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Possible Neuroprotective Effects of Crocin Against Motor And Neurochemical Changes In Rotenone Induced Animal Model Of Parkinson's Disease

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Keywords

- Saffron
- Crocin
- Oxidative stress
- Parkinson like behavior
- DNA damage

Abstract

Parkinson's disease (PD) is considered the second most common neurodegenerative disease subsequent motor and behavioral deficits. Oxidative stress plays a key role in the pathogenesis of AIM: The aim of this study was to investigate the possible neuroprotective effects of crocin on roter induced Parkinson- like behaviors. MATERIALS &METHODS: 70 male adult Wister Albino rats randomly divided into 4 groups: (1) Control (10 rats); (2) Crocin 40 (10 rats); (3) Polyethylene g (PEG) (10 rats) ; (4) Rotenone (40 rats) injected I.P. of 1.5 mg/kg/48 hrs. for 2 weeks [11]; Prelimi behavioral tests and the rats that showed PD features were randomly subdivided into 4 equal grou 10 rats per each. (4A) Rotenone-treated: (4B): Crocin 20 treated. (4C): Crocin 40 treated. (4D) L-D treated- group. The neurobehavioral were done. In serum, the level of 8-hydroxydeoxyguanosi OHdG was estimated. The level of malondialdehyde (MDA), reduced glutathione (GSH), tumor nec factor alpha TNF-α, dopamine, and nitrite/ nitrate levels were measured in the brain tissue. RESU Rotenone induced neurobehavioral deficits with elevation of brain MDA, brain TNF- α, Nitrite/n level and serum 8-OHdG and reduction of GSH, brain tissue dopamine. Crocin (20 or 40) impr these neurobehavioral deficits. Crocin (20 or 40) and L-DOPA decreased MDA, serum 8-OHdG, TN and Nitrite/nitrate level and increased GSH and dopamine level. Crocin 40 had achieved potent e compared with crocin 20. In summary, rotenone-induced Parkinson- like behavior in rats. C achieved a protective effect.

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INTRODUCTION

Parkinson's disease (PD) is considered one of the most popular neurodegenerative disease whose prevalence increases with age [1].

The unique characteristics of PD include static tremor, alpha rigidity and sluggishness of movements. Other non-motor features include depression and sleep abnormalities [2].The main pathological character of PD is neurodegeneration of the nigrostriatal dopaminergic pars compacta (SNc), however, recently, serotonergic, noradrenergic, glutamatergic, GABAergic, and cholinergic systems may be included [3].

Rotenone, especially prolonged exposure, produces degeneration of nigeostriatal dopaminergic (DA) neurons with appearance of behavioral characteristics of PD [4].

A variety of studies had mentioned that oxidative stress possesses a major role in the pathogenesis of PD [5].

Free radicals and other reactive oxygen species (ROS) accumulated from dopamine oxidation and metabolism, lipid peroxidation, altered mitochondrial activity, and reduction of the endogenous antioxidant systems contribute to PD appearance [6].

Crocin is a carotenoid substance which is water soluble and constitutes the active component of saffron. It has been postulated that crocin achieves many pharmacological functions such as anti-oxidative function [7-8], anti-inflammatory [9], reduces the incidence of cardio- vascular morbidities, amelioration of tumor cell proliferation, neuroprotection and hepatoprotection [6].

It has also been shown that the spice saffron, which contains powerful antioxidants such as crocin, protects nigral and retinal dopaminergic cells in an acute mouse model of Parkinson's disease [10].However, the mechanisms explain remains un-cleared and needs further explanation. They studied the neuroprotective effects of alkaloids from Piper longum in a MPTP-induced mouse model of Parkinson's disease.

Taking in mind the antioxidant and anti-inflammatory prosperities of crocin and the role of oxidative stress in the pathogenesis of Parkinsonism, and the presence of missed items in the pathogenesis of Parkinsonism, this study was labored. Therefore, the aim of this study was to study the possible neuroprotective effects of crocin (saffron active compound) on rotenone induced Parkinson- like behaviors.

MATERIALS & METHODS

Experimental animals: 70 Male Wister Albino rats weighting (210±20 g) were purchased from the Faculty of Science Tanta University. All animal experiments were undertaken with the approval of Ethical Animal Research Committee of Tanta University. (The study was done from April 2018 till August 2018). The animals were housed at temperature 22±2 ˚C, exposed to alternate cycles of 12 h dark/light throughout the study and fed chow ad libitum. All rats had free access to distilled water. Animals were kept for 2 weeks for acclimatization.

Chemicals: Crocin extract, Rotenone, Levodopa and Polyethylene glycol (PEG) were obtained from Sigma-Aldrich, Egypt.

Experimental design: 70 male adult Wister Albino rats were randomly divided into 4 groups (1) Control group (10 rats) (normal saline injected intraperitoneally I.P. with the same volume as the remaining groups; (2) Crocin treated group (10 rats) received crocin 40 mg/Kg/day I.P. for 2 weeks; (3) Polyethylene glycol (PEG) group (10 rats) (vehicle of Levodopa); (4) Rotenone group (40 rats) injected I.P. with a dose of 1.5 mg/kg/48 hs. dissolved in 1:1 dimethylsulfoxide (DMSO) and polyethylene glycol (PEG) for 2 weeks [11]; At the end of the experiment, rats of this group were subjected to preliminary behavioral tests and the rats that showed PD features were randomly subdivided into 4 equal groups of 10 rats per each. (4A) Rotenone- treated: The rats of this group did not receive any further medications for another 2 weeks. (4B): Crocin 20 treated group: The rats of this group received crocin 20 mg/Kg/day I.P. for another 2 weeks. [11]; (4C): Crocin 40 treated group. The rats of this group received crocin 40 mg/Kg/day I.P. for 2 another weeks. [11]; (4D) L-DOPA treated group. The rats of this group received Levodopa 10 mg/Kg/day I. for another 2 weeks. [12]. All agents except rotenone were injected intraperitoneally once a day for 2 weeks.

Open –field test

The open-field apparatus, consisted of white wood, had a floor of 100 × 100 cm divided by red lines into 25 equal units of 20 ×20 cm. The walls (50 cm high) were also painted in white. The test room was illuminated at the same intensity at the colony room. To disguise the presence of other animals, after each test, the apparatus was cleaned thoroughly with wet [13].

Bar test for Catalepsy

According to the method described by **Costall and Naylor** [14]., the rats were placed with forelimbs on a horizontal thin bar 9 cm above and parallel to the base with a half rearing position. Removing one paw from the bar was considered the end of the test and the time was noted and recorded.

Forepaw stride length

Rats were trained to walk in a narrow corridor that was lined by a clean white paper. The forepaws were dipped in red ink and rats were allowed to walk along the corridor again. The distance of stride length was estimated through measuring the distance between the fore prints [15].

Then, the animals were anesthetized by injection of a mixture of ketamine (150 mg/kg) and xylazine (15 mg/kg) I.P. and blood samples were collected from the heart by a cardiac puncture into non-anti-coagulant containing tubes to obtain serum. Blood was allowed to clot for 30 minutes followed by centrifugation for 10 minutes at 5000 rpm. Sera were separated and stored in aliquots at – 70 ◦C. Serum oxidative DNA damage (8-hydroxy-2,-deoxyguanosine level was estimated in accordance of the previously described method [16].

Then, all animals were sacrificed by cervical dislocation. The hippocampus and the cortex were dissected bilaterally, washed, wrapped in aluminum foil and stored at -80 ◦C and homogenized for estimation of the lipid peroxidation parameter (MDA) according to the method of **Fernandez et al.1997** [17]. Reduced GSH was estimated according to the method of **Moron et al**.1979 [18]. TNF-α measured in accordance with **Ye & Johnson 1999** [19] . Dopamine level was measured according to the method described by **Jacobowitz and**

Richardson; 1978 [20]. **Nitrite/ Nitrate level was measured by colorimetric method** [21].

Statistical analysis

All values were expressed as mean ± SD. SPSS version 16.0 was used for statistical analysis. Data were statistically analyzed using one-way ANOVA followed by Tukey–Kramer posttest for multiple comparisons. The values less than 0.05 were considered significant.

RESULTS

Descent latency time in the bar test (seconds)

The mean value of the descent latency time in the bar test was 13.9±1.91, 13.7±1.56 and 13.6±2.31 seconds in the control, crocin and PEG groups respectively. Rotenone administration significantly increased the descent latency time compared with the control, crocin and PEG groups**.** Rotenone treated groups with crocin 20mg/Kg and 40 mg/Kg showed a significant decrease of the descent latency time compared with the rotenone group. Crocin 40 mg/Kg showed significant decrease of the descent latency time compared crocin 20 mg/Kg treated group. L-DOPA treated group showed a significant reduction of descent latency time in the bar test compared with the rotenone and rotenone- crocin 20 groups. No significant change was observed when comparing rotenone – crocin 40 and rotenone- L-DOPA groups (Fig.1)

Forepaw stride length (cm)

The mean value of the forepaw stride length was 8±2, 8±2.1 and 8±1.9 cm in the control, crocin and PEG groups respectively. Rotenone group showed significant reduction of forepaw stride length compared with the control, crocin and PEG groups**.** Rotenone treated group with crocin 20mg/Kg and 40 mg/Kg showed significant increase compared with the rotenone group. Rotenone- treated group with crocin 40 mg/Kg showed significant increase of the forepaw stride length compared with crocin 20 treated group. L-DOPA treated group showed a significant increase of the forepaw stride length compared with the rotenone and rotenone- crocin 20 groups. No significant change was observed when comparing crocin 40 mg/Kg treatment with L-DOPA treatment. (Fig.2)

Effect of rotenone and crocin on locomotor activity

Using open field test in the 1st day did not reveal significant changes among the studied groups (Fig.3)

At the end of the experiment, rotenone reduced the central, peripheral and total locomotion compared to the control, crocin and PEG groups. Crocin 20, 40 and L-DOPA plus rotenone increased the peripheral, the central and the total locomotion compared to the control group (Fig. 4)

Lipid peroxidation

Rotenone administration markedly induced a significant increase of MDA level compared with control, crocin and PEG groups. A significant reduction of MDA observed in rotenone treated (crocin 20, crocin 40 and L-DOPA) groups respectively. L-DOPA treated group showed a significant reduction of MDA compared with both crocin 20 and 40 treated groups. (Fig.5)

Effect of crocin on reduced GSH

Rotenone decreased significantly the level of GSH compared with control, crocin and PEG groups. Rotenone treated groups with crocin 20 mg/Kg, 40 mg/Kg and L-DOPA significantly restored the content of GSH. L-DOPA achieved a significant elevation of GSH compared with both crocin 20 and crocin 40 treated groups. (Fig.6)

Effect of crocin on TNF-α

There was a significant increase of TNF-α in rotenone treated group compared with control, crocin and PEG groups. Administration of crocin 20, crocin 40 and L-DOPA reduced significantly this rotenone induced elevation of TNF-α. The TNF-α was still higher after L-DOPA treatment compared with crocin treated groups in both doses. (Fig. 7)

Brain tissue dopamine level

Rotenone decreased significantly the dopamine level compared with control, crocin and PEG groups. Rotenone treated groups with crocin 20 mg/Kg, 40 mg/Kg and L-DOPA significantly restored the content of dopamine. Dopamine level was significantly higher in crocin 40 treated group compared with crocin 20 treated one. Dopamine level restoration was more pronounced in L-DOPA treated group as dopamine level was significantly higher in this group compared with rotenone treated with crocin in both doses. (Fig. 8)

Effect of crocin on DNA damage

Rotenone increased significantly the level of **8-hydroxy- 2 deoxyguanosine** (8-OHdG) compared with control, crocin and PEG groups. Rotenone treated groups with crocin 20 mg/Kg, 40 mg/Kg and L-DOPA significantly reduced this elevation. 8-OHdG level was still significantly higher in rotenone treated with crocin and L-DOPA compared with control, crocin and PEG groups. 8-OHdG was significantly lower in L-DOPA treated group compared with crocin20 and 40 treated groups. (Fig. 9)

Nitrite/nitrate level

Rotenone increased significantly the level of Nitrite/nitrate compared with control, crocin and PEG groups. Rotenone treated groups with crocin 20 mg/Kg, 40 mg/Kg and L-DOPA significantly reduced this elevation. Nitrite/nitrate level was still significantly higher in rotenone treated with crocin and L-DOPA compared with control, crocin and PEG groups. Nitrite/nitrate level was significantly lower in L-DOPA treated group compared with crocin20 and 40 treated groups and in crocin 40 treated compared with crocin 20 treated groups. (Fig. 10)

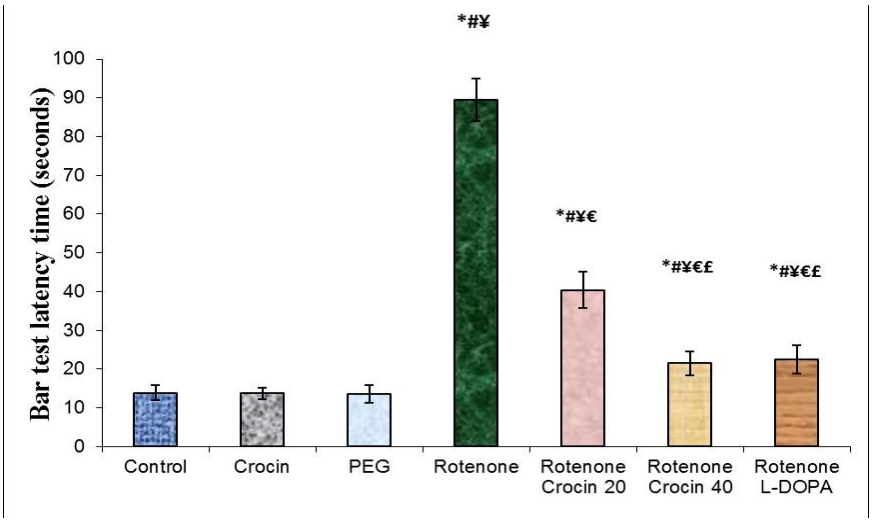

Fig. (1) Effect of crocin on descent latency time in the bar test (seconds)

* Significant compared with control
\# Significant compared with crocin
¥ Significant compared with PEG
€Significant compared with Rotenone
£ Significant compared with Rotenone + Crocin 20
¢ Significant compared with Rotenone + Crocin 40

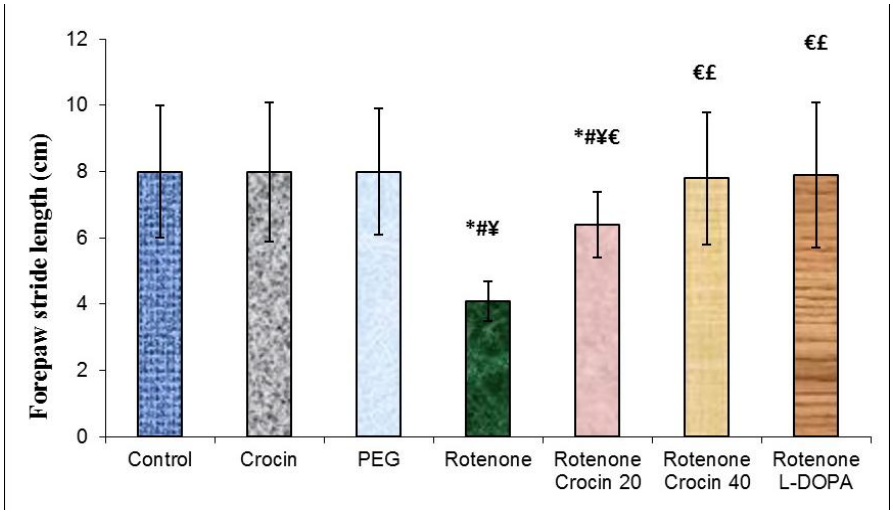

Fig. (2) Effect of crocin on Forepaw stride length (cm)

* Significant compared with control
# Significant compared with crocin
¥ Significant compared with PEG
€Significant compared with Rotenone
£ Significant compared with Rotenone + Crocin 20
¢ Significant compared with Rotenone + Crocin 40

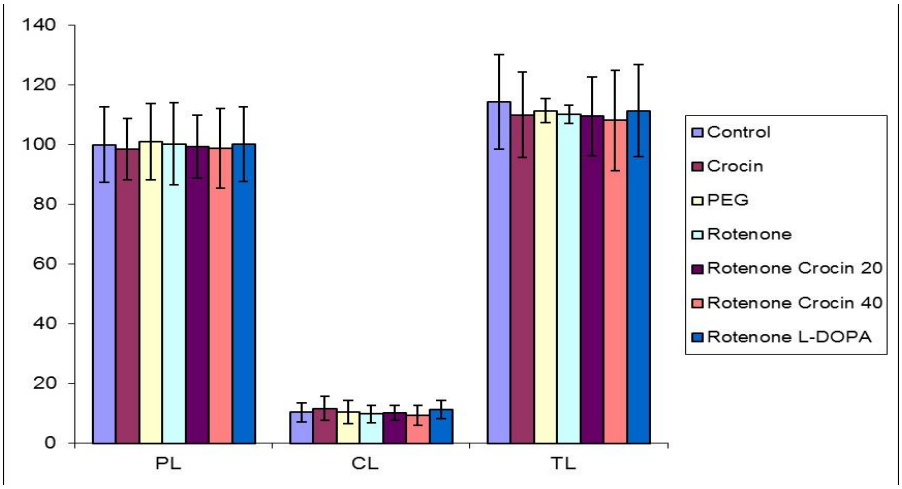

Fig. (3) Effect of rotenone and crocin on total locomotion (TL), central locomotion (CL) and peripheral locomotion (PL) in the 1st day of the experiment

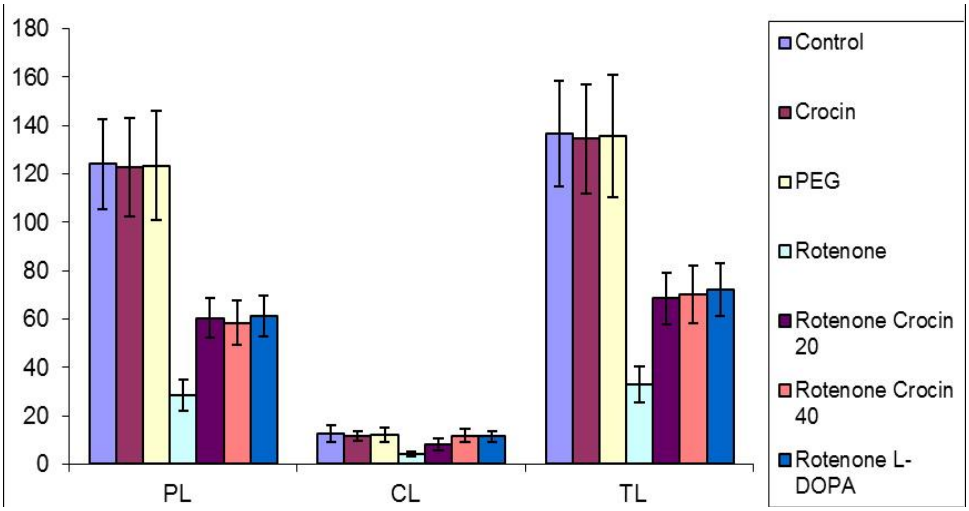

Fig. (4) Effect of rotenone and crocin on total locomotion (TL), central locomotion (CL) and peripheral locomotion (PL) at the end of the experiment

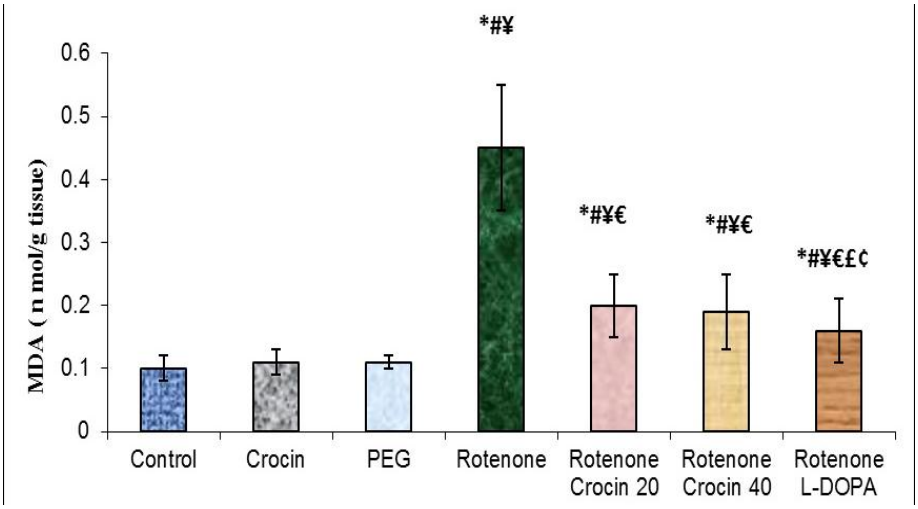

Fig. (5) Effect of crocin on lipid peroxidation

* Significant compared with control
# Significant compared with crocin
¥ Significant compared with PEG
€Significant compared with Rotenone
£ Significant compared with Rotenone + Crocin 20
¢ Significant compared with Rotenone + Crocin 40

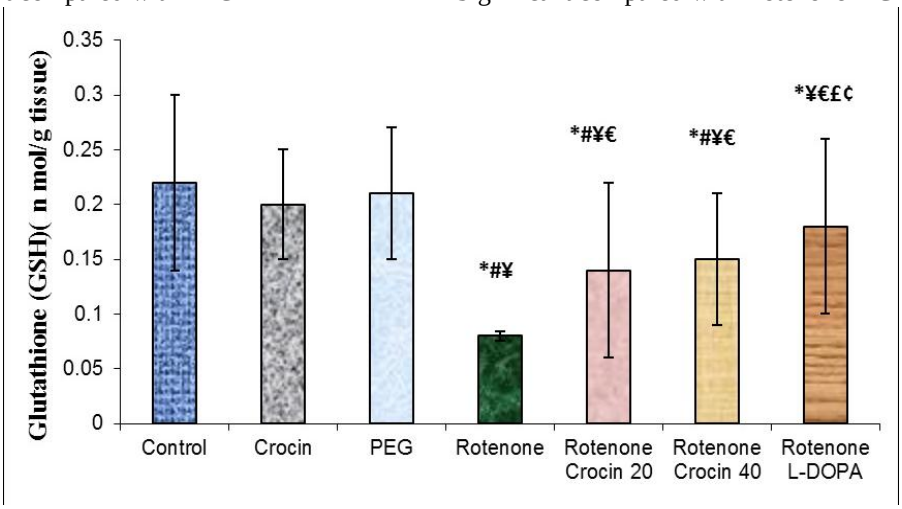

Fig.(6) Effect of crocin on reduced glutathione

* Significant compared with control
# Significant compared with crocin
¥ Significant compared with PEG
€Significant compared with Rotenone
£ Significant compared with Rotenone + Crocin 20
¢ Significant compared with Rotenone + Crocin 40

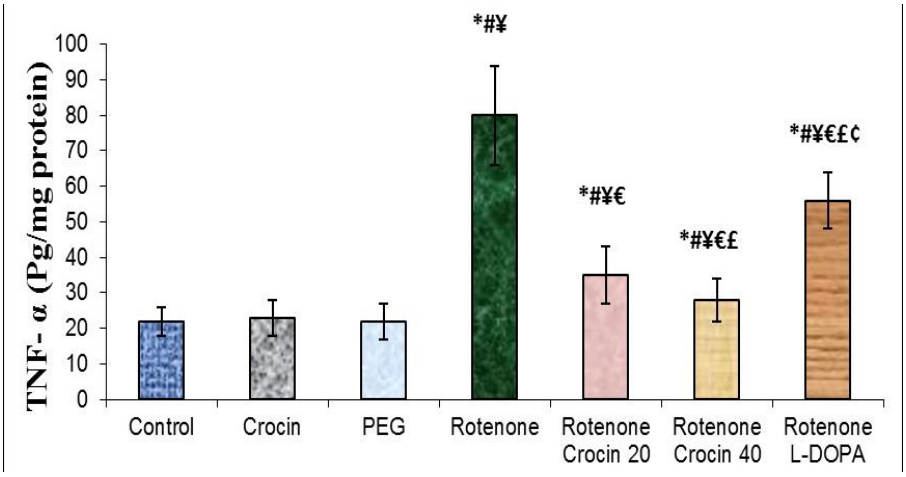

Fig.(7) Effect of crocin on inflammation

* Significant compared with control
# Significant compared with crocin
¥ Significant compared with PEG
€Significant compared with Rotenone
£ Significant compared with Rotenone + Crocin 20
¢ Significant compared with Rotenone + Crocin 40

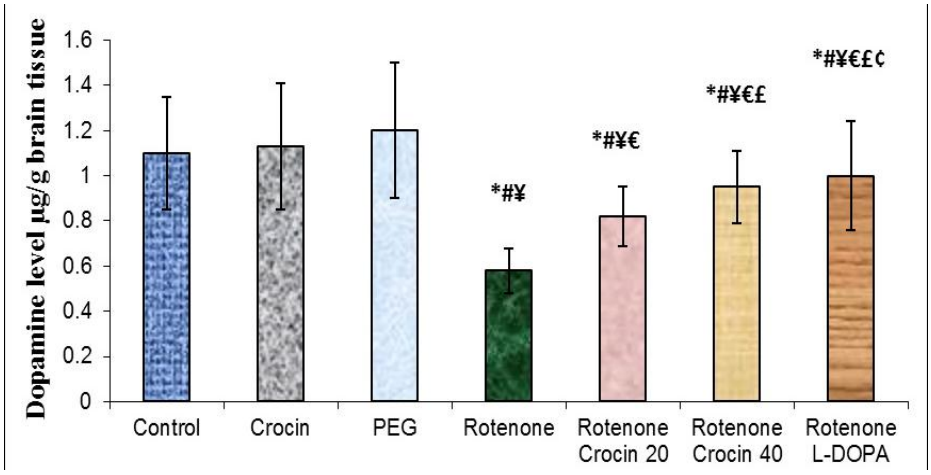

Fig.(8) Effect of crocin on brain dopamine level (µg/g)

* Significant compared with control
# Significant compared with crocin
¥ Significant compared with PEG
€Significant compared with Rotenone
£ Significant compared with Rotenone + Crocin 20
¢ Significant compared with Rotenone + Crocin 40

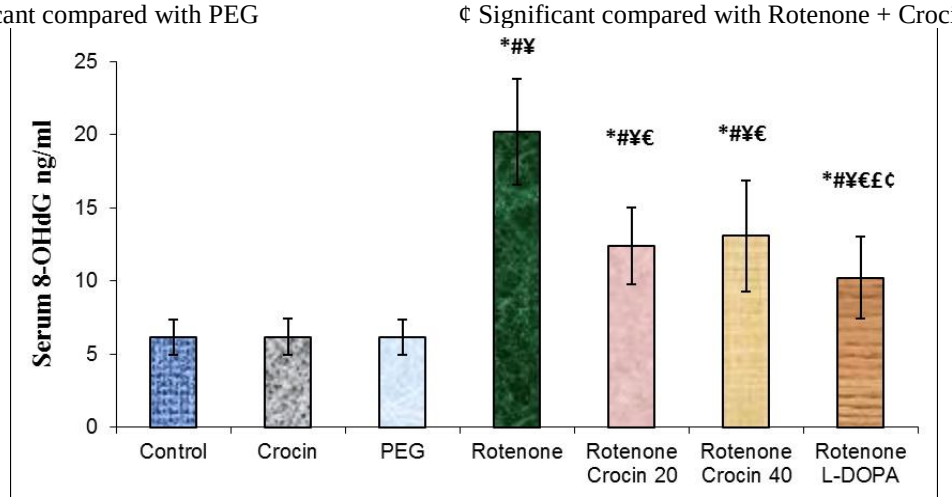

Fig.(9) Effect of crocin on Serum 8-OHdG level

* Significant compared with control
# Significant compared with crocin
¥ Significant compared with PEG
€Significant compared with Rotenone
£ Significant compared with Rotenone + Crocin 20
¢ Significant compared with Rotenone + Crocin 40

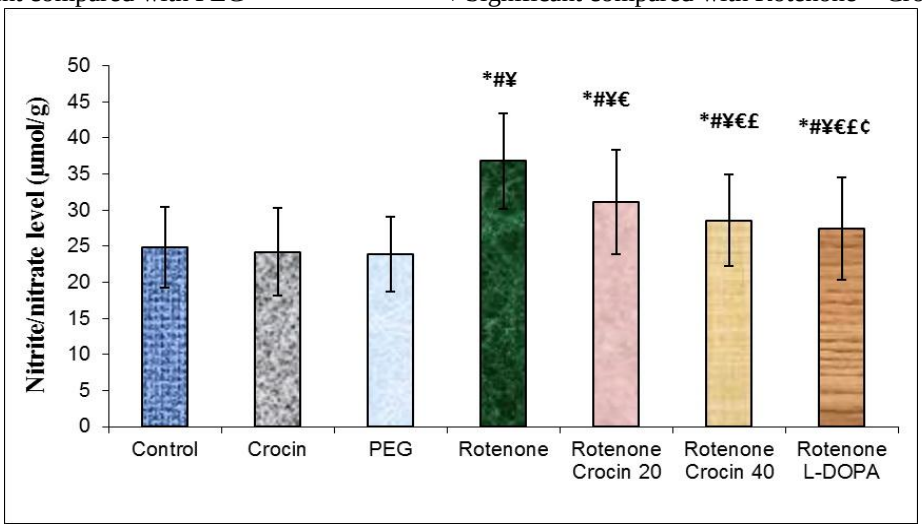

Fig. (10) Effect of crocin on brain nitrite / nitrare level

* Significant compared with control
# Significant compared with crocin
¥ Significant compared with PEG
€Significant compared with Rotenone
£ Significant compared with Rotenone + Crocin 20
¢ Significant compared with Rotenone + Crocin 40

DISCUSSION

This study revealed that rotenone- treated animals created cataleptic behavior and impairment of the motor coordination compared with the control and the crocin treated groups. This confirmed that there was a loss of dopaminergic cells in the substantia nigra by rotenone. The same had been reported by **Dijkstra et al**. [22].

Motor disruption in rotenone rat model of Parkinsonism was attenuated by using antioxidants**.** Catalepsy is considered a critical marker of nigrostriatal damage and can be induced by rotenone. This cataleptic behavior is connected to the dopaminergic nigrostriatal degeneration [23].

The open field test had indicated that rotenone reduced the central, peripheral and total locomotion compared with the control and crocin groups. Crocin 20 and 40 mg/kg as well as L-DOPA treatment had improved this impairment of locomotion. These results were supported by **El-Horany and his followers & Hosseini and his followers** [24-25]. L-DOPA treatment had reversed the deficits related to nigrostriatal degeneration [26].

Oxidative stress is considered an important step in aging and neurological disorders [27]. It can be induced by hypoxiaa, leading to an increased free radical formation. The mitochondria are the main organelles that produce free radicals [28].

Brain tissue is more vulnerable to oxidative stress than other tissues as it incorporates a high metabolic activity, high oxygen consumption and presence of enhanced iron level leading to reduction of H2O2 to form the highly reactive hydroxyl radical [29].

Lipid peroxidation is the process of oxidative destruction leading to impaired membrane integrity and function and inactivation of membrane-bound enzymes. Oxidative stress produced by free radicals and lipid peroxidation was considered an important step in the pathogenesis of Parkinsonism [30].

During oxidative stress, there is accumulation of oxidants and formation of TBARS [31]. The brain has a less pronounced antioxidant mechanism and is rich in polyunsaturated fatty acids so, the brain is sensitive to oxidative damage.

In this study, there was a critical increment in MDA level in rotenone- induced PD. Treatment with crocin reduced this elevation significantly and this effect refers to the crocin antioxidant activity as evidenced by increased enzymatic and GSH availability [32]. Chen and his team had expressed that crocin, the major constituent determined from saffron, has diverse pharmacologic properties such as antioxidant movement [7].

In this consider, there was a critical increment in MDA level in rotenone-induced PD. Treatment with crocin decreased this height all together and this impact alludes to the crocin antioxidant action as prove by expanded enzymatic and GSH accessibility [32]. Chen and his group had communicated that crocin, the major constituent from saffron, has diverse pharmacologic properties such as antioxidant activity [7].

The endogenous antioxidant systems consist of enzymatic (SOD, catalase and GPx) and non-enzymatic GSH that neutralizes the oxygen free radicals that leads to oxidative stress, if the antioxidant system is compromised [33].

Disruptions of the antioxidant system such as GSH have been documented in the PD brain [34-35]. The reduction of the GSH content occurred to minimize the delirious consequences of oxidative damage. A fault of one or more components of the antioxidant systems particularly, GSH is a key factor in the etiology of PD. Rotenone treatment caused diminishment of enzymatic antioxidants. This is in accordance with previous studies stating that SOD and GSH are key antioxidants in the central nervous system (CNS). The diminishment of these antioxidants is produced through their inactivation by ROS [34].

Inflammation is involved in the pathogenesis of Parkinsonism and this is followed by microglial activation. Microglial activation induces a variety of mediators particularly, NO and inflammatory cytokines and all contribute in the pathogenesis of PD and even its progression [25].

Crocin administration significantly reduced the inflammatory markers especially Tumor necrosis factor –alpha (TNF-α) is considered a central cytokine involved in inflammation, immunity and cellular organization. The cytotoxicity of TNF-α is achieved by overproduction of ROS that threatens the cellular components such as protein, lipids, and DNA [36]. Crocin anti-inflammatory activity is through the inhibition of m RNA expression for TNF-α [37].

Rotenone decreased significantly the dopamine level compared with control, crocin and PEG groups. Rotenone- treated groups with crocin 20 mg/Kg, 40 mg/Kg and L-DOPA significantly restored the content of dopamine. Dopamine level restoration was more pronounced in L-DOPA treated group as dopamine level was significantly higher in this group compared with rotenone treated with crocin in both doses.

Crocin produced a pronounced release of dopamine in rat brains. Crocin interacts with NMDA (N-methyl- D- aspartate) glutamate receptor sites of the brain to induce dopamine release. Crocin improves memory, and this may be because of the ability of the extract to induce dopamine and/or glutamate release [36].

Dopaminergic neurons damage is a complicated process and several factors are responsible for this damage including oxidative, and nitrosative stress as evidenced by increased nitrite/nitrate level, mitochondrial dysfunction, inflammation and, cytotoxicity. It has been postulated that reactive nitrogen species (RNS) play an important role in the achievement of dopaminergic damage. Rotenone increased the level of the nitrite, stable NO metabolite. Crocin administration reduced this rotenone- induced elevation. Nam et al. [9] had been reported that crocin attenuated the NO and NOS activity. Crocin could be effective in attenuating nitrosative stress in rotenone animal model of Parkinsonism [25].

Mirmosayyeb et al. had postulated that crocin suppressed lipopolysaccharide (LPS) - induced nitrite from microglial cells with subsequent protection from LPS- induced cytotoxicity [38].

Disruption of homeostasis of inflammatory cytokines especially TNF-α could lead to immune system dysfunction and inflammation. Hyperactivity of B-cells and T-cells results in increased cytokine level [39].

In this study, rotenone increased the DNA damage as manifested by elevated serum 8-OHdG. In general, OHdG is used as a marker of DNA damage in PD. Rotenone induced mitochondrial

dysfunction and oxidative stress as pronounced in our study. The unique property of mitochondria is the procession of their DNA (mt DNA) that is different from nuclear DNA in a way which makes it vulnerable to damage. Essential mitochondrial functions including oxidative phosphorylation, Ca++ buffering, and apoptosis are influenced by mt DNA mutations, resulting in the onset of the CNS diseases particularly PD [40]. Crocin administration reduced the level of serum 8-OHdG and this could be attributed to its antioxidant property or up-regulation and down-regulation of p 53 and other transcription factors [41].

Oxidant-antioxidant disruption is a well-established item to increase the conversion of deoxyguanosine to 8-hydroxy-2-deoxyguanosine (8-OHdG) in DNA. The damaged DNA can be repaired in vivo by endonucleases or by a base-specific glycosylase. The DNA repair products are transported through the blood and excreted into urine without further metabolism. OHdG is a direct adduct of hydroxylation of DNA bases formed when OH reacts with DNA [42-43].

Different factors are included in the development of cognitive, motor and behavioral changes in PD like oxidative, nitrosative stress with imbalance in NO generation, cholinergic system dysfunction, inflammation and apoptosis [44].

Rotenone induced loss of tyrosine hydroxylase (TH) positive neurons and reduced the expression of TH proteins. TH is a rate limiting enzyme of dopamine biosynthesis and converts tyrosine into L-DOPA. At the same time, oxidative stress contributes the occurrence of dopaminergic neurons degeneration. Inflammation and apoptosis had been suggested to be responsible for dopaminergic neurons degeneration. Crocin has a potent anti-inflammatory and anti-oxidative effect [45].

Crocin has the ability to induce dopamine release. DNA damage, oxidative stress and inflammation are the main triggering factors in the pathogenesis of Parkinsonism. Fortunately, this study was unique as it made a bridge between this triad in the pathogenesis of Parkinsonism. Unfortunately, we did not measure the changes of the regional level of neurotransmitters. This needs additional research to clarify this point.

CONCLUSION

Crocin protected against rotenone- model of Parkinsonism due to reduction of oxidative and nitrosative stress, proinflammatory cytokines and DNA damage. Crocin may be a unique item in preventing behavioral and motor deficits accompanying Parkinsonism.

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CONTRIBUTIONS

Concept, design, definition of intellectual content, data acquisition, and statistical analysis: Ahmed A. Abdalfattah and Aber A. Abo Zeid , literature search, manuscript preparation, manuscript review, manuscript editing, and data analysis: Ahmed A. Abdalfattah ; experimental studies : Ahmed A. Abdalfattah and Aber A. Abo Zeid . All authors have read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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