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Original Research Article

The protective influence of saffron and selenium on oxidant disturbances in brain of rats exposed to acrylamide

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Abstract

Acrylamide (ACR) is an industrial neurotoxic chemical that has been recently found in carbohydrate-rich foods cooked at high temperature. ACR is a potent neurotoxic in human and animal models. The present study aimed to recapitulate the potential neuroprotective effect of saffron and selenium in acrylamide-induced neurotoxicity. Seventy male Wistar rats were divided into seven groups served as control group, groups treated orally with selenium (0.04 mg/kg), saffron 30mg/kg, selenium (0.04 mg/kg) and saffron (30 mg/kg), and Acrylamide (50 mg/kg) for 8 days, and groups treated orally with saffron and selenium before and after 8 days of ACR treatment. The results indicated that treatment with ACR alone resulted in a significant increase in serum Lipid Profile, AST, CK, LDH, brain tissues of MDA, GPx and SERT accompanied with reduced in serum SOD, ALT, and GST in brain tissues when compared with control group. Treatment with saffron and selenium before or after ACR treatment reduces or partially antagonized the effects induced by ACR towards the normal values of control. Only weak and transient DNA damage was recorded in the brain homogenate. The treatment in combination of saffron with selenium after acrylamide treatment partially antagonized the effects induced by ACR through an antioxidative mechanism.

Key Words: Acrylamide, Comet assay, Neurotoxicity, Saffron, Selenium, Serotonin transporter

INTRODUCTION

Acrylamide is a vinyl monomer derived from a wide range of foods through the Maillard-Browning reaction during the cooking process (Postles *et al.,* 2013). It is formed when food high in carbohydrates and low in proteins are cooked at high temperature or undergo thermal processing at temperatures of 120ºC or higher (Lineback *et al.,* 2012). Since humans are chronically exposed to ACR at very low levels through consumption of thermally processed carbohydrate rich foods, it is imperative to explore if common dietary components can alleviate the possible neurotoxic impact. Consumption of these foods may result in significant human exposure to ACR. The biological consequences of ACR exposure have chiefly centered on neurotoxicity ever since this effect was observed in humans occupationally exposed to this compound. ACR neurotoxicity may be attributed to its higher affinity to form adducts with glutathione, proteins, and DNA directly or after metabolized to its epoxide, glycidamide (2, 3-epoxy1propanamide), which produce severe lesions. (LoPachin and Gavin, 2008).

The involvement of oxidative stress and inflammatory responses in ACR neurotoxicity are widely accepted (Tareke *et al.,* 2009). Studies of neurotransmitter

distribution and receptor binding in the brain of rats have revealed changes induced by acrylamide (Alturfan *et al*., 2012). ACR-induced oxidative stress in nervous system (brain, spinal cord and sciatic nerve) and sensory and motor dysfunction in rats (Zhu *et al*., 2008). ACR significantly reduced the proliferation of mouse neuronal progenitor cells and induced apoptotic cell death via elevation in the reactive oxygen species (Park *et al.,* 2010).

Food composition and food additives play major role in providing the required antioxidants for the body. In addition, plants with neurological bioactivity can either stimulating or depressive activity on central nervous system have been in certain classes of compounds, namely, alkaloids (Campos *et al.,* 2005), phenolic and polyphenolic compounds (Coleta *et al*., 2006), amino acids and flavonoids (Tarrago *et al.,* 2008). Several researches have shown that spices containing phenolic and flavonoid compounds indicated antioxidant activities (Reddy and Lokesh, 1992). A positive linear correlation among phenolic compounds and flavonoids with antioxidant capacity of spices has also been reported (Zheng *et al.,* 2007).Saffron is one of the most expensive spices in the world, apart from its traditional value as a food additive and herbal medicine. Saffron has been cultivated as a spice for at least 3500 years in Egypt and Middle East (Fernandez, 2004). Beneficial effects of saffron have been demonstrated in models of neurona, and other disorders (Bathaie and Mousavi, 2010). Furthermore, administration of saffron (60 mg/kg body weight) to normal and aged mice for 7 days significantly improved learning and memory as assessed by step-through passive avoidance test and this was correlated with the significant cerebral antioxidant protection (Papandreou *et al.,* 2011).Other studies have also demonstrated neuroprotective effects of saffron and its constituents in vitro and in rodent models of brain disorders (amnesic and ischemic) (Ochiai *et al*., 2004). The first small-scale clinical trials of saffron against depression and mild Alzheimer's disease have brought forward promising results (Akhondzadeh *et al.,* 2010). Shati *et al.* (2011) demonstrated the ameliorative effects of aqueous saffron extract administration against Aluminum-induced neurotoxicity, by presenting changes of brain antioxidant enzymes, serum tumor markers and brain expression of genes.

Selenium (Se) is an essential micronutrient required for cellular antioxidant systems. In addition to acting as an essential nutrient for the immune system and overall body function, it is apparent that selenium also plays a critical role in the operation of the nervous system. Selenium itself is a constituent of selenoproteins, which are primarily involved in antioxidant function and redox status. However, apart from its covalent incorporation into these proteins, selenium also performs neuroprotective actions independent of translational processes.

Furthermore, low selenium intake has detrimental effects on proper brain function, such as epileptic episodes and neuronal cell death, which have, in turn, been shown to be mitigated by higher selenium levels. Understanding the mechanisms of selenium action will be crucial to determining its potential as a preventive and therapeutic agent against excitatory brain damage. In the last 10year, there has been intense interest in Se supplementation and its role in health. Major dietary sources of Se are plant foods (provided the soil is not deficient in Se), animal kidneys, seafood, egg yolk and Brazil nuts. Besides, the soil Se level is reflected in the concentrations seen in plants (Combs *et al.,* 2001). Se is incorporated into proteins to make selenoproteins, which are important antioxidant enzymes [especially, glutathione peroxidase (GSH-Px)]. The antioxidant properties of selenoproteins help prevent cellular damage from free radicals. Free radicals are natural by-products of oxygen metabolism that may contribute to the development of chronic diseases (Burk and Hill, 2005). Glutathione (GSH) could be one of the primary events in ACR-induced neurotoxicity. Se as a component of GSH-Px significantly increased GSH and GSH-Px levels and can partially prevent the biochemical changes of the rats which received ACR. Morphological studies indicated that acrylamide-induced neurotoxic syndrome was associated with nerve damage characterized by distal axonal swelling with neurofilament accumulation and retrograde degeneration in both central and peripheral myelinated axons (Postles *et al.,* 2013). However, the mechanisms underlying these processes are still not well understood. Sickles et al. proposed that acrylamide bound to and inhibited the motor protein kinesin, leading to the inhibition of the anterograde and retrograde transport and Inadequate support of the distal axon or terminals producing the behavioral outcomes (Sickles *et al.,* 2002), others thought that aberrant cell body processing and deficient axonal transport induced by acrylamide decreased Na/K-ATPase activity. Accumulation of Na+ and loss of K+ reversed operation of the Na/Ca exchanger, resulting in the distal axon degeneration (LoPachin and Gravin, 2008). Acrylamide induces genetic damage through binding of its metabolite, glycidamide, with DNA, and causes disturbances in the oxidative status and enzyme activities through the release of large numbers of free radicals in the body (Nixon et al., 2012).

So the present study was carried out to investigate the protective and curative role of saffron with selenium in acrylamide – induced neurotoxicity in rats to determine the possible antioxidant mechanisms.

Material and Methods

Seventy white male Wistar rats weighs 180-220 grams were obtained from the animal facility of King Fahd Medical Research Center, King Abdulaziz University,

Jeddah, Saudi Arabia. The animals were conditioned at room temperature and commercial balanced diet and tap water, ad libitum was provided throughout the experiment. Animals were divided randomly into seven groups and were subjected to the following schedule of treatments. Group 1 (G1): Rats were fed daily by oral gavage with normal saline. Saffron Group (G2): Rats were fed daily by oral gavage with saffron (30 mg/kg) for 8 days. Selenium group (G3): Rats were fed daily by oral gavage with selenium (0.04 mg/kg) for 8 days. Acrylamide Group (G4): Rats were fed daily by oral gavage with Acrylamide (50mg/kg) for 8 days. Treated Group SS (G5): Rats were fed daily by oral gavage with selenium (0.04 mg/kg) and saffron (30 mg/kg) for 8 days. Treated Group, SS → ACR (G6): Rats were fed daily by oral gavage with selenium (0.04 mg/kg) and saffron (30 mg/kg) for 8 days before ACR (50mg/kg) exposure for 8 days. Treated Group, ACR → SS(G7): Rats were fed daily by oral gavage with selenium (0.04 mg/kg) and saffron (30 mg/kg) for 8 days after ACR (50mg/kg) exposure for 8 days. At the end of each specified period, rats were anesthetized using diethyl ether and serum samples were collected. Anesthetized animals were scarified by cervical dislocation and the brains were rapidly dissected out.

Serum Biochemical analysis

Total cholesterol assessed by using enzymatic colorimetric kit as described by (Roeschlau *et al.,* 1974). Enzymatic colorimetric kit used for measured triglycerides as described by (Fossati and Prenape, 1982). An enzymatic colorimetric kit used for the determination of High-Density Lipoprotein Cholesterol (HDL-C) as described by (Lopes-Virella *et al*., 1977). Aspartate Transaminase (AST), Alanine Aminotransferase (ALT), Lactate Dehydrogenase (LDH) and Creatine Kinase (CK) activity were measured using a dichromatic rate technique at 340 nm wave length according to Tietz (2006). Superoxide dismutase (SOD) activity was assessed using a Xanthine oxidase system to generate superoxide radicals (O2 -) as described by Kakkar *et al.* (1995).

Oxidative Stress Markers of Brain Homogenate

The Glutathione S-Transferase (GST) Assay Kit measures total GST activity (cytosolic and microsomal) by measuring the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione (Habig *et al*., 1974). Glutathione peroxidase (GPx) activity was assayed by NADPH oxidation at 340 nm wave length as described by Paglia and Valentaine (1967). Thiobarbituric Acid Reactive Substances TBARS assay kit used to assay Malondialdehyde (MDA) according to (Yoshioka *et al.,* 1979) as a marker for oxidative stress. Serotonin Transporter (SERT) assay kit was measured spectrophotometrically at a wavelength of 450nm ± 10nm (Hsu *et al.,* 1981) and Na^+ K^+ - ATPase activity in brain homogenate was assyed according to the method described by Tsakiris *et al*., (2000).The protein content in the brain tissue was determined according the method described by Lowry *et al*. (1951).

Comet assay (molecular study)

For comet assay, one gram of crushed brain samples was transferred to 1 ml ice-cold phosphate buffer saline. This suspension was stirred for 5 min and filtered and used to evaluate the DNA damage parameters (Tailed %, Untailed %, Tail length, Tail DNA% and Tail moment) according to (Singh and Stephen, 1997).

Statistical analysis

Statistical analyses were performed using Microsoft office excel and SPSS 16.0. The variability degree of the results is expressed as mean ± standard of means (mean ±SD). The significance of the difference between samples was determined using one way ANOVA. The difference was regarded as significance when $p \geq 0.05$, where p is a value for comparing between groups.

RESULTS

The data in Table (1 and 2) indicate that G4 increased significantly ($p \leq 0.05$) in lipid profile, AST, ALT, LDH and CK and significant ($p \leq 0.05$) decrease in SOD and Na^+ K^+ - ATPase activity as compared to G1. G2, G3and G5 showed decline for lipid profile levels and CK, but non-significant ($p > 0.05$) in the mean value of AST , ALT, LDH ,SOD concentration and Na^+ K^+ - ATPase activity as compared to G1. G6 and G7 showed non- significant ($p > 0.05$) change for lipid profile levels and SOD, but significant ($p \leq 0.05$) increase in AST and CK and non-significant ($p \leq 0.05$) reduction in ALT, LDH values and Na^+ K^+ - ATPase activity as compared to G1 .

The data in Table (3) indicate that brain tissues GPx, MDA and SERT levels increased ($p \leq 0.05$) significantly in G4, and significantly ($p \leq 0.05$) decreased in GST as compared to G1. G2 and G3 showed significantly ($p \leq 0.05$) decreased in GPx and SERT, while non-significantly ($p > 0.05$) increased in GST and MDA levels as compared to G1. G5 showed significantly ($p \leq 0.05$) decreased in GPx and MDA level, while, GST and SERT levels concentration has non significantly ($p \leq 0.05$) change as compared to G1. Significantly ($p \leq 0.05$) decreased in GST, while MDA, GPx and SERT concentration has non-significantly ($p > 0.05$) increase

Table 1. Serum lipid profile for groups of Saffron, selenium, both, before and after Acrylamide administration on rats.

Groups \ Parameters	Triglycerides mg/dl	TC mg/dl	HDL-c mg/dl	LDL-c mg/dl	VLDL-c mg/dl
G1	132.5^{c}± 16.9	82.5^{c}±1.84	29.3^{b}± 5.87	13.8^{c}±4.31	34.6^{b}±3.68
G2	164.5^{b}± 7.7 *	88.4^{c}±0.84 N.S	23.9^{c}± 2.68 *	23^{c}±1.69 N.S	34.9^{b}±1.55 N.S
G3	154.5^{b}± 5.4 *	93.4^{b}±0.49 N.S	21.9^{c}± 1.48 *	22^{c}±1.82 N.S	33.9^{b}±2.42 N.S
G4	276.8^{a}± 11. *	180.55^{a}±1.2 *	61^{a}± 1.41 *	68.9^{a}±3.25 *	55.35^{a}±2.19 *
G5	142^{c}± 5.6 N.S	96^{b}±1.41 *	34.3^{b}± 1.69 N.S	27.85^{c}±9.4 N.S	33.4^{b}±1.69 N.S
G6	161.5^{b}± 3.5 N.S	94.3^{b}±0.7 *	32.05^{b}± 3.39 N.S	41.4^{b}±1.12 N.S	32.3^{b}±5.37 N.S
G7	134.5^{c}± 1.5 N.S	110.9^{b}±0.7 *	27.4^{c}± 1.05 N.S	56.45^{b}±8.2 *	25^{c}±0.7 *

G1 = Control G2= SAFG3= SE G4= ACR G5= SS G6= SS → ACRG7= ACR → SS
TG = Triglycerides **TC**= Total Cholesterol
HDL-C = High Density Lipoprotein Cholesterol
LDL-C = Low Density Lipoprotein Cholesterol
VLDL-C = Very Low Density Lipoprotein
Values are expressed as mean value of ± S.D
***** = Significant ($P \leq 0.05$)
N.S = Non significant ($P > 0.05$)

Table 2: Effect of Saffron, selenium, both, before and after acrylamide administration on Liver Enzymes, Cereatin kinase, lactate dehydrogenase, and Superoxide Dismutase in rats

Groups \ Parameters	AST U/L	ALT U/L	CK U/L	LDH U/L	SOD U/L
G1	55.93^{c}±3.25	30^{b}±1.4	39.95^{c}±1.33	252^{c}±2.83	2.12^{b}± 0.14
G2	54.8^{d}±8.38 N.S	29.5^{c}±1.04 N.S	41.4^{c}±6.75 *	262.5^{b}±2.12 N.S	2.06^{b} ± 0.21 *
G3	58.8^{c}±8.38 N.S	31^{b}±0.7 N.S	37.4^{c}±6.43 *	277.5^{b}±3.11 N.S	2.01^{b} ± 0.31 *
G4	145.1^{a}±7.86 *	61.5^{a}±5.65 *	176.55^{a}±16.33 *	915.5^{a}±2.53 *	0.96^{a}± 0.04 *
G5	49^{d}±5.01 *	35^{b}±2.82 N.S	44.83^{c}±4.95 *	243^{c}±2 N.S	2.08^{b} ± 0.02 *
G6	67.26^{b}±5.75 N.S	29.4^{c}±2.5 *	68.19^{b}±8.16 *	270.5^{b}±4.5 *	1.57^{c}± 0.01 *
G7	58.6^{c}±4.75 N.S	33^{b}±4.01 N.S	66.45^{b}±0.58 *	269.5^{b}±6.5 *	2.38^{b} ± 0.03 N.S

G1= Control G2= SAFG3= SE G4= ACR G5= SS G6= SS → ACRG7= ACR → SS
AST= Aspartate Aminotransferase
ALT= Alanine Aminotransferase
LDH = Lactate Dehydrogenase
CK = Ceatine kinase
SOD = Superoxide Dismutase
Values are expressed as mean value of ± S.D
* = Significant ($P \leq 0.05$)
N.S = Non significant ($P > 0.05$)

Table 3. Brain homogenate for oxidative stress markers for groups of Saffron ,selenium, both, and before and after Acrylamide administration

Parameters / Groups	GPx Activity nmol/min/ml	GST Activity nmol/min/ml	MDA µM	SERT ng/ml	$Na^+ K^+$ - ATPase µmol Pi/h /mg protein
G1	322.14^{b}±14.21	430.42^{c}±1.41	5.34^{b}±0.07	0.89^{b}± 0.01	
G2	279.07^{c}±16.01	454.28^{c}±12.65	5.54^{b}±0.07	0.87^{b} ± 0.01	7.94^{b}± 0.34
G3	284.07^{b}±13.01	438.21^{c}±12.65	4.93^{c}±0.05	0.85^{b} ± 0.03	7.84^{b}± 0.28
G4	674.56^{a}±4.59	327.96^{a}±8.06	7.38^{a}±0.42	1.16^{a} ± 0.04	6.82^{a}± 0.19
G5	291.07^{c}±9.01	434.21^{c}±9.12	3.99^{d}±0.03	0.86^{b} ± 0.05	7.88^{b}± 0.18
G6	328.55^{b}±14.41	309.7^{b}±10.65	5.72^{b}±0.06	0.78^{b} ± 0.04	8.01^{b}± 0.22
G7	289.75^{b}±1.8	328.41^{b}±4.62	4.95^{c}±0.21	0.82^{b}± 0.01	6.98^{a}± 0.19

G1= Control G2= SAFG3= SE G4= ACR G5= SS G6= SS → ACRG7= ACR → SS
GST= Glutathione S-transferase
GPx= Glutathione Peroxidase
MDA = Malondialdehyde
SERT= Serotonin Transporter
Values are expressed as mean value of ± S.D
GST= Glutathione S-transferase
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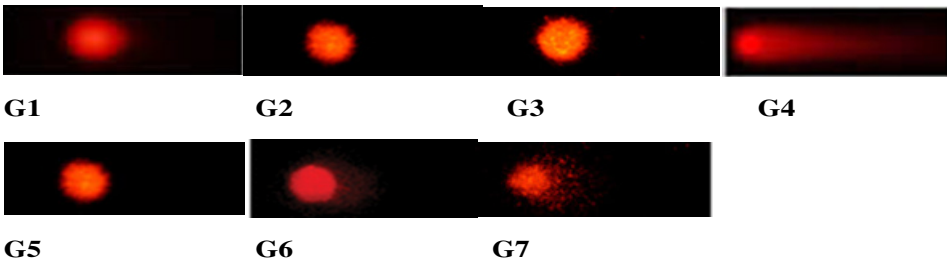

Figure 1. Comet assay of genomic DNA of rats brain cells for control and different treated groups: (G1) control, (G2) SAF, (G3) SE, (G4) ACR, (G5) SS, (G6) SS → ACR, and (G7) ACR → SS

Table 4. Effects of acrylamide alone or in combination with saffron and selenium on genomic DNA of rat's brain cells.

	G1	G2	G3	G4	G5	G6	G7
Tailed%	6.01±1.9	5.88±0.7	6.1±.061	9.05±0.78	6.1±1.1	6.03±0.8	7.3±1.07
Untailed%	95±1	93.62±1	94.7±1	90±1.03	95±1	95±0.9	91.7±1
Taillengthµm	2.58±0.49	2.83±0.62	2.71±.0.2	3.91±0.13	2.76±.56	2.6±0.4	3.16±0.13
Tail DNA%	1.93±0.31	1.87±0.42	1.79±0.35	3.39±.16	1.92±0.3	1.92±0.4	2.8±0.26
Tail moment	4.69±0.9	4.22±1	4.63±.08	13.1±0.92	4.38±.98	5.5±1	6.21±0.62

G1= Control G2= SAFG3= SE G4= ACR G5= SS G6= SS → ACRG7= ACR → SS
Values are expressed as mean value of ± S.D

showed in G6 as compared to G1. G7 showed significantly ($p \leq 0.05$) decreased in GPx, GST, MDA level, SERT concentration and $Na^+ K^+$ - ATPase activity.

Brain homogenate were treated and harvested and life cells are embedded within agarose on a glass slide. Cells were then lysed, and DNA is unwound under alkaline conditions followed by electrophoresis and ethidium bromide staining. Damaged DNA migrates towards the anode, resulting in an appearance of a comet. G1-Digital image of Unteated cells (control) after single cell gel electrophoresis as observed in the fluorescence microscope. Digital image of G2, G3, G4, G5, G6, and G7 treated groups are illustrated respectively (Figure 1). Treatment with ACR produced significant increases in comet assay tail moment in the brain tissue. In acrylamide treated group tailed%, tail length, DNA tail %,

and DNA moments were increased, while untailed % significantly decreased compared with control (Figure 1). These elevations were significantly decreased while untailed % significantly increased in other treated groups with acrylamide in combination with saffron and selenium. No changes in the levels of DNA damage in brain cells were observed between the experimental groups. In the treatment group with saffron and selenium tissues examined, no increase in DNA damage was seen before ACR exposure (Table 4).

DISCUSSION

Risk factors in food are either of chemical or microbiological origin, or a combination of both. Acrylamide (ACR), one such risk factor, is a possible human carcinogen. Acrylamide is a chemical compound used in many technological applications and can be formed naturally when foods, especially those are rich in sugars and low in protein cooked at high temperatures during (e.g. frying, grilling, baking or toasting). It has several harmful health effects including neurotoxicity, carcinogenicity, reproductive toxicity, genotoxicity, and mutagenicity. Humans have chronic contact with acrylamide through eating, e.g. fried potato chips and/or French fries; cereal products, including bread, breakfast cereals, cakes and biscuits; as well as, roasted coffee and probably also from smoking. Based on food contents, the average daily intake of ACR in western countries were estimated to be in the range of 0.2–1.4 mg/kg bw for adults and 3.4 mg/kg bw among younger age groups (Dybing *et al*., 2005). Once absorbed, acrylamide may be conjugated by glutathione-S-transferase (GST) to N-acetyl-S-(3-amino-3-oxopropyl) cysteine or it reacts with cytochrome P450 (CYP450) to produce glycidamide (Sumner *et al.,* 1992). The major metabolite formed in both rat and mouse is N-acetyl-S-(3-amino-3-oxopropyl) cysteine, accounting for approximately 70% of the urinary metabolites observed in the rat and 40% of those observed in the mouse (Sumner *et al.,* 1997). A growing body of evidence indicates that the nerve terminal is a primary site of ACR action and that inhibition of corresponding membrane fusion processes impairs neurotransmitter release and promotes eventual degeneration (LoPachin *et al*., 2004).

The need for nueroprotective drugs with high efficacy and low toxicity has led to studies of putatively protective factors in fruits, vegetables, herbs, and spices. Saffron, which consists of the dry stigmas of the plant *Crocus sativus* L., is used as a spice and a food colorant. In folk medicine, it has been used in the treatment of numerous diseases. 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 (Musaie *et al*., 2013).

Selenium (34Se), an antioxidant trace element, is an important regulator of brain function. These beneficial properties that Se possesses are attributed to its ability to be incorporated into selenoproteins as an amino acid. Several selenoproteins are expressed in the brain in which some of them, e.g. glutathione peroxidases (GPxs), thioredoxinreductases (TrxRs) or selenoprotein P (SelP), are strongly involved in antioxidant defence and in maintaining intercellular reducing conditions (Randjelovic, *et al.,* 2012): Protective effect of selenium on gentamicin-induced oxidative stress and nephrotoxicity in rats. Drug and Chemical Toxicology, 35(2): 141–148.

The current study showed that oral administration of ACR (G4) to rats at dosage levels 50 mg/kg b.wt., for 8 days induced a significant ($p \leq 0.05$) decrease in body weight gain percent when compared to the negative control group (G1). This result are in agreement with the result reported by Wang *et al.* (2010) who suggested that acrylamide exerts detrimental effect on growth and development of immature male rats. Another explanation of body weight retardation may be resulted from total protein deficiency. This is consistent with Abdul-Hamid *et al.* (2007) who suggested that, the reduction of body weight resulted from growth and protein deficiencies due to malnutrition during the development. It also, may have resulted from excessive break down of tissue proteins (Chatterjea and Shinde, 2002) or decreased in both plasma and tissue proteins (Yousef and El-Demerdash, 2006). In relation to brain weight there was significant ($p \leq 0.05$) increase in brain weight of saffron with ACR rats; the results showed that weight of these organs increased when compared to the neurotoxic group. Our results were in agreement with those of Abd El-Azime *et al.* (2014) who reported that Saffron exerts its modulating effect in the organs under investigations due to the presence of associated bioactive compounds with antioxidant properties.

Results of the present study revealed that feeding of rats on ACR (G4) resulted in significant ($p \leq 0.05$) increases in serum levels of TC, TG, LDL-C, HDL-C and VLDL-C levels as compared to the negative control group (G1). Our results agreed with those obtained by Teodor *et al. (*2011), who found that Acrylamide intake is associated with significantly altered levels of total cholesterol, LDL-cholesterol, triglycerides. Seddek et al. (2013) examined the effect of daily ACR 50 mg/kg BW for consecutive 5 days on male albino rats. ACR-treated group showed significant increases in serum levels of TG, TC, LDL-C and VLDL-C, but significant decrease in HDL-C level compared with control and other treated groups. In contrast, Rawi et al. (2012) reported that treatment with ACR did not induce any significant differences in serum levels of HDL-C and LDL-C in male and female rats. Our results revealed that administration of saffron or selenium and both after and before ACR exposure rats decreased in serum levels of TC, TG, LDL-C, VLDL-C and HDL-C. The present results agreed with

Cousins and Miller (1985) who reported that, there were hypolipidemic effects of crocetin by its intraperitoneal injection in rat. A 10-day treatment with crocin significantly reduced serum TG, total cholesterol (TC), LDL and very low-density lipoprotein (VLDL) levels (He *et al.,* 2007). Increased selenium and lipid concentrations could be the consequence of a common exposure. Selenium is incorporated into selenoproteins as selenocysteine. Selenoproteins, including glutathione peroxidases, iodothyronine deiodinases, selenoprotein P, and thioredoxin reductase, are responsible for the biological functions of selenium. Above selenium levels needed to maximize serum selenoprotein concentration and activity (70–90 ng/mL Se in serum or plasma), increases in serum selenium, however, reflect the nonspecific incorporation of selenomethionine replacing methionine in albumin and other serum proteins. The association between selenium and lipid concentrations could then be driven by a common dietary factor or by general over nutrition, but this association was not modified after adjustment for body mass index or use of vitamin-mineral supplements (Bleys *et al.,* 2008).

Serum AST and ALT are the most sensitive biomarkers used in the diagnosis of liver diseases (Pari and Kumar, 2002). Our results revealed that ACR (G4) rats cause significant ($p \leq 0.05$) elevation in serum levels of AST and ALT enzymes. Our results were in agreement with those of Khalil and AbdEl-Aziem, (2005) who showed increment in serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities following ACR treatment in immature male and female rats as compared to their corresponding controls. These results confirmed by the hypothesis recorded by Chinoy and Memon (2001), who attributed the increase in the serum AST and ALT activities to the bipolar nature of ACR, where the CH2=CH part may undergo hydrophobic interactions while the $CONH_2$ part can form hydrogen bonds with the cell compounds. This property may enhance its ability to alter the cell membrane structure and make the parenchymal cell membrane of liver more permeable. The present study showed that oral administration of saffron or selenium and both with neurotoxic rats induced a significant ($p \leq 0.05$) decrease in ALT and AST as compared to ACR rats (G4). The enzymatic activity of G5, G6, and G7 being almost the same as in the case of the control group. These results were coincided with Iranshahi *et al.* (2011) who suggested that aqueous and ethanolic extracts of saffron significantly decreased the levels of AST and ALT in plasma rats induced by CCl_4, and he suggested that aqueous and ethanolic extracts of saffron exhibit hepatoprotective effects against liver damages induced by CCl_4 in mice.). The simultaneous intake of selenium or as a dietary supplement partially prevented some of the biochemical changes in rats which received high doses of acrylamide, evidencing its beneficial role in case of acrylamide intoxication (Ali *et al.,* 2014).

The results showed that there were significantly ($p \leq 0.05$) decreased in LDH level in saffron and/or selenium with ACR treated rats. This result agreed with Hosseinzadeh *et al.* (2009) who indicated that administration of saffron was found to considerably reduce the isoproterenol-induced raise in the activities of lactate dehydrogenase). Excessive oxidative stress in the Se deficiency, as indicated by changes in the GSH-Px/GST activity, which in turn the LDH- level (Capcarova *et al*., 2014).

Shuming *et al.* (2009) reported that CK activity in brain and blood seems to be the most sensitive indicators of acrylamide intoxication. The results found that treatment only with ACR resulted in a significant increase in serum creatine kinase activity. Increase the activity of CK may due to changes of cell membrane and mechanical damage of the muscle fibers. Lactate dehydrogenase (LDH) and creatine kinase (CK) enzymes can increase not only produce energy and lactate, but play effective roles during inflammatory conditions in muscle cells. Therefore, some researchers have attributed LDH and CK levels increment to muscle fibers membrane damage (Choung *et al.,* 2004, González-Garrido *et al.,* 2015). Shuming *et al.* (2009) investigated the protective effects of dark soy sauce against acrylamide (ACR)-induced neurotoxicity in rats, rats were given dark soy sauce (0.5 ml/kg body weight/day) before, after, and during ACR treatment (10 mg/kg body weight/day) for 8 weeks in total. Treatment only with ACR resulted in a significant increase in lactate dehydrogenase and serum creatine kinase activity in brain homogenate. The current results of oral administration of saffron or selenium and both when given with ACR to rats revealed that significant ($p \leq 0.05$) decrease in CK. These results are agreement with Musaie *et al.* (2013) who reported that a significant decrease in CK after 8 days consumption of saffron supplement. Since saffron prevents oxidation of different enzymes by free radicals and reactive oxygen species, its level may remain high and hence reduces CK levels immediately after activity. Tissue damage in animals deficient in both selenium and vitamin E is thought to arise from impaired scavenging of peroxides and oxygen-derived radicals. These radicals cause damage to cell constituents and may result in cell death. However, the cellular antioxidant systems dependent on selenium and vitamin E should not be considered separate from other processes in the cell that may influence the scavenging of potentially damaging free radicals or provision of substrates for peroxidation.

Malondialdehyde (MDA) is a main degradative product of lipid peroxidation. It may indirectly represent the level of lipid peroxidation. Acrylamide inhibited the action of lactate dehydrogenase in brain and serum. These changes were accompanied by increased braindopamine receptors in a concentration- dependent manner. Nonetheless, acrylamide caused increased in the activities of malondialdehyde due to the lipid peroxidation

process induced by free radical caused by acrylamide toxicity. GSH is a major intracellular antioxidant, as well as an important component in the metabolism of many xenobiotics, including ACR. Cellular oxidative stress can either lead to a depletion of GSH and apoptosis. Therefore glutathione-ACR adduct formation can favor cellular oxidative stress, which may be one possible mechanism governing ACR toxicity. In present study, ACR (G4) showed significant ($p \leq 0.05$) reduce in serum SOD and brain tissue GST and significant increase in GPx and MDA levels (marker of lipid peroxidation extent) in brain tissues. This results were agree with Khalil and Abdel Aziem (2005) who reported that ACR administration increased the lipid peroxidation while decreased the activities of superoxide dismutase and glutathione-S-transferase (GST) and increased the activities of glutathione peroxidase (GPx) as a consequence of GSH depletion after ACR exposure. A decrease in SOD means there is an imbalance between prooxidant and antioxidants scavenger system, and this occurs when lipid peroxidation overload take places (Wu and Cederbaum, 2003). Also, ACR binds to iron atom and make iron depletion, which may affect SOD enzyme activity (Burek *et al.,* 1980). Decreased activities of SOD might have been caused by the accumulation of superoxide radicals and H_2O_2, thereby consuming the SOD activity. The present result also, agree with other reports which reported significant decrease in GST activity of rat brain and liver intoxicated with ACR (Shukla-Pradeep *et al*., 2002). This suggests an increased utilization of this antioxidant enzyme with subsequent depletion to counter the increased level of free radicals induced by acrylamide in these tissues. While GPx used GSH as cofactor to remove hydrogen peroxide, the increase in GPx activities could be combat free radical generation during ACR toxicity (Ghorb *et al.,* (2015). Finally, increase in MDA may be an indicator of lipid peroxidation (Diplocke, 1994). The current results of oral administration of saffron or selenium and both when given with ACR to rats revealed that significant ($p \leq 0.05$) increase in SOD, non-significant change in GST ($p > 0.05$), significant decrease ($p \leq 0.05$) in GPx and MDA. These results are agree with Asdaq and Inamdar (2010) who reported that saffron and crocin showed significant fall in elevated levels of MDA and GPx and significant increase in SOD in hyperlipidmic rats. They suggested that both saffron and crocin prevented the elevation of MDA and GPx in serum resulting in potent antioxidant effect. The carotenoids scavenge free radicals, especially superoxide anions and thereby may protect cells from oxidative stress (Ochiai *et al.* 2004). Among the constituent of saffron stigmas, crocins and crocetin derivatives are most abundant with established antioxidant and antitumor effects. The antioxidant enzymes of *Crocus sativus* root was also measured, and quantitatively classified as superoxidedismutase (SOD) (Keyhani and Keyhani, 2007). In contrast to the present study, Ahmad *et al.,* 2005 reported that increased activity of GST in lung and liver tissues from mice treated with crocetin, thereby suggesting that this carotenoid could be influencing host detoxification processes. The administration of Se, as a component of GSH-Px in combination with ACR significantly lowered lipid peroxidation, and enhanced glutathione levels. Our results show that selenium supplement can partially prevent the biochemical changes in the rats which received high doses of acrylamide. There were no significant differences between groups G4, G5 and G6 and control in all hematological parameter (Teodor *et al*., 2011; Ali *et al.* 2014). The serotonin transporter clears the synaptic cleft from serotonin and, therefore, has an important rolein the regulation of serotonergic neurotransmission. Given that the serotonin transporter has a role in clearing extracellular serotonin. Evidence suggests acrylamide (ACR) neurotoxicity is mediated by impaired presynaptic transmission, that ACR-induced synaptic dysfunction involves adduction of presynaptic protein thiol groups and subsequent reduction in neurotransmitter release. The present study showed that oral administration of ACR (G4) induced a significant ($p \leq 0.05$) increase in SERT in brain tissue when compared to the negative control group. No previous literature was available regarding this result (Qusti and Qahtani, 2015). Greater serotonin transporter binding potential may be viewed as a contributing factor for lowering extracellular serotonin levels, which may be particularly important when other factors, such as greater intracellular degradation of serotonin, happen to be present. Independent of the underlying mechanism, a reduction in serotonin transporter numbers is expected to be similar in its consequences to a pharmacologic serotonin transporter blockade, a mechanism of action shared by many antidepressant medications. Since higher serotonin transporter density is associated with lower synaptic serotonin levels. ACR impaired neurotransmitter uptake into striatal synaptic vesicle (LoPachin *et al*., 2008). So, this result is agree with Mannaa *et al.* (2006) who reported that ACR highly significant decrease in whole brain serotonin level in the immature male and female rats following ACR treatment.

The current results revealed that oral administration of saffron or selenium and both with ACR rats significantly ($p \leq 0.05$) decrease in SERT level in brain tissue. In this respect, Ghorb *et al.,* (2015) reported that crocin and safranal inhibit reuptake of dopamine, norepinephrine and serotonin. Saffron improvements in the action of the neurotransmitter serotonin, antioxidant effects, protecting the brain against the damaging effects of ACR. Georgiadou *et al.* (2012) reported that saffron may exert its antidepressant effect by modulating the levels of certain chemicals in the brain, including serotonin (a mood-elevating neurotransmitter). Saffron increases serotonin levels in the brain. saffron extract might inhibit serotonin reuptake in synapses. Inhibiting

synapticserotonin reuptake keeps serotonin in the brain longer, thereby enhancing its positive effects while combating depression. This proposed mechanism is supported by animal studies, which demonstrated antidepressant properties in extracts sourced from multiple parts of the saffron plant. Selenium will influence compounds with neurotransmitters in the brain, and this is postulated to be the reason selenium affects moods in humans and behavior in animals. Nutritional deficit of selenium decreases the brain antioxidant protection in experimental conditions by the decrease in glutathione peroxidase activity. These results suggest that the decrease of brain protection against oxidative damage could induce brain damage by disturbing the turnover rate of some monoamines (Pan *et al*., 2015).

Lehning et al., (1998) stated that decreased Na^+ K^+ - ATPase activity has been considered as possible mechanism for peripheral nerve axon damage induced by ACR. Previous reports indicated that Na^+ K^+ - ATPase is delivered to axon and nerve terminal sites by a kinesin-based rapid anterograde transport (Lomber *et al*., 1986). Yet, in distal tibial nerve axons of severely affected ACR-intoxicanted rats. loPachin and Gravin (2008) found that axolemmal Na^+ K^+ - ATPase was normal with respect to corresponding protein content and enzyme activity. Significant increased were noticed in Na^+ K^+ - ATPase activity in the brain homogenate within treatment groups, although there was an insignificant decrease in the enzyme activity in brain of animals treated with ACR alone. Moreover, the present results suggest that ACR exposure does not alter either anaerobic or aerobic energy production in central nervous tissues (Sickles *et al.,* 1990). No previous literature was available regarding this result. Thus, this study could be considered the first to investigate the effect of saffron in combination with selenium in SERT and Na^+ K^+ - ATPase activity.

Using comet assay, ACR induced DNA damage whereas in the treatment group before ACR exposure, no increase in DNA damage was seen. These results suggest that additional cellular factors beyond adduct formation may affect the DNA damage caused by acrylamide *in vivo* (Ellwanger *et al*., 2015). To explain the mechanism of acrylamide inducing genotoxicity, it has been reported that most sensitive endpoints of genetic toxicity for acrylamide are kinesin inhibition and oxidative stress. These enzymes centre and then segregate the chromosomes and then depolymerise the mitotic spindles. Inhibition of kinesin is consistent with the mitotic inhibition and the aneuploidy observed *in vitro* in fibrosarcoma cells. Therefore, the increase in oxidative stress could enhance the damage of the biological macromolecules such as protein, lipid and DNA (Davis and Recio, 2007). This observation showing induction DNA damage by ACR in previous reported tumor target sites suggests this event may carcinogenicity in the rat. Other potential mechanisms have been suggested for ACR tumorgenicity, such as the induction of oxidative stress. For the latter mechanism, both ACR and glycidamide have shown to conjugate with glutathione, which may lead to depletion of glutathione and resulting oxidative stress (Ibrahim et al., 2015). The DNA-damaging effect of the ACR could be a simple biomarker of acrylamide exposure and genotoxicity. The use of antioxidants to prevent genetic damage induced by physical or chemical agents is currently of considerable interest.

CONCLUSION

Short-term co-administration of saffron extract and selenium at the end of the treatment period beneficially affected mouse brain oxidative stress, antioxidant status markers, and SERT and Na^+ K^+ - ATPase activity that were disturbed by ACR. The decrease in glutathione levels and GSH-Px activity might be one of the primary events in the ACR-induced hematological. The administration of Se as a component of GSH-Px in combination with saffron significantly lowered lipid peroxidation, and enhanced glutathione levels. To prove this hypothesis, intervention with glutathione and glutathione peroxidase should be further investigation and the potential of saffron and selenium combination as neuroprotective agents.

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