RESEARCH PAPER

Acta Neurobiol Exp 2024, 84: 59–69
DOI: 10.55782/ane-2024-2538

Preventive effects of crocin, a natural compound from saffron, against nicotine-induced oxidative stress and neurobehavioral disturbances

Majid Motaghinejad[1]*, Manijeh Motevalian[2]*, Antoni Sureda[3,4]

[1] *Chronic Respiratory Disease Research Center (CRDRC), National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[2] *Department of Pharmacology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran*
[3] *Research Group in Community Nutrition and Oxidative Stress and Health Research Institute of Balearic Islands (IdISBa), University of Balearic Islands, Palma de Mallorca, Spain*
[4] *CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III (ISCIII), Madrid, Spain*
* *Email: Dr.motaghinejad6@gmail.com; Manijeh.motevalian@gmail.com*

Nicotine is a psychostimulant that induces neurochemical and behavioral changes upon chronic administration, leading to neurodegenerative conditions associated with smoking. As of now, no preventive or therapeutic strategies are known to counteract nicotine-induced neurodegeneration. In this study, we explore the neuroprotective effects of crocin, a bioactive agent commonly found in saffron – a spice derived from the flower of *Crocus sativus* – using a rat model. The dose-dependent effects of crocin were evaluated in nicotine-induced neurodegeneration and compared with a control group. Neurobehavioral changes, assessed through the elevated plus maze, the open field test, the forced swim test, and the Morris water maze, as well as oxidative stress in the hippocampus, were evaluated. Interestingly, nicotine administration resulted in depression, anxiety, and abnormal motor and cognitive functions, while crocin treatment protected the rat brain from these abnormalities. The beneficial effects of crocin were associated with reduced oxidative stress biomarkers such as malondialdehyde, along with increases in superoxide dismutase, glutathione peroxidase, and glutathione reductase activities. These results demonstrate that crocin can mitigate nicotine-induced neurodegeneration by reducing oxidative stress, potentially offering a protective measure against neurodegenerative effects in smokers.

Key words: nicotine, crocin, oxidative stress, neurobehavioral disturbances

INTRODUCTION

Nicotine is a central stimulant with numerous detrimental consequences in its applications (Benowitz, 2009; 2010). Previous studies have elucidated the long-term effects of nicotine use, highlighting its adverse neurochemical and psychological impacts (Benowitz, 2010; Bruin et al., 2010). Due to its neurostimulant properties, nicotine possesses an exceptionally high potential for abuse and addiction (Balfour, 1982; Benowitz, 2009). Numerous studies have demonstrated that chronic nicotine administration can lead to neurobehavioral disturbances, including anxiety and depression during withdrawal (Balfour, 1982; Eddins et al., 2009). It has been suggested in previous research that the depletion of specific neurotransmitters, as a result of prolonged nicotine use, and the down-regulation of receptors may contribute to such behaviors (Sudheer et al., 2008; Cardinale et al., 2012). Prolonged use of nicotine may cause behavioral changes, such as anxiety and depression-like behaviors, potentially causing mood-related disturbances in both experimental animals and clinical subjects (Bal-

Received 9 November 2022, accepted 25 January 2024

four, 1982; Eddins et al., 2009). Molecular studies have also indicated that chronic nicotine abuse can result in mitochondrial dysfunction, oxidative stress, and inflammation, ultimately leading to neurodegeneration in certain brain areas, such as the hippocampus (Sudheer et al., 2008).

In recent years, there has been a significant surge in the utilization of herbal and natural compounds as therapeutic agents. Natural flavonoids and their derivatives have garnered attention for their potential therapeutic effects against neurodegenerative diseases/disorders and drug-induced toxicity (Suk, 2005). Among these compounds, crocin, a carotenoid chemical compound present in crocus and gardenia flowers, is noteworthy (Hosseinzadeh & Talebzadeh, 2005; Lee et al., 2005; Yousefsani et al., 2018). Crocin is the primary compound responsible for the distinctive color of saffron (Lee et al., 2005; Chen et al., 2008; Khalili et al., 2010). Previous studies have consistently indicated that crocin possesses both anxiolytic and antidepressant effects, suggesting its potential as a viable alternative in situations where both anxiety and depression coexist (Saleem et al., 2006; Deslauriers et al., 2011; Finley & Gao, 2017). A substantial body of literature has shown the effectiveness of crocin in modulating neurodegeneration, attributed to its anti-apoptotic, antioxidant, and anti-inflammatory properties (Saleem et al., 2006; Finley & Gao, 2017). Its biological effects are mediated by antioxidant, anti-inflammatory, anti-apoptotic, immunomodulatory activities (Hoshyar & Mollaei, 2017). Conversely, the neuroprotective effects of this compound could be crucial against certain neurotoxins and neurodegenerative agents (Asdaq & Inamdar, 2010; Finley & Gao, 2017). Despite the wealth of information on crocin, its effects against nicotine-induced oxidative stress remain unclear. Therefore, the primary objective of this study was to assess the neuroprotective effects of crocin against nicotine-induced oxidative stress and alterations in neurobehavioral parameters. This investigation aims to contribute to a deeper understanding of the mechanisms underlying nicotine toxicity. Specifically, this study endeavors to evaluate the neuroprotective role of crocin against nicotine-induced hippocampal toxicity.

METHODS

Animals

Seventy adult male Wistar rats, weighing between 250-300 g, were purchased from the animal house of Iranian University of Medical Sciences. They were housed under standard conditions, with a room temperature of 22 ± 0.5°C, a 12-hour light/dark cycle, and had *ad libitum* access to food and water. The experimental protocol received approval from the Ethical Research Committee of the Iran University of Medical Sciences (IUMS) (Ethical Code: IR.IUMS.FMD.REC.1398.309). The guidelines of the IUMS were aligned with the procedures outlined in ARRIVE (Animal Research: Reporting of In Vivo Experiments) (Kilkenny et al., 2010).

Experimental design

Animals were randomly assigned to one of six groups, with each group consisting of 10 rats: Group 1 (control): received normal saline (0.2 ml/rat, ip, once daily) for 21 days; Group 2 (nicotine-treated): administered nicotine (10 mg/kg, ip, once daily) for 21 days; Groups 3, 4, 5, and 6: simultaneously treated with nicotine (10 mg/kg, ip, once daily) and crocin at doses of 10, 20, 40, and 80 mg/kg, ip, for 21 days; Group 7: received crocin (80 mg/kg, ip) dissolved in normal saline for 21 days.

It is important to note that in groups treated with nicotine in combination with crocin, crocin was administered first, followed by nicotine after one hour. Additionally, the selection of a high dose of nicotine, known to induce neurotoxicity in hippocampal brain cells, was based on our and other previous studies (Carlson et al., 2000; 2001; Ciani et al., 2005; Motaghinejad et al., 2016; 2017b). The neuroprotective doses of crocin were chosen in accordance with similar previous works (Tamaddonfard et al., 2013; Vakili et al., 2014; Sarshoori et al., 2014; Mohammadzadeh et al., 2018).

From day 17 to 21, the Morris water maze (MWZ) standard behavioral method was employed to evaluate learning and spatial memory in the treated animals. Subsequently, from day 22 to 28, various additional standard behavioral methods were applied to assess anxiety and depression levels in the experimental animals: the elevated plus maze (EPM) was conducted on day 22, the open field test (OFT) was performed on day 24 and, the forced swim test (FST) took place on day 26.

The behavioral studies were conducted over several days deliberately. This approach was adopted considering that each behavior could potentially influence the outcome of another, and, based on this premise, the tests were conducted on separate days. Additionally, adhering to fundamental principles concerning behavioral changes and biochemical parameters in multiple experimental procedures, a minimum of 21 days was deemed necessary after drug administration in experimental models. This duration has been consistently

supported and corroborated in our study, as well as in other relevant previous works (Altinoz et al., 2018; Cryan & Slattery, 2007; Kamyar et al., 2016; Motaghinejad et al., 2017b; Tamaddonfard et al., 2013). From day 22 to 27, following the administration of drugs, various mood and motor activity behaviors were assessed (Feizipour et al., 2020; Kamyar et al., 2016; Keshavarzi et al., 2019; Motaghinejad et al., 2016). On day 28, all animals underwent anesthesia through the administration of 50 mg/kg of thiopental. Subsequently, the brain tissue was extracted, and the hippocampus was isolated from each rat following established guidelines from previous studies. The assessment of hippocampal oxidative stress biomarkers was then conducted (Frank et al., 2006; Motaghinejad et al., 2017a).

Crocin and nicotine were obtained from Sigma-Aldrich (USA) and dissolved fresh in normal saline prior to administration.

Behavior tests

FST is a behavioral test used to evaluate depression-like behavior in the experimental model (Gould et al., 2009). It was carried out in accordance with the previous standard protocols (Gould et al., 2009; Can et al., 2012a; Ghafarimoghadam et al., 2022). The swimming behavior of the animals in the FST is used as an indicator of non-depressive behavior (Gould et al., 2009; Can et al., 2012b; Ghafarimoghadam et al., 2022).

EPM is a behavioral employed to assess anxiety-like behaviors in the experimental model and it was conducted in accordance with established standard protocols (Ghafarimoghadam et al., 2022; Walf & Frye, 2007). In alignment with the methodology, the time spend in open arms of the EPM by the animal was indicative of non-depressive and non-anxiety behavior (Ghafarimoghadam et al., 2022; Walf & Frye, 2007).

In OFT, locomotor changes and anxiety-like behavior were assessed. This test involved the evaluation of behaviors such as rearing, ambulation distances, time spent in the central square entry, and the number of entrances to the central square area (Cryan et al., 2005; Can et al., 2012; Ghafarimoghadam et al., 2022).

MWM, as a standard behavioral test for cognition and spatial memory assessment, was conducted on the basis of prior research (Motaghinejad et al., 2017a; Vorhees & Williams, 2006). In this experiment, parameters such as escape latency, defined as the time of discovery of the hidden platform, the distance traveled by each animal to reach the hidden platform and the proportion of animal involvement in the target quarter were assessed were measured (Motaghinejad et al., 2017a; Vorhees & Williams, 2006).

Molecular studies

As mentioned above, all animals were anesthetized by intraperitoneal injections of 50 mg/kg sodium thiopental and the total hippocampus was isolated from each rat. Subsequently, the mitochondria of the hippocampal cells were isolated and prepared by standard protocol according to previous studies (Kipp & Ramirez, 2001; Zadali et al., 2019).

The degree of lipid peroxidation, determined by measuring malondialdehyde (MDA) levels, and activity of antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) were determined as previously described in the standard protocols (Alhadidi et al., 2018; Hacioglu et al., 2016; Motaghinejad et al., 2016; Ranzolin et al., 2016).

Statistical analysis

The obtained data were analyzed by Graph Pad PRISM v.6 Software and the results of the different experimental groups expressed as mean ± standard error (SEM) for each molecular and behavioral parameter. The analysis of the normality of the data was performed using the Shapiro-Wilk test and the homogeneity of variance with the Levene test. Statistical significance, between control and treatment groups, was then determined for all analyzed parameters by one-way analysis of variance (ANOVA). For those data groups that evidenced statistical differences, a *post-hoc* analysis was performed applying the Tukey test, to determine the groups involved in the differences. The F and P values were reported, and $P<0.05$ was considered significant.

RESULTS

Forced swim test and elevated plus maze

The group of animals treated with nicotine (10 mg/kg) exhibited a shorter swimming period in the forced swim test (FST) ($F_{(6,63)}=7.312$, $P<0.001$) and spent less time in the open arms of the elevated plus maze (EPM) ($F_{(6,63)}=8.637$, $P<0.001$) compared to the control group (Fig. 1A, B). Conversely, crocin at doses of 80 mg/kg significantly mitigated the effects of nicotine in the FST ($F_{(6,63)}=7.312$, $P\leq0.001$), while doses of 20, 40, and 80 mg/kg in the EPM ($F_{(6,63)}=8.637$, $P<0.001$) effectively counteracted the impact of nicotine, leading to increased swimming time in the FST and enhanced presence of animals in the open arms of the EPM, respectively (Fig. 1A, B). Moreover, the group treated with crocin alone (80 mg/kg) did not differ significant-

ly from the control group in terms of swimming time in the FST ($F_{(6,63)}$=7.312) and time spent in the open arms of the EPM ($F_{(6,63)}$=8.637) (Fig. 1A, B).

Open field test

The group treated with nicotine (10 mg/kg) exhibited a decrease in rearing behavior ($F_{(6,63)}$=6.277, P<0.001), ambulation distances ($F_{(6,63)}$=12.96, P<0.001), time spent in central square entry ($F_{(6,63)}$=8.950, P<0.001), and the number of entrances to the central square area ($F_{(6,63)}$=11.54, P<0.001) in the OFT (Fig. 2A-D). Crocin at doses of 40 and 80 mg/kg demonstrated significant increases in ambulation distances ($F_{(6,63)}$=12.96, P<0.001), time spent in the central square entry ($F_{(6,63)}$=8.950, P<0.001), and the number of entrances to the central square area ($F_{(6,63)}$=11.54, P<0.001) in the OFT compared to the nicotine-treated group (Fig. 2A-C). Furthermore, crocin at a dose of 80 mg/kg significantly elevated the rearing number ($F_{(6,63)}$=6.277, P<0.001) in the OFT compared to the nicotine-treated group (Fig. 2D). Importantly, the group treated with crocin alone (80 mg/kg) did not exhibit significant differences from the control group in OFT behaviors (Fig. 2A-D).

Morris water maze

Significant changes in escape latency ($F_{(6,21)}$=7.46, P<0.001) and distance traveled ($F_{(6,21)}$=520.2, P<0.001) during the four days of practice, as well as the proportion of the animal's appearance in the target quarter in the MWM, were observed in the 10 mg/kg nicotine-treated group compared to the control group (Fig. 3A, B). In contrast, crocin statistically inhibited nicotine-induced increases in escape latency (with 40 and 80 mg/kg of crocin, $F_{(6,21)}$=70.46, P<0.001) and distance traveled (with 20, 40, and 80 mg/kg of crocin, $F_{(6,21)}$=520.2, P<0.001) relative to the nicotine-only treated group (10 mg/kg) (P<0.001) (Fig. 3A, B). Crocin at doses of 40 and 80 mg/kg demonstrated the potential to mitigate the impact of nicotine on learning ($F_{(6,63)}$=3.507, P<0.001). This effect was statistically significant compared to the nicotine-only treated group (10 mg/kg) (Fig. 3D). Importantly, swimming speed ($F_{(6,21)}$=17.07) remained unchanged during practice in all animal groups (Fig. 2C). Importantly, the group treated with crocin alone (80 mg/kg) did not exhibit significant differences in MWM parameters (Fig. 3A-D).

Oxidative stress biomarkers

Nicotine administration significantly increased the MDA level with ($F_{(6,63)}$=16.6 and P<0.001) and concurrently decreased the SOD activity with ($F_{(6,63)}$=3.759 and P<0.001), GPx activity with ($F_{(6,63)}$=9.53 and P<0.001) and GR activity with ($F_{(6,63)}$=22.02 and P<0.001) compared to the control group (Table 1). Conversely, crocin (10, 20, 40 and 80 mg/kg) attenuated the nicotine-induced rise in MDA level ($F_{(6,63)}$ =16.6 and P<0.001) (Table 1). Furthermore, crocin (80 mg/kg) inhibited the nicotine-induced decrease in SOD ($F_{(6,63)}$=3.759 and P<0.001) (Table 1). Regarding GPx and GR activity, crocin (40 and 80 mg/kg) mitigated the nicotine-induced decreases in GPx activity ($F_{(6,63)}$=9.53 and P<0.001) and GR activity ($F_{(6,63)}$=22.02 and P<0.001) compared to the nicotine-treated group

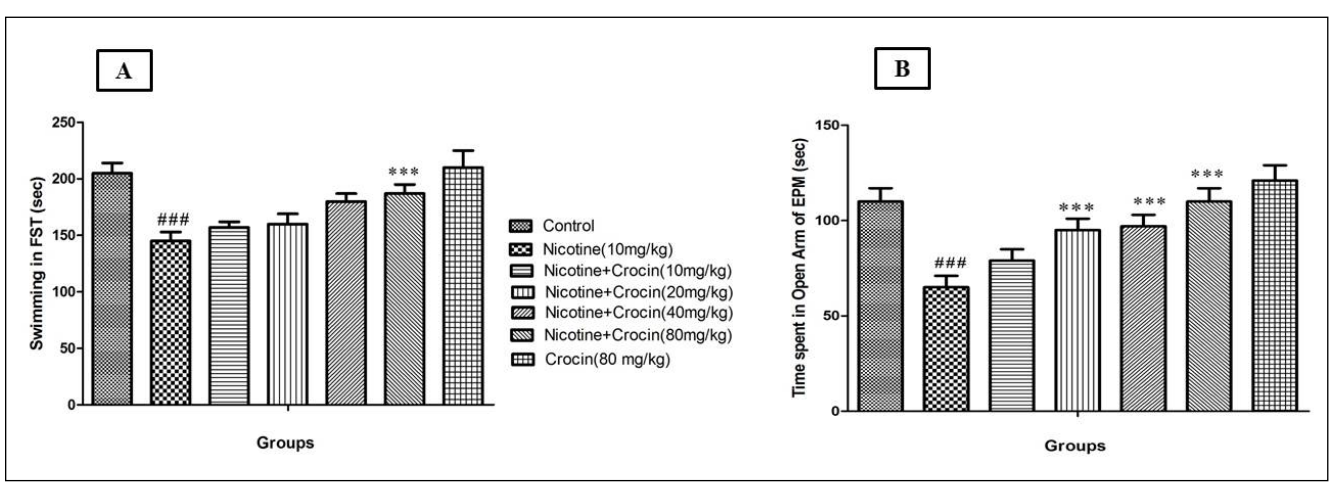

Fig. 1. Animals behavior in the FST and EPM. Swimming time in FST (A); time spent in open arms in EPM (B); in control group, and groups treated concurrently with 10 mg/kg of nicotine and 10, 20, 40 and 80 mg/kg of crocin and also group under treatment with crocin alone (80 mg/kg). All data are expressed as mean ± SEM (n=10). ### P<0.001 *vs.* control group. *** P<0.001 *vs.* 10 mg/kg of nicotine.

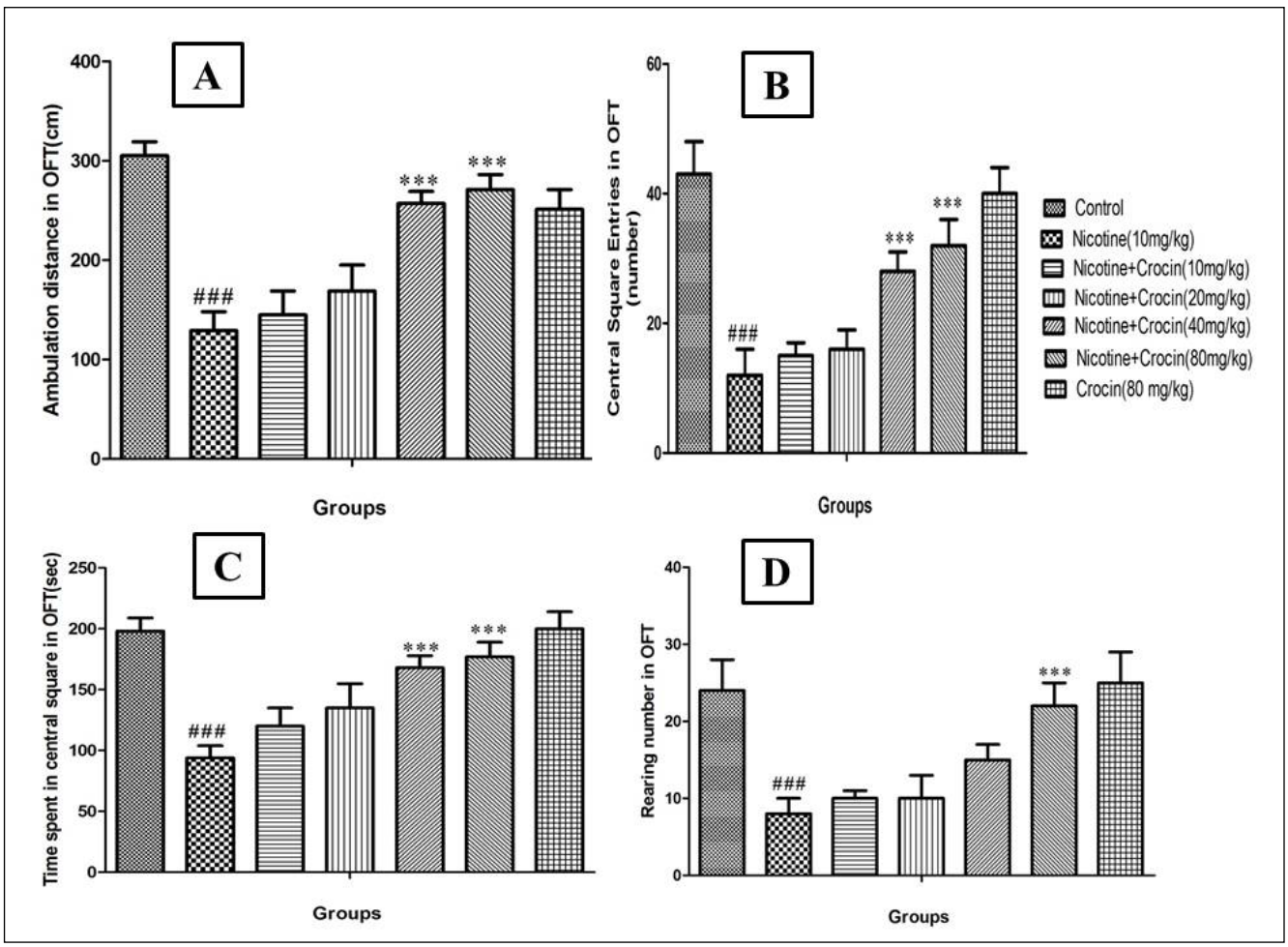

Fig. 2. Animals behavior in the OFT. Ambulation distances (A), number of entrances to central square area (B), time spent in central square entry (C), and rearing number (D) in OFT; in control group, and groups treated concurrently with 10 mg/kg of nicotine and 10, 20, 40 and 80 mg/kg of crocin and also group under treatment with crocin alone (80 mg/kg). All data are expressed as mean ± SEM (n=10). ### P<0.001 *vs.* control group. *** P<0.001 *vs.* 10 mg/kg of nicotine.

(Table 1). Additionally, the group treated with crocin alone (80 mg/kg) did not exhibit significant changes in MDA levels ($F_{(6,63)}$=16.6) and SOD ($F_{(6,63)}$=3.759), GPx ($F_{(6,63)}$=9.53), and also GR ($F_{(6,63)}$=22.02) activities, compared to the control group (Table 1).

DISCUSSION

The main findings of the current study are that different doses of crocin can ameliorate nicotine-induced oxidative stress in the hippocampus, and improve neurobehavioral parameters such as anxiety, depression, and cognition.

The obtained results evidenced that a daily dose of 10 mg/kg of nicotine is capable to decrease the swimming time in the FST. However, crocin administration has the potential to reverse this effect, particularly the immobility sign, and increase swimming time at the higher dose of 80 mg/kg. When administered alone, crocin (80 mg/kg) also demonstrated a reduction in immobility behavior in the FST, although it did not reach statistical significance compared to the control group. The research suggests that chronic nicotine administration may deplete amine-based neurotransmitters implicated in anxiety and depression. It appears that the depletion of these neurotransmitters is responsible for the depressed behavior observed in animals treated with nicotine (Eddins et al., 2009; Benowitz, 2010; Budzynska et al., 2013).

Due to its antidepressant effect, we posit that crocin may increase dopamine levels in brain synapses, thereby regulating depressive behavior in rats and compensating for depleted dopamine (Talaei et al.,

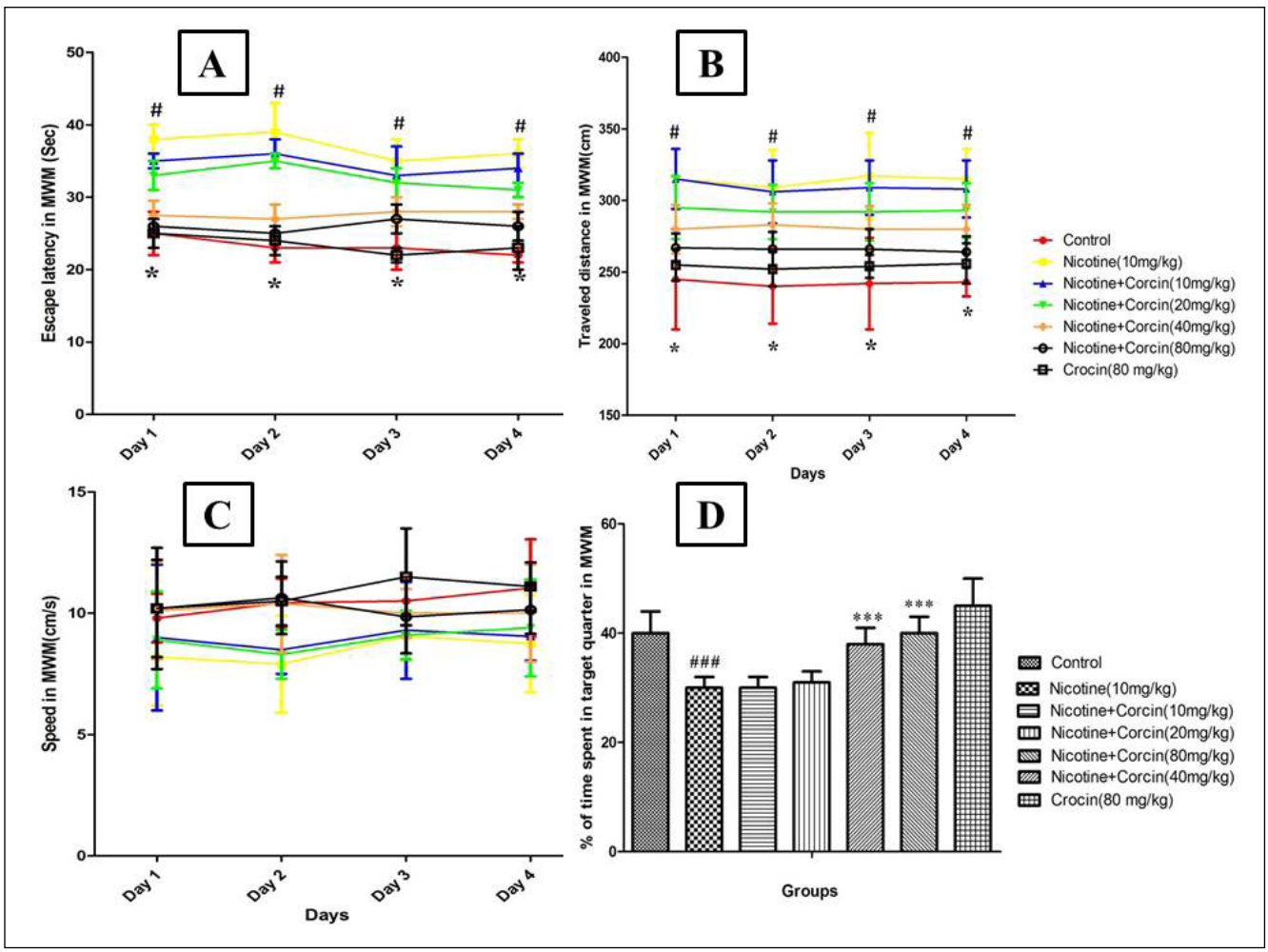

Fig. 3. Animals behavior in the MWM. Mean of escape latency (A), traveled distance (B) and swimming speed (C) during four days of training and also percentage of time spent in target quarter in probe trial (D) in MWM in control group, and groups treated concurrently with 10 mg/kg of nicotine and 10, 20, 40 and 80 mg/kg of crocin and also group under treatment with crocin alone (80 mg/kg). All data are expressed as mean ± SEM (n=10). # P<0.001 *vs.* groups under treatment with 10 mg/kg of nicotine in combination with crocin (40 and 80 mg/kg). * P<0.001 *vs.* 10 mg/kg of nicotine. ### P<0.001 *vs.* control group. *** P<0.001 *vs.* 10 mg/kg of nicotine.

Table 1. The effects of various doses of crocin on alterations of oxidative stress biomarkers in hippocampus of rats treated with nicotine (10 mg/kg/day).

Group	MDA nmol/mg of protein	SOD U/ml/mg protein	GPx U/ml/mg protein	GR U/ml/mg protein
Control	9.2±0.9	71.2±8.2	80.1±2.8	62.2±4.2
Nicotine (10 mg/kg)	27.3±3.3[a]	37.5±5.6[a]	32.1±5.1[a]	23.3±4.2[a]
Nicotine + crocin (10 mg/kg)	17±1.1[b]	50.1±7.2[b]	49.1±7.2	24.6±5.1
Nicotine + crocin (20 mg/kg)	15±2[b]	57.2±5.1	56.2±6.4	43.1±3.3
Nicotine + crocin (40 mg/kg)	12±0.6[b]	63.7±6.8	68.2±4.6[b]	52.2±7.1[b]
Nicotine + crocin (80 mg/kg)	10±0.8[b]	71.3±9.2[b]	75.2±6.2[b]	54.2±5.1[b]
Crocin (80 mg/kg)	7.4±1.4	75.4±6.1	86.1±9.1	67.3±5.2

[a] Showed significant level with P<0.001 *vs.* control group. [b] Showed significant level with P<0.001 *vs.* 10 mg/kg of nicotine. All data are expressed as mean ± SEM (n=10).

2015). Previous studies have demonstrated crocin's antidepressant efficacy in the FST, and the current data further support that the antidepressant and anxiolytic effects of crocin are likely the primary mechanisms of its action (Wang et al., 2010). Crocin is hypothesized to induce an elevation in dopamine levels within the synaptic cleft, and this increase is believed to modulate mood-related behavior in animals treated with crocin (Wang et al., 2010). The current study revealed that the duration of time spent in the open arms is reduced in the EPM due to nicotine, indicating an increase in depressive behavior. In contrast, treatment with crocin at all administered doses (20, 40, and 80 mg/kg) and crocin alone (80 mg/kg) could effectively increase the time spent in the open arms. These findings are consistent with previous laboratory and clinical studies that have reported the induction of anxiety and depressive behaviors by nicotine and similar compounds (Picciotto et al., 2015).

Crocin appears to have the capability to counteract nicotine-induced behavioral changes in the current study and is likely to inhibit nicotine-induced depression and anxiety in the FST. These results align with the overarching concept that various antidepressant and anxiolytic compounds, including crocin and other herbal agents, exhibit the potential to alleviate anxiety- and depression-like behaviors during drug withdrawal periods and other mood-related disorders (Purushothuman et al., 2013). Numerous prior studies have also demonstrated that crocin can mitigate mood-related disorders in both human and animal subjects (Pitsikas et al., 2008; Hosseinzadeh & Noraei, 2009; Dastgerdi et al., 2017). Based on the findings of this study, it can be suggested that crocin could serve as an effective antidepressant and anxiolytic agent. Additionally, it appears to function as a motor activity modulator, showing potential utility against nicotine-induced behavioral disturbances and other substances of abuse.

On the other hand, the present data indicated that nicotine administration can lead to a reduction in four behaviors observed in the OFT. Numerous previous studies support these findings, showing that nicotine abuse in both human and animal subjects can result in motor activity disturbances and anxiety-like disorders (Iniguez et al., 2009; Motaghinejad et al., 2020; 2017b; Naha et al., 2018; Romero & Chen, 2004; Slawecki et al., 2003). This result aligns with the aforementioned concepts regarding the role of nicotine abuse or chronic administration in inducing disturbances in key neurotransmitters in the brain, which are responsible for anxiety, depression, and motor activity behaviors (Abreu-Villaça et al., 2003; Iniguez et al., 2009; Khadrawy et al., 2011; Motaghinejad et al., 2016; Naha et al., 2018; Romero & Chen, 2004; Slawecki et al., 2003). In contrast, the obtained data also indicated that crocin, at doses of 40 and 80 mg/kg, significantly increased ambulation distances, time spent in the central square entry, and the number of entrances to the central square area in the OFT. At the dose of 80 mg/kg, crocin also significantly increased the number of rearings in the OFT. However, crocin alone (80 mg/kg) did not alter OFT behaviors. The neuroprotective effects of crocin on neurobehavioral disorders have been previously established, and our results confirm these findings (Mohammadzadeh et al., 2018; Motaghinejad et al., 2019; Mozaffari et al., 2019; Ahmed et al., 2020; Kermanshahi et al., 2020; Seyedinia et al., 2023). Previous studies have suggested that crocin acts as an anxiolytic and has potential efficacy in modulating motor activity behaviors (Muduli et al., 2023; Seyedinia et al., 2023). Based on this concept, it can be speculated that crocin's protective effects against anxiety, motor activity dysfunction, and other mood-related behaviors may play a role in mitigating nicotine-induced anxiety and motor activity disorders in the OFT standard test (Mohammadzadeh et al., 2018; Motaghinejad et al., 2019; Mozaffari et al., 2019; Ahmed et al., 2020; Kermanshahi et al., 2020; Muduli et al., 2023; Seyedinia et al., 2023).

With respect to the learning time in the MWM, the data suggested that nicotine, at the given dose, may diminish learning activity and decrease the proportion of appearance in the target quadrant on the probe day in the MWM. The findings also indicate that prolonged nicotine injections may result in disturbances in learning and spatial memory in the used experimental animal model. These observations align with previous research that has demonstrated the detrimental effects of long-term nicotine administration on learning and memory in immature rats (Gould & Leach, 2014). On the other hand, the present findings indicated that crocin, particularly at higher doses (40 and 80 mg/kg), can mitigate nicotine-induced impairment in learning and spatial memory. According to our data, high-dose crocin (40 and 80 mg/kg) can prevent nicotine-induced learning deficits throughout all four days of practice and also modulate nicotine-induced memory defects on the day of the test. The neuroprotective properties of crocin, which protects neurons from oxidant agents such as nicotine, as well as its impact on the modulation of amines and their receptors involved in cognitive activity, can be discussed (Talaei et al., 2015; Finley & Gao, 2017). Based on this research, it can be suggested that crocin may act as an effective antidepressant, anxiolytic agent, and cognitive enhancer, providing potential utility against nicotine-induced behavioral disturbances and other abuse drugs.

Consistent with the behavioral outcomes, the biochemical findings indicated that nicotine (10 mg/kg) can induce oxidative stress and neuro-inflammation. The present study revealed that nicotine reduces the activities of SOD, GPx, and GR, while increasing the levels of MDA as a marker of lipid peroxidation in the hippocampus of rats. These data suggest a potential role of nicotine in depleting antioxidant enzymes, inducing lipid peroxidation, and disrupting mitochondrial function (Benowitz, 2010; Cardinale et al., 2012). It can be proposed that some aspects of nicotine-mediated neurodegeneration and neurotoxicity may be attributed to the inhibition of SOD, GPx, and GR activities, along with the induction of lipid peroxidation and disruption of mitochondrial function (Benowitz, 2009; Cardinale et al., 2012). Nevertheless, the exact mechanism of action of nicotine in this context remains unclear, but mitochondrial dysfunction appears to be the primary reason and cause for this type of neurotoxicity (Balfour, 1982; Oliveira-da-Silva et al., 2010). Consistent with previous studies, crocin therapy (at all administered doses) has demonstrated effectiveness in reversing the nicotine-induced increase in MDA levels and restoring the reduction of SOD, GPx, and GR activities in hippocampal tissues (Ochiai et al., 2004; Papandreou et al., 2006). In the current study, crocin at all mentioned doses exhibited antioxidant effects against nicotine-induced effects on MDA levels. Specifically, at doses of 80 mg/kg for SOD activity and at doses of 40 and 80 mg/kg for GPx and GR activities, crocin demonstrated its antioxidant properties against the sequels induced by nicotine. Furthermore, our study revealed that crocin alone (at dose of 80 mg/kg) decreased MDA levels and increased SOD, GPx, and GR activities; however, these changes were not statistically significant when compared to the control group. These data suggest a potential neuroprotective role of crocin, supporting our findings that crocin has neuroprotective properties. Moreover, its metabolism in the body may activate mitochondrial biogenesis and potentially inhibit lipid peroxidation while activating antioxidant enzymes (Zhang et al., 2018). In accordance with this concept, crocin is likely to modulate the neurodegeneration and neurotoxicity induced by nicotine through the activation of antioxidant enzymes or the inhibition of lipid peroxidation. Numerous studies have substantiated the role of crocin in enhancing antioxidant defense and increasing the activity of antioxidant enzymes (Asdaq & Inamdar, 2010; Deslauriers et al., 2011; Kocaman et al., 2019). A crucial aspect of the current results regarding the management of nicotine-induced behavioral and molecular outcomes is that crocin appears to be effective only at high doses, specifically 40 and 80 mg/kg. These doses demonstrate efficacy across various behavioral parameters (OFT, EPM, FST, and MWM) and oxidative stress biomarkers. It seems that low doses of crocin may not exert a significant neuroprotective effect against the molecular and behavioral consequences of nicotine. According to our understanding of biology, any molecular change can potentially lead to alterations in behavior (Galizia & Lledo, 2013; Ghafarimoghadam et al., 2022). Following this concept, the current study suggests that high doses of crocin (40 and 80 mg/kg) may mitigate the detrimental effects of oxidative stress induced by nicotine. This potential effect could contribute to preventing the occurrence of neurodegeneration in brain cells, thereby reducing anxiety, depression, movement, and cognitive disorders observed in the OFT, FST, EPM and MWM (Chen et al., 2015; Zhao et al., 2022). In general, behavioral changes such as anxiety, depression, motor, and cognitive disorders are objective and intuitive parameters of neurodegeneration. It can be reasonably asserted that high doses of crocin (40 and 80 mg/kg) likely exert neuroprotective effects on the consequences of nicotine exposure. This is because, in addition to reducing oxidative stress, these doses also appear to inhibit the aforementioned behavioral disorders (Chen et al., 2015; Ebrahimzadeh et al., 2019; Soeda et al., 2016).

It is noteworthy that, to firmly establish the neuroprotective effects of crocin against nicotine, further research should encompass additional behavioral studies, extensive molecular investigations, in-depth exploration of the relationship between behavioral and molecular changes across various dose scales, utilization of a broader range of doses with meticulous measurements, and evaluation of critical signaling pathways and receptors. Future studies are recommended to prioritize these aspects for a comprehensive understanding. Nevertheless, based on the current findings, it can be inferred that crocin, especially at high doses (40 and 80 mg/kg), along with similar agents, may serve as neuroprotective agents, preserving cell survival and providing neuroprotection, likely through the reduction of oxidative stress and modulation of neurobehavioral disorders in nicotine-dependent rats. These novel findings offer valuable insights into the potential use of therapeutic agents, such as crocin, in mitigating nicotine-induced neurotoxicity in hippocampal cells.

CONCLUSION

The data collected from the present study suggest that chronic administration of nicotine in adult rats may lead to cognitive impairment, motor activ-

ity disorders, mood-related disturbances in behavior, and the induction of oxidative stress. Our findings also indicate that crocin, particularly at high doses (40 and 80 mg/kg), has the potential to counteract nicotine-induced neurobehavioral modifications and oxidative stress. Notably, this study revealed, for the first time, the protective effects of crocin against the deleterious effects induced by nicotine. While these findings provide new insights into the mechanisms underlying nicotine-induced neurodegeneration, further investigation into the precise behavioral, molecular, and cellular aspects of protective mechanisms against nicotine-induced neurodegeneration and neurobehavioral changes, especially in human subjects, appears necessary.

ACKNOWLEDGMENT

This research was financially supported by Research Deputy of Iran University of Medical Sciences, Tehran, Iran (Code number 14561; Ethical Code: IR.IUMS.FMD.REC.1398.309). A. Sureda was supported by the Spanish Government, Institute of Health Carlos III (CIBEROBN CB12/03/30038).

REFERENCES

Abreu-Villaça, Y., Seidler, F. J., & Slotkin, T. A. (2003) Impact of adolescent nicotine exposure on adenylyl cyclase-mediated cell signaling: enzyme induction, neurotransmitter-specific effects, regional selectivities, and the role of withdrawal. *Brain research*, *988*(1–2), 164–172.

Ahmed, S., Hasan, M. M., Heydari, M., Rauf, A., Bawazeer, S., Abu-Izneid, T., et al. (2020) Therapeutic potentials of crocin in medication of neurological disorders. *Food and Chemical Toxicology*, *145*, 111739.

Alhadidi, Q., Nash, K. M., Alaqel, S., Sayeed, M. S. B., & Shah, Z. A. (2018) Cofilin knockdown attenuates hemorrhagic brain injury-induced oxidative stress and microglial activation in mice. *Neuroscience*, *383*, 33–45.

Altinoz, E., Erdemli, M., Gul, M., Aksungur, Z., Gul, S., Bag, H., et al. (2018) Neuroprotection against CCl4 induced brain damage with crocin in Wistar rats. *Biotechnic & Histochemistry*, *93*(8), 623–631.

Asdaq, S. M. B., & Inamdar, M. N. (2010) Potential of Crocus sativus (saffron) and its constituent, crocin, as hypolipidemic and antioxidant in rats. *Applied biochemistry and biotechnology*, *162*(2), 358–372.

Balfour, D. (1982) The effects of nicotine on brain neurotransmitter systems. *Pharmacology & therapeutics*, *16*(2), 269–282.

Benowitz, N. L. (2009) Pharmacology of nicotine: addiction, smoking-induced disease, and therapeutics. *Annual review of pharmacology and toxicology*, *49*, 57.

Benowitz, N. L. (2010) Nicotine addiction. *The New England journal of medicine*, *362*(24), 2295.

Bruin, J. E., Gerstein, H. C., & Holloway, A. C. (2010) Long-term consequences of fetal and neonatal nicotine exposure: a critical review. *Toxicological sciences*, *116*(2), 364–374.

Budzynska, B., Boguszewska-Czubara, A., Kruk-Slomka, M., Skalicka-Wozniak, K., Michalak, A., Musik, I., et al. (2013) Effects of imperatorin on nicotine-induced anxiety- and memory-related responses and oxidative stress in mice. *Physiology & behavior*, *122*, 46–55.

Can, A., Dao, D. T., Arad, M., Terrillion, C. E., Piantadosi, S. C., & Gould, T. D. (2012a) The mouse forced swim test. *JoVE (Journal of Visualized Experiments)* (59), e3638.

Can, A., Dao, D. T., Terrillion, C. E., Piantadosi, S. C., Bhat, S., & Gould, T. D. (2012b) The tail suspension test. *JoVE (Journal of Visualized Experiments)* (59), e3769.

Cardinale, A., Nastrucci, C., Cesario, A., & Russo, P. (2012) Nicotine: specific role in angiogenesis, proliferation and apoptosis. *Critical reviews in toxicology*, *42*(1), 68–89.

Carlson, J., Armstrong, B., Switzer III, Robert C., & Ellison, G. (2000) Selective neurotoxic effects of nicotine on axons in fasciculus retroflexus further support evidence that this a weak link in brain across multiple drugs of abuse. *Neuropharmacology*, *39*(13), 2792–2798.

Carlson, J., Noguchi, K., & Ellison, G. (2001) Nicotine produces selective degeneration in the medial habenula and fasciculus retroflexus. *Brain research*, *906*(1–2), 127–134.

Chen, L., Qi, Y., & Yang, X. (2015) Neuroprotective effects of crocin against oxidative stress induced by ischemia/reperfusion injury in rat retina. *Ophthalmic research*, *54*(3), 157–168.

Chen, Y., Zhang, H., Tian, X., Zhao, C., Cai, L., Liu, Y., et al. (2008) Antioxidant potential of crocins and ethanol extracts of Gardenia jasminoides ELLIS and Crocus sativus L.: A relationship investigation between antioxidant activity and crocin contents. *Food Chemistry*, *109*(3), 484–492.

Ciani, E., Severi, S., Bartesaghi, R., & Contestabile, A. (2005) Neurochemical correlates of nicotine neurotoxicity on rat habenulo-interpeduncular cholinergic neurons. *Neurotoxicology*, *26*(3), 467–474.

Cryan, J. F., Mombereau, C., & Vassout, A. (2005) The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. *Neuroscience & Biobehavioral Reviews*, *29*(4–5), 571–625.

Cryan, J. F., & Slattery, D. A. (2007) Animal models of mood disorders: recent developments. *Current opinion in psychiatry*, *20*(1), 1–7.

Dastgerdi, A. H., Radahmadi, M., Pourshanazari, A. A., & Dastgerdi, H. H. (2017) Effects of crocin on learning and memory in rats under chronic restraint stress with special focus on the hippocampal and frontal cortex corticosterone levels. *Advanced biomedical research*, *6, 157.*

Deslauriers, A. M., Afkhami-Goli, A., Paul, A. M., Bhat, R. K., Acharjee, S., Ellestad, K. K., et al. (2011) Neuroinflammation and endoplasmic reticulum stress are coregulated by crocin to prevent demyelination and neurodegeneration. *The Journal of Immunology*, 187(9), 4788–4799.

Ebrahimzadeh, A., Moghadam, S., Rahimi, H., Motaghinejad, M., Motevalian, M., Safari, S., & Mesrabadi, M. (2019) Crocin acts as a neuroprotective mediator against methylphenidate-induced neurobehavioral and neurochemical sequelae: Possible role of the CREB-BDNF signaling pathway. *Acta Neurobiologiae Experimentalis*, *79*(4), 352–366.

Eddins, D., Petro, A., Williams, P., Cerutti, D. T., & Levin, E. D. (2009) Nicotine effects on learning in zebrafish: the role of dopaminergic systems. *Psychopharmacology*, *202*(1–3), 103.

Feizipour, S., Sobhani, S., Mehrafza, S., Gholami, M., Motaghinejad, M., Motevalian, M., et al. (2020) Selegiline acts as neuroprotective agent against methamphetamine-prompted mood and cognitive related behavior and neurotoxicity in rats: Involvement of CREB/BDNF and Akt/GSK3 signal pathways. *Iranian Journal of Basic Medical Sciences, 23(5), 606–661.*

Finley, J. W., & Gao, S. (2017) A perspective on Crocus sativus L. (Saffron) constituent crocin: a potent water-soluble antioxidant and potential therapy for Alzheimer's disease. *Journal of agricultural and food chemistry*, *65*(5), 1005–1020.

Frank, M. G., Wieseler-Frank, J. L., Watkins, L. R., & Maier, S. F. (2006) Rapid isolation of highly enriched and quiescent microglia from adult rat hippocampus: immunophenotypic and functional characteristics. *Journal of neuroscience methods*, *151*(2), 121–130.

Galizia, C. G., & Lledo, P. M. (2013) *Neurosciences-From Molecule to Behavior: a university textbook*. Springer Science & Business Media.

Ghafarimoghadam, M., Mashayekh, R., Gholami, M., Fereydani, P., Shelley-Tremblay, J., Kandezi, N., et al. (2022) A review of behavioral methods for the evaluation of cognitive performance in animal models: Current techniques and links to human cognition. *Physiology & behavior*, *244*, 113652.

Gould, T. D., Dao, D. T., & Kovacsics, C. E. (2009) The open field test. In *Mood and anxiety related phenotypes in mice* (pp. 1–20). Springer.

Gould, T. J., & Leach, P. T. (2014) Cellular, molecular, and genetic substrates underlying the impact of nicotine on learning. *Neurobiology of learning and memory*, *107*, 108–132.

Hacioglu, G., Senturk, A., Ince, I., & Alver, A. (2016) Assessment of oxidative stress parameters of brain-derived neurotrophic factor heterozygous mice in acute stress model. *Iranian journal of basic medical sciences*, *19*(4), 388.

Hoshyar, R., & Mollaei, H. (2017) A comprehensive review on anticancer mechanisms of the main carotenoid of saffron, crocin. *Journal of Pharmacy and Pharmacology*, *69*(11), 1419–1427.

Hosseinzadeh, H., & Noraei, N. B. (2009) Anxiolytic and hypnotic effect of Crocus sativus aqueous extract and its constituents, crocin and safranal, in mice. *Phytotherapy Research*, *23*(6), 768–774.

Hosseinzadeh, H., & Talebzadeh, F. (2005) Anticonvulsant evaluation of safranal and crocin from Crocus sativus in mice. *Fitoterapia*, *76*(7), 722–724.

Iniguez, S. D., Warren, B. L., Parise, E. M., Alcantara, L. F., Schuh, B., Maffeo, M. L., et al. (2009) Nicotine exposure during adolescence induces a depression-like state in adulthood. *Neuropsychopharmacology*, *34*(6), 1609–1624.

Kamyar, M., Razavi, B. M., Hasani, F. V., Mehri, S., Foroutanfar, A., & Hosseinzadeh, H. (2016) Crocin prevents haloperidol-induced orofacial dyskinesia: possible an antioxidant mechanism. *Iranian journal of basic medical sciences*, *19*(10), 1070.

Kermanshahi, S., Ghanavati, G., Abbasi-Mesrabadi, M., Gholami, M., Ulloa, L., Motaghinejad, M., & Safari, S. (2020) Novel neuroprotective potential of crocin in neurodegenerative disorders: an illustrated mechanistic review. *Neurochemical Research*, *45*, 2573–2585.

Keshavarzi, S., Kermanshahi, S., Karami, L., Motaghinejad, M., Motevalian, M., & Sadr, S. (2019) Protective role of metformin against methamphetamine induced anxiety, depression, cognition impairment and neurodegeneration in rat: the role of CREB/BDNF and Akt/GSK3 signaling pathways. *Neurotoxicology*, *72*, 74–84.

Khadrawy, Y., El-Shamy, K., & Mohamed, S. (2011) Nicotine restores monoamine neurotransmitter changes in the cortex and hippocampus of reserpinized rats as a model of depression. *European Review for Medical & Pharmacological Sciences*, *15*(8), 863–870.

Khalili, M., Roghani, M., & Ekhlasi, M. (2010) The effect of aqueous crocus sativus L. extract on intracerebroventricular streptozotocin-induced cognitive deficits in rat: a behavioral analysis. *Iranian Journal of Pharmaceutical Research*, 185–191.

Kilkenny, C., Browne, W., Cuthill, I., Emerson, M., & Altram, D. (2010) Animal research: Reporting of in vivo experiments, National Centre for the Replacement, Refinement and Reduction of Animals in Research. *Originally published in PLOS biology 8*(6), 1–5.

Kipp, J. L., & Ramirez, V. D. (2001) Effect of estradiol. diethylstilbestrol, and resveratrol on F0F1-ATPase activity from mitochondrial preparations of rat heart, liver, and brain. *Endocrine*, *15*(2), 165–175.

Kocaman, G., Altinoz, E., Erdemli, M., Gul, M., Erdemli, Z., Gul, S., & Bag, H. (2019) Protective effects of crocin on biochemistry and histopathology of experimental periodontitis in rats. *Biotechnic & Histochemistry*, *94*(5), 366–373.

Lee, J., Kim, C. H., Simon, D. K., Aminova, L. R., Andreyev, A. Y., Kushnareva, Y. E., et al. (2005) Mitochondrial cyclic AMP response element-binding protein (CREB) mediates mitochondrial gene expression and neuronal survival. *Journal of Biological Chemistry*, *280*(49), 40398–40401.

Mohammadzadeh, L., Hosseinzadeh, H., Abnous, K., & Razavi, B. M. (2018) Neuroprotective potential of crocin against malathion-induced motor deficit and neurochemical alterations in rats. *Environmental Science and Pollution Research*, *25*(5), 4904–4914.

Motaghinejad, M., Farokhi, N., Motevalian, M., & Safari, S. (2020) Molecular, histological and behavioral evidences for neuroprotective effects of minocycline against nicotine-induced neurodegeneration and cognition impairment: Possible role of CREB-BDNF signaling pathway. *Behavioural Brain Research*, *386*, 112597.

Motaghinejad, M., Fatima, S., Karimian, M., & Ganji, S. (2016) Protective effects of forced exercise against nicotine-induced anxiety, depression and cognition impairment in rat. *Journal of basic and clinical physiology and pharmacology*, *27*(1), 19–27.

Motaghinejad, M., Motevalian, M., Falak, R., Heidari, M., Sharzad, M., & Kalantari, E. (2016) Neuroprotective effects of various doses of topiramate against methylphenidate-induced oxidative stress and inflammation in isolated rat amygdala: the possible role of CREB/BDNF signaling pathway. *Journal of Neural Transmission*, *123*(12), 1463–1477.

Motaghinejad, M., Motevalian, M., Fatima, S., Faraji, F., & Mozaffari, S. (2017b) The neuroprotective effect of curcumin against nicotine-induced neurotoxicity is mediated by CREB–BDNF signaling pathway. *Neurochemical research*, *42*(10), 2921–2932.

Motaghinejad, M., Motevalian, M., Fatima, S., Hashemi, H., & Gholami, M. (2017a) Curcumin confers neuroprotection against alcohol-induced hippocampal neurodegeneration via CREB-BDNF pathway in rats. *Biomedicine & Pharmacotherapy*, *87*, 721–740.

Motaghinejad, M., Safari, S., Feizipour, S., & Sadr, S. (2019) Crocin may be useful to prevent or treatment of alcohol induced neurodegeneration and neurobehavioral sequels via modulation of CREB/BDNF and Akt/GSK signaling pathway. *Medical Hypotheses*, *124*, 21–25.

Mozaffari, S., Yasuj, S. R., Motaghinejad, M., Motevalian, M., & Kheiri, R. (2019) Crocin acting as a neuroprotective agent against methamphetamine-induced neurodegeneration via CREB-BDNF signaling pathway. *Iranian Journal of Pharmaceutical Research: IJPR*, *18*(2), 745.

Muduli, N., Aparna, S., Patri, M., & Sahoo, K. K. (2023) Saffron stigma extract and crocin play an important neuroprotective role in therapeutic measures against benzo[a]pyrene-induced behavioral alterations in zebrafish. *Drug and Chemical Toxicology, 47*, 131–142.

Naha, N., Gandhi, D., Gautam, A., & Prakash, J. R. (2018) Nicotine and cigarette smoke modulate Nrf2-BDNF-dopaminergic signal and neurobehavioral disorders in adult rat cerebral cortex. *Human & experimental toxicology*, *37*(5), 540–556.

Ochiai, T., Ohno, S., Soeda, S., Tanaka, H., Shoyama, Y., & Shimeno, H. (2004) Crocin prevents the death of rat pheochromocytoma (PC-12) cells by its antioxidant effects stronger than those of α-tocopherol. *Neuroscience letters*, *362*(1), 61–64.

Oliveira-da-Silva, A., Manhaes, A., Cristina-Rodrigues, F., Filgueiras, C., & Abreu-Villaca, Y. (2010) Hippocampal increased cell death and decreased cell density elicited by nicotine and/or ethanol during adolescence are reversed during drug withdrawal. *Neuroscience*, *167*(1), 163–173.

Papandreou, M. A., Kanakis, C. D., Polissiou, M. G., Efthimiopoulos, S., Cordopatis, P., Margarity, M., & Lamari, F. N. (2006) Inhibitory activity on amyloid-β aggregation and antioxidant properties of Crocus sativus stigmas extract and its crocin constituents. *Journal of agricultural and food chemistry*, *54*(23), 8762–8768.

Picciotto, M. R., Lewis, A. S., van Schalkwyk, G. I., & Mineur, Y. S. (2015) Mood and anxiety regulation by nicotinic acetylcholine receptors: A potential pathway to modulate aggression and related behavioral states. *Neuropharmacology*, *96*, 235–243.

Pitsikas, N., Boultadakis, A., Georgiadou, G., Tarantilis, P., & Sakellaridis, N. (2008) Effects of the active constituents of Crocus sativus L., crocins, in an animal model of anxiety. *Phytomedicine*, *15*(12), 1135–1139.

Purushothuman, S., Nandasena, C., Peoples, C. L., El Massri, N., Johnstone, D. M., Mitrofanis, J., & Stone, J. (2013) Saffron pre-treat-

ment offers neuroprotection to nigral and retinal dopaminergic cells of MPTP-treated mice. *Journal of Parkinson's disease*, *3*(1), 77–83.

Ranzolin, A., Duarte, A. L. B. P., Bredemeier, M., da Costa Neto, C. A., Ascoli, B. M., Wollenhaupt-Aguiar, B., et al. (2016) Evaluation of cytokines, oxidative stress markers and brain-derived neurotrophic factor in patients with fibromyalgia–A controlled cross-sectional study. *Cytokine*, *84*, 25–28.

Romero, R. D., & Chen, W.-J. A. (2004) Gender-related response in open-field activity following developmental nicotine exposure in rats. *Pharmacology Biochemistry and Behavior*, *78*(4), 675–681.

Saleem, S., Ahmad, M., Ahmad, A. S., Yousuf, S., Ansari, M. A., Khan, M. B., Islam, F. (2006) Effect of Saffron (Crocus sativus) on neurobehavioral and neurochemical changes in cerebral ischemia in rats. *Journal of medicinal food*, *9*(2), 246–253.

Sarshoori, J. R., Asadi, M. H., & Mohammadi, M. T. (2014) Neuroprotective effects of crocin on the histopathological alterations following brain ischemia-reperfusion injury in rat. *Iranian journal of basic medical sciences*, *17*(11), 895.

Seyedinia, S. A., Tarahomi, P., Abbarin, D., Sedaghat, K., Rashidy-Pour, A., Yaribeygi, H., et al. (2023) Saffron and crocin ameliorate prenatal valproic acid-induced autistic-like behaviors and brain oxidative stress in the male offspring rats. *Metabolic Brain Disease*, *38*(7), 2231–2241.

Slawecki, C. J., Gilder, A., Roth, J., & Ehlers, C. L. (2003) Increased anxiety-like behavior in adult rats exposed to nicotine as adolescents. *Pharmacology Biochemistry and Behavior*, *75*(2), 355–361.

Soeda, S., Aritake, K., Urade, Y., Sato, H., & Shoyama, Y. (2016) Neuroprotective activities of saffron and crocin. *Adv Neurobiol, 12*, 275–292.

Sudheer, A. R., Muthukumaran, S., Devipriya, N., Devaraj, H., & Menon, V. P. (2008) Influence of ferulic acid on nicotine-induced lipid peroxidation, DNA damage and inflammation in experimental rats as compared to N-acetylcysteine. *Toxicology*, *243*(3), 317–329.

Suk, K. (2005) Regulation of neuroinflammation by herbal medicine and its implications for neurodegenerative diseases. *Neurosignals*, *14*(1–2), 23–33.

Talaei, A., Moghadam, M. H., Tabassi, S. A. S., & Mohajeri, S. A. (2015) Crocin, the main active saffron constituent, as an adjunctive treatment in major depressive disorder: A randomized, double-blind, placebo-controlled, pilot clinical trial. *Journal of affective disorders*, *174*, 51–56.

Tamaddonfard, E., Farshid, A. A., Ahmadian, E., & Hamidhoseyni, A. (2013) Crocin enhanced functional recovery after sciatic nerve crush injury in rats. *Iranian journal of basic medical sciences*, *16*(1), 83.

Tamaddonfard, E., Farshid, A. A., Asri-Rezaee, S., Javadi, S., Khosravi, V., Rahman, B., & Mirfakhraee, Z. (2013) Crocin improved learning and memory impairments in streptozotocin-induced diabetic rats. *Iranian journal of basic medical sciences*, *16*(1), 91.

Vakili, A., Einali, M. R., & Bandegi, A. R. (2014) Protective effect of crocin against cerebral ischemia in a dose-dependent manner in a rat model of ischemic stroke. *Journal of Stroke and Cerebrovascular Diseases*, *23*(1), 106–113.

Vorhees, C. V., & Williams, M. T. (2006) Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nature protocols*, *1*(2), 848.

Walf, A. A., & Frye, C. A. (2007) The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nature protocols*, *2*(2), 322–328.

Wang, Y., Han, T., Zhu, Y., Zheng, C.-J., Ming, Q.-L., Rahman, K., & Qin, L.-P. (2010) Antidepressant properties of bioactive fractions from the extract of Crocus sativus L. *Journal of natural medicines*, *64*(1), 24.

Yousefsani, B. S., Mehri, S., Pourahmad, J., & Hosseinzadeh, H. (2018) Crocin prevents sub-cellular organelle damage, proteolysis and apoptosis in rat hepatocytes: A justification for its hepatoprotection. *Iranian Journal of Pharmaceutical Research, 17(2), 553–562*.

Zadali, R., Ghareghozloo, E. R., Ramezani, M., Hassani, V., Rafiei, Y., Chiyaneh, S. M., & Meratan, A. A. (2019) Interactions with and membrane permeabilization of brain mitochondria by amyloid fibrils. *JoVE,* (151), e59883.

Zhang, X.-Y., Zhang, X.-J., Xv, J., Jia, W., Pu, X.-Y., Wang, H.-Y., et al. (2018) Crocin attenuates acute hypobaric hypoxia-induced cognitive deficits of rats. *European journal of pharmacology*, *818*, 300–305.

Zhao, T., Lu, H., Li, M., Yan, Q., Gu, J., & Liu, L. (2022) Neuroprotective mechanism of crocin via PI3K/Akt/mTOR signaling pathway after cerebral infarction: an in vitro study. *American Journal of Translational Research*, *14*(5), 3164.