Effect of inositol on ciprofloxacin-induced depression in rats through upregulation of Keap1-Nrf2 system

Abeer Salama[a], Mohamed E. Elhadidy[b], Mahitab El-Kassaby[c], Abdel Razik H. Farrag[d]

Departments of [a]Pharmacology, [b]Research on Children with Special Needs, [c]Medical Physiology, [d]Pathology, National Research Centre, Cairo, Egypt

Correspondence to Abeer Salama, Department of Pharmacology, National Research Centre, 33 El Buhouth Street (Former El-Tahrir Street), Dokki, Cairo 12622, Egypt.
Tel:+20 100 379 3622; fax: +20 2337 0931;
e-mail: berrotec@yahoo.com

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Background
Kelch-like erythroid cell-derived protein 1 (Keap1)/nuclear factor (erythroid-derived 2)-like 2 (Nrf2) are transcription factors that can protect against oxidative stress and inflammation. Its deficiency has a contributory role in depression. Inositol is a nutritional supplement that is linked to various neurotransmitter receptors, such as serotonin. In the current study, we aimed to investigate the influence of inositol on ciprofloxacin (CPX)-induced depression through the upregulation of Keap1/Nrf2 system.

Materials and methods
Animals were divided into four groups as follows: group I: the normal control group that received saline. Group II (depressed group): rats treated daily with CPX (50 mg/kg body weight) for 14 days. Groups III and IV: rats received daily inositol (0.625 and 1.25 mg/kg body weight) for 14 days concurrently with daily dose of CPX. Forced swimming, oxidative biomarkers such as nitric oxide, malondialdehyde, and glutathione-s-transferase, and Keap1-Nrf2 and serotonin brain contents were assessed.

Results and conclusion
CPX-induced oxidative stress, reduced swimming time, and serotonin (5-HT) brain contents and showed severe neural injury in the form of spongiosis, focal gliosis around the degenerating neurons, and injured neurons revealed differences in sizes, vacuolization, shrinking, apoptosis, and lysis. An elevation of swimming time, brain glutathione-s-transferase, serotonin contents with a decrease of nitric oxide and malondialdehyde, and ameliorated histopathological alterations were observed in the inositol-administered group with respect to the CPX group. In conclusion, inositol alleviated neurological toxicity and has antidepressant activity through the downregulation of oxidative stress pathway and upregulation of 5-HT level and Keap1/Nrf2 system.

Keywords:
5-HT, ciprofloxacin, depression, glutathione-s-transferase, inositol, Kelch-like erythroid cell-derived protein 1, nuclear factor (erythroid-derived 2)-like 2

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Introduction

Depression is a psychiatric disorder, which is characterized by persistent and intense sadness, disturbed sleep or appetite, loss of interest or pleasure, feelings of guilt, feelings of tiredness, restlessness or irritability, and trouble with concentration, memory, or making decisions that worsen to thoughts of suicide or death. These feelings have a negative impact on mood and behavior and on various physical functions, including sleeping too much or too little and appetite changes and gaining or losing weight [1]. More than 300 million people suffer from depression at a global level, the number is going up, particularly in lower-income countries [2]. The prevalence of depression has been elevated globally, and it was considered as the second cause for the disease burden by 2020 [3]. In Egypt, according to the Ministry of Health Survey, 2.5% of the population suffers from mental health-related problems. Of them, 30.1% were suffering from depression that was linked to substance abuse. It was estimated that ~50% of patients have a history of at least one suicide attempt during their lifetime [4]. In addition, complex interactions among psychological, social, and biological factors are the reason for diabetic depression [5]. Clinicians and researchers usually criticize antidepressant drugs, while they showed thousands of clinical trials and will continue to know the effective treatment of antidepressant drugs against depressive disorders [6].

Ciprofloxacin (CPX) is a second-generation fluoroquinolone with a broad spectrum of antibacterial activity [7]. Fluoroquinolones are a group of antibiotics

broadly used because of their wide spectrum activity against both Gram-negative and Gram-positive bacteria. It is well tolerated in patients but their uses have been associated with numerous adverse effects, including adverse central nervous system effects and juvenile joint toxicity due to oxidative stress [8]. The prevailing hypothesis of depression over the last 50 years was the monoamine hypothesis, which originated from studies of the serendipitously discovered tricyclic antidepressants and monoamine oxidase inhibitors [9]. Antidepressants such as selective serotonin (5-HT) reuptake inhibitors and selective norepinephrine reuptake inhibitors, which acutely increase the levels of synaptic 5-HT and norepinephrine neurotransmitters [10] give a substantial evidence supporting the role of monoamine systems in the etiology of major depressive disorders. Another hypothesis of depression is the transcription factor Kelch-like erythroid cell-derived protein 1 (Keap1)/nuclear factor (erythroid-derived 2)-like 2 (Nrf2) system that has a vital role in oxidative response and regulates inflammation [11], its deficiency activating nucleotide-binding oligomerization domain, leucine-rich repeat, and pyrine domain-containing protein 3 (NLRP3) inflammasome that has a contributory role in depression [12]. Inositol, a vitamin-like substance, is present in some foods and in all animal tissues especially, the heart and the brain. Inositol is a constituent of the second messenger system, which is linked to 5-HT, glutamate, and dopamine neurotransmitter receptor, altering the level of neurotransmitters in the brain [13]. Low inositol levels were found in the frontal cortex of depressed patients [14], while there is no clear evidence of a therapeutic benefit of inositol in depressed patients [15]. Therefore, the current study was conducted to evaluate the protective effect of inositol against CPX-induced depression in rats through upregulation of the Keap1-Nrf2 system.

Materials and methods

Animals

Adult male Wister albino rats weighing 120–140 g purchased from the animal house colony of the National Research Centre (NRC), Dokki, Giza, Egypt and were kept in the animal house under conventional laboratory conditions. Experiments were performed according to the National Regulations of Animal Welfare and Institutional Animal Ethics Committee (IAEC). All procedures and experiments were performed according to the Ethics Committee of the NRC (Giza, Egypt) and according to the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985).

Chemicals and drugs

CPX was obtained from Memphis Co. for Pharm. & Chem. Ind. (MEMCO), Cairo, Egypt. Inositol was purchased from Fluka AG, CH-9470 Buchs, Switzerland.

Experimental design

Depression was induced by CPX in oral daily doses of 50 mg/kg for 14 days in rats [16]. Twenty-four rats were divided into four groups (n=6) as follows: group I: normal control group received saline. Group II: rats treated daily with CPX (50 mg/kg body weight) for 14 days. Group III: rats received daily inositol (0.625 mg/kg body weight) for 14 days concurrent with a daily dose of CPX. Group IV: rats received daily inositol (1.25 mg/kg body weight) for 14 days [17] concurrent with CPX.

Forced swimming test

The forced swimming test was conducted according to the method described by Porsolt *et al.* [18]. Each rat was placed for 5 min in a cylindrical water tank (70 cm high, 40 cm diameter), where the water level was about 40 cm and the water temperature was maintained at 23–25°C. A 15-min pretest was conducted 24 h before the 5-min swim test. During the test, times for swimming (horizontal movement on the surface of the water) 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.

Determination of oxidative stress biomarkers

One day following the last treatment, the animals were killed by cervical dislocation [19], and the brain of each rat was immediately dissected out, washed with ice-cooled physiological saline, and homogenized in 0.15 M potassium chloride (KCl) solution. Aliquots of the homogenate (20%) were prepared for the assessment of brain contents of nitric oxide (NO), malondialdehyde (MDA), and glutathione-s-transferase (GST) using commercially available kits (Biodiagnostic, Egypt) [20].

Determination of Kelch-like erythroid cell-derived protein 1-nuclear factor (erythroid-derived 2)-like 2 brain contents

Brain contents of Keap1 and Nrf2 were determined using the enzyme-linked immunosorbent assay

(ELISA)kit. We followed the manufacturer's instructions of SinoGeneClon Biotech Co. Ltd, Catalog number SG-20833, China ELISA kits for calculating the results. Standards and samples were pipetted into wells with immobilized antibodies specific for rat Keap1 and Nrf2, and then were incubated for 30 min at 37°C. After incubation and washing, horseradish peroxidase-conjugated streptavidin was pipetted into the wells and incubated for 30 min at 37°C, which were washed once again. Tetramethylbenzidine substrate solution was added to the wells and incubated for 15 min at 37°C; color developed proportionally to the amount of Keap1 and the Nrf2 bound. Color development was discontinued (Stop Solution) and after 10 min the color intensity was measured at 450 nm using a Multisacn FC ELISA reader, Model 1545, Thermo Scientific, China.

Determination of 5-HT neurotransmitter

Serotonin was measured according to the method of Ciarlone [21] using a spectrofluorometer (Jasco FP-6500, JASCO Ltd, Tokyo, Japan) with a 150 W xenon arc lamp as the light source (extinction slit band width of excitation monochromator: 5 nm). Briefly, three external standards for 5-HT were prepared in duplicate, in 0.2 N acetic acid, and to a total volume of 0.2 ml. Then the homogenates and standard of 5-HT tubes were centrifuged at 1000 g for 5 min. All 5-HT tubes, including a reagent blank consisting of 0.2 ml N acetic acid, 1.2 ml of OPT were added and mixed well. All tubes were placed in a boiling water bath for 10 min, cooled in tap water, and read in a spectrophotofluorometer at 470 nm.

Data analysis

All the values are presented as means±SD. Comparisons between different groups were carried out using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Difference was considered significant when P value less than 0.05. Graph ad prism software (version 5) (Inc., San Diego, USA) was used to carry out these statistical tests.

Histological study

At the end of the experiment, the rats were killed. Tissue samples for histology were fixed immediately in 10% formalin solution (100 ml formalin, 8.5 g sodium chloride in 900 ml distilled water). The specimens were fixed for 24 h. After fixation, the specimen was washed carefully with water and passed through ascending grades of ethanol, and cleared with xylene, and then impregnated with molten paraffin wax. After trimming, the paraffin blocks were cut into 4 μm thickness, mounted on slides, and stained with hematoxylin and eosin (×40). All sections were examined using a Olympus light microscope [22].

Results

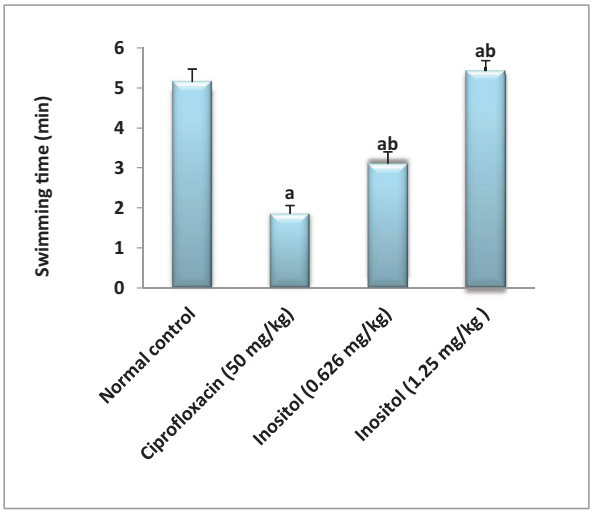
Forced swimming test

In this study, CPX trigger a reduction in swimming time by 64% (1.85±0.21), after 14 days, as compared with normal control (5.14±0.33). Treatments with both doses of inositol were effective in the prevention of swimming time decrement by 65% (3.09±0.31) and 193% (5.42±0.27), respectively, as compared with CPX rats (Fig. 1).

Assessment of brain contents of nitric oxide, malondialdehyde, and glutathione-s-transferase as indicators of oxidative stress

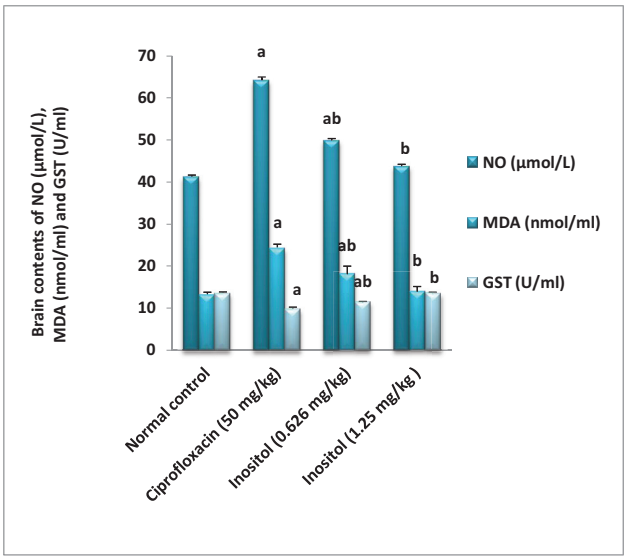
CPX increased brain contents of NO and MDA inducing oxidative stress by 56% (64.07±0.96 vs. 41.13±1.32) and 84% (24.24±0.44 vs. 13.19±0.28), respectively, and decreased brain contents of GST by 28% (9.84±0.40 vs. 13.61±0.23), respectively, after 14 days, as compared with the normal control group. Treatment with both doses of inositol ameliorated oxidative stress induced by CPX through reduction of brain contents of NO by 22% (49.81±1.15) and 32% (43.70±0.84), MDA by 26% (18.00±0.90) and 43% (13.89±0.57) with elevation of GST brain content by 15% (11.36±0.23) and 38% (13.55±0.23) after 14 days as compared with the CPX group (Fig. 2).

Figure 1



Effect of inositol on forced swimming test (FST). Data were expressed as mean±SD (n=6). Statistical analysis was carried out by one-way ANOVA followed by Tukey's HSD test for multiple comparisons. [a]Significantly different from normal control at P value less than 0.05. [b]Significantly different from CPX group at P value less than 0.05.

Figure 2



Effect of inositol on brain contents of NO, MDA, and GST. GST, glutathione-s-transferase; MDA, malondialdehyde; NO, nitric oxide. Data were expressed as mean±SD (n=6). Statistical analysis was carried out by one-way ANOVA followed by Tukey's HSD test for multiple comparisons. [a]Significantly different from normal control at P value less than 0.05. [b]Significantly different from CPX group at P value less than 0.05.

Assessment of brain contents of nuclear factor (erythroid-derived 2)-like 2 and Kelch-like erythroid cell-derived protein 1

CPX reduced brain contents of Nrf2 and Keap1 by 33% (1.98±0.00 vs. 2.96±0.07) and 43% (114.61±0.45 vs. 199.44±1.57), respectively, after 14 days, as compared with the normal control group. Treatment with both doses of inositol elevated Nrf2 brain content by 20% (2.39±0.02) and 33% (2.64 ±0.01) as well as the Keap1 brain content by 26% (144.38±4.27) and 50% (172.36±4.51), respectively, after 14 days, as compared with the CPX group (Fig. 3).

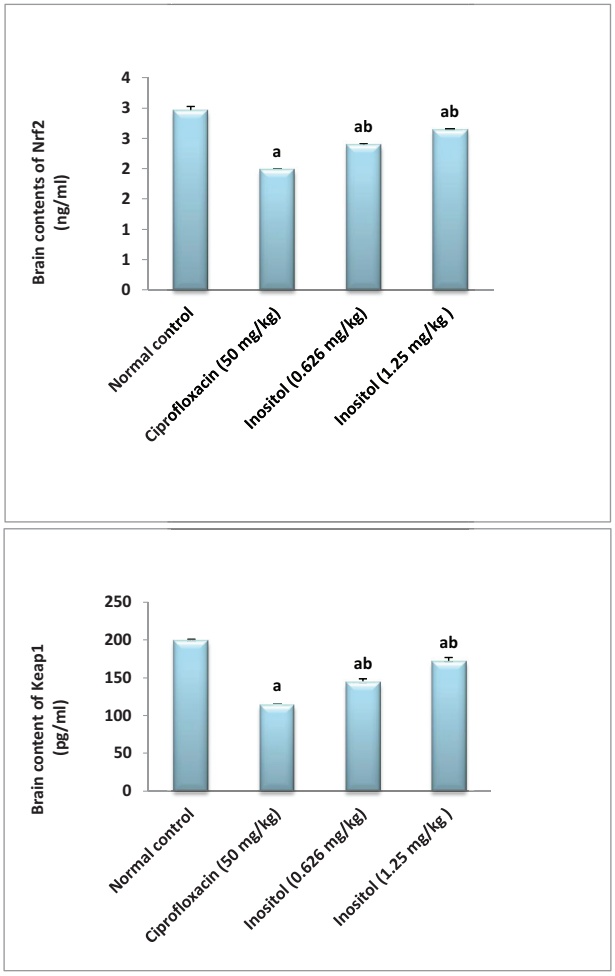
Effect of inositol on 5-HT neurotransmitters

Our results showed that CPX produced a decrease in the levels of 5-HT in the brain by 31% (7.94±0.01 vs. 11.48±0.02) as compared with normal rats, whereas treatment with both doses of inositol produced an increase of brain contents of 5-HT by 20% (9.55 ±0.22) and 42% (11.32±0.01), respectively, as compared with CPX rats (Fig. 4).

Histological results

The normal control group shows normal structure of neurons having enormous pale-stained nuclei, the nuclear chromatin and well-known nuclei disappeared, the adjacent support cells (glial cells) that having small nuclei with heavily stained, strong chromatin with no evident nucleoli and background

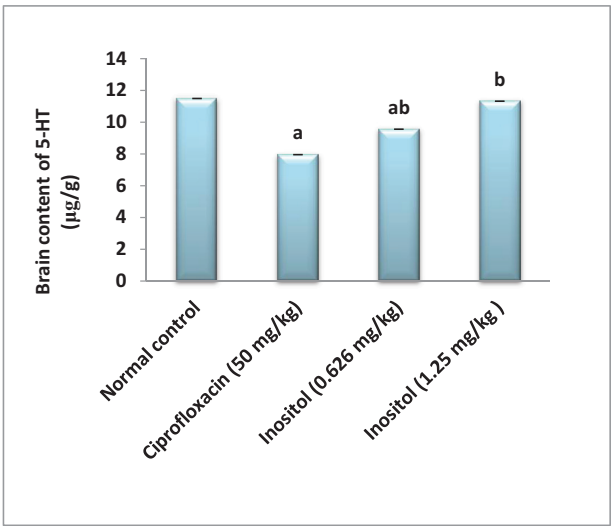
Figure 3



Effect of inositol on brain contents of Nrf2 and Keap1. Keap1, Kelch-like erythroid cell-derived protein 1; Nrf2, nuclear factor (erythroid-derived 2)-like 2. Data were expressed as mean±SD (n=6). Statistical analysis was carried out by one-way ANOVA followed by Tukey's HSD test for multiple comparisons. [a]Significantly different from normal control at P value less than 0.05. [b]Significantly different from CPX group at P value less than 0.05.

matter (neuropil) (Fig. 5a). Microscopic investigation of the cortical tissue of the CPX-administered group showed severe neural injury in the form of spongiosis, focal gliosis around the degenerating neurons, injured neurons revealed differences in sizes, vacuolization, shrinking, apoptosis, and lysis (Fig. 5b). In the case of administration of CPX and low-dose inositol group, histopathological examination showed that the structure of neurons seemed more or less as normal one (Fig. 5c). Moreover, some sections showed multiple vacuolated areas and pyknotic neurons (Fig. 5d). Microscopic examination of the cortical tissue of brain from CPX/high dose of inositol group indicated nearly normal cortical tissue (Fig. 5e). On the other hand, numerous damage and shrinkage areas or vacuolization of neurons have occurred (Fig. 5f).

Figure 4



Effect of inositol on brain contents of 5-HT. Data were expressed as mean±SD (n=6). Statistical analysis was carried out by one-way ANOVA followed by Tukey's HSD test for multiple comparisons. [a]Significantly different from normal control at P value less than 0.05. [b]Significantly different from CPX group at P value less than 0.05.

(a) Normal control group showing normal structure of neurons (short arrow) having enormous pale-stained nuclei, the nuclear chromatin and well-known nuclei disappeared, the adjacent support cells (glial cells) (arrowhead) that having small nuclei with heavily stained, strong chromatin with no evident nucleoli and background matter (neuropil); (b) CPX group showing severe neural injury in the form of spongiosis (arrow), focal gliosis around the degenerating neurons (arrowhead). CPX group showing a collection of injured neurons revealing differences in sizes, vacuolization, shrinking, apoptosis, and lysis; (c) CPX/inositol group at low dose showing the structure of neurons that appeared more or less as normal one; (d) CPX/inositol group at low dose showing multiple vacuolated areas and pyknotic neurons; (e) CPX/inositol group at high dose showing nearly normal cortical tissue, (f) CPX/inositol group at high dose showing numerous damage and shrinkage or vacuolization of neurons (hematoxylin and eosin, ×40, scale bar: 5 µm).

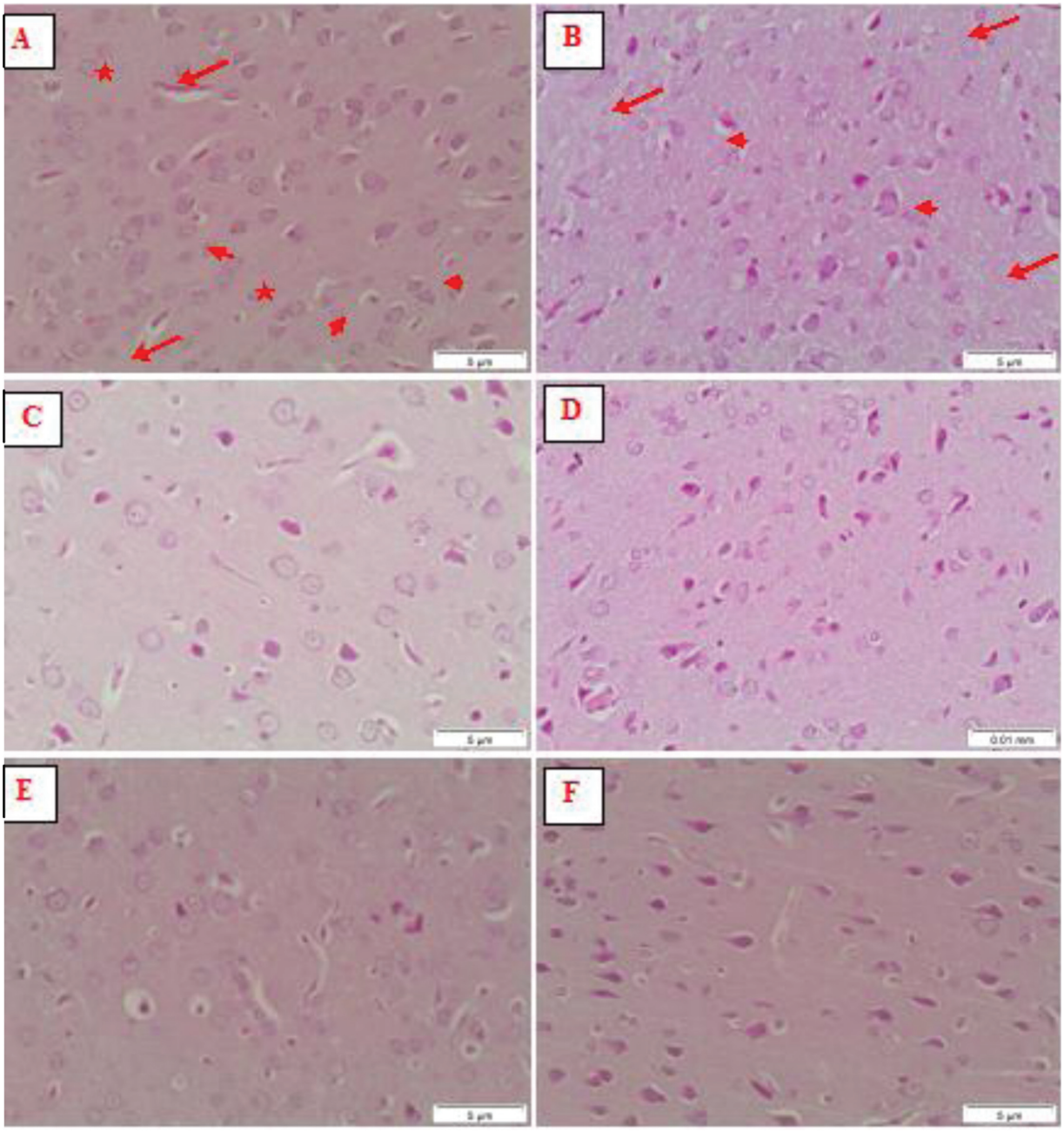
Discussion

Depression is one of the most common mood disorders with a high rate of relapse which shortens one's lifespan [23]. Swimming test was done to test for the psychological state of animals and then confirmed by the assessment of some biochemical parameters such as 5-HT, NO, MDA, GST, and Nrf2 levels in brain homogenates of rats, and finally supported by histopathological examination.

Neurotransmitter dysregulation is one of the major depression pathological hypotheses [24]. 5-HT low concentration was indicated with depression in patients central nervous system [25]. 5-HT deficiency and the disturbance of its receptors lead to electrical activity impairment of the dorsal raphe 5-HT neurons, and inefficient concentration of hippocampal extracellular 5-HT exhibits anxiety and depression [26]. In addition, monoamine neurotransmitters mediate the behavioral abnormalities [27]. Especially, the 5-HT system has an important role in depression and its behavioral abnormalities [28]. The forced swimming test is a behavioral model for potential serotonergic antidepressant evaluation through recording swimming, struggling, and immobility in mice and rats [29]. In the current study, CPX triggered a reduction in the swimming time and diminished neurotransmitter signaling such as 5-HT-induced depression, after 14 days, as compared with normal controls. CPX administration induces anxiety and depression through altering brain 5-HT, dopamine, and noradrenaline levels [30]. Treatments with both doses of inositol elevated the swimming time and ameliorated a reduction of 5-HT neurotransmitter signaling in the brain, as compared with CPX rats. In addition, the present histopathological study showed that inositol treatment showed nearly normal cortical tissue and reversed severe neural injury, focal gliosis, degenerating neurons, apoptosis, and lysis produced by CPX administration, elevating rat brain 5-HT.

Inositol is a vital compound for maintaining cell function and has regulatory roles in some cellular processes, imbalance in inositol-dependent signaling triggers molecular changes and changes in cellular functions including metabolism, transcription, ion channel, calcium homeostasis, energy metabolism, and autophagy. The reduction of inositol levels plays an important part in neurological diseases [31]. The activity of inositol seems to be similar to 5-HT reuptake inhibitor (selective serotonin reuptake inhibitors) antidepressant drugs which act on presynaptic mechanisms, whereas mechanism of action of inositol is related to postreceptor intracellular mechanisms and it serves as a second messenger system for 5-HT2 receptors and other neurotransmitters [13,32]. In addition, exogenous inositol enters the brain and exerts behavioral effects [33].

Figure 5



Photomicrographs of the rat cortex.

Oxidative stress is a critical factor in neuronal structures and functional alterations within the brain regions [34] in neurodegenerative diseases such as depression [35]. Elevated reactive oxygen species levels provoked behavioral, biochemical, and histopathological changes in depressive disorders [36]. Depression induced by levofloxacin or CPX administration produced oxidative stress [37]. Our data revealed that CPX elevated brain contents of NO and MDA and decreased GST brain contents as compared with the normal control group. On the other hand, treatment with both doses of inositol ameliorated oxidative stress induced by CPX through reduction in the level of brain contents of NO and MDA and elevation of GST brain content as compared with the CPX group. Previously, inositol treatment restored GSH contents affecting the oxidative status of erythrocytes in patients with polycystic ovary syndrome [38]. In addition, it decreased hepatic MDA levels in rats with cholestasis [39] and improved the antioxidant status in diabetic rats [40]. For the first time, we study the effect of inositol on activating the Nrf2/Keap1 antioxidant pathway. Keap1-Nrf2 system plays an important role in the pathophysiology of stress resilience, mood disorders, and major psychiatric disorders [41]. Nrf2 is a

transcriptional activator of antioxidant genes and triggers first-line responses against proteostasis, redox metabolism, and inflammation. Nrf2 decrement reduced GSH release and this reduction of GSH are associated with depression and is considered as a promising therapeutic approach in many chronic diseases induced by oxidative stress and inflammation [42,43]. CPX, in current study, reduced brain contents of Nrf2 and Keap1, after 14 days, as compared with the normal control group. Protein expressions of Keap1 and Nrf2 in the hippocampus were lower than the control in depression-like behavior in phenotype mice [44]. Interestingly, Nrf2 knockout mice reduced brain-derived neurotrophic factor expression in the hippocampus releasing pro-inflammatory cytokine and producing depression as compared with normal mice [11]. Treatment with both doses of inositol elevated brain contents of Nrf2 and Keap1, as compared with the CPX group exerting its antidepressant effects.

Conclusion

Inositol modified mood disorders induced by CPX in depressed rats through restoring 5-HT, scavenging reactive oxygen species, and upregulation of Keap1-Nrf2 system. Inositol may be proposed as a potential drug candidate for depression.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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