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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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Abstract

Backgropund: Parkinson's Disease (PD) is considered a second most common neurodegenerative disease with slow and irreversible nigrostriatal degeneration with subsequent motor and behavioral deficits. Oxidative stress plays a key role in the pathogenesis of PD. Rotenone is a common pesticide inducing PD by the generation of oxidative stress.

Aim of Study: The aim of this study was to investigate the possible neuroprotective effects of crocin (saffron active compound) on rotenone induced Parkinson-like behaviors.

Material and Methods: 70 male Wister Albino rats were divided into 7 groups (10) per each. (1) Control group (normal saline); (2) Crocin 40mg/Kg; (3) Polyethylene Glycol (PEG) group (vehicle of Levodopa); (4) Rotenone group; (5) Rotenone + crocin 20mg/Kg; (6) Rotenone + crocin 40mg/Kg; (7) Rotenone + Levodopa 10mg/Kg. All agents were injected intraperitoneally once a day for 4 weeks. The neurobehavioral tests include open field, descent latency time in the bar test (seconds), forepaw stride length (cm) and locomotor activity. In serum, the level of 8-hydroxydeoxyguanosine 8-OHdG was estimated. The level of Malondialdehyde (MDA), reduced glutathione (GSH), tumor necrosis factor alpha TNF-a, dopamine and nitrite/nitrate levels were measured in the brain tissue.

Results: Rotenone induced neurobehavioral deficits with elevation of brain MDA, brain TNF-a, Nitrite/nitrate level and serum 8-OHdG and reduction of GSH, brain tissue dopamine. Crocin (20 or 40) improved these neurobehavioral deficits. Crocin (20 or 40) and L-DOPA decreased MDA, serum 8-OHdG, TNF-a and Nitrite/nitrate level and increased GSH and dopamine level. Crocin 40 had achieved potent effect compared with crocin 20.

Conclusion: Rotenone induced Parkinson-like behavior in rats. Crocin achieved a protective effect through reducing lipid peroxidation, anti-inflammatory and reducing DNA damage together with improvement of neurobehavioral deficits.

Key Words: *Saffron – Crocin – Oxidative stress – Parkinson like behavior – DNA damage.*

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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 antioxidative 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. Taking in mind the role of oxidative and nitrosative stress in the pathogenesis of PD and the antioxidant and anti-inflammatory prosperities of crocin, the present study was labored to evaluate the effects of crocin on animal model of PD. Therefore, the aim of this study was to study the possible neuroprotective effects of crocin (saffron active compound) on rotenone induced Parkinson-like behaviors.

Material and Methods

Experimental animals: 70 Male albino rats of the local strains weighting (210±20g) 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 (from April 2018 till August 2018). The animals were housed at temperature 22±2ºC, exposed to alternate cycles of 12h 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: Rats were randomly divided into 7 groups 10 per each. (1) Control group (normal saline); (2) Crocin 40mg/Kg; (3) Polyethylene Glycol (PEG) group (vehicle of Levodopa); (4) Rotenone group; (5) Rotenone + crocin 20 mg/Kg [10]; (6) Rotenone + crocin 40mg/Kg; (7) Rotenone + Levodopa 10mg/Kg [11]. All agents were injected intraperitoneally once a day for 4 weeks.

Open-field test:

White wood, with a floor of 100 X 100cm divided by red lines into 25 lines equally. The height was 50cm and was white painted. Illumination of the test room and the colony room was the same. Cleaned and dried apparatus was achieved. Placing each rat in the center and observing their behavior. The total number of squares crossed (total locomotion), the outer squares (peripheral locomotion) and inner squares (central locomotion) [12].

Bar test for catalepsy:

According to the method described by Costall and Naylor [13], the rats were placed with forelimbs on a horizontal thin bar 9cm 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 [14].

Then, the animals were anesthetized by injection of a mixture of ketamine (150mg/kg) and xylazine (15mg/kg) I.P. and blood samples were collected from the heart into non-anti-coagulant containing tubes to obtain serum. Blood was allowed to clot for 30 minutes followed by centrifugation for 10 minutes at 5000rbm. Sera were separated and stored in aliquots at –70ºC.

The hippocampus and the cortex were dissected bilaterally, washed and homogenized for estimation of the lipid peroxidation parameter (MDA) according to the method of Fernandez et al., 1997 [15]. Reduced GSH was estimated according to the method of Moron et al., 1979 [16]. TNF-α was measured in accordance with Ye & Johnson 1999 [17]. Dopamine level was measured according to the method described by Jacobowitz and Richardson; 1978 [18]. Nitrite/Nitrate level was measured by colorimetric method [19].

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 group significantly increased the descent latency time compared with the control, crocin and PEG groups. Rotenone treated group with crocin 20mg/Kg and 40mg/Kg showed a significant decrease compared with the rotenone group with significant difference when comparing crocin 20mg/Kg treated group with crocin 40mg/Kg treated group. L-DOPA treated group showed significant reduction of descent latency time in the bar test compared with the rotenone group with insignificant change when comparing crocin 40mg/Kg treatment with L-DOPA treatment.

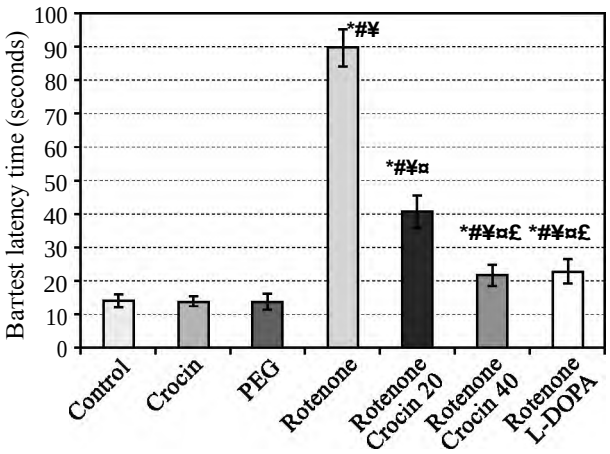

Fig. (1): Effect of crocin on descent latency time in the bar test (seconds).

* : Significant compared with control.
¤ : Significant compared with Rotenone.
: Significant compared with crocin.
£ : Significant compared with Rotenone + Crocin 20.
¥ : Significant compared with PEG.
¢ : Significant compared with Rotenone + Crocin 40.

Forepaw stride length (cm):

The mean value of the forepaw stride length was 8±2, 8±2. 1 and 8± 1 .9cm in the control, crocin and PEG groups respectively. Rotenone group showed a significantly decrease of forepaw stride length compared with the control, crocin and PEG groups. Rotenone treated group with crocin 20mg/Kg and 40mg/Kg showed significant increase compared with the rotenone group with a significant difference when comparing crocin 20mg/Kg treated group with crocin 40mg/Kg treated group. L-DOPA treated group showed a significant increase of the forepaw stride length compared with the rotenone group. No significant change was observed when comparing crocin 40mg/Kg treatment with L-DOPA treatment.

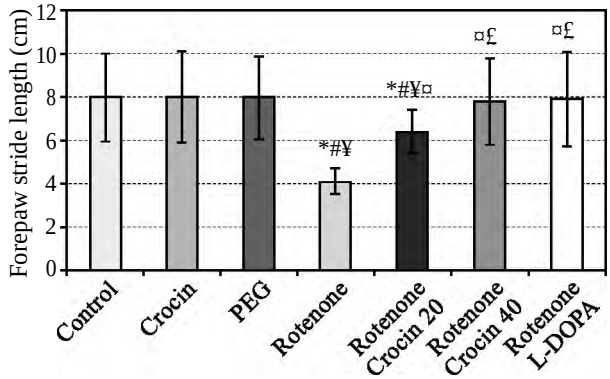

Fig. (2): Effect of crocin on forepaw stride length (cm).

* : Significant compared with control.
¤ : Significant compared with Rotenone.
: Significant compared with crocin.
£ : Significant compared with Rotenone + Crocin 20.
¥ : Significant compared with PEG.
¢ : Significant compared with Rotenone + Crocin 40.

Effect of rotenone and crocin on locomotor activity:

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

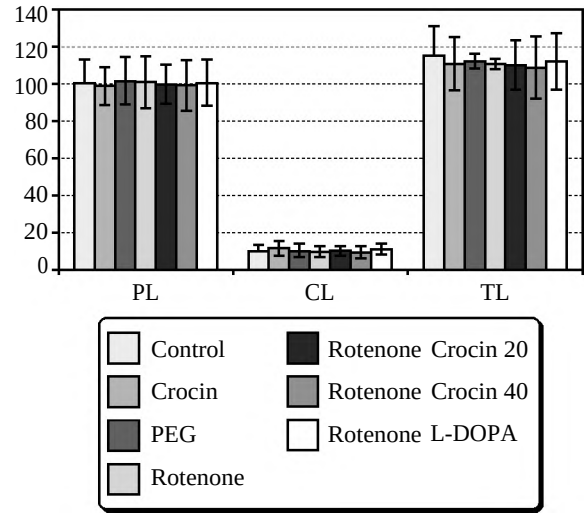

Fig. (3): Effect of rotenone and crocin on Total Locomotion (TL), Central Locomotion (CL) and Peripheral Locomotion (PL) in the 1 st day of the experiment.

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).

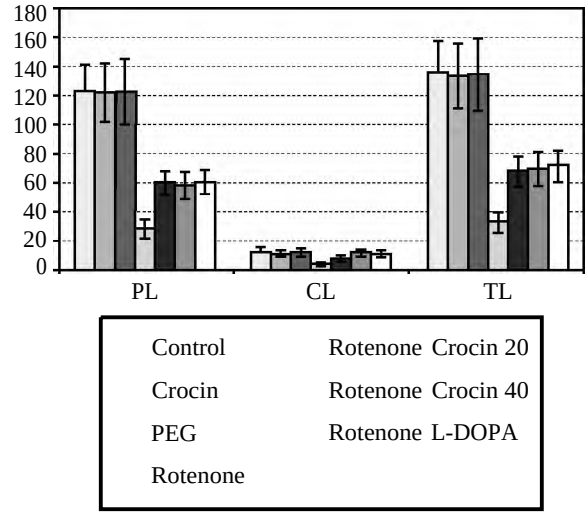

Fig. (4): Effect of rotenone and crocin on Total Locomotion (TL), Central Locomotion (CL) and Peripheral Locomotion (PL) at the end of the experiment.

Lipid peroxidation:

Rotenone administration markedly induced a significant increase of MDA level compared with control, crocin and PEG groups. A significant reduction of MDA was 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.

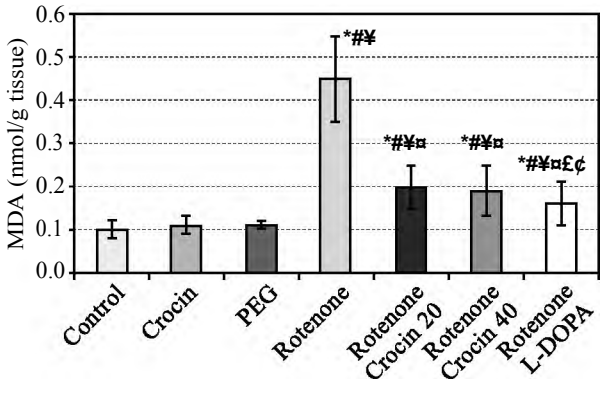

Fig. (5): Effect of crocin on lipid peroxidation.

* : Significant compared with control.
¤ : Significant compared with Rotenone.
: Significant compared with crocin.
£ : Significant compared with Rotenone + Crocin 20.
¥ : Significant compared with PEG.
¢ : Significant compared with Rotenone + Crocin 40.

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 20mg/Kg, 40mg/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.

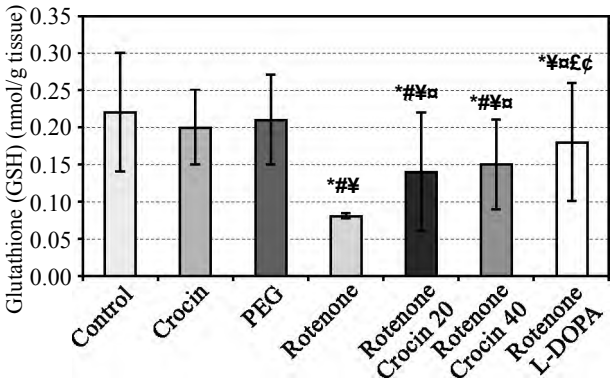

Fig. (6): Effect of crocin on reduced glutathione.

* : Significant compared with control.
¤ : Significant compared with Rotenone.
: Significant compared with crocin.
£ : Significant compared with Rotenone + Crocin 20.
¥ : Significant compared with PEG.
¢ : Significant compared with Rotenone + Crocin 40.

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-ct was still higher after L-DOPA treatment compared with crocin treated groups in both doses.

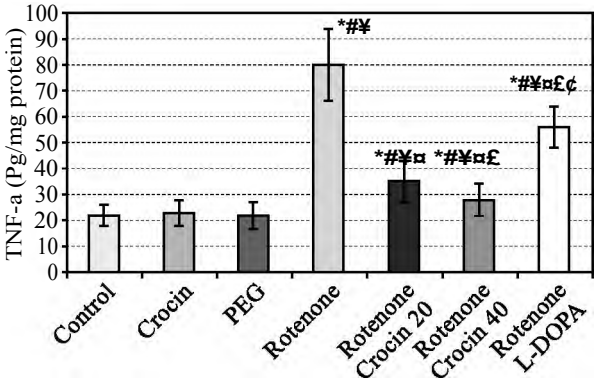

Fig. (7): Effect of crocin on inflammation.

* : Significant compared with control.
¤ : Significant compared with Rotenone.
: Significant compared with crocin.
£ : Significant compared with Rotenone + Crocin 20.
¥ : Significant compared with PEG.
¢ : Significant compared with Rotenone + Crocin 40.

Brain tissue dopamine level:

Rotenone decreased significantly the dopamine level compared with control, crocin and PEG groups. Rotenone treated groups with crocin 20mg/Kg, 40mg/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.

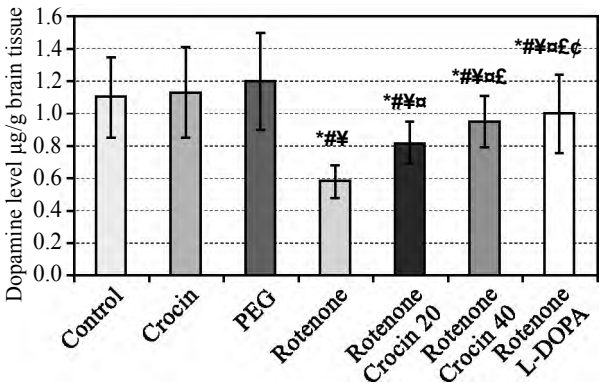

Fig. (8): Effect of crocin on brain dopamine level (µg/g).

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 20mg/Kg, 40mg/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 Crocin 20 and 40 treated groups.

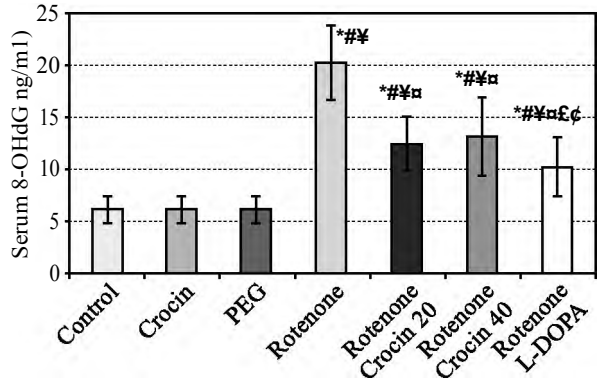

Fig. (9): Effect of crocin on Serum 8-OHdG level.

* : Significant compared with control.
¤ : Significant compared with Rotenone.
: Significant compared with crocin.
£ : Significant compared with Rotenone + Crocin 20.
¥ : Significant compared with PEG.
¢ : Significant compared with Rotenone + Crocin 40.

Nitrite/nitrate level:

Rotenone increased significantly the level of Nitrite/nitrate compared with control, crocin and PEG groups. Rotenone treated groups with crocin 20mg/Kg, 40mg/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 crocin 20 and 40 treated groups and in crocin 40 treated compared with crocin 20 treated groups.

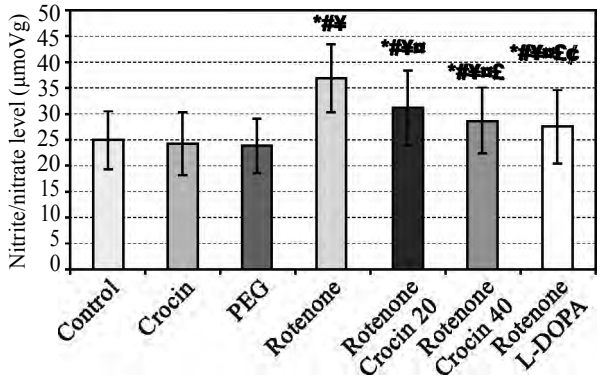

Fig. (10): Effect of crocin on brain nitrite/nitrare level.

* : Significant compared with control.
¤ : Significant compared with Rotenone.
: Significant compared with crocin.
£ : Significant compared with Rotenone + Crocin 20.
¥ : Significant compared with PEG.
¢ : Significant compared with Rotenone + Crocin 40.

Discussion

This study revealed that rotenone treated animals developed 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. The same had been reported by Dijkstra et al. [20].

Motor disruption in rotenone rat model of Parkinsonism was attenuated by using antioxidants [21]. Catalepsy is considered an important marker of nigrostriatal damage and can be induced by rotenone. This cataleptic behavior is linked to the dopaminergic nigrostriatal degeneration.

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 40mg/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 [22,23]. L-DOPA treatment had reversed the deficits related to nigrostriatal degeneration [24].

Oxidative stress is considered an important step in aging and neurological disorders [25]. It can be induced by hypoxia or hyperoxia, leading to an increased free radical formation. The mitochondria is the main organelle that produce free radicals [26].

Brain tissue is more susceptible to oxidative stress than other tissues as it has a high metabolic activity, high oxygen consumption and presence of enhanced iron level leading to reduction of H_2O_2 to form the highly reactive hydroxyl radical [27].

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 [28].

During oxidative stress, there is accumulation of oxidants and formation of TBARS [29]. The brain has 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 significant increase in MDA level in rotenone induced PD. Treatment with crocin reduced this elevation significantly and this effect refers to the corcin antioxidant activity as evidenced by increased enzymatic and GSH availability [30]. Chen and his team had stated

that crocin, the major constituent derived from saffron, has different 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 anti-oxidant system is compromised [31]. Disruptions of the antioxidant system such as GSH have been documented in the PD brain [33]. 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.

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 [23].

NO generation resulting from increased gene expression of induced NOS is followed by iNOS enzyme activation with subsequent contribution to the inflammatory process [34].

Crocin administration significantly reduced the inflammatory markers especially TNF-a. Tumor necrosis factor-alpha is considered a central cytokine involved in inflammation, immunity and cellular organization. The cytotoxicity of TNF-a is achieved by overproduction of ROS that threatens the cellular components such as protein, lipids, and DNA [35]. Crocin anti-inflammatory activity is through the inhibition of m RNA expression for TNF-a [36].

Rotenone decreased significantly the dopamine level compared with control, crocin and PEG groups. Rotenone treated groups with Crocin 20mg/Kg, 40mg/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 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 inhibits neuronal degeneration and this may be because of the ability of the extract to induce dopamine and/or glutamate release [35].

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 [23].

Mirmosayyeb et al., had postulated that crocin suppressed lipopolysaccharide (LPS)-induced nitrite from microglial cells with subsequent protection from LPS-induced cytotoxicity [36].

Disruption of homeostasis of inflammatory cytokines especially TNF-a could lead to immune system dysfunction and inflammation. Hyperactivity of B-cells and T-cells results in increased cytokine level [37].

In this study, rotenone increased the DNA damage as manifested by elevated serum 8-OHdG. In general, very little is known about DNA damage in PD [38].

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 [39]. 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 p53 and other transcription factors [40].

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 [41].

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 [42]. 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.

Crocin has the ability to induce dopamine and glutamate release. 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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الآثار العصبية المحتملة من الكروسين ضد التغييرات الحركية والميكروكيميائية فى نموذج الباركنسون المستحدث بواسطة الريتونون فى الجرذان

مقدمة: يعتبر مرض باركنسون (PD) ثانى أكثر الأمراض العصبية شيوعاً مع تنكس نيغروستيراتى بطئ ولا رجعة فيه مع عجز حركى وسلوكى لاحق. الإجهاد التأكسدى يلعب دوراً رئيسياً فى التسبب فى مرض الباركنسون المستحث بواسطة الروتينون. هو مبيد شائع يحفز الباركنسون عن طريق توليد الإجهاد التأكسدى. بحثت هذه الدراسة التأثيرات المحتملة للكروسين (المركب الفعال للزعفران) على السلوكيات الشبيهة بالباركنسون التى يسببها الروتينيون.

المواد والطرق: أجرى هذا البحث على ٧٠ جرذاً من النوع ويستر البيضاء وتم تقسيم الجرذان إلى ٧ مجموعات (١٠) لكل منهم. (١) مجموعة التحكم، (٢) مجموعة الكروسين ٤٠مج/كجم، (٣) مجموعة البولى إيثيلين جلايكول، (٤) مجموعة روتينيون، (٥) مجموعة الروتينون بالإضافة إلى الكروسين ٢٠مج/كجم، (٦) مجموعة الروتينون بالإضافة إلى الكروسين ٤٠مج/كجم، (٧) روتينون + ليفودوبا ١٠ملغ/كغ. تم حقن جميع المواد بالبريتون مرة واحدة فى اليوم لمدة ٤ أسابيع. الإختبارات السلوكية العصبية بما فى ذلك المجال المفتوح، وقت النزول من على البار (بالثوانى) ومسافة الخطوة والنشاط الحركى. فى مصل الدم، تم تقدير مستوى ٨–هيدروكسى ديوكسى جوانوزين. تم قياس مستوى المالونديالدهايد والجلوتاثيون عامل نخر الورم ألفا ومستويات النترات/النترات والدوبامين فى أنسجة المخ.

النتائج: أظهرت النتائج نقص العلامات السلوكية العصبية مع إرتفاع المالونديالدهايد وعامل التورم النكروزى ألفا ومستوى النتريت/النترات ومستوى ٨–هيدروكسى ديوكسى جوانوزين وإنخفاض مستوى الجلوتاثيون والدوبامين فى أنسجة المخ الحد من. تحسنت العيوب السلوكية العصبية بالكروسين ٢٠ و٤٠مج–كجم مع إنخفاض مستوى المالونديالدهيد و٨–هيدروكسى ديوكسى جوانوزين وعامل التورم النكروزى ومستوى النيتريت والنيترات مع وزيادة مستوى الجلوتاثيون ومستوى الدوبامين. حقق الكروسين ٤٠ تأثيراً قوياً مقارنة مع الكروسين ٢٠.

المختصر: يسبب الريتنون سلوكاً شبيهاً بالباركنسون فى الجرذان. حقق الكروسين تأثيراً وقائياً من خلال الحد من بيروكسيد الدهون، عمله ومضاداً للإلتهابات والحد من تلف الحمض النووى مع تحسين حالات العجز السلوكى العصبى. بالإضافة إلى تحسن الخلل السلوكى العصبى.