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Original Research Article

Neuroprotective role of Saffron against Acrylamide-Induced Neurotoxicity in Rats by Antioxidative Activity

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Abstract

Acrylamide 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 in acrylamide-induced neurotoxicity. Sixty male Wistar rats were divided into six groups served as control group, group treated orally with saffron 300mg/kg for 8 days, group treated orally with ACR 50mg/kg for 8 days, and groups treated orally with saffron for 8 days before, during or after 8 days of ACR treatment. The results indicated that treatment with ACR alone resulted in a significant increase in serum Lipid Profile, AST, ALT, CK, LDH, brain tissues of MDA, GPx and SERT accompanied with reduced in serum SOD and GST in brain tissues when compared with control group. Treatment with saffron before, during or after ACR treatment reduces or partially antagonized the effects induced by ACR towards the normal values of control. Histopathological investigation of brain tissues showed the beneficial effect of saffron on neuron damage induced by ACR. The treatment with saffron before and after acrylamide treatment partially antagonized the effects induced by ACR, and was more effective than when administration at the same time, partly through an anti oxidative mechanism.

Key words: Acrylamide, Neurotoxicity, Saffron, Serotonin transporter

INTRODUCTION

Thermal cooking is an important method of food processing as it increases the palatability and to extend the range of colors, tastes, aromas and textures in foods produced from similar raw materials. Thermal processes also destroy toxic microorganisms and lower the water activity of the food thereby preserving the foods. Baking, toasting, frying, roasting and sterilization result in desired and undesired effects due to various chemical reactions. The major concern arising from heating processes comes from the formation of compounds that are not naturally present in foods, but that may develop during heating or preservation processes and that reveal harmful effects such as acrylamide.

Acrylamide is a dietary contaminant and environmental toxicant. Assessment of the presence of acrylamide is a great concern in many countries. According to the results obtained so far, potato products account for around 50% and baking products and bread for around 20% of human exposure to acrylamide (Keramat et al., 2011).

Several observations have led to the hypothesis that heating of food could be an important source of human exposure to acrylamide. It is formed in foods, if the heating/frying is done in an oven, on a frying pan or by microwave heating, whilst no acrylamide has been detected in boiled food products (Torqvist, 2005). Therefore, detection of acrylamide in food has become a very important issue in food safety. Based on food contents, the average daily intake of ACR in western countries was 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, Children eat more acrylamide than adults

probably because of their higher caloric intake relative to body weight as well as their higher consumption of certain acrylamide-rich foods, such as French fries and potato crisps (Dybing et al., 2005). Acrylamide is rapidly absorbed from the gastrointestinal tract following oral administration and it is rapidly absorbed into the circulation and thereafter distributed to various organs, reacts with cellular DNA, hemoglobin, nerve cells and enzymes (Rayburn and Friedman, 2010). The traditional standard method for acrylamide detection is gas chromatography coupled with mass spectrometry (GC–MS) (Castle, 1993) and-Tandem mass spectrometry (MS/MS)(Hamlet et al., 2004) for the advantages of high accuracy, sensitivity, and selectivity.

The toxicity of acrylamide involves the enhancement of cellular oxidative stress by depleting glutathione which is the major cellular antioxidant, generation of reactive oxygen species and DNA damage (Mei et al., 2008).The acrylamide toxicity is considered to be hepatotoxic and causes lipid peroxidation (Abou Donia et al., 1993). Acrylamide have significant binding capacity to brain (Sumner et al., 1997). Neurotoxicity of acrylamide is well established in humans and experimental models. It is known to affect both central and peripheral nervous system, hampering various sensory and motor functions (LoPachin and Gavin, 2008). Acrylamide neurotoxicity in both laboratory animals and humans is characterized by ataxia and distal skeletal muscle weakness (Yu et al., 2006). However, ACR intoxication in rodent models is associated with selective nerve terminal damage in both central and peripheral nervous systems. 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, 2008). 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). Accordingly, antioxidants protect the body from damaging oxidation reactions by reacting with free radicals and other reactive oxygen species; therefore, diseases linked with free radicals can be prevented by antioxidant therapy. The subject has gained an immense importance and current research trends are directed towards finding naturally occurring antioxidants particularly of plant origin (Ak and Gulcin, 2008).

Saffron is the dried stigmas of Crocus sativus L. (Iridaceae) flowers and it is one of the most precious and expensive spice in the world (Mousavi, 2010). The herb contains orange pigments and typically used as a spice with coloring and flavoring properties in a wide range of culinary, bakery confectionery preparations and as a perfume .The extracts of saffron and its active constituents have anticonvulsant, antidepressant, anti-inflammatory and antitumor effects (Hosseinzadeh and Younesi, 2002). Saffron extract is also reported to be chemopreventive and showed protective effects on genotoxins-induced oxidative stress in animals (Abdullaev et al., 2003).In recent years beneficial effects of saffron have been demonstrated in models of neuronal, 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 conferred (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., 2007). 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 expressionof genes. Therefore, the present study was conducted to investigate neuroprotective effect of saffron aqueous extract on some serum biochemical parameters, brain homogenate parameters and brain histopathological changes in acrylamide rats.

MATERIAL AND METHODS

Sixty white male Wistar rats weighs 190-225 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 six groups (10 rats each in two cages) and were subjected to the following schedule of treatments :Control group (G1): Rats were fed daily by oral gavage with saline for8 days before dissection. Group 2 (G2): Rats were fed daily by oral gavage with saffron (300 mg/kg) for 8 days. Group 3 (G3): Rats were fed daily by oral gavage with Acrylamide (50mg/kg) for 8 days. Group 4 (G4): Rats were fed daily by oral gavage with saffron (300 mg/kg) for 8 days before ACR (50mg/kg) exposure for 8 days. Group 5 (G5): Rats were fed daily by oral gavage with saffron (300 mg/kg) during ACR (50mg/kg) exposure for 8 days. Group 6 (G6): Rats were fed daily by oral gavage with saffron (300 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 cervi-cal dislocation and the brains were rapidly dissected out.

Table 1. Effect of saffron on Serum lipid profile in rats treated with Acrylamide.

Parameters / Groups	TG mg/dl	TC mg/dl	HDL-C mg/dl	LDL-C mg/dl	VLDL-C mg/dl
G1	174± 16.97	86.3±1.84	45.55 ± 5.87	17.05±4.31	34.6±3.68
G2	104.4[a] ± 0.85	66[a]±2.83	28.65[a] ± 0.49	16.8±3.68	20.2[a]±0.85
G3	259.5[a]± 13.44	180.55[a]±1.2	57.8 ± 1.13	68.9[a]±3.25	55.35[a]±2.19
G4	215.4[a] ± 16.4	128[a]±5.66	44.3 ± 5.66	51.2[a, b]±8.2	42.9±2.97
G5	193.9[b]± 14	102.5[a, b, c]±0.7	33.8[b] ± 3.39	25.19[b, c]±1.12	33[b]±5.37
G6	120.5[b, c] ± 7.78	73.4[a, b, c]±3.39	31.55[b] ± 3.18	24.35[b, c]±2.62	24.1[b, c]±1.56

G1= Control **G2**= Saffron **G3**= Acrylamide **G4**= SAF before ACR **G5**= SAF during ACR **G6**= SAF after ACR
TG= Triglycerides **TC**= Total Cholesterol **HDL-C**= High Density Lipoprotein Cholesterol **LDL-C**= Low Density Lipoprotein Cholesterol
VLDL-C= Very Low Density Lipoprotein Cholesterol Values are expressed as mean value of ± S.D
a: Significant ($p \leq 0.05$) from G1; **b**: Significant ($p \leq 0.05$) from G3; **c**: Significant ($p \leq 0.05$) from G4

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). Serotonin Transporter (SERT) assay kit was measured spectrophotometrically at a wavelength of 450nm ± 10nm (Hsu et al., 1981).

Histological Studies

For histological studies, the procedures of preparation and staining were done according to Drury and Wallington (1980).Stained sections were examined and photographed using digital camera, attached to Olympus EX51 light microscope and connected to computer.

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 G3 increased significantly ($p \leq 0.05$) in lipid profile, ALT, AST, and CK and significant ($p \leq 0.05$) decrease in SOD as compared to G1. G2 showed significant ($p \leq 0.05$) decline for lipid profile levels and CK, but non-significant ($p > 0.05$) in the mean value of AST, ALT, LDH and SOD concentration as compared to G1. G4 increased significantly ($p \leq 0.05$) in lipid profile levels, and non-significant ($p > 0.05$) change in ALT, AST and LDH, while CK and SOD has decreased significantly as compared to G1.G5 and G6 showed non-significant ($p > 0.05$) change for lipid profile levels and SOD, but significant ($p \leq 0.05$) increase in

Table 2. Effect of saffron on Liver Enzymes, Ceatine kinase and Superoxide Dismutase in rats treated with Acrylamide.

Enzymes for groups of Saffron before, during and after

Parameters / Groups	AST U/L	ALT U/L	LDH U/L	CK U/L	SOD U/L
G1	57.55 ±2.33	38.5 ±4.95	248±15.13	135.3±2.33	2.12± 0.15
G2	59.35 ±8.98	30 ±2.83	191.27±9.19	69.7^{a}±2.92	1.98± 0.04
G3	148.7^{a} ±6.79	61^{a} ±5.66	804.33^{a}±193.06	165.4^{a}±7.58	1.5^{a}± 0.12
G4	52.35^{b} ±3.32	24.5^{b} ±0.71	243.33^{b}±54	$63.115^{a, b}$±7.59	1.55^{a} ± 0.07
G5	$142.2^{a, c}$ ±9.48	$23.5^{a, b}$ ±0.71	477.67^{b}±18.18	$98.59^{a, b, c}$±7.58	$2.31^{b, c}$ ± 0. 25
G6	$111.05^{a, b, c}$ ±2.47	34.5^{b} ±3.54	440.33^{b}±69.87	$86.2^{a, b}$±7.6	$2.55^{b, c}$ ± 0.07

G1= Control **G2**= Saffron **G3**= Acrylamide **G4**= SAF before ACR **G5**= SAF during ACR **G6**= SAF after ACR
AST= Aspartate Aminotransferase **ALT**= Alanine Aminotransferase
LDH = Lactate Dehydrogenase **CK** = Ceatine kinase
SOD = Superoxide Dismutase
Values are expressed as mean value of ± S.D
a: Significant ($p \leq 0.05$) from G1; **b**: Significant ($p \leq 0.05$) from G3; **c**: Significant ($p \leq 0.05$) from G4

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

Parameters / Groups	GPx Activity in nmol/min/ml	GST Activity in nmol/min/ml	MDA concentration in µM	SERT ng/ml
G1	362.94±16.21	430.42±1.41	5.34±0.07	0.89 ± 0.01
G2	275.07±18.01	454.28±12.65	5.54±0.07	0.67^{a} ± 0.01
G3	654.56^{a}±3.59	339.96^{a}±14.06	6.38^{a}±0.42	1.06^{a} ± 0.04
G4	$235.59^{a, b}$±34.22	315.11^{a}±9.84	5.34^{b}±0.07	0.88^{b} ± 0.02
G5	$328.55^{b, c}$±14.41	$384.7^{a, b, c}$±12.65	$6.72^{a, c}$±0.06	0.88^{b} ± 0.04
G6	$189.75^{a, b}$±1.8	$365.81^{a, c}$±5.62	4.95^{b}±0.21	0.92^{b}± 0.01

G1= Control **G2**= Saffron **G3**= Acrylamide **G4**= SAF before ACR **G5**= SAF during ACR **G6**= SAF after ACR
GST= Glutathione S-transferase **GPx**= Glutathione Peroxidase **MDA** = Malondialdehyde
SERT= Serotonin Transporter
Values are expressed as mean value of ± S.D
a: Significant ($p \leq 0.05$) from G1; **b**: Significant ($p \leq 0.05$) from G3; **c**: Significant ($p \leq 0.05$) from G4

AST and significant ($p \leq 0.05$) reduction in ALT, CK and LDH values as compared to G1.

The data in Table (3) indicate that GPx, MDA and SERT levels increased ($p \leq 0.05$) significantly in G3, and significantly ($p \leq 0.05$) decreased in GST as compared to G1. G2 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. GPx and GST in G4 showed significantly ($p \leq 0.05$) decreased, while MDA and SERT concentration has non-significantly ($p > 0.05$) changed as compared to G1.G5showed significantly ($p \leq 0.05$) decreased in GPx, GST and SERT levels, while MDA concentration has significantly ($p \leq 0.05$) increase

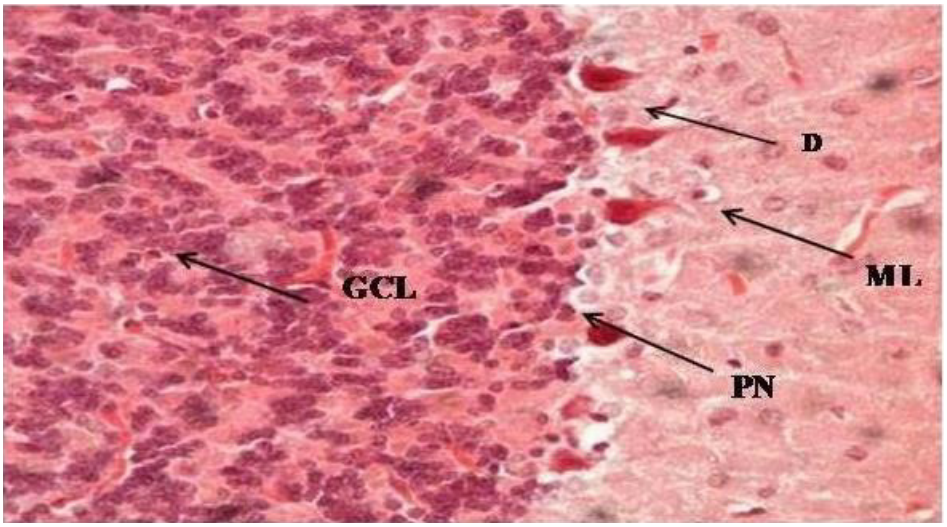

Figure 1. Section of (G1) in Rat brain. B:The slide showing Neurons(N), Axon (AX) and Nucleus (nu). C: The slide showing Purkinje Neurons (PN), Dendrites (D), Molecular Layer (ML) and Granule Cell Layer. (H and E X4

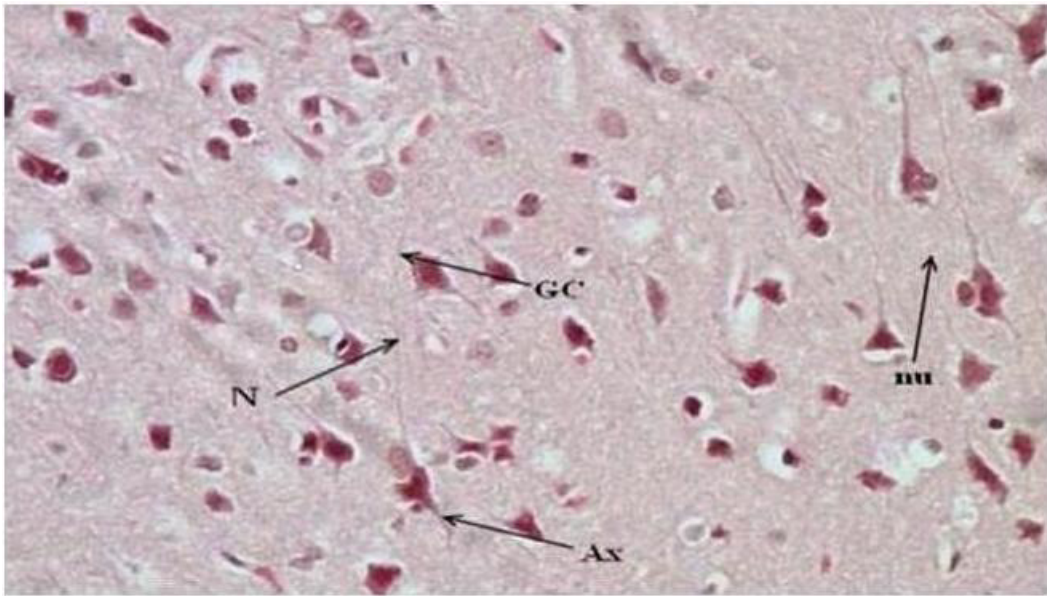

Figure 2. Section of (G2) in Rat brain. A: The slide showing Normal Neurons cells (N) with Nucleus (nu), Axon (Ax) and Glial cells (GC). (H and E X100) B: The slide showing Normal Neurons cells (N) with Nucleus (nu), Axon (Ax) and Glial cells (GC). (H and E X40)

as compared to G1. Significantly ($p \leq 0.05$) decreased in GPx and GST, while MDA and SERT concentration has non-significantly ($p > 0.05$) increase showed in G6as compared to G1.

Microscopically, brain from G1 showed the normal histological Structure of Neurons, Glial cells, Astrocytes, Neurons, Axon, Nucleus, Purkinje Neurons, Dendrites, Molecular Layer and Granule Cell Layer without alterations (Figure 1). Brain tissues in G3 showing Hemorrhage, Shrinkage in Neurons and there are disappearances of the nucleus. Ischemic injury: the sine qua non of ischemic damage to the nervous system is the so-called red neuron, as well as Hemorrhage and there are disappearances of the nucleus in Neurons. The soma (cell body) is shrunken, the cytoplasm is intensely eosinophilic, and the nucleus is pyknotic with no discernible nucleolus. (Figure 3)

Brain tissues in G2 showed apparent normal histological structure showing normal Neurons cells with Nucleus Axon and Glial cells (Figure 2). G5 showing

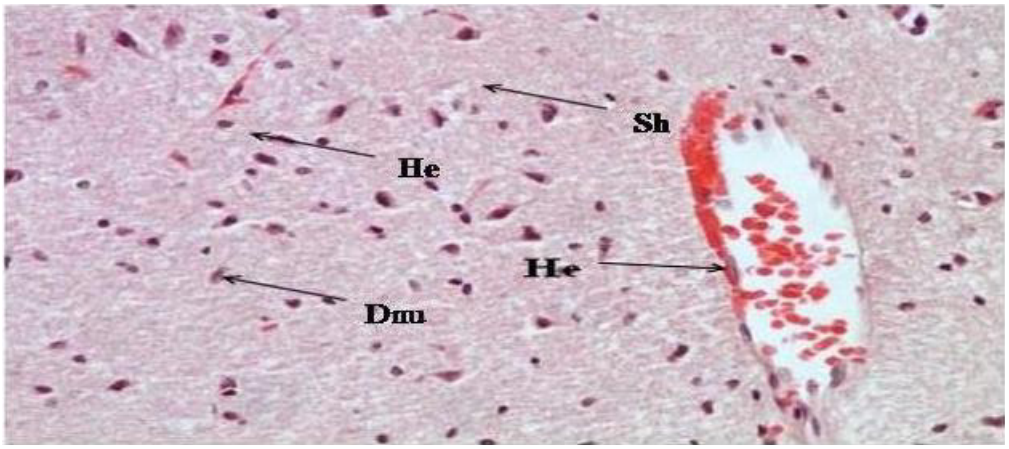

Figure 3. Section of (G3) in Rat brain. The slide showing Hemorrhage (He) and there are Disappearance of the nucleus (Dnu) in Neurons. The soma (cell body) (head of row) is shrunken, the cytoplasm is intensely eosinophilic, and the nucleus is pyknotic with no discernible nucleolus. (H and E X100)

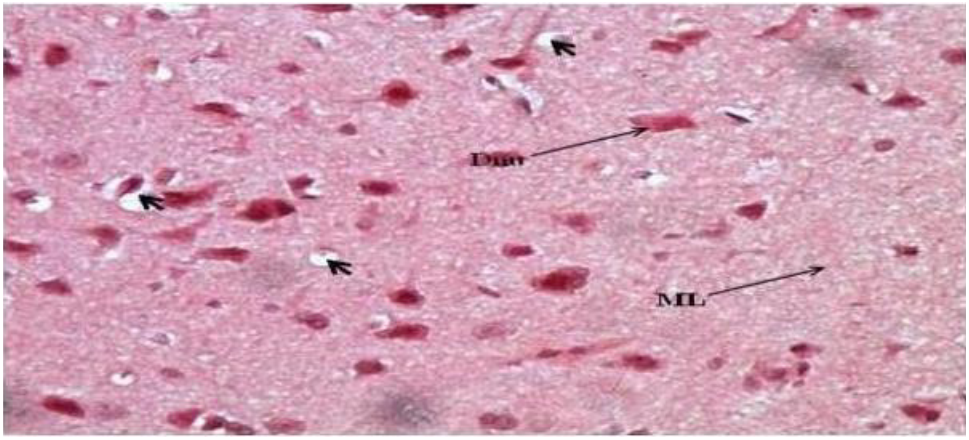

Figure 4. Section of (G4) in Rat brain. A: The slide showing Disappearance of the nucleus (Dnu) in Neurons. The soma (cell body) (head of row) is shrunken, the cytoplasm is intensely eosinophilic, and the nucleus is pyknotic with no discernible nucleolus and showing up normal Molecular Layer (ML). (H and E X40).

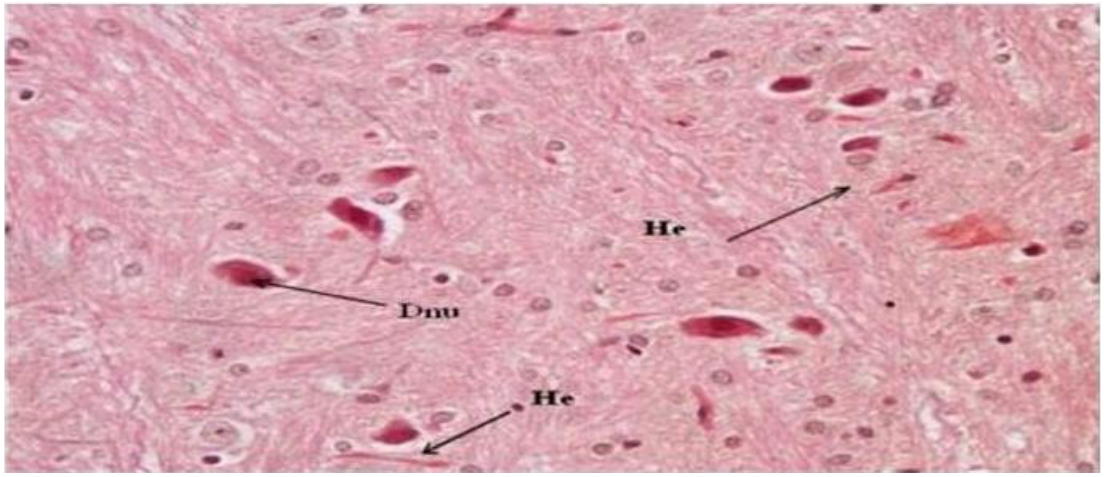

Figure 5. Section of (G5) in Rat brain. A: The slide showing mild improvement in tissue but there some Hemorrhage (He) and Disappearance of the nucleus (Dnu) in Neurons. (H and E X40).

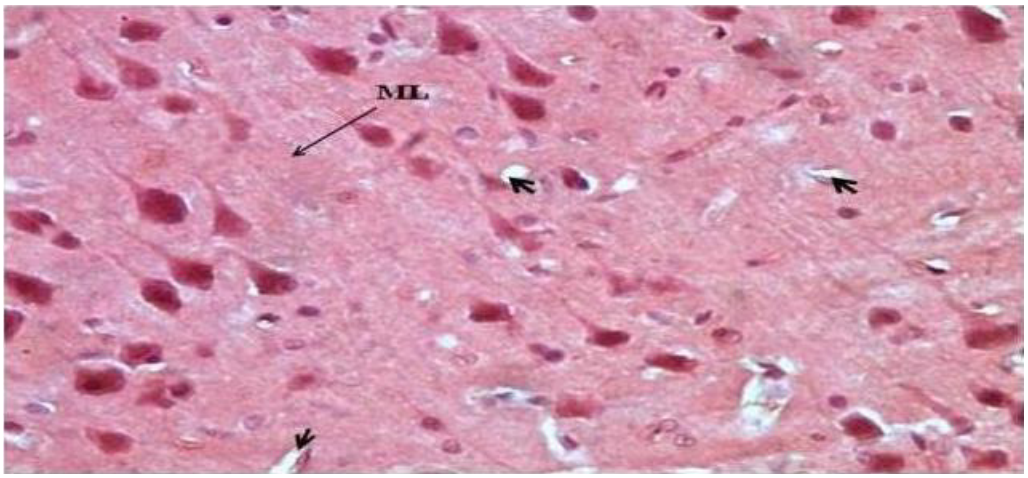

Figure 6. Section of (G6) in Rat brain. A: The slide showing semi normal tissue in Molecular Layer (ML) but there are the soma (cell body) (head of row) is shrunken. (H and E X40)

maild improvement in tissue but there some Hemorrhage (He) and disappearance of the nucleus (Dnu) in Neurons (Figure 5). G4 showing disappearance of the nucleus (Dnu) in Neurons. The soma (cell body) (head of row) is shrunken, the cytoplasm is intensely eosinophilic, and the nucleus is pyknotic with no discernible nucleolus and showing up normal Molecular Layer (Figure 4). In G6 showing semi normal tissue in Molecular Layer (ML) but there are the soma (cell body) is shrunken (Figure 6).

DISCUSSION

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. Favorable results have been obtained with many natural compounds in recent years, suggesting the possible utility of further investigation. Dietary factors, such as spices, are attractive sources of nueroprotective agents. Saffron, which consists of the dry stigmas of the plant Crocus sativus L., is used as a spice and a food colorant.

Results of the present study revealed that feeding of rats on ACR (G3) 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. These results indicated that changes in plasma lipoproteins can serve as sensitive and simple markers for rats liver disorders caused by acrylamide. The marked decrease serum triglycerides (TG) concentration in presence of acrylamide may be explained on the basis of decrease formation of plasma lipoproteins at an early stage after the liver injury.

Highly intracellular levels of ROS can lead to the damage of mitochondria, DNA modification, elevated cytokine production, and even cell death. Also, ROS reacts with protein thiol moieties to produce a variety of sulfur oxidations, thus diminishing the insulin receptor signal and inhibiting cellular uptake of triacylglycerol from the blood, so it will trigger the lipogenesis and accumulates lipid in blood and tissues. When lipid peroxidation increases, this triggers insulin resistance, which in turn stimulates lipogenesis and tissue lipid accumulation (Zhu et al., 2008).

Our results revealed that administration of saffron with ACR rats significantly ($p \leq 0.05$) decreased in serum levels of TC, TG, LDL-C, VLDL-C and HDL-C. The present results agreed with He et al., 2007, who reported that, there were hypolipidemic effects of crocetin by its intraperitoneal injection in rat.

There has been conflicting reports on the effect of high cholesterol diet on serum biochemical parameters related to hepatic function (AST and ALT). Our results revealed that ACR (G3) rats cause significant ($p \leq 0.05$) elevation in serum levels of AST and ALT enzymes. 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 CONH2 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 with neurotoxic rats. With ACR rats induced a significant ($p \leq 0.05$) decrease in ALT and AST as compared to ACR rats (G3). These results were coincided with Iranshahi et al. (2011) who suggested that aqueous and ethanolic extracts of saffron signific-antly decreased the levels of AST and ALT in plasma rats

induced by CCl4, and he suggested that aqueous and ethanolic extracts of saffron exhibit hepatoprotective effects against liver damages induced by CCl4 in mice. Cam et al., 2003 who reported that the hypoglycemic and antioxidant of saffron prevent oxidative stress and preserve liver function as was observed by low rates in AST and ALT. Crocetin also reduced the increased as partateand alanine aminotransferases (AST and ALT) in rat.

Results of the present study showed that there were significant ($p \leq 0.05$) increased in lactate dehydrogenase (LDH) serum in ACR treatment. This result may due to acute injuries to hepatocytes. The present results agreed with the results obtained by Awad et al. (1998) who reported that LDH activity was significantly elevated in ACR treated rats and that increase indicated the increased permeability, damage, and/or necrosis of cells. The results showed that there were significantly ($p \leq 0.05$) decreased in LDH level in saffron with ACR treated rats. This result agreed with Hosseinzadeh et al. (2012) who indicated that administration of saffron was found to considerably reduce the isoproterenol-induced raise in the activities of lactate dehydrogenase.

Matsuoka et al. (1990) reported that CK activity in brain and blood seems to be the most sensitive indicators of acrylamide intoxication. The results of current study showed that there were significant increases in CK serum in ACR treatment. This finding agreed with the previous work of Shuming et al. (2009) which 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 (Grusard et al., 2003). The current results of oral administration of saffron 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.

Free radicals are continuously produced in vivo and there are a number of protective antioxidant enzymes (SOD, GST, and GPx) for dealing with these toxic substances. The delicate balance is critical for maintenance of the biological function (Sridevi et al., 1998). Oxidative stress is usually caused by the increase of intracellular prooxidant species such as hydrogen peroxide, hydroxyl radicals, and superoxide anion radicals .In present study, ACR (G3) 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) Wu and Cederbaum, 2003, 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. Also, ACR binds to iron atom and make iron depletion, which may affect SOD enzyme activity. Decreased activities of SOD might have been caused by the accumulation of superoxide radicals and H2O2, 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 (Wu and Cederbaum, 2003). Finally, increase in MDA may be an indicator of lipid peroxidation (Diplocke, 1994).

The current results of oral administration of saffron when given with ACR to rats revealed that significant ($p \leq 0.05$) increase in SOD, non-significant changein 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 (Silva et al., 2001).

The serotonin transporter clears the synaptic cleft from serotonin and, therefore, has an important role in the regulation of serotonergic neurotransmission. Given that the serotonin transporter has a role in clearing extracellular serotonin. The present study showed that oral administration of ACR (G3) 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. Thus, this study could be considered the first to investigate the effect of ACR in SERT.

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 (LoPachinet al., 2006). 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 decrease in serotonin in ACR-treated rat supported the other findings reported by other investigators that ACR induces severe nerve damage in the central nerve system (Ali et al., 1983).

The current results revealed that oral administration of saffron with ACR rats significantly ($p \leq 0.05$) decrease in SERT level in brain tissue. In this respect, Karimi et al. (2001) 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 .

Lopresti and Drummond (2014) reported that affron's antidepressant effects potentially are due to its serotonergic, antioxidant, anti-inflammatory, neuro-endocrine and neuroprotective effects. Although the saffron compounds are likely to be able to effectively cross the blood-brain barrier (Hosseinzadeh et al., 2002), more detailed pharmacokinetic studies are needed to support their use in neurotoxic models under in vivo conditions.

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. Noorbala et al., (2005) determined that saffron extracts were effective in treating mild to moderate depression similar to fluoxetine (the antidepressant, Prozac)after 30 mg/day intake for six weeks.

The histopathological changes seen in the present study showed hemorrhage, shrinkage in neurons and there are disappearances of the nucleus. Ischemic injury: the sine qua non of ischemic damage to the nervous system is the so-called red neuron, and there were disappearance of the nucleus in Neurons. The soma (cell body) was shrunken, the cytoplasm is intensely eosinophilic, and the nucleus is pyknotic with no discernible nucleolus. This result is agree with Allam (2013) who reported that the most striking features of acrylamide toxicity in the medulla oblongata in the ACR treated groups were pyknosis. This damage may have resulted from the metabolic and biochemical alterations caused by acrylamide and its metabolites.Ibrahim et al. (2008) examined the effect of ACR on rat tissues. Histological examination showed a variable damage of tissues in different organs in rats received acrylamide. Hemorrhage was pronounced in liver, kidney, heart, spleen and Intestine. Vacuolar degeneration of hepatocytes of nearly all animals. Stomach revealed sever vacuolar degeneration of the epithelial lining stomach mucosa.ACR appeared to induce changes in neurofilaments distribution and function in CNS (Chauhan et al., 1993) Shrinkage of the neurons in both spinal cord and cerebellum.

Oral administration of saffron with ACR rats showed semi normal tissue in Molecular Layer (ML) but there some Hemorrhage (He) and soma (cell body) was shrunken. Soeda et al. (2001) showed that crocin not only has the anti-apoptotic effect, but also prevents their death. These findings suggest that crocin inhibits neuronal death induced by both an internal and an external apoptotic stimulus in ischema (highly differentiated neurons cells). The effectiveness of crocin on ischemia/reperfusion injury in mice micro vesseles was investigated by Zheng et al. (2007) and the beneficial effect of its pretreatment (20 and 10 mg/Kg) against cerebral ischemia through the inhibition of oxidizing reactions and modulation of the ultra-structure of cortical microvascular endothelial (CMEC) was shown in mice. Moreover, saffron is beneficial for curing nervous pains and brain damages (Bisset and Wichtl, 2001).

Saffron exhibited a neuroprotective and hepatoprotective action against ACR-induced oxidative stress in rats and treatment with saffron before and after ACR exposure was more effective protective role than during ACR treatment.

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CONCLUSION

Saffron exhibited a neuroprotective action against ACR-induced oxidative stress in rats and treatment with saffron after ACR exposure was more effective than before and during ACR treatment. Further research should be done to investigate the active components of saffron and identify the type of phenolic compounds responsible for their neuroprotective effects.

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