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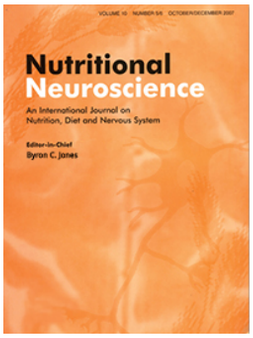

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Evaluation of modulatory effects of saffron (*Crocus sativus* L.) aqueous extract on oxidative stress in ischemic stroke patients: a randomized clinical trial

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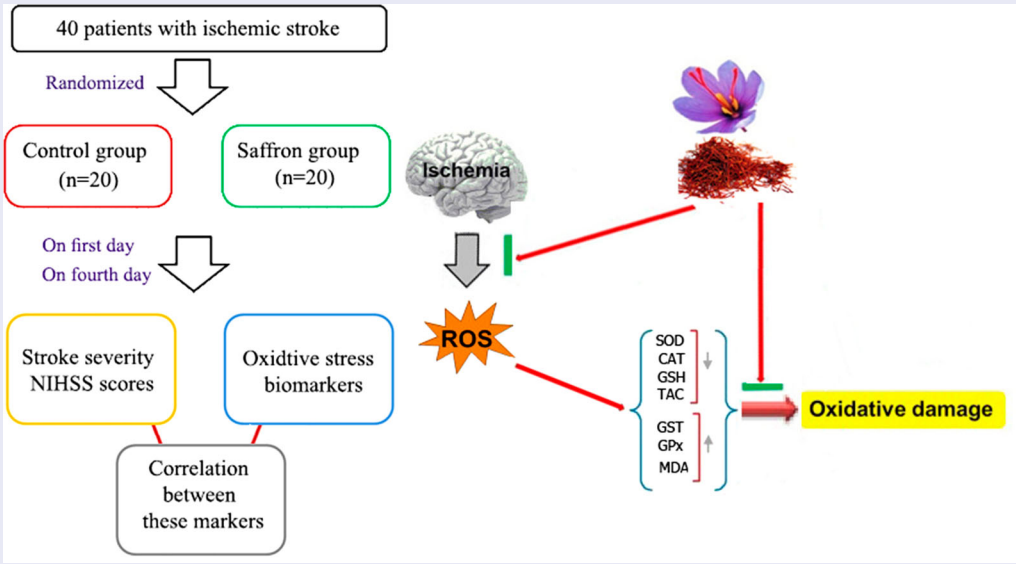
ABSTRACT

Objectives: Saffron (*Crocus sativus L.*) has been widely used in traditional medicine as a treatment of nervous disorders. Saffron as an antioxidant can be considered effective for treatment of oxidative stress in ischemia stroke. Therefore, the aim of the present study was to investigate the role of aqueous extract of saffron in reducing oxidative stress in ischemic strokes patients.

Methods: Forty patients with acute ischemic stroke were randomly divided into two groups including control group and saffron group. During 4 days of experiment, control group received routine stroke care and saffron group received routine care plus capsule of saffron 400 mg/day (200 mg twice per day). Then, two groups were compared using the National Institute of Health Stoke Scale (NIHSS) and serum oxidative stress biomarkers, at the time of hospital admission and 4 days later as well.

Results: On the fourth day after ischemic stroke onset, antioxidant enzymes activities and glutathione (GSH) and total antioxidant capacity (TAC) levels were higher in the saffron group compared to the control group, while malondialdehyde (MDA) level was lower. In addition, the severity of stroke, based on the NIHSS scores, was significantly reduced after 4 days in the saffron group. The severity of stroke was negatively correlated with the levels of GSH and TAC and positively correlated with MDA level.

Conclusions: Saffron has modulatory effects on ischemic-induced oxidative stress due to its free radical scavenging and antioxidant properties. Thus, saffron extract can be considered as a potential candidate therapy of the ischemic brain.



KEYWORDS
Ischemic stroke; saffron; National Institutes of Health Stroke Scale; oxidative stress; serum; strok severity; antioxidant enzymes; glutathion; Malondialdehyde

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Introduction

Stroke, as a serious medical problem, is caused by a sudden interruption of blood flow to a particular area of the brain, which leads to the loss of neurologic function and even death. It is the third common cause of death after cancer and myocardial infarction in western countries [1,2]. According to the World Health Organization, 15 million people worldwide have a stroke each year, of which 5 million die and 5 million are permanently disabled. It should be noted that strokes are pathologically classified into ischemic and hemorrhagic types. Eighty-seven per cent of the strokes occur as a result of thrombotic or embolic occlusion of a cerebral artery called ischemic strokes (IS), which causes blood not to reach the brain [3,4]. It is also a common cause of permanent disability among adults worldwide [5].

Oxidative stress plays an important role in the pathogenesis of ischemic brain injury [6]. Ischemia leads to the increased production of reactive oxygen species (ROS) and impaired antioxidant defenses, so that all of them result in overt oxidative stress and subsequent neuronal injury. High levels of ROS make a number of direct detrimental effects, such as lipid peroxidation and protein and DNA oxidation, as well as multiple cell signaling effects resulting in the initiation of inflammation and cell death pathways [7,8]. For this reason, supplementations with exogenous source of antioxidants detoxify excessive ROS and inhibit oxidative stress-mediated brain injury in stroke [9]. In this regard, herbal medicines derived from plant extracts with natural antioxidant and pharmacological activities are being increasingly utilized to treat a wide variety of clinical diseases [10,11]. Saffron (*Crocus sativus L.*) is a member of Iridaceae family that is widely used as an herbal medicine. It has been originaly used as a spice for flavoring and coloring food and various energizing drinks for years. This plant is cultivated in Iran, Greece, Turkey, Spain, India, China and Egypt [10,12,13]. In Iran, it has been cultivated in the south Khorasan province from ancient times [12].

Saffron has been used in traditional medicine in various countries as an antispasmodic, nerve sedative, anticonvulsant, diaphoteric, expectorant, stimulant and stomachic. It has also been utilized in the treatment of learning and memory impairment, inflammatory disease and respiratory, hepatic and cardiovascular disorders [10,13,14]. One of the most important properties of saffron is its exhilarant and anti-depressant activity, which leads to the sense of happiness [12]. In Indian traditional medicine (Ayurveda), it is used as an adaptogen (considered to strengthen the body against stresses such as trauma, anxiety and fatigue) [15]. In Iranian traditional medicine, saffron has been used to cure obstructions inside brain to protect it from oxygen deprivation. In addition, the topical use of saffron in the boiling water is good for severe headaches and insomnia [14].

Saffron has the ability to scavenge free radicals because of its main constituents including carotenoids, safranal, picrocrocin, crocetin, crocin and quercetin. It has also the inhibitory effects on lipid peroxidation of membrane and, thus, protects cells from oxidative stress [10,11,16]. Vakili et al. reported the protective effects of saffron against ischemia/reperfusion injury, cerebral edema and decreased infarct volume in a rat model of stroke [17]. Several studies have been demonstrated that saffron has antioxidant activity and reduces oxidative damages in hippocampus [18,19] and muscle skeletal [20] during ischemia/reperfusion-induced oxidative damage in rats.

It should be noted that some of the positive effects of saffron on the antioxidant status have been established using in vitro or in vivo animal studies, but whether these effects are identical in human still remains unclear [21]. Thus, further clinical studies are necessary to elucidate its potential benefits in human. To our knowledge, this is the first study investigating the effect of saffron extract on the antioxidant status of the patients with acute IS. The aim of the present study was to evaluate these effects on serum oxidative stress biomarkers in IS patients and, also, find any correlation between these markers and the patients' clinical status (stroke severity) using the National Institutes of Health Stroke Scale (NIHSS).

Methods

Patients

This clinical trial study was conducted at Imam Hussein hospital of the Shahid Beheshti University of Medical Sciences, Tehran, Iran. Forty patients with middle cerebral artery (MCA) ischemic stroke participated in this study, who had been referred to the emergency unit of the neurology center of hospital between December 2017 and July 2018. The incidence of cerebral ischemic stroke was initially diagnosed by an attendant neurologist using the clinical examination and brain computed tomography (CT) scan or magnetic resonance imaging (MRI) immediately on admission to hospital. The severity of the neurologic deficit was measured by the NIHSS scores at the time of hospital admission and discharge (on the first and fourth days of hospitalization). NIHSS is a 24-point scale (11 items) and categorized as mild (1–4), moderate

(5–15), moderate to severe (16–20), and severe (21–24) [22]. The inclusion criteria were the patients' NIHSS scores between 5 and 20 on admission (severity of stroke: moderate and moderate to severe) and less than 24 h since the onset of the stroke. The exclusion criteria included suffering from liver and kidney diseases, hemorrhagic stroke, acute ischemic heart stroke in the last 48 h, NIHSS>20 during hospital stay, smoking, history of previous neurological diseases, using medications with antioxidant properties in the previous two months, central nervous system tumor and recent head trauma. Prior to the study, all patients were asked to complete a checklist of variables including age and gender by a research physician. Besides that, the participants or their relatives completed and signed an informed consent form.

All of the participants with IS were randomly divided into the control ($n = 20$) and saffron ($n = 20$) groups using random Graph pad software. Both groups received standard treatment during the study, including statin, warfarin, aspirin, clopidogrel, ticlopidine and dipyridamole. The saffron group received routine care plus capsule of saffron 200 mg twice per day during four days of hospitalization after the stroke. The control group received only the standard treatment. The mean hospital length of stay for all patients was four days. The patients and their families received stroke education. In addition, daily physiotherapy services were also done for patients. The ethics committee of the Baqiyatallah University of Medical Sciences approved the study protocol (IR.BMSU.REC.1397.028). The number of clinical trial assignment is IRCT201 80806040716N1.

Preparation of saffron capsules

Saffron stigmas were purchased from Talakaran-e-Mazraeh Company, Torbate-Heydarieh, South Khorasan, Iran. It was identified by botanists in the herbarium of the Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences (specimen number: P-408). The stigmas of saffron were air-dried in the shadow and, then, milled into fine powder before extraction. 100 g milled saffron stigma were mixed with 1000 ml of distilled water. After that, it was kept for 48 h at 30–33°C for drying. The yield of extraction was 24 g of freeze-dried powder for 100 g of the dry stigma. The extract was then delivered to the Niak Darou Company (Tehran, Iran) to make the capsules containing 50 mg of saffron extract and 150 mg starch. Notably, the extract analysis showed that it contains 20% crocin, used as the base for filling capsules. Saffron delivery was based on the crocin content in each capsule.

Quantification of saffron extract

The chemical compounds in the saffron stigma were analyzed according to the ISO 3632-2. The quantity of crocin (dye agent-yellow dye), picrocrocine (bitter agent- flavour expressed as bitterness) and safranal (volatile agent) with values of 259, 96.9 and 41.5 were detected at 440, 257 and 330 nm, respectively [20].

Blood samples

Fasting venous blood samples were taken from all patients on the first and fourth days of hospitalization. After clot forming, the serum was separated from the coagulated blood by centrifugation at 1500×g for 15 min at 4°C. Samples were then transferred into 0.5 ml microtubes and stored at –80°C until analysis.

Biomarkers of oxidative stress

Serum superoxid dismutase (SOD) activity was measured based on the inhibition of the reduction of nitro-blue tetrazolium by superoxide at 560 nm. Catalase (CAT) activity was determined by monitoring the initial rate of disappearance of H_2O_2 at 240 nm. Glutathione S-transferase (GST) activity was assayed based on monitoring the formation of the thioether product of the reaction between glutathione (GSH) and 1-chloro-2, 4-dinitrobenzene at 340 nm. GSH level was performed at 412 nm by the use of 5, 5'-dithiobis 2-nitrobenzoic acid [23]. Serum glutathione peroxidase (GPx) and glutathione reductase (GR) activities were determined based on the decrease in absorbance of NADPH at 340 nm using KiaZist Company kits (Tehran-Iran). The serum total antioxidant capacity (TAC) was measured by ferric reducing/antioxidant power (FRAP) method using commercially available kit (ZellBio GmbH, Germany). Malondialdehyde (MDA) level as an indicator of lipid peroxidation was determined by condensation with thiobarbituric acid at 532 nm using Teb Pazhouhan Razi Company kit (Tehran-Iran).

Statistical analysis

The statistical analysis of the data was conducted using the IBM SPSS software package version 22 (SPSS Inc., Chicago, USA). Chi-square test was used for analysis of patients' gender and severity of stroke and independent samples *t*-test was used in comparison of age between the two groups. Statistical differences of NIHSS scores were evaluated with the Mann–Whitney U test for comparison across two groups, followed by

Wilcoxon test. Data were tested for normal distribution with the Kolmogorov–Smirnov test. Two-way repeated-measures analysis of variance (ANOVA) test was used to test within and between subject differences in oxidative stress biomarkers levels. Correlation between the the severity of stroke and oxidative stress biomarkers were estimated by Pearson's Correlation coefficients. Results were expressed as mean ± SD. Statistically signifcant values were considered when $P < 0.05$.

Results

Clinical characteristics of stroke patients in two groups

In the control group, there were 20 IS patients, mean age 72.25 ± 10.24 years. Among them, 16 were males (80%) and 4 were females (20%). Besides that, among 20 IS patients in the saffron group, there were 12 males (60%) and 8 females (40%), mean age of 70.20 ± 11.11 years. Based on the NIHSS scores, the severity of stroke was significantly reduced on the fourth day in the saffron group (7.85 ± 3.28) compared to the fourth day in the control group (10.65 ± 4.40, $P = 0.040$) and the first day in the saffron group (11.35 ± 3.77, $P = 0.007$). Notably, the NIHSS score on the fourth day ($P = 0.000$) was significantly lower than the first day in both control and saffron groups (Figure 1).

Parameters of oxidative stress

After four days, serum SOD (17.35%, $P = 0.002$) and CAT (16.53%, $P = 0.026$) activities in the control group were significantly lower than the first day. Besides that, SOD (22.93%, $P = 0.000$) and CAT (24.34%, $P = 0.004$) activities in the saffron group was significantly higher than the control group (Figure 2).

On the fourth day of IS, serum GPx (29.18%, $P = 0.000$) and GST (25.54%, $P = 0.034$) activities were higher than the first day in the control group. In addition, after four days of the treatment with capsules of saffron, GPx ($P = 0.000$) and GST ($P = 0.003$) activities were significantly increased compared to the first day in both control and saffron groups. There was no significant difference in

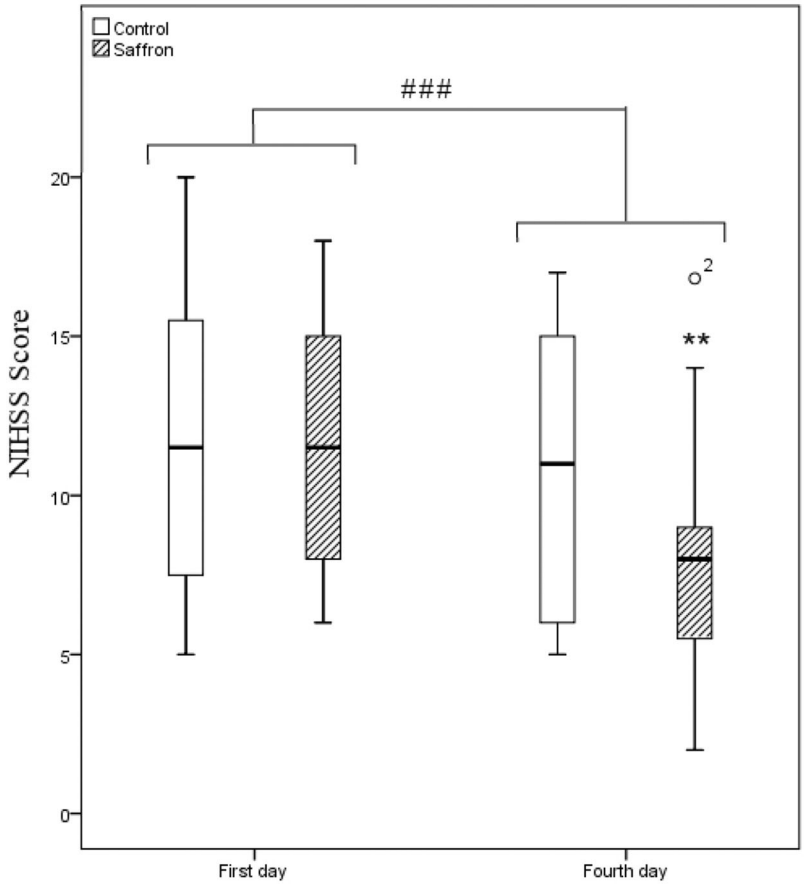

Figure 1. The severity of stroke based on the NIHSS score in patients with acute ischemic stroke in the control and saffron groups on the first and fourth days ($n = 20$/group). $^{**}P < 0.01$ vs. the first day in the saffron group and $^{###}P < 0.001$ vs the first day in both control and saffron groups.

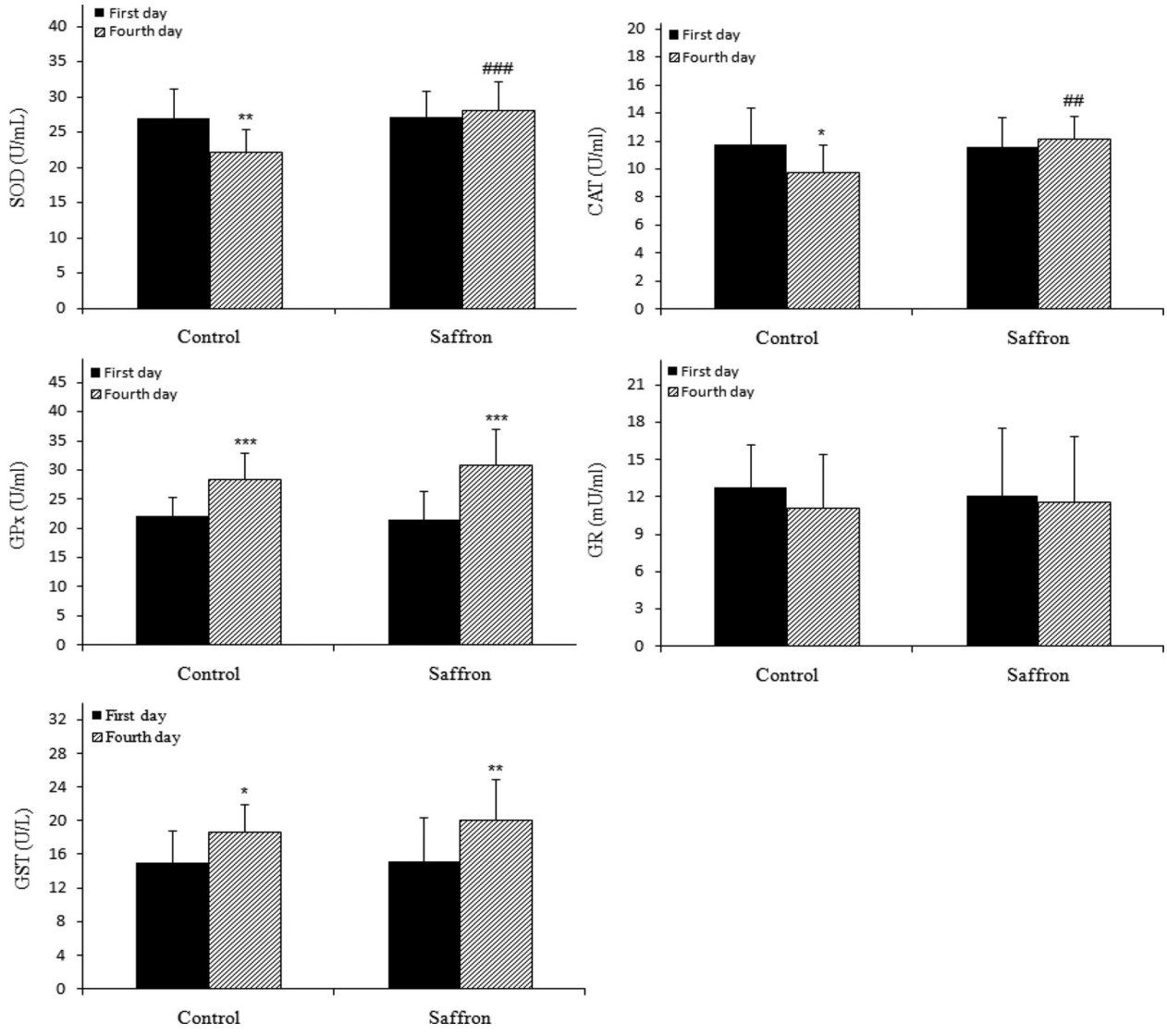

Figure 2. Serum antioxidant enzymes activities in patients with acute ischemic stroke in the control and saffron groups on the first and fourth days. Values are expressed as mean ± SD ($n = 6$). *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ vs. the control and saffron groups on the first day; $^{\#\#}P<0.01$ and $^{\#\#\#}P<0.001$ vs the control group on the fourth day. SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; GR: glutathione reductase; GST: glutathione S-transferase.

activity of serum GR between the first and fourth days in the two stroke groups (Figure 2).

On the fourth day after IS, serum GSH (12.02%, $P = 0.023$) and TAC (12.52%, $P = 0.026$) levels were lower than the first day in the control group of IS patients. After four days, GSH (19.30%, $P = 0.001$) and TAC (16.49%, $P = 0.008$) levels in the saffron group was higher than the control group. In addition, serum MDA level were also significantly higher than the first day in the control group (13.48%, $P = 0.011$). On the other hand, MDA level in the saffron group after 4 days was significantly lower than that of the control group on the fourth day (20.33%, $P = 0.000$) and the saffron group on the first day (11.09%, $P = 0.044$) (Figure 3).

Correlations between parameters

According to Pearson correlation analysis, the severity of stroke based on the NIHSS score was negatively correlated with the levels of GSH ($r = -0.233$, $P = 0.038$) and TAC ($r = -0.250$, $P = 0.025$). On the other hand, it is positively correlated with MDA level ($r = 0.257$, $P = 0.021$) in IS patients (Figure 4). A significant positive correlation was observed between MDA level and SOD ($r = -0.336$, $P = 0.002$) and CAT ($r = -0.235$, $P = 0.036$) activities. However, no significant correlation was observed between NIHSS score and the activities of SOD ($r = -0.110$, $P = 0.333$), CAT ($r = -0.043$, $P = 0.707$), GPx ($r = -0.166$, $P = 0.142$), GST ($r = -0.160$, $P = 0.157$) and GR ($r = -0.049$, $P = 0.667$).

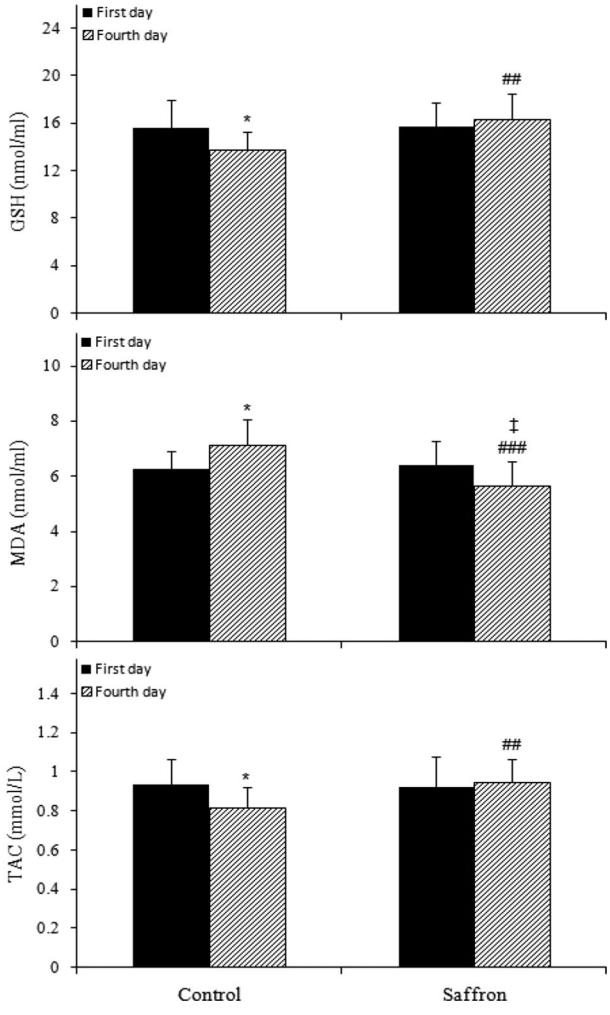

Figure 3. Serum glutathione (GSH), malondialdehyde (MDA) and total antioxidant capacity (TAC) levels in patients with acute ischemic stroke in the control and saffron groups on the first and fourth days. Values are expressed as mean ± SD ($n =$ $P < 0.05$ vs. the control group on the first day; $^{\#\#}P < 0.01$* .(20 and $^{\#\#\#}P < 0.001$ vs the control group on the fourth day and $^{\ddagger}P < 0.05$ vs. the saffron group on the first day.

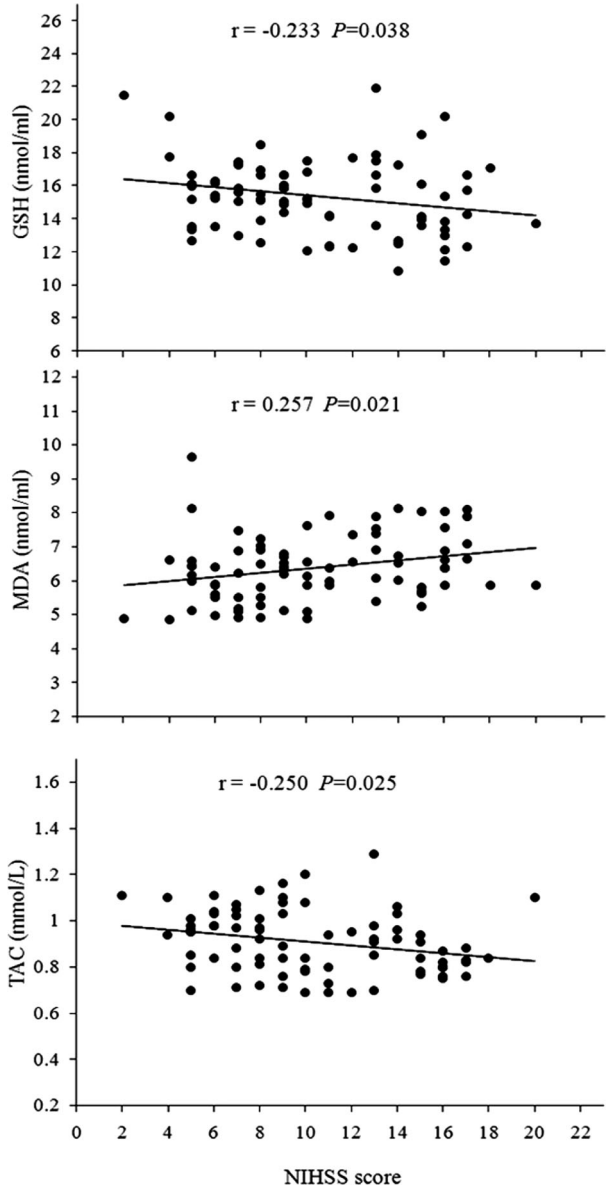

Figure 4. Correlation of the severity of stroke based on the NIHSS score with oxidative stress biomarkers using determining Pearson correlation coefficients.

Discussion

Herbal medicines derived from plant extracts are being increasingly utilized to prevent oxidative damages caused by ROS through clinical diseases such as stroke [10,11]. Saffron is one of these plants with a beneficial neuroprotective activity on brain ischemia in the rat [19,24] and human [22]. The results of our study confirmed the modulatory effects of saffron capsules on oxidative changes in patients with IS. In fact, saffron's effect is made through alleviation of antioxidants depletion and inhibition of lipid peroxidation, all of which led to the functional improvement of the patients assessed by NIHSS score.

Oxidative stress affects the onset, progression, and complications of acute ischemic stroke [4,6]. It is characterized by the excess production of ROS via enzymes of NADPH oxidase, xanthine oxidase and cyclooxygenase, which can damage macromolecules in cellular components. The brain is particularly vulnerable to oxidative damage due to its high oxygen consumption, intense production of ROS, low levels of endogenous antioxidant enzymes, high content of polyunsaturated fatty acids and high levels of iron, all acting as pro-oxidants under pathological conditions [1,8,9]. The activity of antioxidant enzymes such as SOD and CAT may be an essential factor providing protection against neurological damage [6]. SOD dismutases superoxide anion into hydrogen peroxide (H_2O_2). CAT degrades H_2O_2 to water and oxygen, and, thus,

increases the overall efficacy of SOD [3,25]. Our results indicated that the activities of antioxidant enzymes including SOD (17.35%) and CAT (16.53%) on the fourth day were significantly lower than the first day in IS patients, which may be a consequence of the increased ROS production during acute IS [6]. In fact, the decreased SOD activity might increase the endogenous superoxide anion, which inhibits CAT activity. The depletion of CAT activity leads to the accumulation of H_2O_2, which may inhibit SOD activity. Both superoxide anion and H_2O_2 can generate hydroxyl radical through the Haber–Weiss and Fenton reactions. Besides, superoxide anion can also react with NO to form peroxynitrite radical, all of which rapidly cause lipid peroxidation and oxidative stress [25,26]. Previous reports on the role of antioxidant enzymes activities in acute IS patients with acute ischemic stroke are contradictory. Several studies reported decreased SOD and CAT activities in plasma and erythrocytes at 1 and 7 days, in patients with acute IS [27,28]. On the other hand, others showed the increase of SOD and CAT activities at 1 and 7 days [1,29], while others found no significant differences [3,30]. Milanlioglu et al. [6] indicated that serum SOD activity on days 1, 5 and 21 and CAT activity on day 5 were significantly lower in IS patients. They also showed that SOD activity was significantly lower on both 5th and 21st days than the 1st day. These contradictory results might be due to the time after stroke, type of stroke and the existence of different measurement methods and isoenzymes [3]. In our study, treatment of patients with capsule of saffron could return these antioxidant enzymes activities to normal values, which might have occurred due to the decreased ROS and restoring GSH level (Figure 3), and led to the reduction of oxidative damage and brain infarction [26]. High antioxidant activity of this plant can be attributed to crocin, safranal and crocetin compounds which have high reducing power and scavenging potential for ROS [11]. Several studies showed the protective roles of saffron and its carotenoid components in neuronal cell injuries *in vivo* and *in vitro,* through the formation of peroxidized lipids inhibition, SOD activity restoration and neuronal morphology maintenance [31,32]. Treatment with saffron was also effective in reducing hippocampal MDA level and increasing hippocampal antioxidant status. There is another possibility that saffron extract acts as a hormetin leading to the activation of antioxidative enzymes [18].

GSH is a powerful antioxidant and free radical scavenger that plays an important role in the maintenance of cellular redox environment and acts as a substrate for several enzymes including GPx, GST and GR [26,33]. GPx degrades H_2O_2 to water and oxygen in the presence of adequate amount of GSH. It has a much higher affinity for H_2O_2 than CAT [6]. GST catalyzes the conjugation of ROS to GSH, producing less toxic forms, and also reduces lipid peroxides [9,34]. In the current study, a significant decrease in GSH level (12.02%) as well as an increase in GST (25.54%) and GPx (29.18%) activities were observed on the fourth day without any changes in GR activity, compared to the first day in IS patients. The elevated activities of these antioxidant enzymes are probably due to the cellular adaptive response to the increased ROS production [34]. These findings are in agreement with the results of the previous reports [1,28,30]. Cojocaru et al. showed that, in acute IS patients, plasma GSH level was lower at 1 and 7 days, while GPx was lower in the first 24 h and higher at 7 days [27]. Two other studies have shown that GSH level in serum and erythrocytes was significantly increased within 24 and 48 h post-stroke [30,35]. Several studies have indicated that GPx, GST and GR activities did not significantly differ at 1, 5, 7 and 21 days after stroke [3,6]. Our results also revealed a significant increase in GSH level as well as GPx and GST activities of patients after daily treatment with saffron capsule. It is indicating that saffron could scavenge the reactive free radicals eventually leading to the reduced oxidative damage in the tissues [11]. Elevated GSH levels could be a part of defense mechanisms against oxidative stress, providing neuroprotection against amino acid excitotoxicity in stroke [35]. Several studies have shown that saffron and its active constituents increased the activities of GST, GPx and level of GSH in rat model of IS [18,36].

MDA is an end-product of lipid peroxidation. It is produced due to the oxidation of polyunsaturated fatty acid side chains of membranes, which changes their functional properties [37]. Our results showed a significant increase in MDA level (13.48%) in serum of IS patients on the fourth day compared to the first day. The increase in lipid peroxidation was associated with a decrease in GSH level in IS patients, which could severely impair tissue antioxidant function and make the tissue more susceptible to potential oxidative stress. The same results have been obtained by different studies at days 1, 2, 3 and 7 after stroke [27,35,38]. According to these studies, serum MDA level was not significantly different within 1,3, 5, 7 and 21 days after stroke onset [6,30]. Notably, according to our results, the administration of saffron capsules significantly decreased the MDA level, suggesting that saffron possesses potential anti-peroxidant properties (Figure 3). In fact, the constituents of saffron have a potential antioxidant activity and allow free radicals to attract a

hydrogen atom from the antioxidant molecule rather than from polyunsaturated fatty acids. Thus, it leads to breaking of the chain of free radical reactions and the resulting antioxidant radical becomes a relatively nonreactive specie [39]. The saffron extract prevents tissues injury by free radicals scavenging activity through the increase in cellular antioxidants levels such as SOD, CAT and GSH. Therefore, it leads to the prevention of ROS accumulation, stabilization of cell membrane and suppression of cellular oxidative stress [34]. Previous studies have demonstrated that saffron and its active constituents could decrease lipid peroxidation in hippocampus during ischemia/reperfusion-induced oxidative damage in rats [18,19].

Measurement of TAC evaluates the efficiency and ability of all antioxidants present in the biological fluids to protect against oxidative damage to membranes and other cellular components. In fact, TAC is used as a new clinical biomarker for diagnosis, prognosis and prevention of many diseases [40]. Our study demonstrated less serum TAC level in IS patients on the fourth day, suggesting a disturbance in prooxidant-antioxidant balance in the patients [41]. This finding supports a strong relationship between impairment of antioxidant defense in the pathogenesis of IS. Our results are in accordance with the previous reports showing the decrease of TAC at 1 and 7 days after acute ischemic stroke [3,27]. Gariballa et al. showed that plasma levels of TAC at 2, 3 and 7 days were lower than the first day in patients with acute IS [38]. The reduced TAC level may be due to the lower plasma ascorbic acid level in stroke patients, which was correlated with the degree of neurological impairment after IS [38,42]. However, the increase in the TAC level in saffron group indicated that saffron increases antioxidants levels and, therefore, may protect against the adverse effects of free radical production during IS [42]. Previous studies have reported the beneficial effects of antioxidant supplementation in acute IS [28,42].

Our results have demonstrated that the severity of the neurological deficit assessed by the NIHSS score on day four was significantly lower than the first day in the saffron-treated stroke patients. A significant positive correlation between NIHSS score and serum GSH, MDA and TAC levels in this study suggests a strong antioxidant defense system in saffron group, which, in turn, leads to further decrease in lipid peroxidation as well as stroke severity. However, no significant correlation was found between NIHSS score and antioxidant defense enzymes activities. Several studies have reported that SOD and GPx activities and MDA level are significantly correlated with infarct size and stroke severity [30,35]. However, some studies showed no significant correlation between NIHSS score of patients, serum antioxidant enzymes activities, TAC level and GSH levels at the time of hospitalization and 1 week later [3,4,6].

It is possible that the changes in enzymatic and non-enzymatic antioxidants coupled with increased lipid peroxidation following IS, have been part of a signaling pathway, which leads to the apoptotic cell death and neuronal damage. Apoptosis is induced by the production of ROS during IS through a direct interaction with nuclear factor κB (NF-κB) and subsequent activation of the MAPK/JNK pathway of cell death. NF-κB is known to activate the redox state of the cell in a number of disease pathologies. It increases the expression of nitric oxide synthase, cyclooxygenase-2 (COX-2), matrix metalloproteinase-9 and cytokines genes, all of which are involved in a number of detrimental pathways of cerebral ischemia such as apoptosis, blood–Brain Barrier breakdown and inflammation [8,9]. Inflammatory pathways may lead to the depletion of GSH and increase in MDA, perhaps through increasing oxidative stress [37]. In addition, overproduction of ROS in the tissue is accompanied with the depletion of ATP, accumulation of intracellular Ca^{2+}, the release of cytochrome c from mitochondria to cytosol, the activation of caspases and the increase in catabolic enzymes such phospholipases. Notably, it eventually causes necrosis or apoptosis of the cells [7,9]. However, many of these pathways can be blocked using some antioxidants such as N-acetyl-cysteine, flavonoids and polyphenols [8,9]. The mentioned data suggests that saffron extract may protect the brain by preventing free-radical damage and oxidant-radical release through its prevention of proinflammatory processes. Numerous studies have indicated that saffron extract and its derivative forms have anti-inflammatory and antiapoptotic properties through the NF-κB modulation, suppression of COX-2 expression and activation of caspase 3, inhibition of cytokines production and release of cytochrome c to the cytosol [43,44]. Crocin inhibits neuronal PC-12 cell death induced by both internal and external apoptotic stimuli [45]. Finally, we suggest that the modulatory effect of saffron extract on inflammation and cell death in the patients with IS should be investigated further.

Conclusion

According to our results, oxidative stress plays an important role in the pathogenesis of acute IS as indicated by decreased GSH level and TAC levels and antioxidant enzymes activities as well as increased lipid

peroxidation. In this regard, saffron has found to have beneficial effects against ischemic injury-induced oxidative stress through increasing reduced antioxidants levels and attenuating lipid peroxidation. The mentioned effects are because of free radical scavenging and antioxidant properties of its constituents. Therefore, saffron extract can be a potential candidate for ischemic stroke therapy. However, further researches are needed to illustrate its exact mechanism of action on IS and better confirm our findings.

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Disclosure statement

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