Crocin Protects Malathion-induced Parkinson-like Disease by Inhibiting Apoptosis and α-synuclein Accumulation in Rats' Striatum

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Abstract

Background

Long-term exposure to organophosphates might result in neurodegenerative diseases, comprising Parkinson's disease. Malathion is an organophosphate pesticide with high neurotoxicity. Oxidative stress, apoptosis, and α-synuclein accumulation are important underlying mechanisms in Parkinson's disease. According to studies, crocin, an active constituent of saffron, has anti-apoptotic, anti-inflammatory, and anti-oxidant properties. Thus, the effect of crocin on malathion-induced Parkinson-like disease in rats was investigated in this study.

Materials and methods

6 groups of male Wistar rats were used: 1. Control (normal saline), 2. Malathion (100 mg/kg/day, i.p), 3. Crocin (10 mg/kg/day, i.p) + malathion, 4. Levodopa (10 mg/kg/day, i.p) +malathion, 5. Crocin (40 mg/kg/day, i.p), and 6. Polyethylene glycol (PEG) (vehicle of levodopa) groups. The drugs were administered for 28 days. The amounts of Bcl-2, Bax, caspases 3, 8, 9 proteins in the striatum were measured by western blotting. Also, the amounts of protein and mRNA level of the α-synuclein in striatum tissue were measured by western blotting and RT-qPCR methods.

Results

Malathion induced apoptosis by increasing the amount of Bax/Bcl2 ratio, caspase 3 and 9 proteins in rat striatum tissue. It also increased the protein and mRNA level of α-synuclein in striatal tissue. Co-administration of crocin or levodopa with malathion inhibited the toxic effects of malathion on striatal tissue.

Conclusion

Crocin ameliorates the neurotoxic effect of malathion by its anti-apoptotic activity and regulating the expression of proteins involved in Parkinson's disease pathogenesis. As a result, crocin has the potential to be used as a treatment for malathion-induced neurotoxicity.

Introduction

Parkinson's disease is the second most common chronic neurological disease. The loss of dopaminergic neurons in the substantia nigra pars compacta over time defines this condition (Emamzadeh and Surguchov 2018). Motor (hypokinesia, bradykinesia, tremor, swallowing, and speech disorder) and nonmotor (sleep and cognitive disorder) symptoms are common in Parkinson's disease (Connolly and Lang 2014). Oxidative stress, inflammatory factors, aquaporin 4, α-synuclein accumulation, and apoptotic pathways appear to have an impact on the pathogenesis of Parkinson's disease (Anglade et al. 1997; Emamzadeh and Surguchov 2018; Hughes et al. 1992; Jenner 2003; Zucca et al. 2017). According to evidence, genetics and environmental factors can both play a part in the disease progression (Warner

and Schapira 2003). Pesticides, herbicides, and heavy metals are some of the most well-known environmental pollutants inducing Parkinson's disease (Agim and Cannon, 2015).

Malathion (O,O'-dimethyl dithiophosphate of diethyl mercaptosuccinate) is an organophosphate insecticide that binds to cholinesterase enzymes in animals such as acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Malathion is a pesticide used in agriculture including beans, lettuce, broccoli, cherries, strawberries, and peaches and all over the world, as well as in homes for mosquito control (Venkatesan et al. 2017). In the rat brain, increased caspase-3 activity and the beginning of toxic consequences are the results of malathion toxicity (Varol et al. 2015). Furthermore, after exposure to malathion, Gibco® Human Astrocytes (GHA cells) showed cell cycle arrest, reactive oxygen species (ROS) formation, a weakening of cellular antioxidant defenses, and apoptosis (Bcl-2, Bax, and cleaved caspase-9/caspase-3) (Shieh et al. 2019).

Parkinson's disease has a multifactorial etiology, and there is no single medication that can stop the disease from progressing. The only treatments for motor problems that are currently available are symptomatic. For example, levodopa is a common medicine that is also the most effective treatment for Parkinson's disease (Jankovic and Stacy 2007). However, it has been documented that consumption of levodopa results in various complications (Thanvi and Lo 2004; Toth et al. 2008).

Today, the beneficial effects of various medicinal plants including *Curcuma longa* (Ma and Guo 2017), *Gingko Biloba* (Yu et al. 2020), *Rosmarinus officinalis* L. (Rahbardar and Hosseinzadeh 2020a, b), and *Camellia sinensis* (Malar et al. 2020) have been demonstrated in the treatment of Parkinson's disease and other age-related disorders (Amro et al. 2018; Srivastav et al. 2017).

Crocus sativus belongs to the Iridaceae family of plants. It's known as saffron, and its main monomer component, crocin, has been used to treat several ailments for decades (Hosseinzadeh et al. 2004; Razavi et al. 2021), memory deficits (Shahidani et al. 2019), Parkinson’s disease (Mohammadzadeh et al. 2018; Tang et al. 2020), anxiety (Hosseinzadeh and Noraei 2009), and other neurodegenerative diseases (Khalatbari-Mohseni et al. 2019; Shaterzadeh-Yazdi et al. 2018). The therapeutic effects of crocin are due to its antioxidant (Hosseinzadeh et al. 2009; Margaritis et al. 2020), anti-inflammatory (Hassani et al. 2014; Zeinali et al. 2019), anti-apoptotic (Mohammadzadeh et al. 2020), antitumor (Rastgoo et al. 2013), and neuroprotective (Hosseinzadeh et al. 2012; Mehri et al. 2015; Mehri et al. 2012) properties. Crocin has also been shown to lower the amount of α-synuclein in rats with rotenone-induced Parkinson's disease (Salama et al. 2020).

As a result, in our previous research, the protective effect of crocin (10, 20, 40 mg/kg, 28 days, i.p.) against malathion-induced Parkinson-like behavior, oxidative stress, and inflammation was examined in rat striatum. In this study, our team evaluated the effect of 28 days of intraperitoneal administration of crocin (10 mg/kg/day) on the malathion-induced Parkinson-like disease on rats by assessing the levels of Bcl-2, Bax, caspases 3, 8, 9, and the amounts of protein and mRNA level of α-synuclein in striatum tissue by Western blotting and RT-qPCR methods.

Materials And Methods

Animals

Male Wistar rats weighing 200 to 250 g were acquired from the animal department of the School of Pharmacy, Mashhad, Iran. Animals were kept at 22 to 25 ° C and 12 hours in light and 12 hours in darkness. There were no restrictions on water and food consumption. All animal studies were carried out in accordance with the norms of the Ethics Committee of Mashhad University of Medical Sciences (No: 931316, 15.03.2017).

Chemicals

For the current research, levodopa and malathion (purity 96%) were obtained from Ramopharmin, Iran, and Ariashimi factory, Zahedan, Iran, respectively. Xylazine and ketamine were attained from Alfasan Pharmaceutical Co., Woerden, Netherlands. Stigmas of *C. sativus* L. were bought from Novin Saffron and were evaluated according to the ISO/TS 3632-2. The isolation and purification of crocin were performed as described previously (Hadizadeh et al. 2010).

Study Protocol

Male Wistar rats were randomly assigned to one of seven groups (n=4):

Group 1: Normal saline was injected intraperitoneally to the rats of this group.

Group 2: Malathion (100 mg/kg/day, i.p.) (Delgado et al. 2006).

Group 3: Malathion (100 mg/kg/day, i.p.) with crocin (10 mg/kg/day, i.p.) (Dorri et al. 2015).

Group 4: Crocin (40 mg/kg/day, i.p.)

Group 5: Malathion (100 mg/kg/day, i.p.) with levodopa (10 mg/kg, i.p.) (Sandhu and Rana 2013).

Group 6: Polyethylene glycol (PEG) group (vehicle of levodopa).

For 28 days, agents were injected. The weight of rats was recorded before the study and any weight change was calculated in the dosage calculation.

Western blot analysis

Striatum tissue was homogenized in a lysis buffer containing 2mM Ethylenediaminetetraacetic acid (EDTA), 50 mM Tris-HCl (pH: 7.4), 10mM NaF, 2mM ethylene glycol tetraacetic acid (EGTA), 10 mM β-glycerophosphate, 1mM sodium orthovanadate (Na3VO4), 0.2% W/V sodium deoxycholate, 1 mM phenylmethylsulfonyl fluoride, as well as complete protease inhibitor cocktail (Sigma Aldrich, USA). The protein concentration of various samples was determined using the Bradford assay kit (Bio-Rad, USA).

The samples were then combined with 2X Sodium Dodecyl Sulfate (SDS) blue buffer, boiled for 5-7 minutes, aliquoted to smaller sample quantities, and stored at -80°C.

To determine the amount of various proteins, samples were electrophoresed on an SDS polyacrylamide gel and transferred to a polyvinylidene difluoride (PVDF) membrane (Bio-Rad, USA). On a rocker, the blots were blocked for 2 hours at room temperature with 5% skimmed milk in Tris-buffered saline tween 20 (TBST). The blots were then washed three times with TBST before being incubated for two hours at room temperature with rabbit polyclonal antiserum against Bax (Cell Signaling #2772, 1:1000), rabbit monoclonal antiserum against Bcl-2 (Cell Signaling #2870, 1: 1000), and rabbit polyclonal antiserum against Bcl-2 (Cell Signaling #2870, 1: 1000), rabbit monoclonal anti-serum against caspase-3 (Cell Signaling #9665, 1: 1000), rabbit monoclonal anti-serum against caspase-8 (Abcam, #32397, EJ), and rabbit monoclonal anti-serum against caspase-9 (Abcam, #32539, E23). Blots were incubated for 1.5 hours at room temperature with anti-rabbit IgG labeled with horseradish peroxidase (Cell Signaling, #7074, 1:3000) or anti-mouse IgG labeled with horseradish peroxidase (Cell Signaling, #7076, 1:3000) after three washes with TBST. Enhanced chemiluminescence was used to detect the peroxidase-coated bands (Pierce, USA). Alliance 4.7 Gel doc (UK) was used to assess band optical densities, and UVITEC software was used to perform densitometric analysis on protein bands (UK). The protein levels were compared to the equivalent bands of β-actin, which served as a control protein.

RNA isolation and RT qPCR

Tripure Isolation Reagent (Roche, Cat #11667157001) was used to extract total RNAs from different samples, as directed by the manufacturer. Nanodrop (NanoDropTM 2000, USA) was used to assess the quality (260/280 and 260/230 ratios) and amount of extracted RNAs, and samples were stored at -80°C until use. Transcript levels were determined using the Express one-step SYBR Green ERTM kit (Invitrogen, Cat #11780–200) and the Phase one thermal cycler (ABI). BioSoft's beacon modeling program was used to generate protein and -actin primers (Table 1). The uniformity of primers and products was assessed using melting curve analysis. Target gene expression was normalized in comparison to β-actin. The ΔΔCT method was used to compute the fold increase of genes compared to the control group (Razavi et al. 2013).

Data analysis and statistical calculations

Prism software program 6 was used for statistical calculations. One-way ANOVA and the Tukey-Kramer post-test were used to compare different groups in different experiments. The results were expressed as mean± SEM, with $p<0.05$ considered statistically significant.

Results

The effect of malathion and crocin on the amount of Bax and Bcl-2 proteins

Comparing with the control group, malathion (100 mg/kg) caused a substantial increase in the ratio of Bax/Bcl-2 proteins in rat striatum tissue after 4 weeks ($p<0.05$). In comparison to the malathion group, crocin 10 mg/kg ($p<0.001$) and levodopa 10 mg/kg ($p<0.01$) administration with malathion resulted in a significant decrease in the ratio of Bax/Bcl-2 proteins in rat striatum tissue.

The effect of administration of crocin 40 mg/kg alone or PEG on the Bax/Bcl-2 protein ratio was similar to the control group (Fig. 1).

The effect of malathion and crocin on the amount of cleaved caspase-3 protein

Comparing with the control group, malathion (100 mg/kg) caused a substantial increase in cleaved caspase-3 protein concentration in rat striatum tissue after four weeks ($p<0.001$). In comparison to the malathion group, crocin (10 mg/kg) or levodopa (10 mg/kg) administration with malathion significantly reduced the amount of cleaved caspase-3 protein in rat striatum tissue ($p<0.001$).

Crocin 40 mg/kg alone or PEG had a similar effect on the amount of cleaved caspase-3 protein as the control group (Fig. 2).

The effect of malathion and crocin on the amount of caspase-8 protein

Comparing with the control group, the effect of malathion (100 mg/kg) on cleaved caspase-8 and pro-caspase-8 protein levels in rat striatum tissue after four weeks was not significant.

Crocin 40 mg/kg alone or PEG had a similar effect on the amount of cleaved and pro-caspase 8 protein as the control group (Fig. 3).

The effect of malathion and crocin on the amount of cleaved caspase-9 protein

Comparing with the control group, malathion (100 mg/kg) caused a substantial increase in cleaved caspase-9 protein concentration in rat striatum tissue after four weeks ($p<0.05$).

When crocin 10 mg/kg ($p<0.01$) or levodopa 10 mg/kg ($p<0.001$) was administered with malathion, the amount of cleaved caspase-9 protein in rat striatum tissue was much lower than when malathion was given alone (Fig. 4).

On the amount of cleaved caspase-9 protein, 40 mg/kg crocin alone or PEG had a similar effect as the control group.

The effect of malathion and crocin on the α-synuclein protein level

Comparing with the control group, malathion (100 mg/kg) induced a substantial increase in the amount of α-synuclein protein in rat striatum tissue after four weeks ($p<0.05$) (Fig. 5).

In comparison to the malathion group, the treatment of crocin 10 mg/kg ($p<0.001$) or levodopa 10 mg/kg ($p<0.01$) with malathion resulted in a significant decrease in the amount of α-synuclein protein in striatum

tissue.

On the amount of α-synuclein protein, 40 mg/kg crocin alone or PEG had a similar effect as the control group.

The effect of malathion and crocin on the α-synuclein mRNA level

Comparing with the control group, malathion (100 mg/kg) caused a substantial rise in α-synuclein mRNA levels in rat striatum tissue after four weeks ($p<0.001$). In comparison to the malathion group, the administration of crocin (10 mg/kg) or levodopa (10 mg/kg) with malathion reduced the level of α-synuclein mRNA in rat striatum tissue ($p<0.001$).

Crocin 40 mg/kg alone or PEG had a similar effect on the expression of the α-synuclein gene as the control group (Fig. 6).

Table 1.Sequences of different primers used for RT qPCR reactions.

Gene	Primer	Sequence
α-Synuclein	Forward	GCCTAAGAATGTCGTTGT
	Reversed	TGGAACTGAGCACTTGTA

Discussion

Our goal in this study was to find out if crocin may protect rats from developing Parkinson-like disease due to increased apoptotic factors and α-synuclein accumulation caused by malathion. The data disclosed that administration of crocin (10 mg/kg) and malathion (100 mg/kg) to animals for 28 days reduced the Bax/Bcl-2 ratio and the amount of cleaved caspase-3 and 9. It also attenuated both protein and mRNA levels of α-synuclein. Furthermore, when levodopa (10 mg/kg) was administered concurrently with malathion, as a reference drug (Sandhu and Rana, 2013), the amount of apoptotic factors (Bax/Bcl-2 ratio, cleaved caspase-3 and 9), as well as protein and mRNA levels of α-synuclein, were reduced.

In our previous study, our team investigated the neuroprotective effect of crocin in contradiction of the malathion-induced neurochemical changes and motor deficits in rats by performing behavioral tests (open field, rotarod, and catalepsy), assessing AChE level in serum, the levels of malondialdehyde (MDA), reduced glutathione (GSH), tumor necrosis factor-alpha (TNF-α), and Interleukin 6 (IL-6) in the striatum. The obtained data illustrated that sub-chronic malathion exposure resulted in Parkinson-like disease. Crocin protected against malathion-induced Parkinson's disease by lowering lipid peroxidation, ameliorating motor deficits, and revealing antioxidant and anti-inflammatory properties (Mohammadzadeh et al. 2018). Therefore, our team decided to assess other mechanisms to unveil the therapeutic potential of crocin against Parkinson-like disease.

Apoptosis has been discovered as a significant factor in the development of several brain diseases (Singh et al. 2019). Apoptosis is triggered by a multitude of executioner and initiator caspases, and it can happen in either intrinsic or extrinsic pathways. The intrinsic pathway, known as the mitochondria-mediated pathway, is mediated by activating caspase-9, the initiator caspase. Activation of initiator caspase-8, on the other hand, promotes the cell death receptor-mediated extrinsic apoptotic pathway. Both initiator caspases culminate on a common executioner caspase pathway including caspase-3. The executioner caspases are activated, resulting in morphological characteristics associated with apoptosis, for instance, DNA fragmentation and cleavage. Bax and other pro-apoptotic factors have been linked to neuronal cell death in Parkinson's disease (Erekat 2018). Members of the Bcl-2 family influence apoptosis in numerous systems, with the Bax/bcl-2 ratio acting as a rheostat to determine cell apoptosis susceptibility (Korsmeyer 1999). The results of the present study illustrated that malathion induces apoptosis in the striatum by increasing Bax/Bcl-2 ratio. The amount of cleaved caspase-3 was also measured to verify the induction of apoptosis by malathion. Besides, caspase 8 and 9 protein levels were measured to determine the pathway of malathion-induced apoptosis. In this part of the study, malathion did not change the amount of caspase-8 and caused apoptosis by increasing cleaved caspase-9 (via the intrinsic pathway) and cleaved caspase-3 in the striatum.

Crocin administration along with malathion reduced the Bax/Bcl-2 ratio, according to our findings. Crocin also inhibited malathion-induced caspases 3 and 9 in the striatum. The effect of other pesticides on apoptosis in the nervous system has been demonstrated in different *in vitro* and *in vitro* models. For example, a study showed that chlorpyrifos (100 μmol, 24 hours) induced apoptosis in the dopaminergic neuronal components of PC12 cells by activation of caspases 3 and 9 (Lee et al. 2012). Another study reported that intraperitoneal injection of a single dose of paraquat (10.96 mg/kg) triggered apoptosis in rat hippocampus and striatum (Wu et al. 2013).

In an earlier investigation, subacute administration of malathion (2.5, 5, 10 mg/kg, 28 days) to rats increased caspase-3 levels in brain tissue cells (Varol et al. 2015). In a study carried out on lymphocytes taken from rats, it was shown that malathion exposure at 0.1 and 0.25 of LC_{50} concentration for 2, 4, 8, and 12 hours increased the levels of caspase 3 and 9 (Ojha and Gupta 2017).

Moreover, the anti-apoptotic property of crocin has previously been demonstrated in several studies. For instance, the oral administration of crocin (40 mg/kg, 10 days) inhibited apoptosis in the brain tissue. Apoptosis was triggered in rats by cerebral ischemia, but receiving crocin decreased the amount of caspase-3 (Oruc et al. 2016). Another research stated that administering crocin intraperitoneally (25, 50 mg/kg, 4 weeks) decreased the amount of caspase 3 and 9, as well as Bax in heart tissue in diazinon-induced cardiac toxicity in rats (Razavi et al. 2016).

Other molecular mechanisms involved in the development of Parkinson's disease following exposure to pesticides include the formation of protein clusters called Lewy bodies, which have been specifically attributed to the death of neurons in association with Parkinson’s disease. α-synuclein is a protein that is found in high concentrations in Lewy bodies and its amount increases in Parkinson's disease (Thakur

and Nehru 2014). Studies have shown that numerous pesticides, such as dieldrin, rotenone, and paraquat can stimulate the production of α-synuclein fibrils, a mechanism that has been suggested as the molecular basis for Parkinson's disease (Uversky et al. 2001). It has also been suggested that subcutaneous injection of low doses of rotenone increases the α-synuclein amount in nigrostriatal neurons (Sherer et al. 2003). Furthermore, it has been shown that paraquat significantly accelerated the production of fibril α-synuclein *in vitro* in a dose-dependent manner. Mice exposure to paraquat also increases the amount of this substance in the brain. The results of this study indicate the increased expression of α-synuclein, as a result of exposure to the toxic agent and also direct interaction between protein and environmental factors, as potential mechanisms in neurodegenerative disorders including Parkinson's disease (Manning-Boğ et al. 2003).

The increased amount of α-synuclein expression impairs mitochondrial function. In other words, impaired mitochondrial activity is the result of increased α-synuclein content and expression. Many reports illustrated that an increased amount of α-synuclein expression causes complex 1 damage in mitochondria (Chu et al. 2014).

The results of the current research showed that malathion increased the level of α-synuclein and its mRNA, while crocin suppressed the increased malathion-induced α-synuclein levels in the striatum.

The obtained data also indicated that levodopa (10 mg/kg, 28 days, i.p.) inhibited apoptotic pathways and the aggregation of α-synuclein induced by malathion in rats' striatum. In line with our study, previous research has shown that levodopa administration can improve α-synuclein DNA methylation in Parkinson's disease patients (Schmitt et al. 2015) and ameliorate the symptoms of this disorder (Mohammadzadeh et al. 2018).

Conclusion

The current study concludes that malathion administration can cause Parkinson-like disease in rats by activating the apoptotic pathway and increasing α-synuclein accumulation in the striatum. Crocin can prevent malathion-induced changes in rats' brains. As a result, more clinical trials are needed to confirm the protective effect of crocin against malathion-induced Parkinson-like disease and to develop a new strategy for treating chronic malathion poisoning.

Declarations

Data availability statements

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICAL STATEMENT:

Ethics approval and consent to participate

All animal studies were carried out in accordance with the norms of the Ethics Committee of Mashhad University of Medical Sciences (No: 931316, 15.03.2017).

Consent for publication

All authors have agreed to the contents and approved the final version for publication.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no conflicts of interest.

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Authors' contributions

HH and BMR were supervisors, designed the work, revised it critically for important intellectual content, and approved the version to be published. LM did the experiment and MGR wrote the manuscript.

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Figures

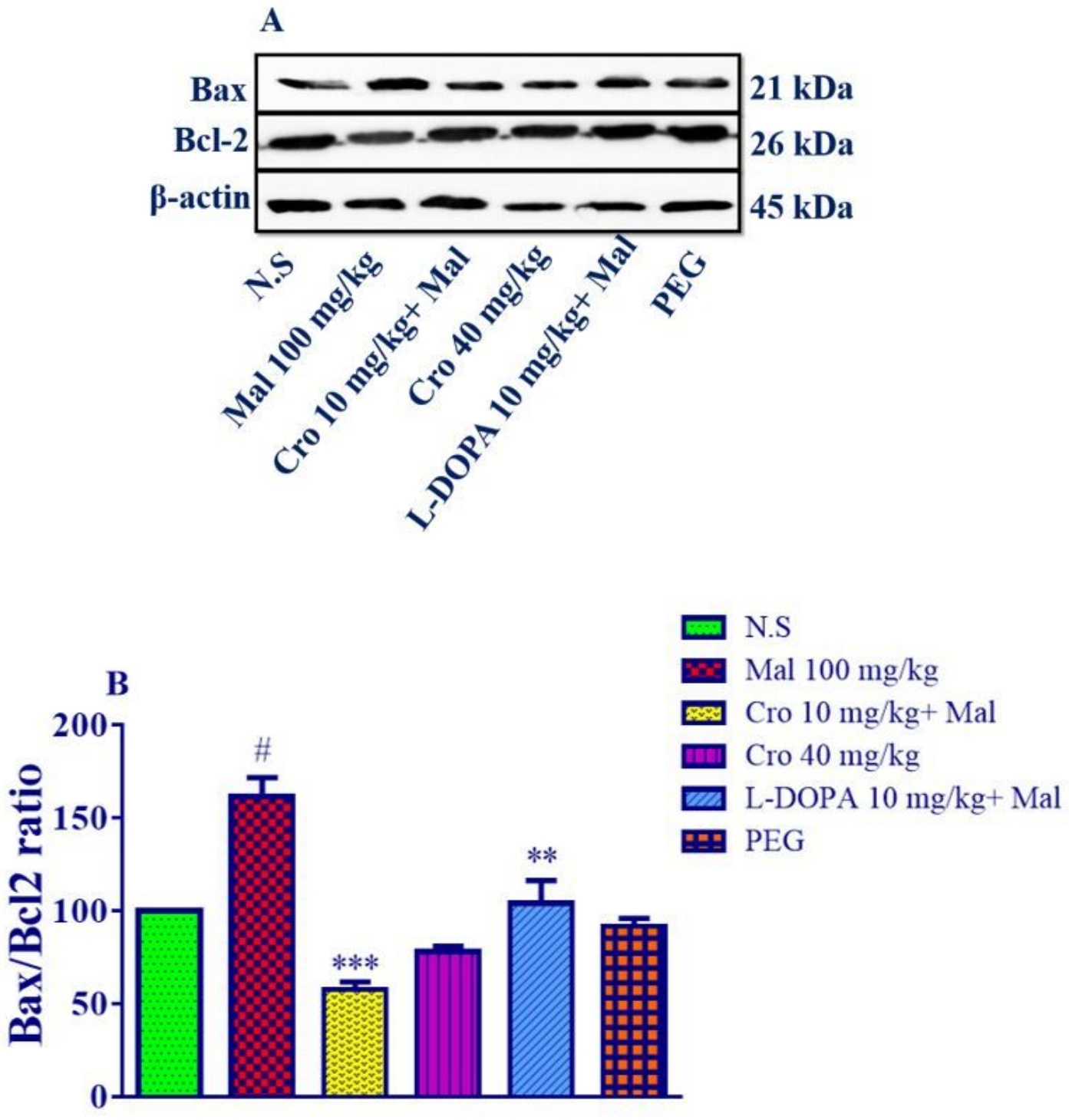

Figure 1

The effect of malathion and crocin on the amount of Bax and Bcl-2 proteins in rat striatum after 4 weeks by Western blotting. A: Bax, Bcl-2, and β-actin (internal control) specific bands. Equal amounts of protein samples (100 µg) obtained from striatum tissue homogeneity were loaded in each row. These bands represent 4 independent experiments. B: Densitometric results of Bax/Bcl-2 ratio. Results were expressed as mean ± SEM. Tukey-Kramer statistical test was used to evaluate the level of statistical differences

between the groups compared to the malathion group. # p<0.05 compared to the control group, *** p<0.001, and ** p<0.01 compared to the malathion group. Mal: Malathion, Cro: Crocin: N.S: Normal Saline, L-DOPA: Levodopa, PEG: Polyethylene glycol

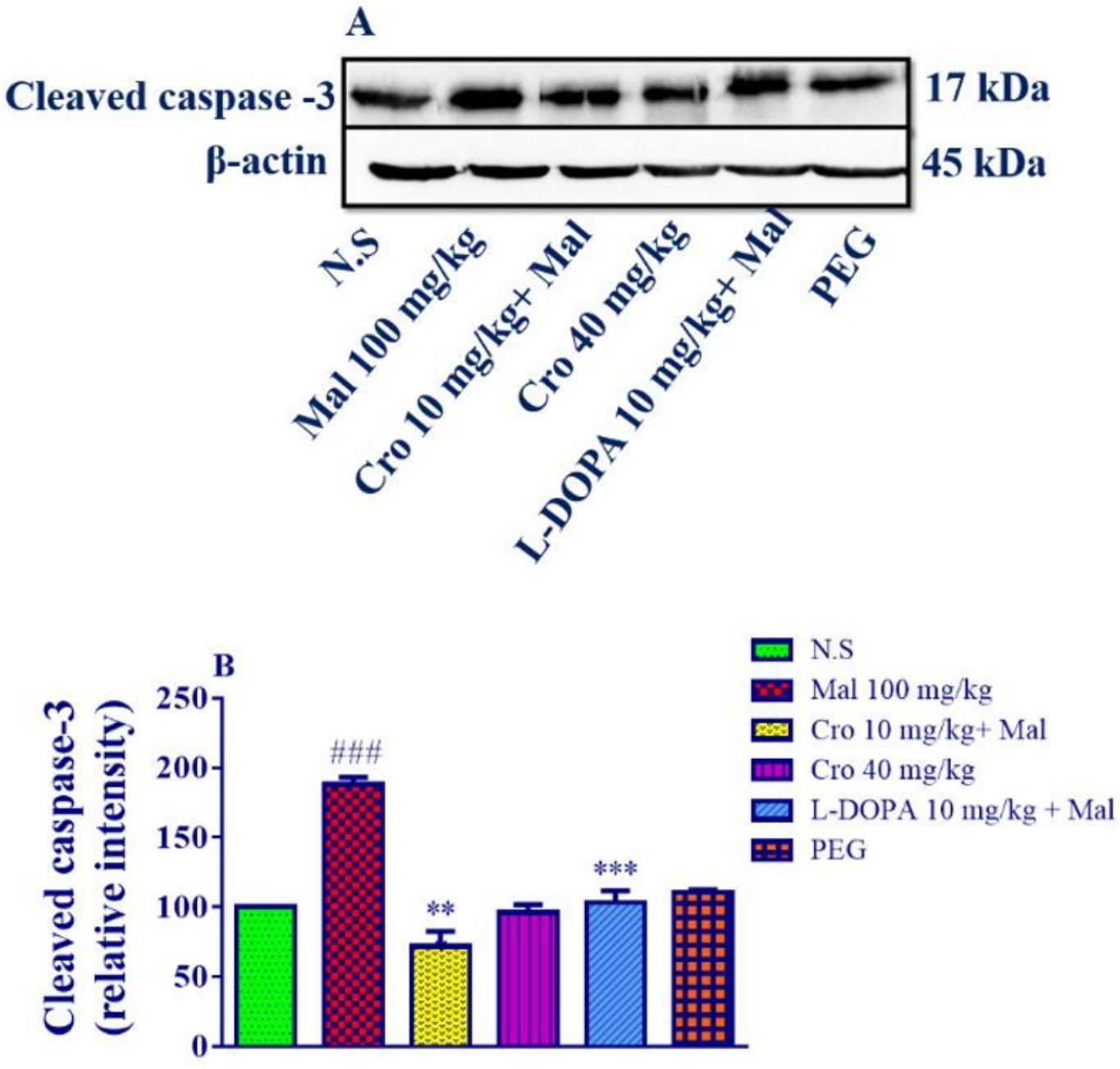

Figure 2

The effect of malathion and crocin on cleaved caspase-3 protein in rat striatum after 4 weeks by Western blotting. A: Cleaved caspase-3 and β-actin (internal control) specific bands. Equal amounts of protein samples (100 µg) obtained from striatum tissue homogeneity were loaded in each row. These bands represent 4 independent experiments. B: Densitometric results of cleaved caspase-3. Results were expressed as mean ± SEM. Tukey-Kramer statistical test was used to evaluate the level of statistical

differences between the groups compared to the malathion group. ### p<0.001 compared to the control group, *** p<0.001 compared to the malathion group. Mal: Malathion, Cro: Crocin: N.S: Normal Saline, L-DOPA: Levodopa, PEG: Polyethylene glycol

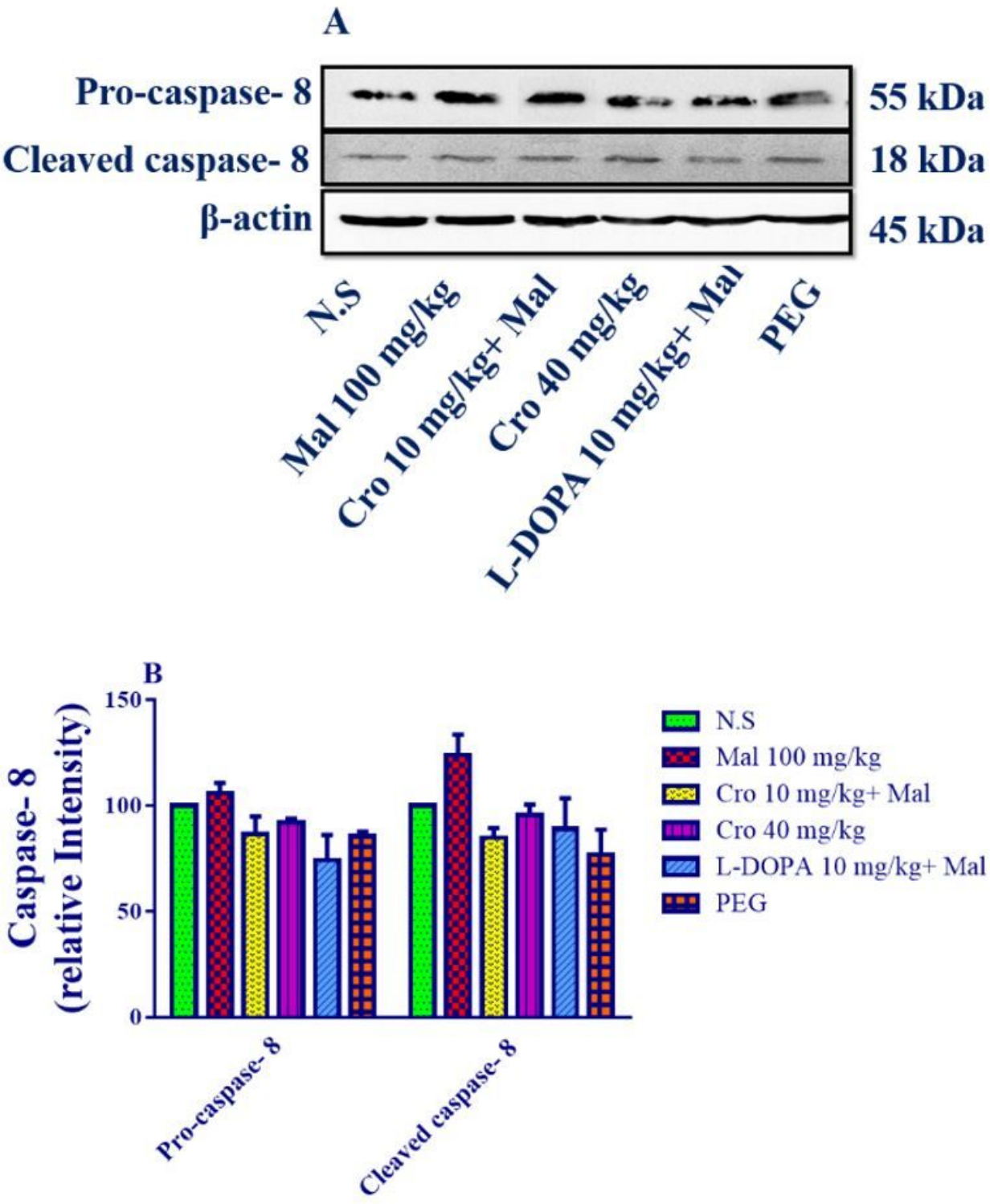

Figure 3

The effect of malathion and crocin on cleaved and pro caspase-8 proteins in rat striatum after 4 weeks by Western blotting. A: Cleaved caspase-8, Pro caspase-8, and β-actin (internal control) specific bands. Equal

amounts of protein samples (100 µg) obtained from striatum tissue homogeneity were loaded in each row. These bands represent 4 independent experiments. B: Densitometric results of cleaved caspase-8, Pro caspase-8. Results were expressed as mean ± SEM. Tukey-Kramer statistical test was used to evaluate the level of statistical differences between the groups compared to the malathion group. Mal: Malathion, Cro: Crocin, N.S: Normal Saline, L-DOPA: Levodopa, PEG: Polyethylene glycol

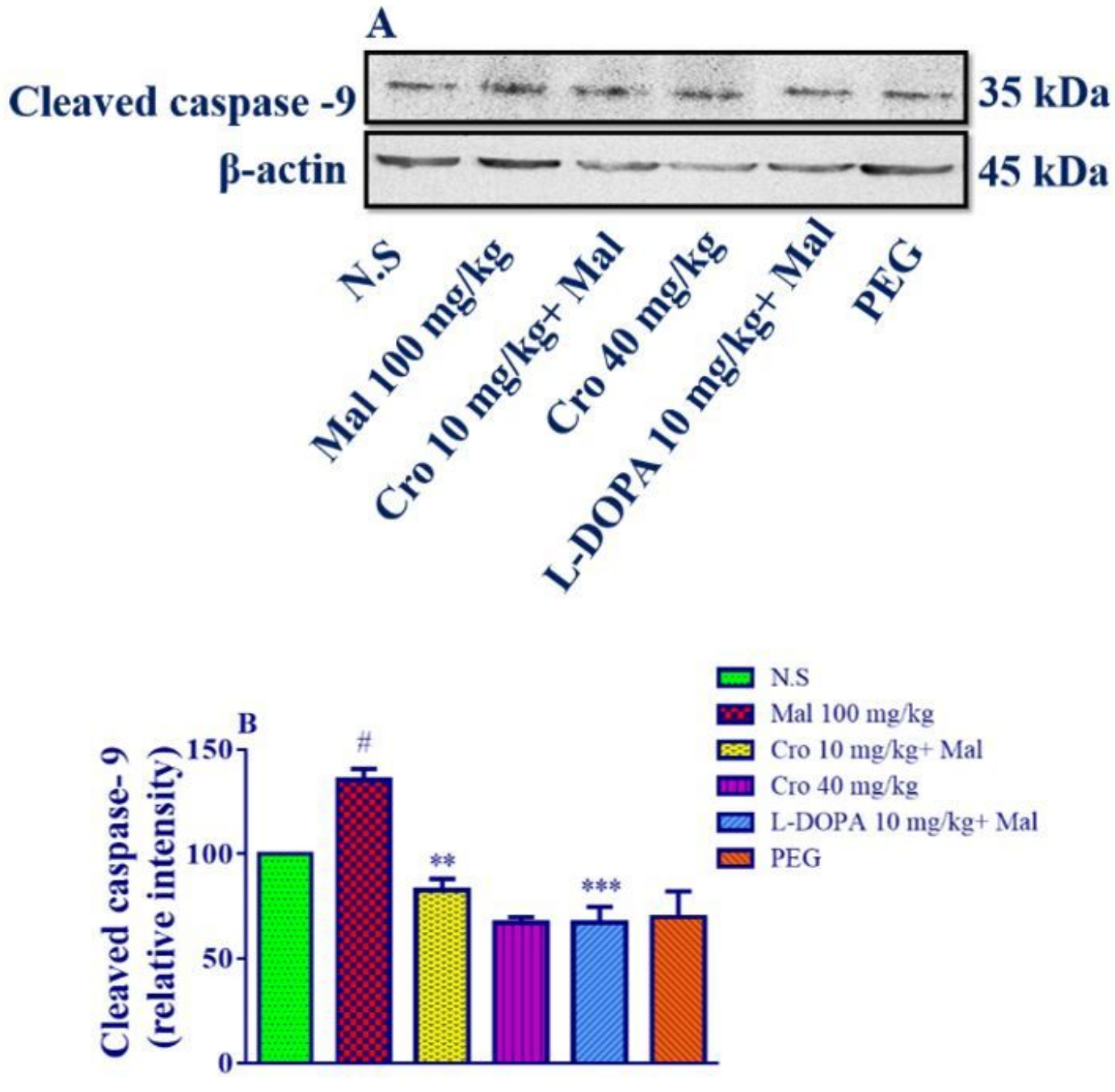

Figure 4

The effect of malathion and crocin on cleaved caspase-9 protein in rat striatum after 4 weeks by Western blotting. A: Cleaved caspase 9 and β-actin (internal control) specific bands. Equal amounts of protein

samples (100 µg) obtained from striatum tissue homogeneity were loaded in each row. These bands represent 4 independent experiments. B: Densitometric results of cleaved caspase-9. Results were expressed as mean ± SEM. Tukey-Kramer statistical test was used to evaluate the level of statistical differences between the groups compared to the malathion group. # p<0.05 compared to the control group, ***p<0.001 and ** p<0.01 compared to the malathion group. Mal: Malathion, Cro: Crocin: N.S: Normal Saline, L-DOPA: Levodopa, PEG: Polyethylene glycol

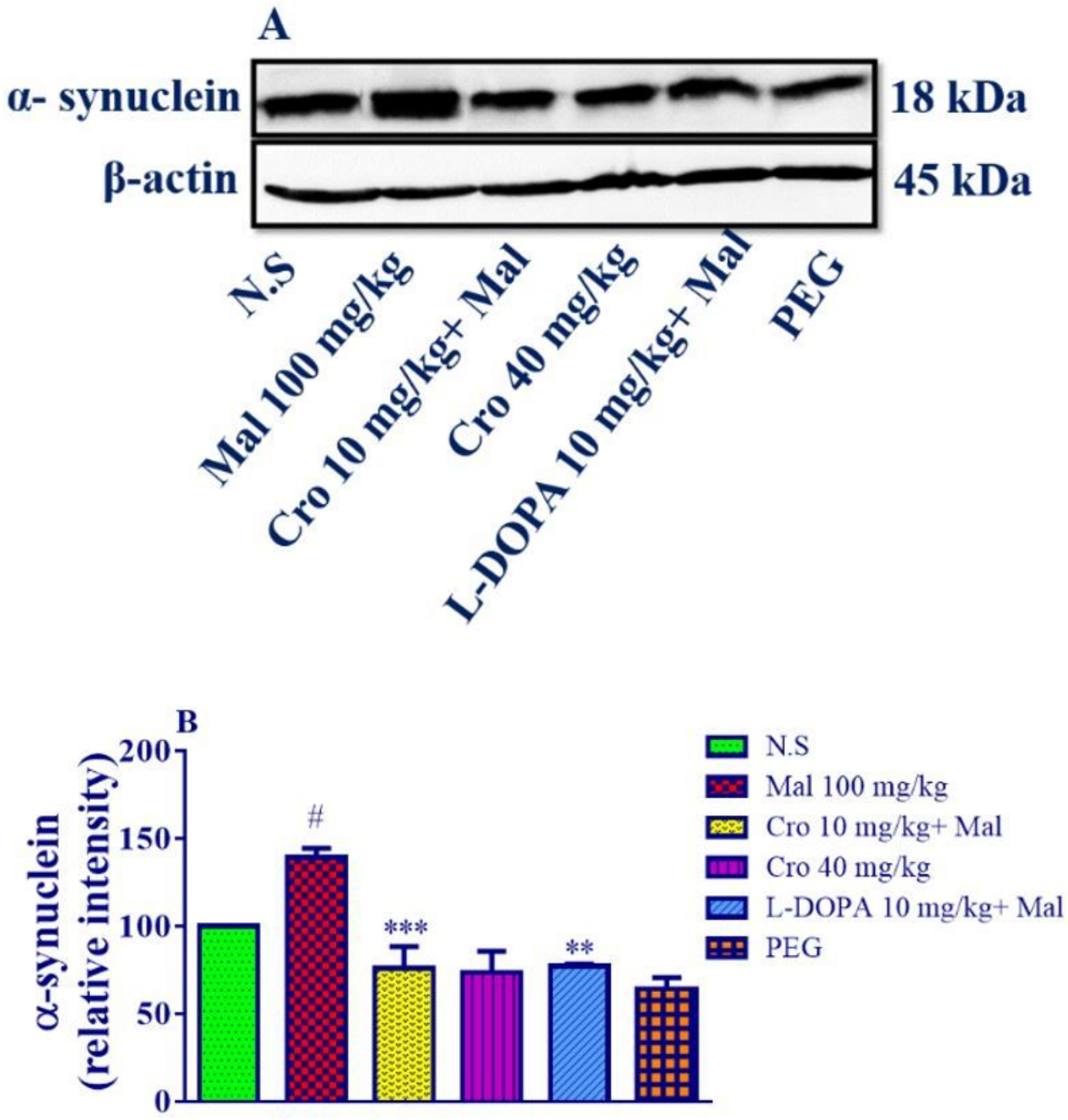

Figure 5

The effect of malathion and crocin on α-synuclein protein in rat striatum after 4 weeks by Western blotting. A: Specific α-synuclein and β-actin (internal control) specific bands. Equal amounts of protein samples (100 µg) obtained from striatum tissue homogeneity were loaded in each row. These bands represent 4 independent experiments. B: α-synuclein densitometric results. Results were expressed as mean ± SEM. Tukey-Kramer statistical test was used to evaluate the level of statistical differences between the groups compared to the malathion group. # p<0.05 compared to the control group, ***p<0.001 and ** p<0.01 compared to the malathion group. Mal: Malathion, Cro: Crocin, N.S: Normal Saline, L-DOPA: Levodopa, PEG: Polyethylene glycol

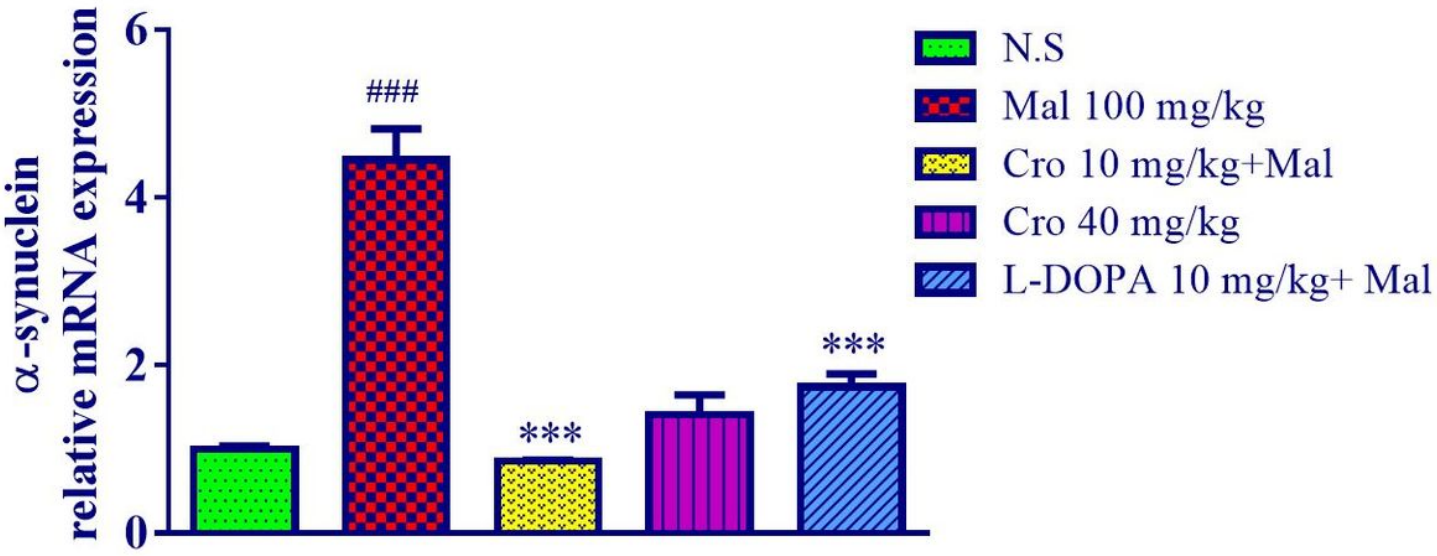

Figure 6

The effect of malathion and crocin on the α-synuclein mRNA level in rat striatum after 4 weeks by RT-qPCR. Expression was normalized by β-actin gene expression. mRNA values were expressed relative to the control group cells and based on obtained mean ± SEM from 4 independent experiments. Tukey-Kramer statistical test was used to examine the level of statistical differences between the groups compared to the malathion group. ### p<0.001 compared to control group, *** p<0.001 compared to the malathion group, Mal: Malathion, Cro: Crocin, L-DOPA: Levodopa, PEG: Polyethylene glycol