Investigation of the neuroprotective action of saffron (Crocus sativus L.) in aluminum-exposed adult mice through behavioral and neurobiochemical assessment

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Abstract

In the present study, the possible reversal effects of saffron against established aluminum (Al)-toxicity in adult mice, were investigated. Control, Al-treated (50 mg AlCl₃/kg/day diluted in the drinking water for 5 weeks) and Al + saffron (Al-treatment as previously plus 60 mg saffron extract/kg/day intraperitoneally for the last 6 days), groups of male Balb-c mice were used. We assessed learning/memory, the activity of acetylcholinesterase [AChE, salt-(SS)/detergent-soluble(DS) isoforms], butyrylcholinesterase (BuChE, SS/DS isoforms), monoamine oxidase (MAO-A, MAO-B), the levels of lipid peroxidation (MDA) and reduced glutathione (GSH), in whole brain and cerebellum. Brain Al was determined by atomic absorption spectrometry, while, for the first time, crocetin, the main active metabolite of saffron, was determined in brain after intraperitoneal saffron administration by HPLC. Al intake caused memory impairment, significant decrease of AChE and BuChE activity, activation of brain MAO isoforms but inhibition of cerebellar MAO-B, significant elevation of brain MDA and significant reduction of GSH content. Although saffron extract co-administration had no effect on cognitive performance of mice, it reversed significantly the Al-induced changes in MAO activity and the levels of MDA and GSH. AChE activity was further significantly decreased in cerebral tissues of Al + saffron group. The biochemical changes support the neuroprotective potential of saffron under toxicity.

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1. Introduction

Aluminum (Al) is the third most abundant element in nature, making human exposure unavoidable (Verstraeten et al., 2008). The main entry sites of Al into the body are the gastrointestinal and respiratory tract and the skin (Verstraeten et al., 2008), enabling distribution and accumulation in several tissues, like spleen, lungs, liver, kidneys, heart, bone and brain (Kumar and Gill, 2009), through systemic circulation. It has been demonstrated that Al crosses the blood–brain barrier, implicating metal binding to transferrin in the blood and a subsequent transferrin receptor-mediated mechanism of brain Al influx (Yokel et al., 1999).

High brain levels of Al induce cognitive deficiency and dementia and, thus, Al is a widely accepted neurotoxin (Kawahara and Kato-Negishi, 2011). Its implication in the pathogenesis of neurodegenerative diseases has been suggested decades ago, but is seriously debated till now (Kawahara and Kato-Negishi, 2011; Tomljenovic, 2011). However, some researchers consider that the model of chronic Al-induced neurotoxicity best describes Alzheimer’s disease, since it manifests many of the pathobiological hallmarks (Walton and Wang, 2009; Yokel, 2006; Zhang et al., 2003).

Al neurotoxicity is manifested through several behavioral and neurochemical alterations, which display diversity depending on the animal species in question, the administration route and the chemical form of Al administered (Erasmus et al., 1993; Kumar and Gill, 2009). It has been demonstrated that Al promotes oxidative stress in the cerebral cortex and hippocampus of young and aged rats, and damage of lipids, membrane-associated proteins and...
(Na+-K-ATPase and protein kinase C) and endogenous antioxidant enzymes (Sethi et al., 2008; Sharma et al., 2009; Tripathi et al., 2009). Negative impacts of Al administration have been also observed in neurotransmitter systems of rodent brain regions, including serotoninergic (Kumar, 2002) and cholinergic systems (Julka et al., 1995). The effects of Al on AChE activity remain controversial as both inhibition and activation have been reported (Julka et al., 1995; Sharma et al., 2009), whereas the respective data concerning BuChE activity are limited, though BuChE enzyme is considered to play a supportive functional role in acetylcholine hydrolysis (Lan et al., 2006). Finally, behavioral deficits assessed with different behavioral tasks, have been observed in rats following Al exposure (Julka et al., 1995; Sethi et al., 2008; Tripathi et al., 2009).

2.2. Animals

Male adult (4 month-old) Balb-c mice were used in this study. The animals were housed in groups in standard laboratory cages (5 mice per cage) in a temperate controlled room (20 ± 2 °C) with a 12 h light/dark cycle. Food in form of dry pellets (feed composition: grain and grain by-products, oil seed products, minerals, vitamins and trace elements from Altromin Spezialfutter GmbH & Co. KG, Lage, Germany) was available ad libitum. Animals were randomly divided into three groups (n = 10/group); Control group: mice had access to normal drinking water over the experimental period. Al treated group: mice received orally AlCl3 (aluminum chloride anhydrous, purity >99%, Sigma-Aldrich, St. Louis, USA) (50 mg/kg body weight/day) dissolved in normal drinking water for a period of 5 weeks. Body weight and liquid intake were measured regularly to adjust the dose and achieve a constant intake of Al. Al + saffron treated group: mice received orally AlCl3 (50 mg/kg body weight/day) dissolved in normal drinking water for 5 weeks and saffron extract (60 mg/kg body weight/day, 30 μL injection volume) intraperitoneally for the last 6 days of the 5-week Al treatment.

AlCl3 solution was freshly prepared every 4 days over the experimental period, while saffron solution was prepared fresh daily, just before administration, by dissolving the dry extract in saline. During the 6-day saffron treatment, control and Al-treated mice received intraperitoneally 30 μL of saline. Al and saffron dosage and schedule of exposure were based on the literature (Jyoti and Sharma, 2006) and on our previous study (Papandreou et al., 2011), respectively. All procedures were in accordance with Greek National Laws (Animal Act, PD 160/91).

2.3. Behavioral testing: step-through passive avoidance task

This test is based on negative reinforcement to examine long-term memory (Kaneto, 1997). A two-compartment passive avoidance apparatus (white/dark, separated by a guillotine door) was used. Mice were subjected to single trial passive avoidance task according to previously described procedures (Kaneto, 1997; Otano et al., 1999; Papandreou et al., 2009). Briefly, on day 5 of saffron treatment an acquisition trial was performed 1 h after a 100-s long habituation trial, while a retention trial was performed 24 h after the training session. Results were expressed as the mean initial (IL, max. time 120 s) and step-through (STL, max. time 300 s) latency time values recorded once mice crossed into the dark compartment, before and 24 h after the delivery of a mild foot shock to their paws. All training and testing sessions were carried out during the light phase (08:00–14:00 h), one hour after intraperitoneal administration of saffron.

2.4. Preparation of tissue homogenates

On the completion of the 5-week treatment period, all animals were sacrificed by cervical dislocation. Brain was excited immediately, and cerebellum was separated from the remaining whole brain. Liver was also removed and used as a reference peripheral tissue. All tissues were rinsed with saline immediately. From the tissue samples isolated, only six tissues per group were used for the biochemical assessments. The others were assayed for Al content by atomic absorption spectrometry. The tissues for biochemical measurements and crocin determination were weighed and homogenized (103, w/v) with a glass-Teflon homogenizer in ice-cold 30 mM Na2HPO4, pH 7.6. The homogenates were then centrifuged at 15,000g for 20 min at 4 °C. Supernatants constituted the salt-soluble (SS) fractions and were stored at −75 °C. The pellets were re-suspended in an equal volume of 1% (w/v) Triton X-100 (in 30 mM Na2HPO4, pH 7.6) and then centrifuged at 15,000g for 20 min at 4 °C. Supernatants, the detergent-soluble (DS) fractions, were collected and stored at −75 °C. This double-extraction method was performed in order to cover the cytosolic (SS fraction) and membrane-bound (DS fraction) isoforms of cholinesterases (Das et al., 2005). Protein concentrations were determined by the Bradford assay (Bradford, 1976).

2.5. Aluminum determination by atomic absorption spectrometry

2.5.1. Digestion of tissues

The whole brain (-ce), cerebellum and liver samples from control, Al-treated and Al + saffron treated mice were analyzed for Al concentration. Glassware was not used during sample preparation in order to avoid the possible contamination. Eppendorf tubes were used after immersing in a solution of nitric acid (HNO3): ethanal (1:9, v/v) for 48 h and washing with ultra-pure water (Milli-Q) 3 times with ultra-pure water (Milli-Q) 3 times. Sample preparation was performed as previously described (Gulya et al., 1995) with modifications. The dry weight of the tissues was measured after heating at 100 °C for 20 h. Then, the samples were digested with 1 ml/100 mg wet tissue (for whole brain) and 1 ml/25 mg wet tissue (for cerebellum and liver) of nitric acid: sulphuric.
acetic acid (HNO₃:H₂SO₄:1:1 v/v) at room temperature overnight. Mixtures were centrifuged at 10,000g for 10 min to remove insoluble material. Al levels were determined in the supernatants.

2.5.2. Atomic absorption spectrometry

Al content in the supernatants was measured employing Atomic Absorption Spectrometry (Perkin Elmer, Analyst 300 equipped with a HGA 700 Graphite Furnace and an AS-70 autosampler). The temperature of the furnace was programmed as described elsewhere (Johnson and Treble, 1992). Absorbance was measured at 309.3 nm.

Preparation of samples for Al analysis: All samples were prepared in 1:1 v/v of acetic acid. Concentrations of 0.5, 1.0, 1.5, 2.0 and 3.0 µg/mL were prepared by dilution from a stock standard solution (1000 µg/mL, Al, Merck, Darmstadt, Germany). The spiked concentration ranged from 0 (unspiked sample) to 60 ppb. The volume of sample was kept equal for all samples by means of addition of the matrix modifier. Preliminary tests assured that the overall concentration of Al was within the linear range of the technique.

Chemical analyses: Absorbance for each sample was measured three times. A calibration curve for each supernatant aliquot was constructed (OriginPro 8). The results are expressed as µg Al/g wet tissue.

2.6. HPLC determination of crocetin in brain

Crocetin determination in whole brain (−ce) of Al + saffron treated mice was performed by an isocratic reversed-phase liquid chromatographic method developed by Chrysanthi et al. (2011) for plasma analysis, with slight modifications. In detail, a high volume (3.5 mL) of the whole brain homogenate (100 mg/mL, SS fraction) was submitted to solid phase extraction on reversed phase Strata-X cartridges (5 mg/3 mL) consisting of a surface modified with styrene–divinylbenzene polymer that were obtained from Phenomenex (Torrance, CA, USA). The eluate was evaporated to dryness in a speed Vac system and the dry residue was redissolved in 30 µL of the HPLC mobile phase. The samples were chromatographed on a Luna C-18 column (4.6 × 250 mm, 5 µm) with a mobile phase consisting of methanol–water–trifluoroacetic acid (75:2.5:2.5 v/v) at a flow rate of 1.0 mL/min. The crocetin eluate was evaporated to dryness in a speed Vac system and the dry residue was redissolved in 30 µL of the HPLC mobile phase. The temperature of the furnace was programmed as described elsewhere (Mokrasch and Teschke, 1984). The crocetin eluate was evaporated to dryness in a speed Vac system and the dry residue was redissolved in 30 µL of the HPLC mobile phase. The samples were chromatographed on a Luna C-18 column (4.6 × 250 mm, 5 µm) with a mobile phase consisting of methanol–water–trifluoroacetic acid (75:2.5:2.5 v/v) at a flow rate of 1.0 mL/min. The crocetin quantification was performed by an isocratic reversed-phase liquid chromatographic method developed by Chrysanthi et al. (2011) for plasma analysis, with slight modifications. The standard addition method was employed for the analysis of crocetin. The standard fluorescence curve of 4-hydroxyquinoline (1–100 µM) was determined in the supernatants.

2.7. Biochemical assays

2.7.1. Determination of cholinesterase activity

The activity of SS and DS isoforms of AChE and BuChE in brain and liver samples, was determined spectrophotometrically based on Ellman’s assay and previously described protocols (Ellman et al., 1961; Lassiter et al., 1998; Papandreou et al., 2009). Total ChE activity was measured by acetylthiocholine iodide (ATCh, Sigma–Aldrich, UK) as substrate, whereas BuChE activity was determined by 5-butyrylthiocholine iodide (BuTCh, Sigma–Aldrich, Switzerland) as substrate, since BuTCh is hydrolyzed only by BuChE (Lassiter et al., 1998). AChE activity was then calculated by subtracting BuChE activity from total ChE activity. Enzyme activity is expressed as µmol of substrate hydrolyzed/min/g of tissue.

2.7.2. Determination of monoamine oxidase activity

The activity of both isoforms of MAO, MAO-A and MAO-B, was assessed in mouse whole brain (−ce) and cerebellum by a fluorometric assay based on previously described protocols (Mahmood et al., 1994; Xu et al., 2010). MAO is a mitochondrial membrane-bound enzyme, the DS fractions of brain tissues homogenates were used. Briefly, 50 µL of brain sample was preincubated at 37 °C for 20 min with 100 µL of either 1 µM K+-or L-Deprenyl hydrochloride (Deprenyl, MAO-B inhibitor, Sigma–Aldrich, USA) or 1 µM N-Methyl-N-propargyl-1H-2,4-dichlorophenoxypyrol-2-propionyl hydrochloride (Clorgyline, MAO-A inhibitor, Sigma–Aldrich, USA) in 100 µL 30 mM NaH₂PO₄, pH 7.6. Then 10 µL of 3.07 mM kynurenine hydrochloride (Sigma–Aldrich, USA) was added to the reaction mixture as substrate. The mixture was then incubated at 37 °C for 15 min again. After incubation, the reaction was terminated by adding 100 µL of 0.6 M perchloric acid and centrifuging the mixture at 1500g for 10 min to remove precipitated proteins. An aliquot of 0.3 mL of the supernatant was added to 2 mL of 1 N NaOH. The fluorescence intensity of the produced 4-hydroxyquinoline was detected at an excitation wavelength of 315 nm and an emission wavelength of 380 nm, using a fluorescence spectrometer. The concentration of product was estimated from a corresponding standard fluorescence curve of 4-hydroxyquinoline (1–100 µM) (Sigma–Aldrich, USA). MAO activity is expressed as nmol of 4-hydroxyquinoline formed/g tissue/min.

2.7.3. Estimation of lipid peroxidation and reduced glutathione levels

Malondialdehyde (MDA) levels, an index of tissue lipid peroxidation, were measured in cerebral and liver samples (SS fractions of tissue homogenates) following a previously described fluorimetric assay (Papandreou et al., 2009). Reduced glutathione (GSH) content of brain and liver samples (SS fractions of tissue homogenates) was determined fluorometrically according to the procedure of Mokrasch and Teschke (1984). GSH levels are expressed as µmol/g of tissue protein.

2.8. Statistical analysis

Data are presented as mean ± S.E.M. Statistical analysis was performed with GraphPad Instat 3 software using the nonparametric Mann–Whitney test for evaluating statistically significant differences (p < 0.05) between the experimental groups.

3. Results

3.1. General observations

HPLC analysis showed that saffron extract contains a variety of crocins: trans-crocin-4 constitutes 46% of total crocin content of the crude extract, trans-crocin-3, 26%; cis-crocin-4, 12%; and trans-crocin-2, 7%, as previously described (Papandreou et al., 2006; Tarantilis et al., 1995).

All animals developed normally during the experimental period and no mortality was recorded during Al treatment. Also, there were no differences on body weight gain and liquid intake, between the experimental groups.

3.2. Effect of saffron on learning and memory ability of Al-exposed mice

Passive avoidance task was performed to examine long-term memory. The initial latency (IL) time, measured at training trial, is an indicator of motor activity, and step-through latency (STL) time, measured at testing trial, indicates memory of the aversive experience. Al intake resulted in impaired long-term memory of mice, as evidenced by the significantly lower (59%) mean STL time of Al-treated animals compared to mean STL time of the controls (Fig. 1). The lack of difference of mean STL time between Al + saffron- and Al-treated groups (Fig. 1), shows that saffron extract co-administered with Al during the last 6 days of the treatment period was ineffective in reversing Al-induced memory...
The impact of Al or saffron treatment on the activities of both isoforms (SS, DS) of AChE and BuChE in whole brain, cerebellum and liver of adult mouse is presented in Table 2. Al intake decreased significantly ($p < 0.05$) the activity of both SS and DS isoforms of AChE and BuChE in mouse whole brain (-ce) (26%, 17% and 21%) and cerebellum (23%, 15% and 22%, 19%) compared to the controls, except of the activity of DS-BuChE of whole brain (-ce) that remained unchanged. Further significant ($p < 0.05$) reduction of the SS- and DS-AChE activity in whole brain (-ce) (15%, 28%) and cerebellum (24%, 27%) was observed in Al + saffron treated mice in comparison with Al treated group, whereas saffron treatment did not further affect the activity of cerebral BuChE. Also, Al-treated mice displayed significantly ($p < 0.05$) decreased liver BuChE (11% SS, 21% DS) and SS-AChE (25%) activities compared to the controls, while Al + saffron treated mice displayed further inhibition of liver BuChE (27% SS, 19% DS) and SS-AChE (31%) compared to Al-treated mice.

3.5. Effect on brain MAO activity

The effect of administration of either Al alone or in the presence of saffron extract at the end of the treatment period, on MAO isoforms activity of mouse whole brain (-ce) and cerebellum, is presented in Fig. 2. Al-treated mice displayed significantly ($p < 0.05$) higher activities of MAO-A (19%) and MAO-B (10%) in their whole brain (-ce) and significantly lower MAO-B activity (14%) in cerebellum, compared to the controls. Short-term co-administration of saffron restored MAO-A and MAO-B activity of whole brain (-ce) (decrease by 14% and 10%, respectively) and MAO-B activity (17% increase) of cerebellum, to normal levels.

3.6. Effect on brain and liver oxidant/antioxidant indices

MDA and GSH levels, as indicators of lipid peroxidation and cellular antioxidant defense, respectively, were measured in mouse brain tissues and liver of all experimental groups. As shown in Fig. 3a, Al intake increased (18%) significantly ($p < 0.05$) MDA levels only in whole brain (-ce) in comparison with controls, while the subsequent short-term co-administration of saffron restored (decrease by 22%) MDA levels to normal. Short-term co-administration of saffron also improved the antioxidant status of cerebellum and liver by significantly lowering the levels of MDA, even though these tissues remained unaffected by Al treatment (Fig. 3a). Additionally, Al-treated mice displayed remarkable reduction in GSH content of whole brain (-ce) (44%), cerebellum (16%) and liver (29%), compared to the control animals, whereas Al + saffron treated mice exhibited elevated levels of GSH (29%, 14% and 22%, respectively) compared to Al-exposed group (Fig. 3b).

4. Discussion

The present study investigates the neuroprotective potential of a 6-day i.p. administration of saffron extract to adult mice that...
were previously exposed to high Al intake through their drinking water for a 5-week period. To this end, cognitive behavior and brain cholinergic, monoaminergic and oxidative indices were assessed. Our findings showed that long-term Al intake induced learning/memory decline in adult mice. Impaired performance of rats on passive avoidance task has been shown by Bhalla et al. (2010) after long-term administration of a double dose of AlCl₃ in drinking water, while Sethi et al. (2008) showed declined spatial learning abilities of rats in Morris-water maze test after administration of the same dose of AlCl₃ (50 mg/kg/day) in drinking water for 6 months. Saffron co-administration at the end of the treatment period failed to reverse Al-induced cognitive deficits. In our previous study, short-term saffron administration enhanced learning/memory in healthy adult and aged mice in passive avoidance task (Papandreou et al., 2011). However, in the current report the administered dosage of saffron extract and/or the duration of exposure (which are the same as in the study of Papandreou et al., 2011) were insufficient to counteract Al-induced memory deficit.

Al levels were determined in whole brain (−ce), cerebellum and liver of all animal groups prior to biochemical evaluation. The values of Al found in cerebral tissues, are comparable with those of other studies that use Al as neurotoxic agent, although different Al forms, doses and routes of administration are investigated (Esparza et al., 2005; Kaizer et al., 2008; Sánchez-Iglesias et al., 2007). Our findings showed high concentrations of Al in cerebellum of control mice (higher than liver), but none or below detection limits in whole brain (−ce), in accordance with Bellés et al. (1998). In the current study, long-term AlCl₃ intake through drinking water resulted in significantly increased Al concentrations in whole brain, cerebellum and liver of adult mice. Our results are

![Effect of saffron administration on monoamine oxidase activity (MAO-A and MAO-B) of Al-exposed adult mouse brain. Al-induced activation of both MAO isoforms in whole brain (−ce), inhibition of MAO-B in cerebellum and reversal effects of saffron are evident. Data are the mean ± SEM (n = 6 animals/group). *p < 0.05 vs. the control group, #p < 0.05 vs. Al treated group (non-parametric Mann–Whitney test).](image)

![Effect of saffron administration on (a) lipid peroxidation (MDA) and (b) reduced glutathione (GSH) levels of Al-exposed adult mouse brain and liver. Al-induced increase in MDA levels of whole brain (−ce), decrease in GSH levels of whole brain (−ce), cerebellum and liver, and reversal effects of saffron are evident. Data are the mean ± SEM (n = 6 animals/group). *p < 0.05 vs. the control group, #p < 0.05 vs. Al treated group (non-parametric Mann–Whitney test).](image)
in accordance with previous studies where AlCl₃ is administered to rodents (Bhalla and Dhawan, 2009; El-Maraghy et al., 2001). The entry of Al into the brain through the blood–brain barrier has long been established (Yokel et al., 1999) and is also confirmed by our results. The accumulation of Al in brain is consistent with the observed impaired learning/memory ability of mice in passive avoidance test. However, cerebral Al levels did not differ significantly between Al- and Al + saffron treated mice, showing that short-term saffron administration in the end of the treatment period did not affect Al bioavailability.

Additionally, for the first time, we determined crocetin, the major metabolite (aglycon) of the bioactive crocin glycosides of saffron, in whole brain of Al + saffron treated mice, which was absent from the brain of control mice. The detection of crocetin in mouse brain demonstrates for the first time that this compound crosses the blood–brain barrier when saffron extract is administered for a short term through intraperitoneal route. In recent report (Chryssanthi et al., 2011), the presence of crocetin in human plasma was shown after 2 and 24 h of saffron tea consumption, while only Yoshino et al. (2011) have demonstrated the distribution of crocetin in rat brain after a single oral administration of pure crocetin, up to now.

The fact that saffron short-term co-administration failed to reverse the cognitive decline raises questions on the dosage, the period and the route of administration, before definite conclusions can be drawn about its effectiveness. However, our study presents some remarkable brain biochemical alterations that followed saffron co-treatment.

Determination of ChE activity showed that the DS-AChE was predominant in cerebral tissues, while approximately equal values of the activity of the two BuChE isoforms were recorded. These observations are consistent with previous findings (Das et al., 2005; Fernández-Gómez et al., 2008). In accordance with other studies (Lassiter et al., 1998; Li et al., 2000), AChE activity constitutes the major fraction of total ChE activity in whole brain (-ce) and cerebellum, whereas BuChE activity predominates in liver. In the current work, Al intake resulted in significant decrease of the activity of AChE and BuChE isoforms in mouse whole brain and cerebellum. Region-specific inhibition of the activity of rat cerebral membrane-bound AChE and BuChE after Al intoxication, has been shown by others (Julkla et al., 1995). Previous studies have shown necrotic rat hippocampal ultrastructural changes following the same Al administration protocol (Jyoti and Sharma, 2006), providing an explanation for our cholinotoxic and memory impairment results. However, Al has been suggested to interfere with the action of metabolotropic glutamate receptors, enhancing excitotoxicity and neuronal injury (Blaylock, 2012). Also, Al impairs hippocampal long-term potentiation (LTP) in rats (Platt et al., 1995). Thus, besides the cholinotoxic effects of Al in the present study, the involvement of possible Al-induced glutamatergic neurotransmission disturbances in the observed memory decline, should also be considered. In liver, both BuChE isoforms’ activity (the main liver ChE fraction) was significantly decreased following Al administration, while only SS-AChE activity was decreased significantly. However, increased activity of soluble and membrane-bound forms of rat liver BuChE after Al intake through diet for 100–115 days (Dave et al., 2002), has been previously demonstrated, but the Al dose and period of exposure are different.

Saffron short-term co-administration caused further significant reduction of cerebral AChE and liver BuChE isoforms activity. Cerebral BuChE activity was not further affected and only SS-AChE activity of liver was further significantly decreased after saffron co-treatment. The inhibitory effect of saffron on the activity of brain SS- and DS-AChE in adult healthy mice has been demonstrated in our previous study (Papandreou et al., 2011). However, the impact of saffron administration on brain BuChE and liver ChE activities, has not been studied before. The considerable higher percentage of inhibition of DS-AChE in whole brain of Al + saffron treated mice compared to SS-AChE, suggests greater susceptibility of the enzyme's DS isoform to saffron treatment. Geromichalos et al. (2012) have recently demonstrated, for the first time, that crocetin inhibits AChE by binding at two different loci, the catalytic center and the peripheral anionic sites, as assessed by in vitro enzymatic and molecular docking studies. Thus, the observed significant cerebral AChE inhibitory activity conferred by saffron short-term co-administration, may be attributed to a direct interaction of crocetin with the enzyme, since crocetin was detected in brain of Al + saffron treated mice and was absent in controls’ brain; however, it remains to be investigated whether the crocetin concentration is sufficient for such an effect. Furthermore, other indirect mechanisms cannot be excluded.

Monoamine oxidase (MAO, types A and B) is a mitochondrial membrane-bound enzyme which catalyzes the oxidative deamination of monoamines, thus regulating monoaminergic neurotransmission. Increased brain MAO activity has been recorded in neurodegenerative process (Hanish Singh et al., 2011; Mallajosyula et al., 2008). Our results showed significant increase of whole brain MAO isoforms activity after Al intoxication, and an opposite effect on cerebellum MAO-B activity. Bhalla et al. (2010) have also presented increased and decreased MAO activity in rat cerebrum and cerebellum, respectively, after long-term oral administration of AlCl₃. Activation of MAO isotypes in rat brain by prolonged intake of Al has been also previously reported (Huh et al., 2005), but the underlying mechanisms have not yet been clarified.

Saffron co-treatment completely reversed Al-induced activation of brain MAO isoforms and inhibition of cerebellar MAO-B. The effect of saffron administration on cerebral MAO activity has not been studied before. MAO inhibitors have long been well characterized for their antidepressant properties (Bortolato et al., 2008). The first small-scale clinical trials of saffron in the treatment of mild to moderate depression, have shown significant benefits in the mood of patients after a 6-week treatment (Akhoundzadeh et al., 2005; Noorbalal et al., 2005). The antidepressant efficacy of saffron could, at least in part, correlated with brain MAO inhibitory activity of saffron extract and the presence of crocetin in brain that are presented in our current study.

Oxidative stress has long been implicated in the initial and later stages of neuronal degeneration (Melo et al., 2011). In the present study, Al intake elevated significantly lipid peroxidation in whole brain and decreased GSH content in both cerebral tissues and liver. Other reports have also demonstrated increased lipid peroxidation levels and decreased GSH content in rat brain regions after long-term oral administration of AlCl₃ (Jyoti and Sharma, 2006; Nehru and Bhalla, 2006). The resistance of liver to Al-induced lipid peroxidation, indicated by us and other studies (Kaneko et al., 2004), is expected, as it constitutes the major site of detoxification in living organism. Although Al is not a redox-active metal, there is extensive experimental evidence on oxidative stress-mediated Al neurotoxicity (Kumar and Gill, 2009). It has been demonstrated that Al induces oxidative stress in cultured rat hippocampal neurons by potentiating iron-mediated oxidative injury (Xie et al., 1996). Also, in vitro studies (Oteiza, 1994) have shown that Al stimulates iron-induced lipid peroxidation in isolated membrane fractions through binding to the membrane and promotion of changes in the arrangement of membrane lipids. Considering the byproduct (hydrogen peroxide) of MAO-mediated reactions, Al-induced activation of brain MAO isoforms provided by our results could, at least in part, contribute to the observed increased brain oxidative stress.

Reversal effects of saffron short-term co-administration were recorded against Al-induced brain lipid peroxidation and GSH content reduction in cerebral tissues and liver. In our previous study
(Papandreou et al., 2011), saffron administration significantly decreased MDA levels and increased GSH content in the brain of healthy adult and aged mice. Shati et al. (2011) also showed that intraperitoneal co-administration of saffron aqueous extract with AlCl₃ ameliorated the disturbances in mouse brain lipid peroxidation and antioxidant enzymes’ activity induced by the metal when injected alone. Additionally, in our previous in vitro work (Papandreou et al., 2011) both saffron extract and its crocin component provided strong protection against H₂O₂-induced toxicity in human neuroblastoma SH-SYSY cells, by repressing reactive oxygen species production. It has been reported that crocin, the di-gentiobiosyl ester of crocetin, promotes mRNA expression of γ-glutamylcysteinyl synthase, the rate-limiting enzyme of GSH synthesis, in PC12 cells under hypoxic conditions (Ochiai et al., 2007). Accordingly, crocin may mediate the observed brain antioxidant protection of saffron extract under Al neurotoxicity.

5. Conclusions

Our findings show that long-term intake of a relative high dose of AlCl₃ through drinking water resulted in metal accumulation in adult mouse brain tissues and exerted neurotoxic effects, as evidenced by the declined learning and memory capacity, metal-induced inhibition of ChEs, changes of MAO and the production of oxidative damage. On the other hand, short-term co-administration of saffron extract at the end of the treatment period beneficially affected mouse brain oxidative stress and antioxidant status markers and MAO activity that were disturbed by Al. The bioavailability of crocin in mouse brain after saffron extract administration through intraperitoneal route demonstrated in the present study, supports the implication of this bioactive component in the observed neurochemical alterations. However, the particular saffron treatment scheme was ineffective in reversing cognitive defects induced by the metal, although it had beneficial action on biochemical markers of brain function. The findings support further investigation of the potential of saffron and its crocin constituents as neuroprotective agents.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fct.2012.11.016.

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