enzymatic co-substrates such as GSH are involved to repress the formation of oxygen radicals (Li et al., 2010a). In case of excessive production of ROS and/or depletion of antioxidants, the balance between the generation and clearance of ROS will be destroyed bringing about oxidative damage (Li et al., 2010b). SOD is an essential oxygen radical scavenger in tissues that changes the superoxide anion radical to hydrogen peroxide and water (Nordberg and Arner, 2001). GSH-Px acts as a scavenger of hydrogen peroxide and other hydroperoxides (Gate et al., 1999).

In the present investigation, the activity of SOD and GSH-Px enzymes was inhibited in the brain of rats following chronic exposure to PCZ. These findings illustrate that regardless of the fact that the brain has a powerful mitochondrial oxidative metabolism to deal with the high ATP demand for neural processing (Li et al., 2010b,c), it has a relatively low antioxidant defense system (Mates, 2000) and exposure to PCZ had brought about induction of oxidative stress in the brain of rats and exhaustion of its antioxidants. PCZ-impelled ROS formation can oxidize the majority of cell constituents, for example, DNA, proteins, and lipids, bringing about damage to these molecules, reducing enzymatic activity and influencing cellular integrity (Klaunig and Kamendulis, 2004). The results of the current study are in line with our previous study that revealed that exposure to PCZ had resulted in a reduction in the activity of CAT enzyme and GSH content and increased MDA concentration in the brain of rats (Noshy et al., 2018). Yet again, PCZ was found to induce oxidative stress and inhibit the antioxidant defense system in the brain of fish (Li et al., 2010b, 2011; Tabassum et al., 2016) as well as the liver of mice (Nesnow et al., 2011a; Bruno et al., 2009). On the other hand, the antioxidant effect of CAR was clearly demonstrated as there was a significant improvement in the activity of antioxidant enzymes in the brain of rats after synchronous administration of CAR with PCZ. In agreement with our results, a large number of trials including our previous study had confirmed the antioxidant role of CAR in neural tissue of rats and mice where it increased the activity of antioxidants and attenuated ROS production (Celik et al., 2013; Deng et al., 2013; Baluchnejadmojarad et al., 2014; Li et al., 2016; Samarghandian et al., 2016; Wang et al., 2017; Noshy et al., 2018).

The comet assay, also known as single cell gel electrophoresis (SCGE), is a rapid, sensitive and quantitative method by which DNA damage in eukaryotic cells can be detected. This assay depends on the measurement of broken DNA strands extending from the cell nucleus during electrophoresis (Liao et al., 2009). In the present study, exposure to PCZ had induced DNA damage in the neurons of rats where all the comet assay parameters were significantly and adversely affected following exposure to PCZ. This may be due to oxidative stress and increased ROS production caused by PCZ. As mentioned before, these unstable free radical species, ROS, can attack the cellular components, inducing damage to lipids, proteins, and nuclear and mitochondrial DNA as well as alter cell growth regulatory pathways (Klaunig and Kamendulis, 2004; Mori et al., 2007). In line with our results, Transcriptomic analyses identified a number of over-expressed genes associated with DNA damage, apoptosis, and general genotoxic stress,

Table 3
Effects of PCZ and/or CAR on different parameters measured during the comet assay.

Group	Control	PCZ	CAR	PCZ + CAR
Comet (%)	7.240 ± 0.449	25.740 ± 2.372*	6.540 ± 0.396+	13.360 ± 0.758*,+
Tail length (px)	1.682 ± 0.105	11.694 ± 1.068*	1.471 ± 0.096+	4.125 ± 0.348*,+
DNA in tail (%)	4.202 ± 0.241	15.144 ± 1.384*	3.616 ± 0.211+	7.487 ± 0.451*,+
Tail moment (arbitrary unit)	0.145 ± 0.009	1.012 ± 0.092*	0.123 ± 0.008+	0.337 ± 0.030*,+

Values are presented as mean ± SE. (n = 15 rats/group).
* Indicates statistically significant difference from control group.
+ Indicates statistically significant difference from PCZ group at $p < 0.05$.

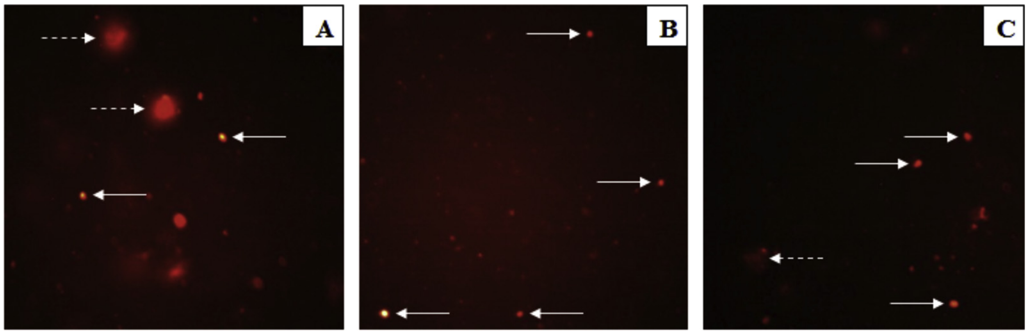

Fig. 5. Comet assay in brain tissue of rats showing the effects of exposure to PCZ and/or CAR on DNA damage: (A) Rats exposed to PCZ alone; (B) Rats exposed to CAR alone; (C) Rats exposed to both PCZ and CAR. A dashed arrow indicates a cell with a comet, while a continuous arrow indicates an intact cell.

namely Gadd45α and Gadd45β, in liver of mice fed diets containing 2500 ppm PCZ for 4–90 days (Allen et al., 2006; Ward et al., 2006; Nesnow et al., 2009, 2011a,b).

On the other hand, CAR had ameliorated the DNA damage induced by PCZ in the nerve cells where the entire comet assay parameters were significantly improved in rats treated concurrently with CAR and PCZ compared to PCZ-exposed rats. In agreement with our results, CAR was found to reduce the DNA damage in lymphocytes induced by peroxide or other proven genotoxins (i.e. 2-amino-3-methylimidazo[4,5-*f*]-quinoline and mitomycin C) (Ipek et al., 2003; Aydin et al., 2005a,b). In addition, CAR showed an ameliorative effect against H_2O_2-induced genotoxicity in human colonic Caco-2, hepatoma HepG2 and leukemic K562 cell lines (Horvathova et al., 2007; Slamenova et al., 2007). Moreover, the antigenotoxic activity of CAR was demonstrated in both in vivo and in vitro studies where CAR attenuated the damage of DNA in the hepatocytes of D-galactosamine-treated rats (Aristatile et al., 2011) or primary hepatocytes exposed to visible-light excited methylene blue (Slamenova et al., 2011). Furthermore, the protection against H_2O_2-induced DNA damage in hepatic and testicular tissues was higher in rats given CAR in drinking water (at 15–60 mg/kg for 7–14 days) (Slamenova et al., 2008).

5. Conclusions

In summary, the present findings confirm the adverse effects of PCZ on the behavior and neurological functions of rats where there was an increase in anxiety, in addition to impairment of cognitive memory and motor functions as demonstrated by behavioral evaluation. Moreover, PCZ not only had prompted oxidative stress in the brain of rats as evident by reduced activity of antioxidant enzymes but also brought about DNA damage as indicated by the comet assay. On the other hand, CAR had exhibited its potent neuroprotective effect and ameliorated most of the neurobehavioral toxic effects of PCZ due to its anxiolytic, antioxidant and anti-genotoxic activities. Future research on the effects of different doses of PCZ and CAR on various biological systems might prove important.

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Conflicts of interest

The authors declare no conflicts of interest.

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