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Trans-sodium crocetinate ameliorates Parkinson-like disease caused by bisphenol A through inhibition of apoptosis and reduction of α-synuclein in rats

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ABSTRACT

Objective(s): Trans-sodium crocetinate (TSC) is one of the crocetin derivations that is more soluble and stable than crocetin and its cis form. It easily crosses the blood-brain barrier. TSC has neuroprotective effects. Bisphenol A (BPA) is an endocrine-mimicking compound that induces Parkinson-like disease by impacting the dopaminergic system. In this research, the effects of TSCs on BPA-induced Parkinson-like symptoms via behavioral and molecular assays have been investigated.
Materials and Methods: Male Wistar rats received BPA (75 mg/kg, gavage), TSC (10, 20, and 40 mg/kg), and levodopa (L-dopa) (10 mg/kg) via intraperitoneal injection (IP) for 28 days. Parkinsonian-like motor features were evaluated using bar test, rotarod, and open field experiments. Malondialdehyde (MDA) and glutathione (GSH) levels were also measured as the most important indicators of oxidative stress. Western blotting was performed for the molecular assays of alpha-synuclein (α-syn), Bcl-2, Bax, caspase-3, Beclin, and LC3 I/II proteins.
Results: Our analyses indicated that treatment with TSC at high dose reduces MDA levels and protects GSH reserves. TSC can also increase anti-apoptotic Bcl-2 and decrease pro-apoptotic Bax and caspase-3 proteins. While it does not affect autophagy markers, TSC decreased α-syn protein expression, reduced the catalepsy time, and improved the time spent staying on the rotating bar and the locomotor activity.
Conclusion: Overall, TSC likely ameliorates BPA-mediated Parkinson's-like symptoms by suppressing oxidative stress inhibition. This leads to reduced α-syn expression, which ultimately results in apoptosis inductions. Therefore, TSC can serve as a promising exploratory target for future research aimed at controlling Parkinson's disease.

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Introduction

Saffron (*Crocus sativus* L.) is a herbaceous plant cultivated in various countries, including Iran, India, and Greece (1). The most important compounds of saffron are crocin, crocetin, safranal, and picrocrocin. Studies have reported that crocin effectively improves Parkinson-like symptoms in rats by reducing apoptosis and α-synuclein expression (2, 3). Crocetin (8,8′-diapocarotene-8,8′-dioic acid) is a low-molecular-weight carotenoid with diverse therapeutic effects (4) such as anticancer (4), antihyperlipidemia (5), antiatherosclerosis (6), and anti-inflammatory (7). The underlying mechanisms of therapeutic effects of crocetin include antioxidant properties, increasing oxygenation in hypoxic tissues, suppression of pro-inflammatory mediators, inhibition of proliferation, and induction of apoptosis in cancerous cells (8). It has been reported that crocetin protects neurons and prevents Parkinson's disease through its antioxidant properties (9, 10). In addition,

crocetin suppressed the destruction of dopaminergic neurons caused by 6-hydroxydopamine and prevented the decrease of dopamine levels (10).

Trans sodium crocetinate (TSC), as one of crocetin derivatives, reduces plasma resistance by changing the intermolecular forces of water molecules. It also increases oxygen diffusion through the plasma, enhancing oxygen supply to the tissues. TSC differs from other carotenoids due to polar groups at both ends of its hydrophobic carbon chain (11). TSC has neuroprotective effects. It has been reported that crocetin clears amyloid-beta by inducing autophagy through the AMPK pathway, and TSC can increase the degradation of amyloid-beta in monocytes of Alzheimer's patients (12).

Among common neurodegenerative disorders, Parkinson's disease (PD) holds the second rank worldwide, following Alzheimer's disease. It affects around ten million people globally. The destruction of dopaminergic neurons

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in the substantia nigra region of the brain is identified as the primary cause of this disease. These neurons release dopamine, a neurotransmitter that regulates motor function by transmitting messages from the substantia nigra to the motor areas of the brain (13, 14). Destruction of 60–80% of dopamine-secreting cells leads to the onset of Parkinson's motor symptoms (15). It has been reported that factors such as oxidative stress, inflammatory factors, aquaporin 4, α-synuclein (α-Syn) accumulation, and apoptotic pathways play roles in the pathogenesis of Parkinson's disease(16, 17). Autophagy has also been linked to Parkinson's disease through α-synuclein (2). α-Syn is a soluble protein that accumulates in Lewy bodies as insoluble masses (18). The overexpression of α-Syn, a hallmark observed in both familial and sporadic cases of PD, as well as in several severe neurodegenerative diseases, inhibits autophagy at the early stages of autophagosome formation (19). Additionally, α-Syn can induce progressive degeneration of dopaminergic neurons and apoptosis in rodents (20).

PD is a progressive chronic disease that ultimately leads to death. Therefore, finding a therapeutic approach to control or treat this disease is paramount. Various clinical strategies such as pharmacotherapy, surgery, deep brain stimulation, and gene therapy have been described for the treatment of PD. Among them, pharmacotherapy, especially L-dopa, is the most common (2, 21). However, L-dopa has been documented to have several side effects (21). Therefore, exploring the potential of natural products, with their therapeutic effects or supporting roles for existing medicines, is considered for treating PD (22).

Bisphenol A (BPA) is among several endocrine-disrupting compounds that mimic the action of hormones (23, 24). Additionally, BPA is a fundamental component in the production of plastics, including polycarbonate and epoxy resins (25). This compound exerts toxicity by various mechanisms such as oxidative stress leading to DNA and cell membrane damage, the inhibition of nerve transmission resulting in the inhibition of proliferation and differentiation of neural stem cells, and the induction of cell death (26-28). Conditions such as increased heat and acidity can trigger hydrolysis of the ester bond between bisphenol monomers, ultimately causing its release from containers and equipment into food and the environment (29). BPA induces apoptosis by activating caspase 3, increasing the expression of the pro-apoptotic Bax gene, and triggering the c-Jun N-terminal kinases (JNKs) pathway due to elevated levels of free radicals (30, 31). Furthermore, this compound can disrupt synaptic function, significantly affecting memory processes (32, 33). Chronic exposure to BPA is directly associated with changes in the central nervous system, especially the dopaminergic system (13, 34).

Considering the positive effects of saffron and its compounds on the dopaminergic system, along with the favorable physicochemical properties and neuroprotective effects of TSC, the present study was conducted to investigate the impact of TSC on Parkinson-like disease induced by BPA in rats.

Materials and Methods
Chemicals
BPA (Ca NO. 803546), thiobarbituric acid (TBA) (Ca NO. 108180), glycine (Ca NO. 104201), tris (hydroxymethyl) aminomethane (CaN. 108387), tris hydrochloride (Tris HCL) (Ca NO. 648317), and acrylamide (Ca NO. 800830)

were purchased from Merck, Germany. The TSC (Ca NO. t59123009908) was obtained from Tinab Chem Middle East Company, Iran. The 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) (Ca NO. D218200) was purchased from Sigma-Aldrich, and other compounds were supplied from Merck, Germany.

Animal
In this study, we used male Wistar rats at 10–12 weeks of age purchased from the Center of Reproduction and Maintenance of Laboratory Animals (Mashhad University of Medical Sciences, Mashhad, Iran). Rats were grouped (a set of 7 rats per cage) and, one week before experiment initiation, acclimated to the new home cage under temperature (22–24 °C) and humidity (40–60%) conditions with a dark/light cycle of 12 hr. Animal maintenance conditions and all the steps for conducting experiments were met following the ethics committee guidelines of the Mashhad University of Medical Sciences (ethics code: IR.MUMS.AEC.1401.046).

Compounds including Olive oil (Vehicle) and BPA (75 mg/kg bw) were gavaged orally, and distilled water, TSC (10, 20, and 40 mg/kg bw) were injected intraperitoneal (35). Based on previous studies, L-dopa (10 mg/kg bw) was also injected intraperitoneal (36). BPA dose was extrapolated using a pilot test (at the 75 mg/kg dose, all rats showed Parkinsonian-like symptoms) (37). All compounds were prepared fresh daily and used for 28 days. Behavioral tests were performed 24 hours after receiving the last dose of compounds. After completing the behavioral tests, the animals were anesthetized using ketamine and xylazine (70/10 mg/kg bw) (38), the brain was removed, and the striatum region was isolated. A part of the isolated tissue was used to measure lipid peroxidation and GSH content, and another part was saved at -80 °C for western blot analysis.

Behavioral experiments
Catalepsy test
On the 29th day of the experiment, a catalepsy test was performed to evaluate muscle rigidity caused by PD. The apparatus used in this test was a wooden box with a stainless bar in the middle. The longer the time to hold the bar, the more severe the catalepsy (39).

Rotarod performance test
The Rotarod test is a common test to assess coordination and movement balance. The speed gradient of the rod used in the rotarod apparatus is adjusted from 5 to 30 rpm. This test includes two parts: 1) the training stage and 2) The test phase. The average animal movement on the rotating rod was estimated as a criterion for maintaining balance and muscle stiffness (40, 41).

Open field test
The open field test was conducted using a Plexiglas open field, including a square with 100 x 100 cm2 dimensions and walls 50 cm in height. Then, factors such as the number of passes through the central zone (50 x 50 cm), the peripheral areas, and the total number of passes were measured. The extent of stopping and the distance traveled show the severity of muscle contraction and stiffness (38).

Biochemical experiments
Lipid peroxidation
This method is based on the reaction of malondialdehyde (MDA), which is a lipid peroxidation product, with thiobarbituric acid (TBA) and the formation of a colored complex. To perform this method, phosphoric acid and

TBA (0.6%) were added to the 10% homogenized samples. Then, the complex was placed in a boiling water bath for 45–90 min. After cooling at room temperature, n-butanol was added, and finally, the absorbance of the organic portion was measured at 532 nm. The concentration of MDA in the samples was extrapolated using the MDA standard curve and expressed as nmol/g tissue (42, 43).

Glutathione content

The basis of the method is the reaction of sulfhydryl groups with 5, 5'-dithiobis(2-nitrobenzoic acid) (DTNB) reagent. DTNB (0.04%) reacts with sulfhydryl groups, creating a colored complex with maximum absorption at 412 nm. Briefly, a 10% homogenate was first prepared with phosphate buffer, and 10% TCA was added to the samples. Then, after the samples were centrifuged, the supernatants were separated. Eventually, DTNB was added, the samples' absorption was evaluated, and the concentration of GSH was extrapolated using the GSH standard curve and expressed as nmol/g tissue (42, 44).

Western blotting

After removing the brain and separating the striatum, the samples were immediately stored at -80 °C for more analysis by immunoblotting technique. Before performing western blotting, all tissue proteins were extracted using lysing buffer (50 mM Tris base, 2 mM EDTA, 2 mM EGTA, 10 mM NaF, 1 mM $Na_3VO_4 \cdot 2H_2O$, 10 mM β-Glycerophosphate, 10 mM 2-Mercaptoethanol and Sodium deoxycholate 0.2 w/v), 0.1 mM phenylmethylsulfonyl fluoride (PMSF), and 1X protease inhibitor cocktail. Then, protein absorption was measured by the Bradford method at a wavelength of 595 nm, and the total protein concentration was calculated using the calibration curve. Protein separation was performed by loading equal quantities of each sample onto a 12 (for Bax, Bcl-2, Beclin, and α-synuclein proteins) and 15% (for caspase-3 and LC3 I/II proteins) polyacrylamide gel containing 10% SDS. Then, electro-transfer was conducted to transfer separated proteins onto a polyvinylidene fluoride (PVDF) membrane (Biorad, USA). Next, the PVDF membrane was imbedded in the Tris-buffered saline with 0.1% Tween (TBST) and overnight incubated at 4 °C. Afterward, primary antibodies, including rabbit anti-Bcl-2 (1:1000 dilution, Cell Signaling #2870), anti-Bax (1:1000 dilution, Cell Signaling #2772), anti-Caspase-3 (1:1000 dilution, Cell Signaling #9665), anti-Beclin-1 (1:1000 dilution, Cell Signaling #3495), anti Lc3 (1:1000 dilution, Cell Signaling #12741), anti α-synuclein (1:1000 dilution, Cell Signaling #4179), and internal control, Mause anti β-actin (1:1000 dilution, Cell Signaling #3700), were used to detection of targeting peptides. After overnight incubation, primary antibodies were removed, and secondary antibodies such as horseradish peroxidase-conjugated anti-mouse (1:3000 dilution, Cell Signaling #7076) and anti-rabbit (1:3000 dilution, Cell Signaling #7074) antibodies were added to the membrane and incubated at room temperature for 2 hr. Eventually, after three washes, the blot appeared using a chemiluminescent (Gel Doc Alliance 4.7, UK) detection method, and the density of the blot was used to assay the protein expression level. The final concentration of each sample protein level was calculated by comparing the blot density of each sample with the blot density of its internal references (β-actin).

Statistical analysis

This study used GraphPad Prism version 9 software for data analysis. All data were obtained from at least three independent experiments, and the triplicated values of each experiment were expressed as mean ± SD. The one-way ANOVA test with Tukey as a post hoc test was used to compare more than two experimental groups. Statistically, the lowest significance level was identified with the P-value<0.05.

Results

The effects of TSC on the BPA- induced Behavioral changes
TSC improved the catalepsy induced by BPA

Our data in Figure 1 demonstrated that BPA-induced severe catalepsy was significantly decreased with TSC 40 mg/kg (P<0.001) compared to the BPA alone group. The catalepsy time in the rats receiving BPA alone was significantly increased compared to the control (P<0.001). The BPA-alone group also showed a significant increase in catalepsy time compared to the L-dopa and BPA groups (P<0.001). However, the group that received TSC 40 mg/kg and BPA did not exhibit a significant difference in catalepsy time compared to the L-dopa and BPA group. Moreover, the group that received TSC 40 mg/kg alone did not show a significant difference compared to the control group.

TSC improved the BPA-induced imbalance

Groups exposed to BPA alone showed a significant decrease in the time spent on the rotarod bar compared to the control (P<0.001). Our results indicated that groups receiving TSC at a dose of 40 mg/kg along with BPA exhibited a notable increase in dwell time on the rotating rod compared to the BPA-only group (P<0.001). There was no significant difference between the group receiving TSC 40 mg/kg with BPA and the group receiving L-dopa 10 mg/kg with BPA. In the rats that received L-dopa 10 mg/kg and BPA, the time spent on the rotarod bar significantly improved compared to those receiving BPA alone (P<0.001). Furthermore, the group that received TSC 40 mg/kg alone
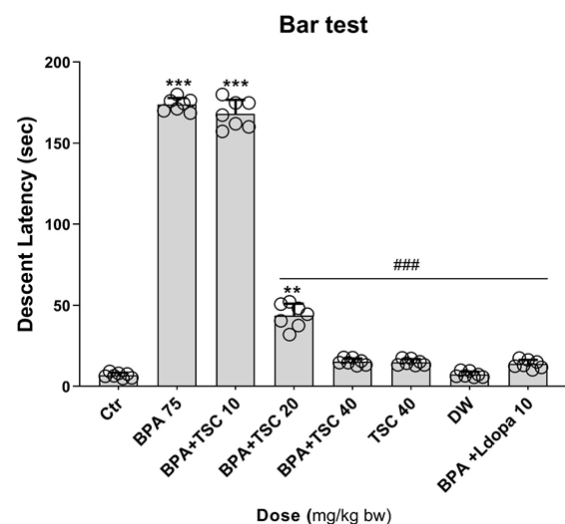

Figure 1. The effect of Bisphenol A (BPA) and Trans sodium crocetinate (TSC) on catalepsy in bar test in rat

Decent latency of the bar test was evaluated 24 hr after the last dose of BPA, TSC, and L-dopa, as well as olive oil and distilled water (DW) as vehicle. n=7 were used to calculate the mean. The one-way ANOVA and Tukey's post hoc test were used for data analysis, and the chart shows the values as means ± SD; **P<0.01 and ***P<.001 indicate significant differences between control and other treated groups; ###P<0.001 indicates significant differences between BPA and other treated groups.
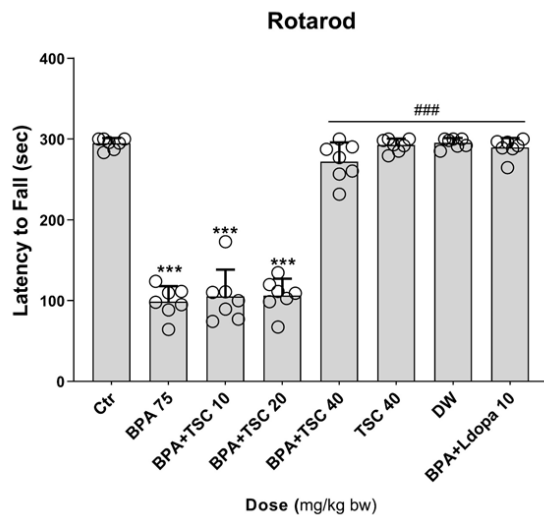
Figure 2. The effect of Bisphenol A (BPA) and Trans sodium crocetinate (TSC) on balance and coordination of locomotor in the rotarod test in rat
Difficulty moving and inability to balance by rotarod evaluated 24 hr after the last dose of BPA, TSC, and L-dopa, as well as olive oil and distilled water (DW) as the vehicle. n=7 were used to calculate the mean. The one-way ANOVA and Tukey's *post hoc* test were used for data analysis, and the chart shows the values as means ± SD; ***$P<0.001$ indicates significant differences between control and other treated groups; ###$P<0.001$ indicates significant differences between BPA and other treated groups.

Figure 3. The effect of Bisphenol A (BPA) and Trans sodium crocetinate (TSC) on locomotor activities in the open field test in rat
The slowness of movements in the open field was evaluated 24 hr after the last dose of BPA, TSC, and L-dopa, as well as olive oil and distilled water (DW) as vehicle. n=7 were used to calculate the mean. The one-way ANOVA and Tukey's *post hoc* test were used for data analysis, and the chart shows the values as means ± SD; ***$P<0.001$ indicates significant differences between control and other treated groups; ###$P<0.001$ indicates significant differences between BPA and other treated groups.

did not show a significant difference compared to the control group (Figure 2).

TSC ameliorated the locomotor activities of rats exposed to BPA
Rats in groups that received BPA alone showed a statistically significant decrease in traveled distance compared to the control group ($P<0.001$), as measured by the number of squares traversed. Our analyses revealed that rats receiving TSC at 40 mg/kg and BPA exhibited a significant increase in the number of squares traversed compared to the group exposed to BPA alone. The traveled distance in the group exposed to L-dopa at 10 mg/kg and BPA significantly improved compared to the BPA-only group ($P<0.001$). The difference in distances traversed by the group receiving TSC at 40 mg/kg alone compared to control rats was not statistically significant. Furthermore, our analyses indicated that in rats receiving TSC at 40 mg/kg and BPA, there was no significant difference in the
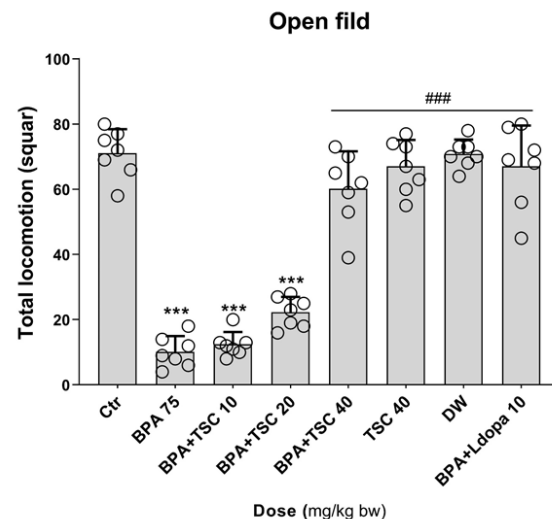
number of squares traversed compared to the L-dopa at 10 mg/kg and BPA group (Figure 3).

Oxidative stress indices
TSC preserved the GSH level in the brain of rats exposed to BPA
Examination of thiol groups in the striatum of rats revealed a statistically significant decrease in glutathione levels in the groups receiving BPA alone compared to the control ($P<0.001$). The changes in glutathione levels in the group receiving TSC 40 mg/kg alone were not significantly different from those in the control group. TSC 40 mg/kg alongside BPA significantly preserved glutathione resources compared to the group receiving BPA alone ($P<0.001$). The glutathione levels were also significantly preserved in the rats that received L-dopa at 10 mg/kg along with BPA alone group ($P<0.001$). However, the group that received TSC 40 mg/kg and BPA did not show a significant difference compared to the L-dopa at 10 mg/kg and BPA group (Figure 4a).
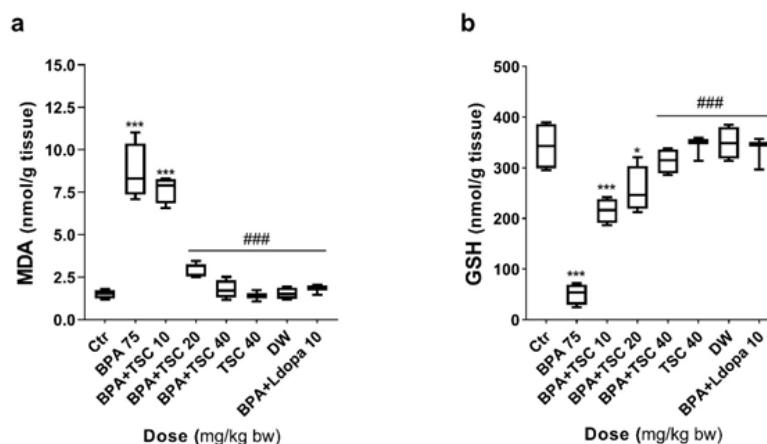

Figure 4. The effect of Bisphenol A (BPA) and Trans sodium crocetinate (TSC) on stress oxidative indexes in the rat brain
In the striatum region of brain tissues of rats, a) Box plot shows results for malondialdehyde (MDA) measuring using thiobarbituric acid (TBA), and b) Box plot shows results for GSH measuring using 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB). At least 3 samples were used to calculate the mean. The one-way ANOVA and Tukey's post hoc test were used for data analysis, and values are expressed as means ± SD; *$P<0.05$, ***$P<0.001$ indicate significant differences between control and other treated groups; ###$P<0.001$ indicates significant differences between BPA and other treated groups.
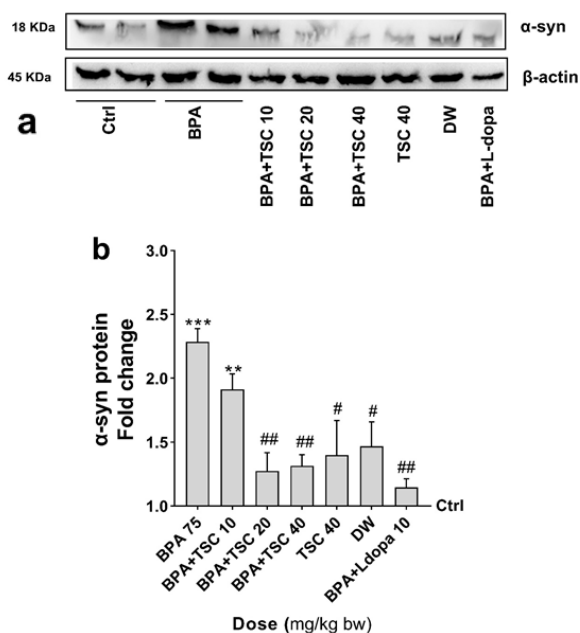
Figure 5. The effect of Bisphenol A (BPA) and Trans sodium crocetinate (TSC) on alpha-synuclein (α-syn) protein level in the rat brain
Western blots performed using striatum region of brain tissues of rats exposed to olive oil and distilled water (DW) (vehicle-treated controls), Bisphenol A (BPA), Trans sodium crocetinate (TSC), and L-dopa for the detection of α-syn protein; a) 18 kDa and 45 kDa bands show results for α-syn and β-actin proteins respectively, b) graph shows analysis results for α-syn protein. At least 3 samples were used to calculate the mean. The one-way ANOVA and Tukey's *post hoc* test were used for data analysis, and values are expressed as means ± SD; **$P<0.01$, ***$P<0.001$ indicate significant differences between control and other treated groups; #$P<0.05$, ##$P<0.01$ indicate significant differences between BPA and other treated groups.

TSC attenuated BPA-induced lipid peroxidation levels

The assessment of MDA levels using the TBA reaction (Figure 4b) revealed a significant increase in MDA levels in the group exposed to BPA alone compared to the control ($P<0.001$). Treatment with TSC (20 and 40 mg/kg) or L-dopa (10 mg/kg) alongside the BPA group decreased MDA levels compared to the BPA-only group ($P<0.001$). However, the group receiving TSC (40 mg/kg) alone did not show significant differences in MDA levels compared to the control group ($P<0.05$). Moreover, the groups treated with TSC (20 or 40 mg/kg) and BPA exhibited no statistically significant differences in MDA levels compared to the L-dopa (10 mg/kg) and BPA group ($P<0.001$).

Blotting analysis
TSC ameliorates BPA-induced α-synuclein protein expression

The results of the blotting analysis in Figure 5 indicated a significant increase in α-syn protein levels in rats exposed to BPA alone compared to the control ($P<0.01$). The level of α-syn protein in rats receiving TSC (20 and 40 mg/kg) or L-dopa (10 mg/kg) alongside BPA significantly decreased compared to rats receiving BPA alone ($P<0.01$). However, the level of α-syn protein in the groups receiving TSC 40 mg/kg alone did not show significant changes compared to the control group. Additionally, α-syn protein levels in the groups receiving TSC 20 or 40 mg/kg and BPA did not significantly differ from those receiving L-dopa and BPA.

TSC ameliorates BPA-induced pro-apoptotic Bax and Caspase-3 proteins and induces anti-apoptotic Bcl-2 protein expression

The results of blot analysis revealed that in the striatum

of rats exposed to BPA alone, the levels of pro-apoptotic Bax (Figure 6a,c) and Caspase-3 (Figure 6a,d) proteins significantly increased compared to the control group ($P<0.001$). In rats receiving BPA plus TSC (10, 20, and 40 mg/kg) or L-dopa 10 mg/kg, the levels of Bax and Caspase-3 proteins significantly decreased compared to the BPA alone group ($P<0.001$). Additionally, the anti-apoptotic Bcl-2 protein expression (Figure 6a,b) in the BPA alone group significantly decreased compared to the control group. Meanwhile, Bcl-2 protein levels in the groups receiving TSC (10, 20 ($P<0.001$) and 40 ($P<0.01$) mg/kg) or L-dopa 10 mg/kg ($P<0.001$) alongside BPA were significantly lower compared to rats exposed to BPA alone. However, there was no significant difference in the levels of Bax (Figure 6a,c), Caspase-3 (Figure 6a,d), and Bcl-2 (Figure 6a,b) proteins between the group receiving TSC 40 mg/kg alone and the control group. Additionally, the changes in the levels of Bax (Figure 6a,c), Caspase-3 (Figure 6a,d), and Bcl-2 (Figure 6a,b) proteins were not significant in rats treated with BPA and TSC (10, 20, and 40 mg/kg) compared to the L-dopa 10 mg/kg and BPA group.
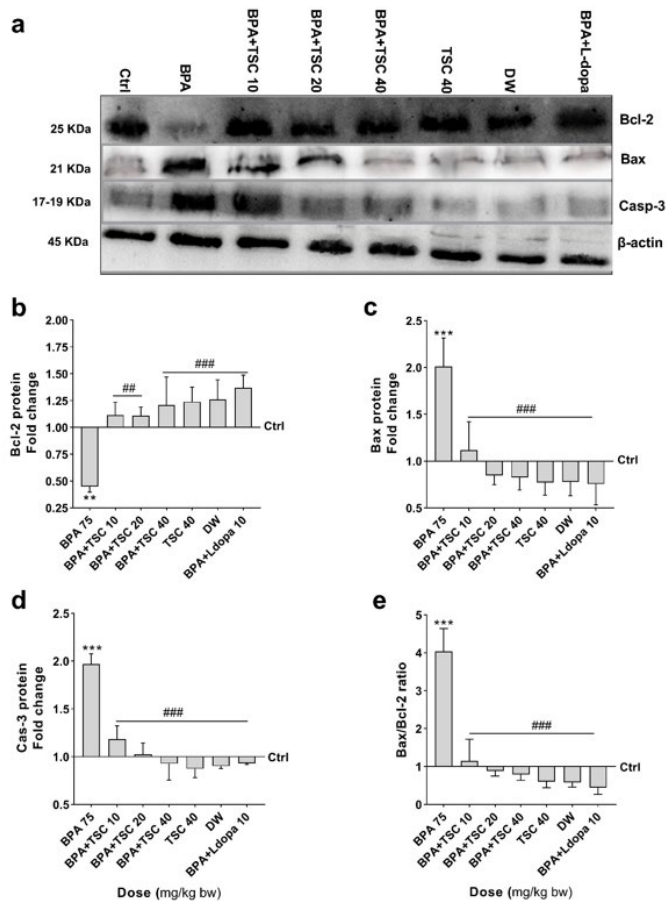

Figure 6. The effect of Bisphenol A (BPA) and Trans sodium crocetinate (TSC) on Bcl-2, Bax, and Caspase-3 protein levels in the rat brain
Western blots performed using striatum region of brain tissues of rats exposed to olive oil and distilled water (DW) (vehicle-treated controls), Bisphenol A (BPA), Trans sodium crocetinate (TSC), and L-dopa for the detection of apoptotic marker proteins; a) 25 kDa, 21 kDa, 17-19 kDa, and 45 kDa bands show results for Bcl-2, Bax, Caspase-3, and β-actin proteins respectively, b) graph shows analysis results for anti-apoptotic Bcl-2 protein, c) graph shows analysis results for pro-apoptotic Bax protein, d) graph shows analysis results for pro-apoptotic Caspase-3 protein, and e) graph shows analysis results for Bax/Bcl-2 proteins ratio. At least 3 samples were used to calculate the mean. The one-way ANOVA and Tukey's *post hoc* test were used for data analysis, and values are expressed as means ± SD; ***$P<0.001$ indicates significant differences between control and other treated groups; ##$P<0.01$, ##$P<0.001$ indicate significant differences between BPA and other treated groups.
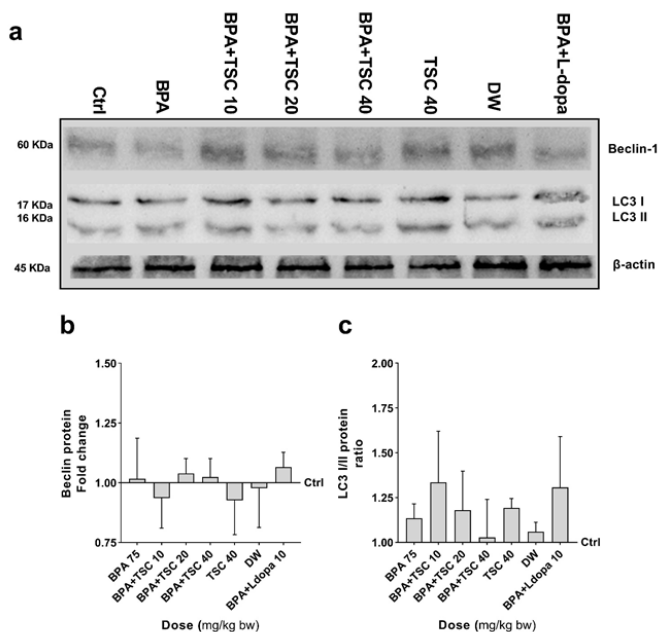
Figure 7. The effect of Bisphenol A (BPA) and Trans sodium crocetinate (TSC) on Beclin, LC3 I/II proteins levels in the rat brain
Western blots performed using striatum region of brain tissues of rats exposed to olive oil and distilled water (DW) (vehicle-treated controls), Bisphenol A (BPA), Trans sodium crocetinate (TSC), and L-dopa for the detection autophagy marker proteins; a) 60 kDa,16-17 KDa, and 45 KDa bands show results for Beclin, LC3 I/II, β-actin proteins respectively, b) graph shows analysis results for pro-autophagy Beclin protein, and c) graph shows analysis results for LC3 I/II protein ratio. At least 3 samples were used to calculate the mean. The one-way ANOVA and Tukey's *post hoc* test were used for data analysis, and values are expressed as means ± SD.

TSC and BPA effects on the autophagy pathway proteins Beclin and LC -3 I/II expression

The expression levels of Beclin and LC-3 I/II in the groups treated with BPA, TSC, and L-dopa did not show noticeable changes compared to the control group, and there were no significant differences among these groups (Figure 7).

Discussion

Although many factors are involved in the occurrence of Parkinson's disease, the main factor in the occurrence of this neurodegenerative disorder is still not known. One of the strongest hypotheses for PD occurrence is the destruction of dopaminergic neurons located in the substantial nigra region resulting in decreased levels of dopamine. The death of dopaminergic neurons occurs following the accumulation of Lewy bodies containing insoluble protein α-syn in the striatum. Our findings show that TSC can ameliorate the motor symptoms of BPA-induced Parkinson-like disease by reducing the α-syn protein level and inhibiting apoptosis (Figure 8).

Motor evaluations show that TSC has successfully prevented Parkinsonian-like symptoms such as catalepsy and movement disorders caused by involuntary muscle contractions. These observed properties of TSC were consistent with the Ajzashokouhi *et al.* study, which showed that TSC improved acrylamide-induced behavioral impairment in rats (45). The movement symptoms associated with Parkinson's disease include tremors, muscle stiffness, slowness of movements, and difficulty in moving and walking, mainly due to a decrease in dopamine secretion resulting from the death of dopaminergic neurons in the substantia nigra region (46). Two main pathological features characterize PD. First, there is apoptosis of dopaminergic neurons caused by oxidative stress and ROS production, leading to motor stiffness, slowness, and an inability to maintain stature. Second, protein coils are formed in masses known as Lewy bodies (LBs). The primary component of these protein coils is the α-syn protein (19, 47). There is documented evidence supporting the notion that LBs,
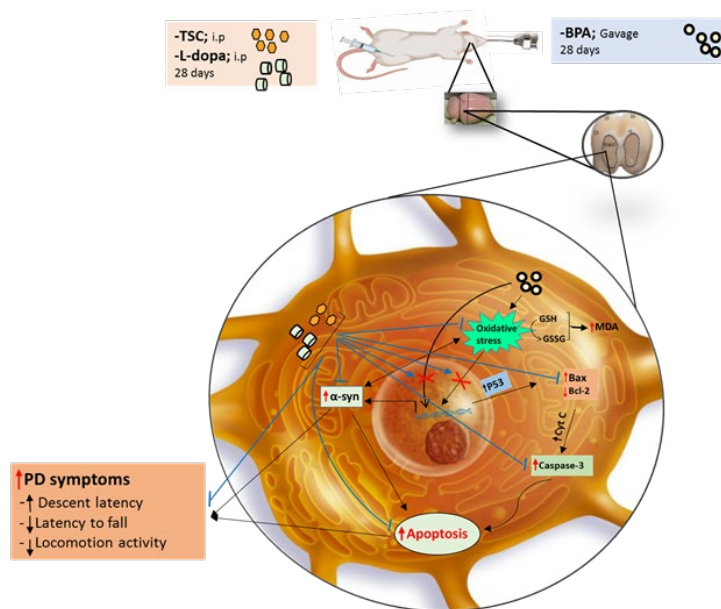

Figure 8. Schematic description of the mechanistic effects of Trans sodium crocetinate (TSC) and L-dopa against Bisphenol A (BPA)-induced Parkinson-like disease in the rat brain
Glutathione (GSH), malondialdehyde (MDA), α-Synuclein (α-Syn), B cell lymphoma-2 (BCL-2), Bcl-2-associated X-protein (Bax), Caspase-3.

containing misfolded and accumulated α-syn proteins, induce apoptosis of dopaminergic neurons (48, 49).

The occurrence of cytoplasmic inclusions or LBs, along with the decrease of dopaminergic neurons, are two important pathological symptoms of Parkinson's disease (13). Destruction of dopaminergic neurons plays an important role in various neurological and locomotion disorders (50). It seems that TSC prevents the destruction of dopaminergic neurons by reducing the level of α-syn and oxidative stress and inhibiting the induction of apoptosis. In accordance, TSC has compensated for decreased locomotor activity, imbalance, and increased muscle contractions caused by BPA and prevents Parkinson's-like symptoms.

Following TSC treatment, protein α-syn levels decreased dramatically. α-syn, which can bind to phospholipids and play a role in lipid metabolism, is a presynaptic protein of 140 amino acids implicated in PD. α-syn is highly expressed in the central nervous system (CNS), particularly in presynaptic nerve terminals. The measurement of this protein in different brain regions is used to evaluate damage caused by Parkinson's (51). In the present study, we showed that exposure to BPA leads to an uncontrolled increase in α-syn protein levels, and treatment with TSC reverses this elevated level to the basal state.

Accumulation of α-syn, a key component of Lewy bodies, in multiple brain regions indicates the creation of intracellular toxicity in the brain tissue. Mutations or increased protein expression are associated with Parkinson's disease (52, 53). Lewy bodies, eosinophilic cytoplasmic spheres, lead to the aggregations of proteins such as α-syn in various brain areas, causing damage to intracellular signals and cellular respiration. However, the precise role of Lewy bodies in neuronal death is not understood (51). It seems that the use of TSC has improved BPA-induced Parkinson-like symptoms by reducing α-syn protein levels in rats. The potential mechanism of how TSC ameliorates the increased α-syn protein levels induced by BPA will be discussed in the following.

Based on the results of the present study, TSC has reversed the disturbance of BPA-induced oxidative stress indices. As shown in Figure 4a and b, TSC dramatically diminished and elevated the increased level of lipid peroxidation and decreased sulfhydryl groups caused by BPA, respectively. BPA induces ROS production by reducing the activity of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione and increasing lipid peroxidation (54-56). Increased α-syn protein levels can be another possible mechanism that contributes to BPA-induced ROS production. A mutual relationship exists between α-syn levels and oxidative damage, mitochondrial cytochrome c release, and mitochondrial dysfunction (13, 57). Therefore, one hypothesis is that α-syn aggregation is increased due to the reduction of ROS-scavenging enzyme activity by BPA. This is consistent with previous studies (58, 59). It was reported that mitochondrial cytochrome c release can also be involved in α-syn aggregation (60). Excessive oxidative stress also can play a pivotal role in the pathogenesis of α-syn by promoting the α-syn misfolding and its intracellular aggregation (60-62). Conversely, abnormal a-syn aggregation can lead to an imbalance in Ca2+ homeostasis, increase cytosolic calcium and reactive oxygen and nitrogen species production, and activate calcium-dependent proteases (51, 63). However, BPA induces oxidative stress followed by an increase in α-syn accumulation, increasing free radicals. TSC can abrogate the effects of BPA on a-syn

production and aggregation.

In response to the question regarding the bilateral increase of oxidative stress and the effects of α-syn on the survival of striatum region cells, this study investigated the indices of apoptosis and autophagy. The results indicate that TSC strongly suppresses BPA-induced apoptosis through a significant decrease in the pro-apoptotic proteins Bax and caspase-3 and a substantial increase in the anti-apoptotic protein Bcl-2. BPA may induce apoptosis in the cells of the striatum, both directly through excessive ROS levels and indirectly by increasing α-syn production and aggregation (55, 56, 59, 60, 62).

BPA might stimulate ROS-induced apoptosis in dopaminergic neurons by increasing the aggregation of α-syn proteins (49). Excessive oxidative stress, a known initiator of apoptosis in various cells and animal models, is also implicated in the development of several neurodegenerative disorders, including Parkinson's and Alzheimer's disease (64). Studies have shown that any method reinforcing the antioxidant system and contributing to oxidative homeostasis balance can be considered a potential treatment approach against PD (65, 66). The antioxidant effects of the main compounds of the saffron plant, such as crocin and crocetin, have been well studied. Notably, crocin has improved Parkinson 's-like symptoms by reducing apoptosis and α-syn protein levels in rats (2). Studies have reported that both crocin and crocetin exert their neuroprotective effects by scavenging and reducing the production of free radicals and safeguarding energy-producing pathways (2, 67, 68). Consistent with prior research, which showed that TSC is potentially a potent antioxidant and anti-apoptotic compound (69), our study indicates that TSC, an active derivative of crocetin, may prevent the onset of BPA-mediated Parkinson-like symptoms by maintaining oxidative homeostasis, preventing apoptosis, and suppressing the production and accumulation of α-syn protein.

The investigation of autophagy markers Beclin and LC3 proteins in the present study showed that removal by the autophagy process is not the mechanism involved in destroying the striatum area cells. Almost 30 genes regulate autophagy, among which LC3 and Beclin-1 genes play a pivotal role. Beclin-1 is involved in the signaling pathways and in the initiation phase of autophagosome formation, where the interaction with PI3PK and hvp34 is necessary (70-72). LC3 consists of a soluble (cytosolic) form, LC3I, and a lipid form, LC3II, expressed as three isoforms (LC3A, LC3B, LC3C) in mammalian tissues. The LC3I interacts with phosphatidylethanolamine to form the LC3II, specifically localizing on the autophagosome (71, 72). Although crocetin has been shown to induce amyloid-beta clearance by triggering autophagy through the STK11/LKB1-mediated AMPK pathway (12), the use of TSC in the present study did not have significant effects on the autophagy marker proteins. TSC apparently regulates autophagy by increasing the BCL-2 protein level, which can interact with Beclin-1.

Bcl-2/ Beclin-1 complex can prevent the development of autophagy (73). An increased expression of caspase protein caused by BPA, contributing to the apoptosis process, is also proposed as a mechanism for BPA-mediated autophagy abrogation and induced apoptosis (74). The activation of caspases cleaved the Beclin-1 proteins and inhibited the development of autophagy. The overexpression of α-syn

by BPA is probably another stronger mechanism to abolish autophagy. Several studies have shown that overexpression of α-syn inhibits autophagosome synthesis and maturation (19, 75). α-Syn inhibits autophagosome synthesis by disrupting Rab1 a, a vital regulator of the secretory pathway, secretion, and homeostasis (19). Abnormal actin stabilization by α-syn is also a mechanism documented to impair autophagosome maturation (75). TSC Probably reversed the inhibitory effect of BPA on autophagy but not significantly and prevented the induction of apoptosis by ameliorating the α-syn level in the striatum cells.

Conclusion

Taken together, TSC prevents motor-associated functions such as locomotor activity, balance, and coordination disorder, which are the Parkinson-like symptoms caused by BPA, by inhibiting the incidence of apoptosis and neural cell death in the striatum area of the rat's brain via 1(Prevention of ROS overproduction) and 2(Inhibition of excessive expression of α-syn protein). Therefore, considering natural productions such as TSC as a therapeutic strategy for the treatment of Parkinson's disease can be of interest to researchers.

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Authors' Contributins

H H and BM R have made major contributions to study design, evaluation, drafting, and managing the submission. MK has made major contributions in study design, data collection and evaluation, drafting, and statistical analysis. All authors edited and approved the final version of this paper for submission.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Declaration

During the preparation of this work, the author(s) used AI-assisted technologies to rephrase to reduce plagiarism and improve language and grammar. After using this tool/ service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Ethical Approval

Animal maintenance conditions and all the steps in conducting experiments were met in accordance with the ethics committee guidelines of the Mashhad University of Medical Sciences (ethics code: IR.MUMS.AEC.1401.046).

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Availability of Data and Materials

The data supporting this study's findings are available upon request from the corresponding author.

References

1. Hosseinzadeh H, Modaghegh MH, Saffari Z. *Crocus sativus* L.(Saffron) extract and its active constituents (crocin and safranal) on ischemia-reperfusion in rat skeletal muscle. J Evid Based Complementary Altern Med 2009; 6:343-350.

2. Mohammadzadeh L, Ghasemzadeh Rahbardar M, Razavi BM, Hosseinzadeh H. Crocin protects malathion-induced striatal biochemical deficits by inhibiting apoptosis and increasing α-synuclein in rats' striatum. J Mol Neurosci 2022; 72:983-993.

3. Rios J, Recio M, Giner R, Manez S. An update review of saffron and its active constituents. Phytother Res 1996; 10:189-193.

4. G Gutheil W, Reed G, Ray A, Anant S, Dhar A. Crocetin: An agent derived from saffron for prevention and therapy for cancer. Curr Pharm Biotechnol. 2012; 13:173-179.

5. Lee I-A, Lee JH, Baek N-I, Kim D-H. Antihyperlipidemic effect of crocin isolated from the fructus of *Gardenia jasminoides* and its metabolite crocetin. Biol Pharm Bull 2005; 28:2106-2110.

6. Zheng S, Qian Z, Sheng L, Wen N. Crocetin attenuates atherosclerosis in hyperlipidemic rabbits through inhibition of LDL oxidation. J Cardiovasc Pharmacol. 2006; 47:70-76.

7. Yan J, Qian Z, Sheng L, Zhao B, Yang L, Ji H, *et al.* Effect of crocetin on blood pressure restoration and synthesis of inflammatory mediators in heart after hemorrhagic shock in anesthetized rats. Shock 2010; 33:83-87.

8. Hashemi M, Hosseinzadeh H. A comprehensive review on biological activities and toxicology of crocetin. Food Chem Toxicol 2019; 130:44-60.

9. Mohammadzadeh L, Hosseinzadeh H, Abnous K, Razavi BM. Neuroprotective potential of crocin against malathion-induced motor deficit and neurochemical alterations in rats. Environ Sci Pollut Res 2018; 25:4904-4914.

10. Ahmad AS, Ansari MA, Ahmad M, Saleem S, Yousuf S, Hoda MN, Islam F. Neuroprotection by crocetin in a hemi-parkinsonian rat model. Pharmacol Biochem Behav 2005; 81:805-813.

11. Gainer J, Stennett A, Murray R. The effect of trans sodium crocetinate (TSC) in a rat oleic acid model of acute lung injury. Pulm Pharmacol Ther 2005; 18:213-216.

12. Wani A, Al Rihani SB, Sharma A, Weadick B, Govindarajan R, Khan SU, *et al.* Crocetin promotes clearance of amyloid-β by inducing autophagy via the STK11/LKB1-mediated AMPK pathway. Autophagy 2021; 17:3813-3832.

13. Dauer W, Przedborski S. Parkinson's disease: Mechanisms and models. Neuron 2003; 39:889-909.

14. Przedborski S. Pathogenesis of nigral cell death in Parkinson's disease. Parkinsonism Relat Disord 2005; 11:S3-S7.

15. Pakkenberg B, Møller A, Gundersen HJ, Dam AM, Pakkenberg H. The absolute number of nerve cells in substantia nigra in normal subjects and in patients with Parkinson's disease estimated with an unbiased stereological method. J Neurol Neurosurg Psychiatry 1991; 54:30-33.

16. Javed H, Nagoor Meeran MF, Azimullah S, Adem A, Sadek B, Ojha SK. Plant extracts and phytochemicals targeting α-synuclein aggregation in Parkinson's disease models. Front Pharmacol 2019; 9:1555.

17. Grotemeyer A, McFleder RL, Wu J, Wischhusen J, Ip CW. Neuroinflammation in Parkinson's disease–putative pathomechanisms and targets for disease-modification. Front Immunol 2022; 13:878771.

18. Dettmer U, Newman AJ, Soldner F, Luth ES, Kim NC, Von Saucken VE, *et al.* Parkinson-causing α-synuclein missense mutations shift native tetramers to monomers as a mechanism for disease initiation. Nat Commun 2015; 6:7314.

19. Winslow AR, Rubinsztein DC. The Parkinson disease protein α-synuclein inhibits autophagy. Autophagy 2011; 7:429-431.

20. Yasuda T, Mochizuki H. The regulatory role of α-synuclein and parkin in neuronal cell apoptosis; possible implications for the pathogenesis of Parkinson's disease. Apoptosis 2010; 15:1312-1321.

21. Jankovic J, Tan EK. Parkinson's disease: Etiopathogenesis and treatment. J Neurol Neurosurg Psychiatry 2020; 91:795-808.

22. B Mythri R, Harish G, M Bharath M. Therapeutic potential of natural products in Parkinson's disease. Recent Pat Endocr Metab Immune Drug Discov 2012; 6:181-200.

23. Fowler PA, Bellingham M, Sinclair KD, Evans NP, Pocar P, Fischer B, *et al.* Impact of endocrine-disrupting compounds (EDCs) on female reproductive health. Mol Cell Endocrinol 2012; 355:231-239.

24. Nordkap L, Joensen UN, Jensen MB, Jørgensen N. Regional differences and temporal trends in male reproductive health disorders: semen quality may be a sensitive marker of environmental exposures. Mol Cell Endocrinol 2012; 355:221-230.

25. Vandenberg LN, Maffini MV, Sonnenschein C, Rubin BS, Soto AM. Bisphenol-A and the great divide: A review of controversies in the field of endocrine disruption. Endocr Rev 2009; 30:75-95.

26. Chang H-Y, Ho Y-L, Sheu M-J, Lin Y-H, Tseng M-C, Wu S-H, *et al.* Anti-oxidant and free radical scavenging activities of *Phellinus merrillii* extracts. Bot Stud 2007; 48:407-417.

27. Ishido M, Suzuki J. Quantitative analyses of inhibitory effects of bisphenol A on neural stem-cell migration using a neurosphere assay *in vitro*. J Health Sci 2010; 56:175-181.

28. Wu H-J, Liu C, Duan W-X, Xu S-C, He M-D, Chen C-H, *et al.* Melatonin ameliorates bisphenol A-induced DNA damage in the germ cells of adult male rats. Mutat Res Genet Toxicol Environ Mutagen 2013; 752:57-67.

29. Steinmetz R, Mitchner NA, Grant A, Allen DL, Bigsby RM, Ben-Jonathan N. The xenoestrogen bisphenol A induces growth, differentiation, and c-fos gene expression in the female reproductive tract. Endocrinol 1998; 139:2741-2747.

30. Iida H, Maehara K, Doiguchi M, Mōri T, Yamada F. Bisphenol A-induced apoptosis of cultured rat Sertoli cells. Reprod Toxicol 2003; 17:457-464.

31. Benachour N, Aris A. Toxic effects of low doses of bisphenol-A on human placental cells. Toxicol Appl Pharmacol 2009; 241:322-328.

32. Leranth C, Szigeti-Buck K, MacLusky NJ, Hajszan T. Bisphenol A prevents the synaptogenic response to testosterone in the brain of adult male rats. Endocrinol 2008; 149:988-994.

33. Fathi F, Hatami H, Ali Hemmati A, Banan Khojasteh S. Effects of sesame oil on improving spatial memory in alzheimers disease. J Babol Univ Med Sci 2014; 16:34-41.

34. Musachio EAS, Araujo SM, Bortolotto VC, de Freitas Couto S, Dahleh MMM, Poetini MR, *et al.* Bisphenol A exposure is involved in the development of Parkinson like disease in Drosophila melanogaster. Food Chem Toxicol 2020; 137:111128.

35. Aminifard T, Mehri S, Rahbardar MG, Rajabian F, Rad AK, Hosseinzadeh H. Trans-sodium crocetinate suppresses apoptotic and oxidative response following myoglobin-induced cytotoxicity in HEK-293 cells. Iran J Basic Med Sci 2024; 27:768.

36. Ahn S, Song T-J, Park S-U, Jeon S, Kim J, Oh J-Y, *et al.* Effects of a combination treatment of KD5040 and L-dopa in a mouse model of Parkinson's disease. BMC Complement Altern Med 2017; 17:1-14.

37. Eweda SM, Abdou HM, Abd Elkader H-TAE, El-Gendy AH. Neurotoxicity and Neuroinflammatory Effects of bisphenol A in male rats: The neuroprotective role of grape seeds proanthocyanidins. Environ Sci Pollut Res Int 2022; 29:9257–9268.

38. Heidari R, Jamshidzadeh A, Niknahad H, Mardani E, Ommati MM, Azarpira N, *et al.* Effect of taurine on chronic and acute liver injury: Focus on blood and brain ammonia. Toxicol Rep 2016; 3:870-879.

39. Wang Y, Yan J, Xi L, Qian Z, Wang Z, Yang L. Protective effect of crocetin on hemorrhagic shock–induced acute renal failure in rats. Shock 2012; 38:63-67.

40. KINNARD Jr WJ, CJ C. A preliminary procedure for the evaluation of central nervous system depressants. J Pharmacol Exp Ther 1957; 121:354-361.

41. Dunham N, Miya T. A note on a simple apparatus for detecting neurological deficit in rats and mice. J Am Pharm Assoc 1957; 46:208-209.

42. Ghasemzadeh Rahbardar M, Hemadeh B, Razavi BM, Eisvand F, Hosseinzadeh H. Effect of carnosic acid on acrylamide induced neurotoxicity: *In vivo* and *in vitro* experiments. Drug Chem Toxicol 2022; 45:1528-1535.

43. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95:351-358.

44. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. BBA Gen Subj 1979; 582:67-78.

45. Ajzashokouhi AH, Razavi BM, Sadeghian H, Hosseinzadeh H. *In vivo* and *in vitro* effects of crocetin and its amide derivative on acrylamide-induced neurotoxicity. Avicenna J Phytomed 2024; 14:78-89.

46. Thomas B, Beal MF. Molecular insights into Parkinson's disease. F1000 medicine reports 2011; 3.

47. Subramaniam SR, Chesselet M-F. Mitochondrial dysfunction and oxidative stress in Parkinson's disease. Prog Neurobiol 2013; 106:17-32.

48. Lau YS, Patki G, Das-Panja K, Le WD, Ahmad SO. Neuroprotective effects and mechanisms of exercise in a chronic mouse model of Parkinson's disease with moderate neurodegeneration. Eur J Neurol 2011; 33:1264-1274.

49. Xu B, Goulding EH, Zang K, Cepoi D, Cone RD, Jones KR, *et al.* Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. Nat Neurosci 2003; 6:736-742.

50. Cohen AD. Role of exercise and GDNF in an animal model of parkinson's disease: implications for neuroprotection: University of Pittsburgh; 2006.

51. Tuon T, Valvassori S, Lopes-Borges J, Luciano T, Trom C, Silva L, *et al.* Physical training exerts neuroprotective effects in the regulation of neurochemical factors in an animal model of Parkinson's disease. Neurosci 2012; 227:305-312.

52. Ellis CE, Murphy EJ, Mitchell DC, Golovko MY, Scaglia F, Barceló-Coblijn GC, Nussbaum RL. Mitochondrial lipid abnormality and electron transport chain impairment in mice lacking α-synuclein. Mol Cell Biol 2005; 25:10190-10201.

53. Winklhofer KF, Haass C. Mitochondrial dysfunction in Parkinson's disease. BBA- Mol Basis Dis 2010; 1802:29-44.

54. Aydoğan M, Korkmaz A, Barlas N, Kolankaya D. Pro-oxidant effect of vitamin C coadministration with bisphenol A, nonylphenol, and octylphenol on the reproductive tract of male rats. Drug Chem Toxicol 2010; 33:193-203.

55. Hassan ZK, Elobeid MA, Virk P, Omer SA, ElAmin M, Daghestani MH, AlOlayan EM. Bisphenol A induces hepatotoxicity through oxidative stress in rat model. Oxid Med Cell Longev 2012; 2012; 2012:194829.

56. Lee S, Suk K, Kim IK, Jang IS, Park JW, Johnson VJ, *et al.* Signaling pathways of bisphenol A–induced apoptosis in hippocampal neuronal cells: Role of calcium-induced reactive oxygen species, mitogen-activated protein kinases, and nuclear factor–κB. J Neurosci Res 2008; 86:2932-2942.

57. Henchcliffe C, Beal MF. Mitochondrial biology and oxidative stress in Parkinson disease pathogenesis. Nat Clin Pract Neurol 2008; 4:600-609.

58. Scudamore O, Ciossek T. Increased oxidative stress exacerbates α-synuclein aggregation *in vivo*. J Neuropathol Exp Neuro 2018; 77:443-453.

59. Ruf RA, Lutz EA, Zigoneanu IG, Pielak GJ. α-Synuclein conformation affects its tyrosine-dependent oxidative aggregation. Biochem 2008; 47:13604-13609.

60. Hashimoto M, Takeda A, Hsu LJ, Takenouchi T, Masliah E. Role of cytochrome c as a stimulator of α-synuclein aggregation in Lewy body disease. J Biol Chem 1999; 274:28849-28852.

61. Hsu LJ, Sagara Y, Arroyo A, Rockenstein E, Sisk A, Mallory M, *et al.* α-Synuclein promotes mitochondrial deficit and oxidative stress. Am J Pathol 2000; 157:401-410.

62. Giasson BI, Duda JE, Murray IV, Chen Q, Souza JM, Hurtig HI,

et al. Oxidative damage linked to neurodegeneration by selective α-synuclein nitration in synucleinopathy lesions. Science 2000; 290:985-989.

63. Conte A, Pellegrini S, Tagliazucchi D. Synergistic protection of PC12 cells from β-amyloid toxicity by resveratrol and catechin. Brain Res Bull 2003; 62:29-38.

64. Zhang P, Tian B. Metabolic syndrome: An important risk factor for Parkinson's disease. Oxid Med Cell Longev 2014; 2014:1-7.

65. Clark J, Silvaggi JM, Kiselak T, Zheng K, Clore EL, Dai Y, *et al.* Pgc-1α overexpression downregulates Pitx3 and increases susceptibility to MPTP toxicity associated with decreased Bdnf. PloS one 2012; 7:e48925.

66. Uppalapati D, Das NR, Gangwal RP, Damre MV, Sangamwar AT, Sharma SS. Neuroprotective potential of peroxisome proliferator activated receptor-α agonist in cognitive impairment in Parkinson's disease: Behavioral, biochemical, and PBPK profile. PPAR Res 2014; 2014:1-9.

67. Karimi P, Gheisari A, Gasparini SJ, Baharvand H, Shekari F, Satarian L, Ader M. Crocetin prevents RPE cells from oxidative stress through protection of cellular metabolic function and activation of ERK1/2. Int J Mol Sci 2020; 21:2949-2972.

68. Yoshino F, Yoshida A, Umigai N, Kubo K, Masaichi C-iL. Crocetin reduces the oxidative stress induced reactive oxygen species in the stroke-prone spontaneously hypertensive rats (SHRSPs) brain J Clin Biochem Nutr 2011; 49:182-187.

69. Rajabian F, Mehri S, Razavi BM, Rad AK, Rahbardar MG, Hosseinzadeh H. Effect of trans-sodium crocetinate on contrast-induced cytotoxicity in HEK-293 cells. Iran J Basic Med Sci 2023; 26:148-156.

70. Eskelinen E-L, Saftig P. Autophagy: A lysosomal degradation pathway with a central role in health and disease. BBA-MolCell Res 2009; 1793:664-673.

71. Kabeya Y, Mizushima N, Ueno T, Yamamoto A, Kirisako T, Noda T, *et al.* LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. EMBO J 2000; 19:5720-5728.

72. Klionsky DJ, Abeliovich H, Agostinis P, Agrawal DK, Aliev G, Askew DS, *et al.* Guidelines for the use and interpretation of assays for monitoring autophagy in higher eukaryotes. Autophagy 2008; 4:151-175.

73. Fairlie WD, Tran S, Lee EF. Crosstalk between apoptosis and autophagy signaling pathways. Int Revcell Molr Biol 2020; 352:115-158.

74. Zhao G-X, Pan H, Ouyang D-Y, He X-H. The critical molecular interconnections in regulating apoptosis and autophagy. Ann Med 2015; 47:305-315.

75. Sarkar S, Olsen AL, Sygnecka K, Lohr KM, Feany MB. α-synuclein impairs autophagosome maturation through abnormal actin stabilization. PLoS Genet 2021; 17:e1009359.