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RESEARCH ARTICLE

Ciprofloxacin-induced neurotoxicity: evaluation of possible underlying mechanisms

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Abstract

Ciprofloxacin (CPX) is a fluoroquinolone antibiotic used for treating respiratory, urinary tract, gastrointestinal and abdominal infections. There are only a limited number of studies related to neurological adverse effects of this drug in therapeutic doses. Therefore, in the present study, we aimed to investigate the influence of CPX, when administered at pharmacological doses, on behavioral parameters of rats and the probable underlying mechanisms. CPX was administered in single oral daily doses of 20 and 50 mg/kg for 14 days in rats. CPX-induced depression and anxiety were evaluated by modified forced swimming test and elevated plus maze test, respectively. Also, spontaneous locomotor activity and motor coordination were assessed by activity cage and Rota-rod apparatus. Effects of CPX administration on brain serotonin, dopamine, γ-amino-butyric acid (GABA), glutamate, adrenaline and noradrenaline levels were determined by high performance liquid chromatography (HPLC) analysis. Contribution of oxidative stress to the changes induced by CPX administration was evaluated by measuring brain catalase, superoxide dismutase, glutathione (GSH) and malondialdehyde (MDA) levels. Our results indicated that depression-like and anxiety-like behaviors were observed only in the 50 mg/kg CPX-administered group with simultaneous decreases in the brain serotonin and GABA levels. In addition, in the brain homogenates of CPX-administered groups, increased MDA as well as decreased GSH and catalase activity with respect to their controls, indicated enhanced oxidative stress and weakened antioxidant defense system. In conclusion, repeated pharmacological doses of CPX were found to induce neurological toxicity. Also, altered brain neurotransmitter levels and increased oxidative stress observed in our study were thought to be the possible underlying mechanisms of ciprofloxacin-induced neurotoxicity.

Keywords

Anxiety, ciprofloxacin, depression, fluoroquinolone, oxidative stress

History

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Introduction

Ciprofloxacin (CPX) is one of the fluoroquinolone antibiotics, which is very effective against various pathogenic bacteria, including a wide range of gram-negative and a number of gram-positive organisms (Naora et al., 1999). According to oral antibiotic prescriptions data (US, 2010), CPX was the fourth (20.4%) among the most frequently prescribed antibiotic agents (Hicks et al., 2013).

CPX usage has been associated with many adverse drug reactions including neurological ones (Ahmed et al., 2011). Encephalopathy (Al-Ghamdi, 2002), dizziness, somnolence, confusion, agitation, delirium, acute organic psychosis, seizure, headache, abnormal vision (Al-Ghamdi, 2002; Azar et al., 2005; Kim et al., 2009), chorea (Al-Ghamdi, 2002), hemiballism (Kim et al., 2009), catatonia (Denysenko & Nicolson, 2011), major depression (Ahmed et al., 2011; Al-Ghamdi, 2002), anxiety (Rollof & Vinge, 1993), manic episode (Bhalerao et al., 2006) have been reported so far due to the use of CPX.

High dosage (Al-Ghamdi, 2002; Kim et al., 2009), female gender (Kim et al., 2009), young age (<45 years) (Ahmed et al., 2011; LaSalvia et al., 2010), renal failure (Al-Ghamdi, 2002; Azar et al., 2005; LaSalvia et al., 2010), pre-existence of a central nervous system disease (Al-Ghamdi, 2002; LaSalvia et al., 2010) have been defined as the risk factors for neurotoxic effects. Drug interactions between non-steroidal anti-inflammatory drugs, theophylline, caffeine and CPX have also been known as important factors in the context of toxicity (Azar et al., 2005; De Sarro et al., 1999). Magnesium chelator activity and oxidative stress inducing capacity of this antibiotic have been reported as possible mechanisms inducing these side effects. Besides, impairment in the GABAergic/glutamergic balance of brain

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has also been suggested as another factor contributing to the neurotoxic effects (Stahlmann & Lode, 1999; Talla & Veerareddy, 2011).

Although there are a few case reports presenting CPX-induced behavioral adverse reactions, neurotoxic effects caused by CPX administration have not been defined in the previous experimental studies. For this reason, we aimed to investigate the effects of repeated CPX administration, in two different pharmacological doses, on behavioral parameters of rats. We also determined levels of brain γ-amino-butyric acid (GABA), glutamate, dopamine, serotonin, adrenalin and noradrenalin, which are endogenous mediators regulating mood and behaviors, for the purpose of identifying possible underlying mechanisms related to neurotoxicity. Moreover, brain glutathione (GSH), superoxide dismutase, catalase and malondialdehyde (MDA) levels were measured in order to evaluate the possible contribution of oxidative stress to the CPX-induced neurotoxicity.

Materials and methods

Materials

CPX was a kind gift from IE Ulagay-Menarini Group, Istanbul, Turkey. Brain superoxide dismutase and catalase levels were determined by ELISA kits from Cayman Chemical Company (Ann Arbor, MI). MDA level was assayed by ELISA kit from Cusabio Biotech Co. Ltd. (Hubei, P.R. China). GSH, L-glutamate, *o*-phthalaldehyde (OPA), β-mercaptoethanol (βME), sodium tetraborate, potassium phosphate, adrenaline, noradrenaline, dopamine, homovanilic acid and serotonin were obtained from Sigma (Taufkirchen, Germany); acetonitrile and methanol from Merck GmbH (Darmstadt, Germany); GABA from Acros Organics (New Jersey, NY). Ultrapure water was produced by a Millipore Synergy Water Purification System (Rotterdam, The Netherlands).

An Agilent 1260 Infinity LC system (Waldbornn, Germany) was used for the determination of neurotransmitters, equipped with a fluorescence detector, an auto-sampler, and a column oven and a binary pump. For the determination of the catecholamines, a coulometric detector with graphite carbon cells was used (Coulochem III, Thermo Fisher Scientific Inc., Waltham, MA). Data acquisition and peak processing were performed with ChemStation for LC 3D systems, Rev. B.04.03(16), (Waldbornn, Germany).

Animals

Female Wistar rats weighing 250–300 g were obtained from Anadolu University Research Center for Animal Experiments. Rats were housed under controlled temperature (24 °C) and lighting (12/12-h light dark cycle) with free access to food and water. The experimental protocol was approved by the Local Ethical Committee on Animal Experimentation of Anadolu University, Eskisehir, Turkey.

The experimental groups of animals were as follows: Group 1, control animals treated orally with saline solution at a volume of 1 ml/100 g for 14 days ($n = 10$) (C); Group 2, animals treated orally with 20 mg/kg CPX at a volume of 1 ml/100 g for 14 days ($n = 10$) and Group 3, animals treated orally with 50 mg/kg CPX at a volume of 1 ml/100 g for 14 days ($n = 10$). The doses of CPX were determined according to the previous studies (Channa et al., 2008, 2012; Kaita et al., 1998; Nduka et al., 2013; Saraçoğlu et al., 2009; Sen et al., 2007). Behavioral tests were performed 60 min after the last dose of CPX.

Behavioral tests

Elevated plus-maze tests

Potential effect of CPX on anxiety behavior was evaluated by performing the elevated plus-maze test, as described previously (Can et al., 2011). At the start of the session, the rats were individually placed on a central platform facing an open arm. The number of entries and the time spent in both of the closed and open arms were recorded during a 5-min observation period. An ''arm entry'' was defined as the entry of all four paws into an arm. The percentage of open arm entries (POAE) and the percentage of time spent in the open arms (PTOA) for each animal were calculated using the following formulas:

$$\mathrm{POAE} = \frac{\text{number of open arm entries}}{\text{number of total arm entries}} \times 100,$$

$$\mathrm{PTOA} = \frac{\text{time spent in the open arm}}{\text{time spent in total arms}} \times 100.$$

Before and between tests, the apparatus was carefully cleaned with a wet tissue paper (10% ethanol solution) to remove any residue or odor of the animals.

Modified forced swimming test

Potential effect of CPX on learned helplessness behavior was evaluated by performing the modified forced swimming test (MFST), as described earlier (Can et al., 2009; Cryan et al., 2002). The rats were forced to swim individually in a glass cylinder. A 15-min pre-test was conducted 24 h before the 5-min swim test. During the test, times for swimming (horizontal movement on the surface of the water), climbing (upward-directed movements of the forepaws along the side of the cylinder) and immobility (movement required just to keep the head above the water) were recorded using a stopwatch.

For each animal, the water in the cylinder was changed after the test to avoid the influence of alarm substances. Following the training and the test sessions, the animals were dried in a heated enclosure.

Activity cage tests

Spontaneous locomotor activities of rats were monitored in an activity cage apparatus (Ugo Basile, no. 7420) (Can & Ozkay, 2012; Can et al., 2010). Total number of horizontal and vertical activities was recorded for 5 min.

Rota-rod tests

The effect of the CPX on motor coordination of rats was examined by Rota-rod test (Ugo Basile, no. 47 600), as described previously (Can et al., 2010). The latency to fall from the rotating mill was recorded for each rat tested.

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Following the behavioral tests, the animals were killed by decapitation with a guillotine, brain tissues were dissected and were frozen as quickly as possible in liquid nitrogen.

The brain tissues were washed with phosphate buffered saline (PBS), pH 7.4. They were diluted at the ratio of 1:20 (w:v) with PBS solution and homogenized. The homogenates were centrifuged at 10 000× g for 15 min at +4 °C and the supernatants were removed for assaying.

The supernatants were used for determining GABA, glutamate, serotonin, dopamine, noradrenaline and GSH levels with a standard analysis method by high performance liquid chromatography (HPLC) and catalase, superoxide dismutase and MDA levels with ELISA kits.

Determination of brain neurotransmitter levels

Determination of GSH, glutamate and GABA

Compounds were measured as their OPA/βME derivatives according to Lunn and Hellwig (1998). Derivatization was performed by mixing 9 µl of derivatization solution (5 mM OPA and 2 mM βME in 0.1 M borate buffer; pH 9.3) with 12 µl microdialysate and by holding 1 min before injection. The procedure was performed automatically by the auto-sampler. The derivatives were detected at the excitation wavelength of 230 nm and the emission wavelength of 450 nm. The analytical column was a Zorbax, Extend-C18 (150 mm× 3 mm, particle size 3.5 µm). A gradient elution consisting of solvent A: (methanol–acetonitrile–40 mM potassium phosphate buffer; pH 6.7), (20:2:78) and solvent B: (methanol–acetonitrile–40 mM potassium phosphate buffer; pH 6.7), (50:10:40) was used. The gradient elution was: 0–4 min, 0% B; 4–15 min, linear from 0% to 100% B; 15–20 min, holding 100% B; 20–25 min, linear from 100% to 0% B; 25–28 min, initial conditions (0% B) for equilibration of the column. Efficient and symmetrical peaks were obtained at 30 °C at a flow rate of 0.6 ml/min with a sample injection volume of 20 µl after the derivatization procedure.

Determination of adrenaline, noradrenaline, dopamine and serotonin

Adrenaline, noradrenaline, dopamine and serotonin were analyzed with ion-pair liquid chromatography using an electrochemical detector. The analytical column (C18, 50 mm× 3 mm, 3 µm particle size) and the isocritic mobile phase (0.03 M NaH_2PO_4 pH 4.11, 2.4×10^{-3} M SDS, 6×10^{-5} M EDTA, 7% MeOH) were used for chromatographic separation. The flow rate of the mobile phase, column temperature and the ejection volume of the samples were 0.8 ml/min and 35 °C and 10 µl, respectively. For the monitoring of the compounds, an electrochemical coulometric detector with graphite carbon cells was used. A guard cell was placed just before auto-sampler in the HPLC system in order to decrease background noise and to increase sensitivity. The working cell and the guard cell were applied with 400 and 600 mV oxidation potentials, respectively. Under the analysis condition, retention time of the adrenaline, noradrenaline, dopamine and serotonin were recorded as 2.12, 3.19, 8.05, 10.02 and 13.23 min, respectively.

Biochemical measurements

The supernatants were assayed for catalase, superoxide dismutase and MDA activity according to the manufacturer's instructions.

Statistical analysis

The data used in statistical analyses were obtained from 10 animals for each of the groups. Statistical analyses of the experimental data were performed using GraphPad Prism 3.0 software (GraphPad Software, San Diego, CA). Comparisons between experimental groups were performed by one-way ANOVA followed by Tukey's test. The results were expressed as mean ± standard error of mean. Differences between data sets were considered as significant with p value was less than 0.05.

Results

Effects of CPX in elevated plus-maze tests

Oral administration of 50 mg/kg CPX significantly decreased the POAE and PTOA values of rats with respect to control group. The POAE and PTOA values did not differ in 20 mg/kg CPX administered group compared to the control group. There was no statistical difference between the CPX-administered groups (Figure 1).

Effects of CPX in MFSTs

Data obtained from the MFST indicated that administration of 50 mg/kg CPX induced a significant increase in the immobility and decrease in the swimming time of rats, without any

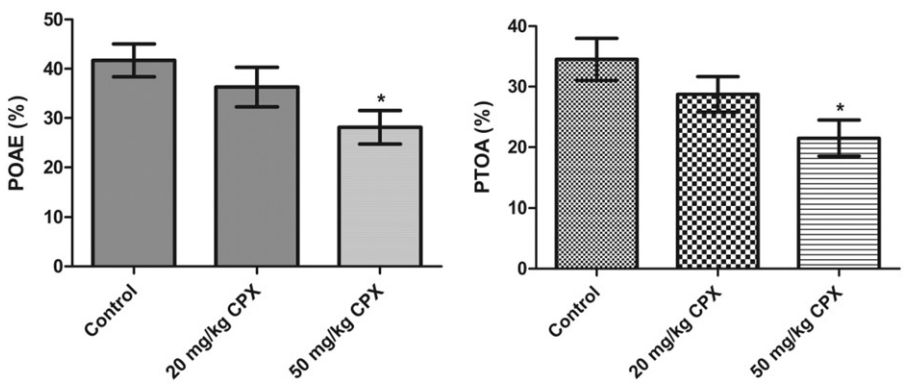


Figure 1. The effects of CPX administration on the (A) POAE % and (B) PTOA % in the elevated plus-maze test. 20 mg/kg CPX: 20 mg/kg CPX-treated rats for 14 days group; 50 mg/kg CPX: 50 mg/kg CPX-treated rats for 14 days group. *Different from control group ($p < 0.05$).

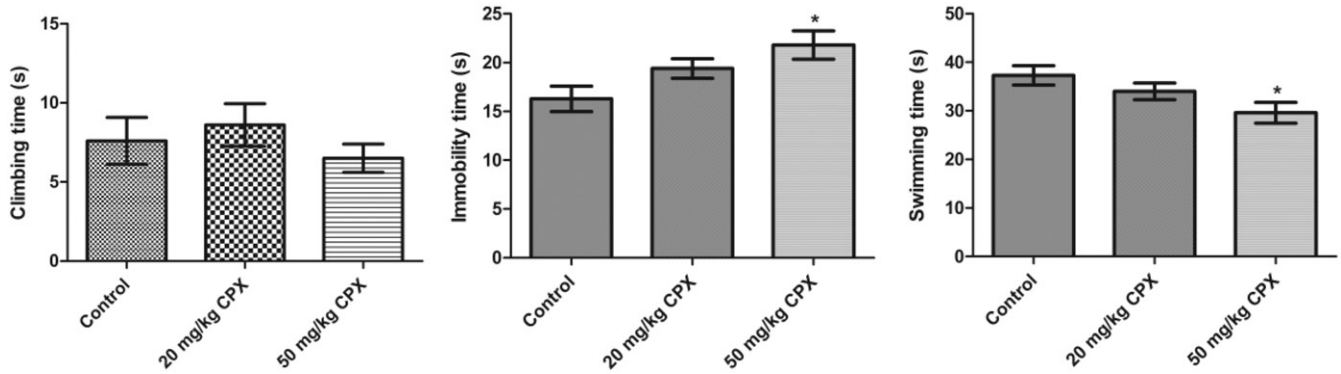
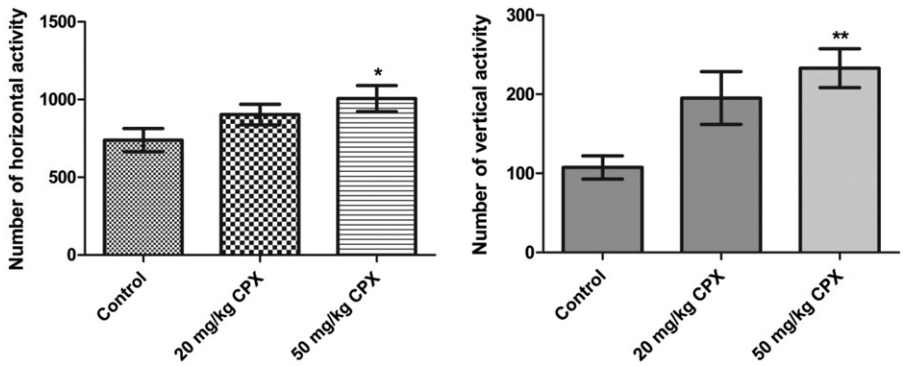


Figure 2. The effects of CPX administration on climbing, immobility and swimming time of rats in the MFST. 20 mg/kg CPX: 20 mg/kg CPX-treated rats for 14 days group; 50 mg/kg CPX: 50 mg/kg CPX-treated rats for 14 days group. *Different from control group ($p < 0.05$).

Figure 3. The effects of CPX administration on total number of horizontal and vertical activities of rats in the activity cage tests. 20 mg/kg CPX: 20 mg/kg CPX-treated rats for 14 days group; 50 mg/kg CPX: 50 mg/kg CPX-treated rats for 14 days group. *Different from control group ($p < 0.05$). **Different from control group ($p < 0.01$).



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alteration in the climbing duration (Figure 2), whereas these parameters were not significantly different in 20 mg/kg CPX-administered group when compared with control group. There was no statistical difference between the CPX-administered groups (Figure 2).

Effects of CPX in the activity cage tests

Effect of CPX on spontaneous locomotor activity of rats in the activity cage tests is shown in Figure 3. Total amount of horizontal or vertical activities increased only in the 50 mg/kg CPX-administered group. In contrast, administration of 20 mg/kg CPX did not cause a significant change spontaneous locomotor activity of rats. There was no statistical difference between the CPX-administered groups.

Effects of CPX in the Rota-rod test

CPX administration did not change motor coordination of rats when assessed in the Rota-rod test (Figure 4).

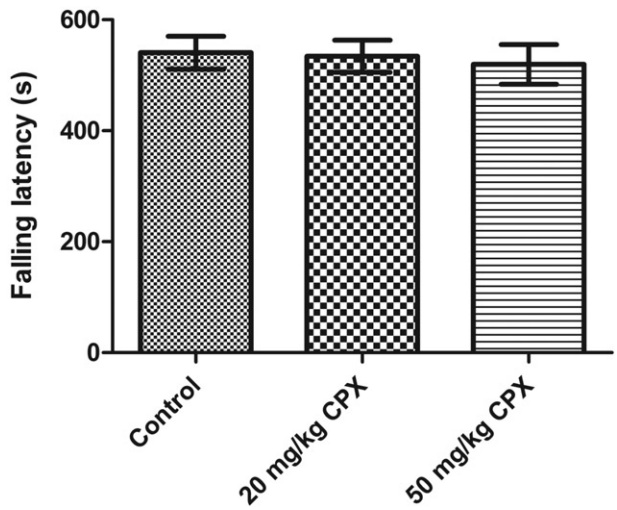

Figure 4. The effects of CPX administration on falling latency time of rats in the Rota-rod tests. 20 mg/kg CPX: 20 mg/kg CPX-treated rats for 14 days group; 50 mg/kg CPX: 50 mg/kg CPX-treated rats for 14 days group.

Neurotransmitter content in the brain

Results of the HPLC analysis revealed that neither administration of 20 mg/kg nor 50 mg/kg CPX induced significant alterations in the dopamine and noradrenaline levels measured in the brain homogenates (Figure 5).

Adrenaline content in the brain was found to be decreased in the 20 mg/kg CPX-administered group with respect to control and 50 mg/kg CPX-administered groups (Figure 5).

However, administration of 50 mg/kg CPX, significantly decreased serotonin levels in the brain with respect to control values. Also, values obtained from 20 mg/kg CPX-administered group was also lower than the control group, however, this difference was not statistically

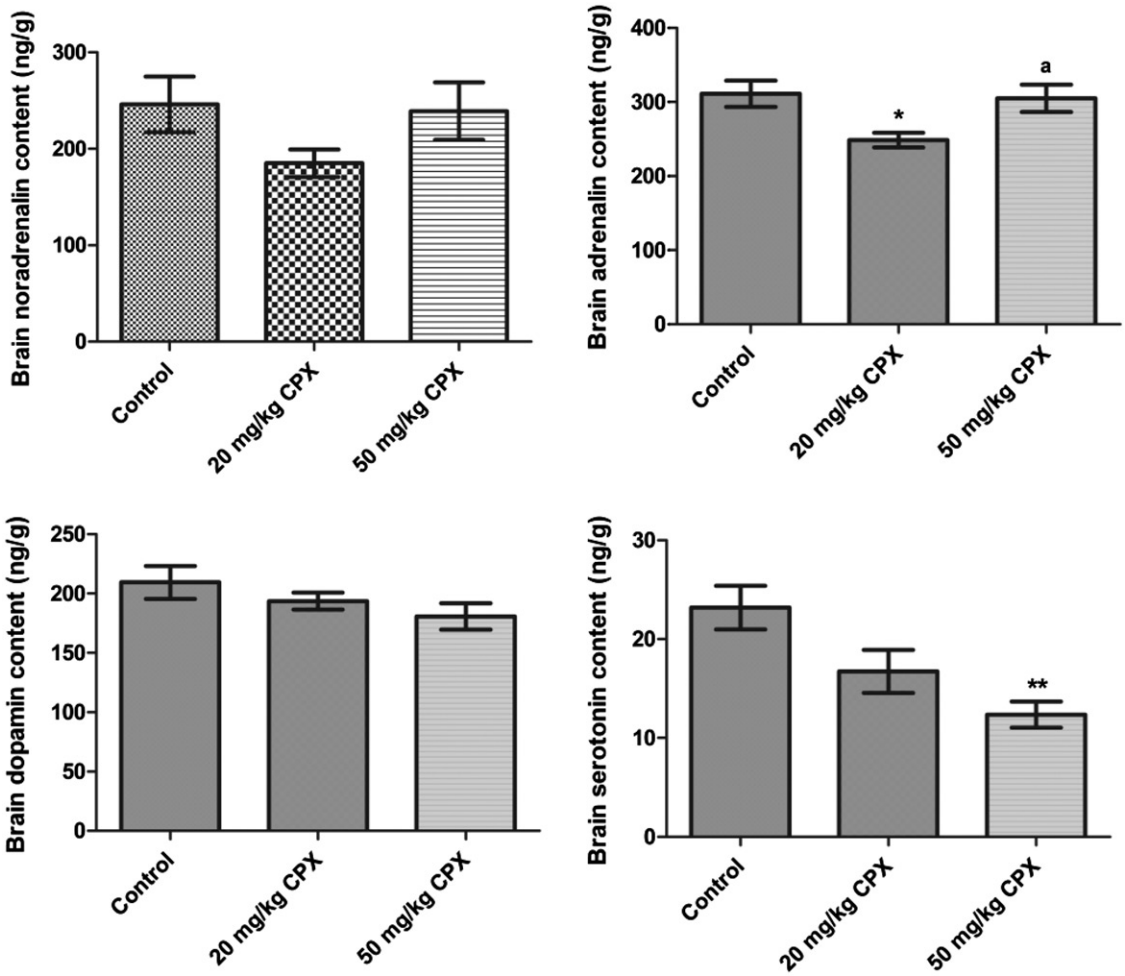

Figure 5. The mean serotonin, dopamine, adrenalin and noradrenaline levels in brain tissues of rats. 20 mg/kg CPX: 20 mg/kg CPX-treated rats for 14 days group; 50 mg/kg CPX: 50 mg/kg CPX-treated rats for 14 days group. *Different from control group ($p < 0.05$). **Different from control group ($p < 0.01$). [a]Different from 20 mg/kg CPX ($p < 0.05$).

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significant (Figure 5). There was no statistical difference between the CPX-administered groups.

Brain GABA content in CPX-administered groups was decreased when compared with the control group, but the differences were found to be significant only in the 50 mg/kg CPX-administered group. There was no statistical difference between the CPX-administered groups (Figure 6).

Furthermore, glutamate levels slightly increased in CPX-administered groups with respect to the control group, but the difference was not significant. There was no statistical difference between the CPX-administered groups (Figure 6).

Biochemical measurements

GSH level

It was found that GSH levels were significantly decreased in the CPX-administered groups with respect to the control group. No significant differences were found between the CPX-administered groups (Table 1).

Catalase level

Catalase activity was found to be significantly lower in the CPX-administered rats with respect to the controls. Also, no significant differences were found between the CPX-administered groups (Table 1).

Superoxide dismutase level

Brain superoxide dismutase activities were not different in animals treated with CPX with respect to the controls. Similarly, no difference was found between the CPX-administered groups (Table 1).

MDA level

Brain MDA levels were higher in the CPX-administered rats compared to the control animals. However, the difference was significant only in the 50 mg/kg CPX-administered group. There was no difference between the CPX-administered groups (Table 1).

Discussion

The aim of this study was to examine probable neurotoxic effects induced by repeated pharmacological doses of CPX and to clarify possible underlying mechanisms. For this purpose, behavioral parameters of rats were evaluated together with the changes of neurotransmitter levels and oxidative status of brain.

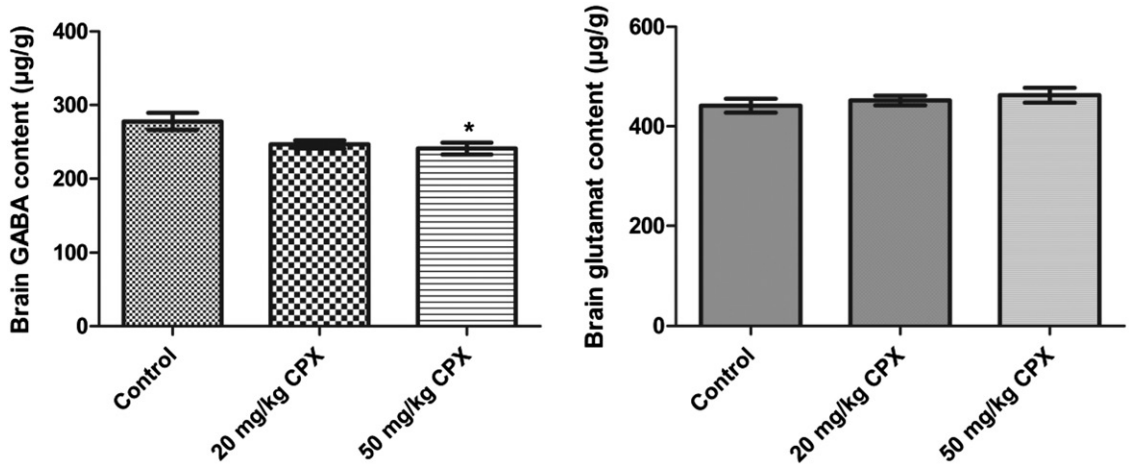

Figure 6. The mean GABA and glutamate levels in brain tissues of rats. 20 mg/kg CPX: 20 mg/kg CPX-treated rats for 14 days group; 50 mg/kg CPX: 50 mg/kg CPX-treated rats for 14 days group. *Different from control group ($p<0.05$).

Table 1. The mean GSH, MDA, SOD and CAT levels in brain tissues of rats.

	C	20 mg/kg CPX	50 mg/kg CPX
MDA (pmol/ml)	799.81 ± 90.41	846.19 ± 73.51	927 ± 112.41*
GSH (µg/g Tissue)	148.44 ± 15.95	125.44 ± 14.32*	117.24 ± 13.17*
SOD (Uml)	1.60 ± 0.48	1.14 ± 0.15	1.63 ± 0.16
CAT (nmol/min/ml)	329.55 ± 43.05	268.23 ± 39.86*	244.93 ± 45.16*

Definition of abbreviations: C: control group; 20 mg/kg CPX: 20 mg/kg CPX-treated rats for 14 days group; 50 mg/kg CPX: 50 mg/kg CPX-treated rats for 14 days group; GSH: glutathione; CAT: catalase; SOD: superoxide dismutase; MDA: malondialdehyde.
*Different from C ($p<0.05$).

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Effects of CPX on depression and anxiety parameters of rats were determined by performing MFST and elevated plus maze test, respectively. Furthermore, CPX-induced changes in the locomotor activity and motor coordination were assessed by the activity cage and the Rota-rod apparatus.

MFST is a well-known method for assessing depression-like/antidepressant-like effect of the pharmacological agents, based on the notion of learned helplessness. The basic parameters measured in this test are duration of immobility, climbing and swimming behaviors of animals in a tank filled with water. In MFST, increase in immobility time indicates a depression-like response, whereas decrease in immobility time points out an antidepressant-like effect (Cryan et al., 2002). In this study, immobility time increased in the 50 mg/kg CPX-administered group. Moreover, swimming time of the animals was decreased, without any change in climbing durations. Prolonged immobility and shortened swimming time was observed by CPX administration, which indicated a depression-like behavioral response. These data were also supported by the previous studies which reported CPX-induced depression cases (Ahmed et al., 2011; Grassi et al., 2001).

In MFST, climbing behaviors of rodents were widely associated with catecholaminergic system, whereas swimming behaviors were associated with serotonergic system of the brain (Cryan et al., 2002; Pesarico et al., 2014). Based on this consideration, it can be hypothesized that shortened swimming time observed with 50 mg/kg CPX administration in the present study, may be related to the decreased serotonin levels of the brain. Results of the HPLC, indicating lower brain serotonin level in the 50 mg/kg CPX-administered group with respect to the controls, also supported this idea. Furthermore, based on the afore-mentioned consideration of Cryan et al. (2002), unchanged climbing time in the MFST may also be explained by unchanged levels of catecholamines (noradrenaline and dopamine) measured in the brain homogenates (Cryan et al., 2002). When all these findings are interpreted together, CPX-induced depression-like behavior appears to be specifically related to the observed decrease in the serotonergic rather than noradrenergic neurotransmission.

In this study, anxiety levels of animals were assessed by elevated plus maze test (Zanoli et al., 2005). This test depends on behavioral inhibition associated with certain features (open, elevated arms) of the test apparatus. Two basic parameters evaluated in this test are ''the POAE'' and the ''PTOA'' values. Our data exhibited that both of the POAE and PTOA values of 50 mg/kg CPX-administered groups were significantly decreased with respect to the control group. These findings pointed out a CPX-induced anxiety-like behavior in rats. These findings were supported by the previous pre-clinical studies which reported anxiogenic effect of CPX (Prabhu & Rewari, 1998; Sen et al., 2007). In clinic, an anxiety case has also been reported depending on the combined use of CPX with a non-steroidal anti-inflammatory drug and chloroquine (Rollof & Vinge, 1993).

Possible underlying mechanisms of this anxiety-like state were evaluated by measuring levels of brain GABA, glutamate and noradrenaline levels, which are closely related with anxiety mechanisms (Garabadu & Krishnamurthy, 2014;

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Jacobson-Pick et al., 2013; Sen et al., 2007; Stangherlin & Nogueira, 2014). Obtained data showed that GABA levels in the brains of 50 mg/kg CPX-administrated group were decreased significantly with respect to the control group. Previous studies exhibiting CPX-induced inhibition in the GABAergic neurotransmission supported this finding (Abdel-Zaher et al., 2012; Arafa et al., 2013; Halliwell et al., 1995). Since no alteration was observed in the brain glutamate and noradrenalin levels, it may be suggested that anxiogenic effects induced by CPX administration seem to be related with the decrease in the GABAergic neurotransmission rather than changes in glutamatergic or noradrenergic ones.

Activity cage results indicated that the number of spontaneous locomotor activity was increased in the 50 mg/kg CPX-administered group. Increased locomotor activity of mice has also been reported for CPX administration, previously (Strzelec et al., 1999). As a general knowledge, increase in the horizontal and vertical movements is associated with the enhancement of dopaminergic neurotransmission (Can et al., 2012). In our study, CPX administration did not induce significant differences in the brain dopamine level; however, repeated administration of this drug might have an effect on receptor levels or on post-receptor processes. These hypotheses must be examined with further studies in detail.

When assessed in the Rota-rod test, it was observed that CPX administration did not cause any changes in the falling latencies of the rats. Similar findings have been reported for CPX-administered mice by Nakamura et al. (2003). Unchanged falling latencies of the rats indicated that CPX did not have any significant effect on motor coordination of the animals. A potential interpretation of this finding was that the observed performances on behavioral tests did not cause a possible change in the motor coordination of the animals.

In this study, another important point to be emphasized was the role of oxidative stress in the development of neurotoxic effects. In our study, it was determined that brain GSH levels and catalase activities were decreased and MDA levels were increased in the CPX-administered groups. These results suggested an enhanced oxidative stress/damage with weakened oxidative defense system. Previous pre-clinical and clinical studies which reported CPX-induced oxidative stress also supported our results (Abdel-Zaher et al., 2012; Hincal et al., 2003; Qin & Liu, 2013; Talla & Veerareddy, 2011). The induction of oxidative stress with CPX administration may be associated with the increase in electrophilic metabolite production due to the CYP450-mediated metabolism of this drug (Gurbay et al., 2001; Talla & Veerareddy, 2011) or CPX-induced structural and functional changes of catalase enzyme, which protects cells from oxidative damage by reactive oxygen species (Qin & Liu, 2013).

Enhanced lipid peroxidation and weakened antioxidant defense system may be the contributing factors to the behavioral changes observed in the present study; since oxidative stress has a vital role in the prognosis of depression (El-Naga et al., 2014; Maes et al., 2011) and anxiety (Ng et al., 2008; Shahzad et al., 2014). Exposure to oxidative stress has also been associated with neurodegenerative diseases and cognitive damage (Merzoug et al., 2014). Oxidative stress-induced abnormal structural changes in different parts of the brain, in cellular proteins, in membrane lipids, in DNA and RNA have been reported as some of the main mechanisms underlying oxidative stress-induced neurotoxicity (El-Naga et al., 2014; Maes et al., 2011; Ng et al. 2008; Shahzad et al., 2014). Furthermore, increased oxidative stress-induced impairment of ongoing production and function of the neurotropic factors, which are responsible for the growth and survival of developing neurons and the maintenance of mature neurons, has been defined as another important factor damaging the health of central nervous system (Gardiner et al., 2009; Koponen et al., 2005; McManus et al., 2014) and causing emotional disorders (Castrén & Rantamäki, 2010; Gardiner et al., 2009; Green et al., 2013; Koponen et al., 2005; Pencea et al., 2001).

In conclusion, it has been shown that CPX administration in repeated pharmacological doses, triggered depression and anxiety behaviors in our study. Based on our data, these negative emotional effects seemed to be related with the decreased brain serotonin and GABA levels. Furthermore, enhanced oxidative stress was observed as another underlying mechanism of CPX-induced neurotoxic effects. At this point, further detailed *in vitro* and *in vivo* studies may be suggested to investigate the role of CPX on serotonergic and GABAergic pathways. In addition, the relationship between CPX-induced alterations of neurotransmitter levels and oxidative stress may be evaluated by assaying each neurotransmitter in its specific region of brain in further studies to identify the cause and effect relationship of them in CPX-induced neurotoxicity.

Declaration of interest

The authors declared no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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