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Research Article
Subchronic Toxicity of Acrylamide in Fried Rice and Preventive Effect of Grape Leaves

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Abstract
Acrylamide is well known to be a Central Nervous System (CNS) toxicant. In this study the effects of dietary acrylamide formed in fried rice on the hematological values, thyroid hormones levels, liver, kidney functions and lipids profile in serum have been investigated. Male Albino rats were divided to three groups, G1 (control), G2 (fed on fried rice) and G3 (fed on fried rice+grape leave). Histopathological examinations of liver, kidney, brain, pancreases, lymph nodes and thyroid gland were carried out. Our results indicated that acrylamide administration in fried rice (G2) significantly decreased daily body weight gain, HB, RBCs, WBCs, HCT, platelet, T3, T4 and HDL cholesterol. However, TSH, liver, kidney function and lipid profile were significantly increased, while, a decreasing in these parameters has been noted in G3, which received grape leave in combination with fried rice. Besides, T3 and T4 analysis have been increased in G3. It can be stated that acrylamide effects on the thyroid hormones, which affects other body hormones and appeared on experimental rats. It was noted that grape leaves reduced the effect of acrylamide and led to improved physiology.

Key words: Acrylamide, fried rice, grape leaves, biochemical analysis, histopathology

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Acrylamide monomer may form in certain foods cooked at high temperatures (Surdyk *et al*., 2004). The highest concentrations of acrylamide have been identified in potato and grain-base foods that are cooked at very high temperatures, e.g., frying, grilling or backing (Tareke *et al*., 2002). Acrylamide is thought to form in food principally from the interaction of amino acid asparagine with glucose or other carbohydrates (Taubert *et al*., 2004). Therefore, the general population can be exposed to acrylamide through their diets. Acrylamide has been reported to be neurotoxic (Seale *et al*., 2012), toxic to the reproductive system (Ma *et al*., 2011) and carcinogenic in experimental animals (Hogervorst *et al*., 2010). Acrylamide have significant binding capacity to liver, kidney, brain and erythrocyte (Sumner *et al*., 1997). The US EPA and IARC have classified acrylamide as B2, a probable carcinogen and as 2B, a possible human carcinogen, respectively (Friedman *et al*., 1995). A decreasing pattern was also found in the haematological parameters like haemoglobin (Hb) content, erythrocyte count and haematocrit value. In this way, acrylamide completely disturbed the equilibrium of haematological and thyroid hormonal status. Rawi *et al*. (2012) carried out a study to investigate the hematological, biochemical, neurological and histopathological effects of acrylamide on immature male and female rats. The results obtained indicate that acrylamide administration induced some behavioral disorders in the movement of immature male and female rats as well as loss of body weight. Sharma and Jain (2008) reported that acrylamide induced a significant decrease in hemoglobin (Hb), erythrocytes (RBCS), haematocrit (HCT) and lymphocyte levels of young female rats. Acrylamide significantly increased total cholesterol and triglycerides concentrations of both immature male and female rats. While, significant increase in the total urea concentration was noticed only in the immature male rats following acrylamide administration. Moreover, acrylamide induced marked increase in the activities of aspartate aminotransferases (AST) and alanine aminotransferases (ALT) in the immature male and female rats. Also, effects of acrylamide on thyroid hormone levels and haematological parameters in Swiss albino mice were investigated by Sharma and Jain (2008). A dose dependent decrease in the T3 and T4 levels and a consequent increase in TSH were observed. The thyroid hormones control the skeletal and mental growth along with cell respiration, thus the above morphological toxic effects can be synchronized with the above results of T3, T4 and TSH.

Grape (*Vitis vinifera*) leaves have been used in medicine due to various biological activities for example grape leaves stop bleeding, reduce inflammation and pain (Baytop, 1999), hepatoprotective, spasmolytic, hypoglycemic and vasorelaxant effects as well as antibacterial, antifungal, anti-inflammatory, antinociceptive, antiviral and particularly antioxidant properties (Kosar *et al*., 2007; Orhan *et al*., 2009; Xia *et al*., 2010). In addition, Orhan *et al*. (2009) reported that *V. vinifera* leaves have role in the formulation of dietary antioxidant supplements. Thus the evaluation of acrylamide effects on biochemical and pathological parameters becomes vital. The objective of the present study is to investigate the effects of acrylamide formed in fried rice on biochemical and pathological parameters in Albino rats fed on this fried rice. Also, the preventive effect of feeding grape leaves as a source of antioxidant was also studied.

MATERIALS AND METHOD

Materials: Fried rice used as a source of dietary acrylamide (1302.36 μg kg^{-1}), which was determined according to Osman *et al*. (2015), in this work 250 g of fine Egyptian rice (purchased from local market) was fried in one litter of pure sunflower oil at 180°C for 10 min. The 1 Kg of fresh grape leaves (*Vitis vinifera*) was provided from local market and it was air dried in shade at room temperature for 3 days (give 250 g dry weight) and the fiber content in the diet has been replaced by grape leaves for animal diet, which was used in feeding experiment. The plants were identified by Mohamed Osama El-Segaee, Professor of Taxonomy, Faculty of Agriculture, Cairo University, Cairo, Egypt. The voucher specimens at the Herbarium Market of the National Research Centre (NRC), Cairo, Egypt.

Experimental animal: This study has been done using 15 male Albino Spargue-Dawely rats with a mean weight of 83.93±1.83 g. The animals were provided by the animal house of the Egyptian Organization of Biological products and vaccines, Cairo, Egypt. They were raised in the animal house of Biology Laboratory, Regional Center for Food and Feed, Agricultural Research Center, Giza, Egypt. The animals were divided into three equal groups of 5 rats each and housed individually in stainless steel cages with wire mesh bottoms and maintained at 25±2°C, relative humidity of 50-60% and 12/12 h light/dark cycle throughout the experiment for a week before the initiation of the experiment.

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the University of Cairo.

Animal diet: Basal diet was prepared according to A.I.N. 93M, Reeves *et al.* (1993). Composition of basal diet (g kg^{-1}): casein 140, corn starch 465.7, sucrose 100, soybean oil 40, cellulose 50, mineral mixture 35, vitamin mixture 10, 1.0 cystine 1.8, choline chloride 2.5 and tert. butylhydroxy quinone 0.008.

Design of the biological experiment: The groups were fed for four week as follows:

- **Group 1:** Control group were fed on the basal diet supplemented with 30% rice (replaced starch)
- **Group 2:** Rats were fed on the basal diet supplemented with 30% fried rice (equivalent to 0.11 mg kg^{-1} body weight daily acrylamide)
- **Group 3:** Rats were fed on the basal diet supplemented with 30% fried rice (equivalent to 0.11 mg kg^{-1} body weight daily acrylamide) and 5% dried grape leave (replaced cellulose)

At the end of the experiment (After 28 days), all the animals were scarified by cervical decapitation. Blood samples from each rat of each group were collected in two clean dry sterile and labeled centrifuge tubes. The first one was used for the determination of blood hemoglobin, Red Blood Cells (RBCs), White Blood Cells (WBCs) and hematocrit (HCT), the 2nd tube was centrifuged at 2500 rpm at 37°C for 15 min to separate the serum, which was kept in the deep freezer for the subsequent investigation. Also, body weight, food consumption were recorded day after day. Organs were excised, rinsed with cold saline, blotted dry and weighted, then stored in formalin.

Biochemical analysis: All the listed biochemical parameters from blood and serum were done in five replicates.

Hematological parameters: Hematological parameters including hemoglobin (Hb), Red Blood Cell (RBCs) count, White Blood Cell (WBCs) count, hematocrit and platelet count were determined according to Henry *et al.* (1974), Schalm (1965), Perkins (2004), Schalm *et al.* (1975) and Brecher and Cronkite (1950), respectively.

Liver functions: Also aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured spectrophotometrically in serum according to the method described by Reitman and Frankel (1957).

Kidney functions: Blood urea and serum creatinine contents were determined according to First (2003) and Faulkner and King (1976), respectively.

Lipid analysis: Triglycerides (TG), Total Cholesterol (TC), Low Density Lipoprotein Cholesterol (LDLC) and High Density Lipoprotein Cholesterol (HDLC) were determined according to Fossati and Prencipe (1982), Allain *et al.* (1974), Levy (1981) and Burstein *et al.* (1970), respectively. Atherogenic Index (AI) was calculated according to Lee and Niemann (1996) using following equation:

$$\text{Atherogenic index} = \frac{\text{Total cholestrol} - (\text{HDL} - \text{C})}{\text{HDL} - \text{C}}$$

Thyroid hormones: Tri-iodohyronine (T3), thyroxine or tetraiodothyronine (T4) and Thyroid Stimulating Hormone (TSH) were determined according to Chopra *et al.* (1971, 1972) and Young *et al.* (1975), respectively.

Pathological examination: Autopsy samples were taken after careful postmortem examination from the liver, kidney, brain, pancreas and lymph node of rats in different groups and fixed in 10% buffered neutral formaline for 24 h. Processed for paraffin wax embedding with the automatic tissue processor (SAKURA FINE TECH, Netherlands) by dehydrating through 70, 90 and 95%. Two changes of absolute ethanol for 90 min each. Clearing was achieved through changes of xylene twice for 2 h each, infiltrating through two changes of paraffin wax at 70°C and embedded in paraffin wax. Sections were cut at 4 µm with the rotary microtome (SAKURA FINE TECH, Netherlands) and mounted on glass slides and dried at 65°C for 45 min and then stained with hematoxylin and eosin stain and then examined by the light microscope (Carleton *et al.*, 1967).

Grape leaves preparation: The leaves were cleaned and dried in shade at room temperature for 3 days and coarsely powdered with the help of a hand-grinding mill. Twenty gram dried powder of plant leave was weighed and transferred into a beaker. Hundred milliliter of solvent (ethanol 70%) was added into the beaker and the mixture was shaken using mechanical shaker (Thermo, Canada) for12 h at room temperature. Each extract was filtered using Whatman No. 1 filter paper. The filtrate was collected and the residue was re-extracted twice. Then 0.2 mL of the mixture was diluted with 2 mL of ethanol and 1µL of final solution was injected in the GC/MS/MS.

Evaluation of radical scavenging activity: Radical scavenging activity of grape leaves extract against the stable DPPH radical was determined spectrophotometrically (Brand-Williams *et al.*, 1995). The colorimetric changes (from deep-violet to light-yellow), when DPPH$^{\cdot}$ is reduced, were measured at 517 nm on a UV-visible light spectrophotometer. The antioxidant activity of test solution was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH$^{\cdot}$. Fifty microliters of various concentrations (100, 200, 400, 600 and 800 µg mL^{-1}) of the test solution in dimethyl sulphoxide (DMSO) as well as ascorbic acid (as standard antioxidant compound) were put into appropriate tubes and 5 mL of 0.004% methanolic solution of DPPH$^{\cdot}$ was added to each tube to give final concentrations (100, 200, 400, 600 and 800 µg mL^{-1}). Absorbance measurements commenced immediately. The decrease in absorbance at 517 nm was determined after 1 h for all samples. Methanol was used to zero the spectrophotometer. Absorbance of the DPPH radical without antioxidant, i.e., the control was measured. Special care was taken to minimize the loss of free radical activity of the DPPH$^{\cdot}$ radical stock solution. The DPPH$^{\cdot}$ radical by the samples was calculated according to the formula of Yen and Duh (1994):

$$\text{Inhibition\%} = \frac{A_{C(o)} - A_{s(t)}}{A_{C(o)}} \times 100$$

where, $A_{C(o)}$ is the absorbance of the control at t = 0 min and $A_{s(t)}$ is the absorbance of the antioxidant at t = 1 h.

The percentage of scavenging activity was plotted against the grape leaves extract to obtain the Inhibitory Concentration (IC_{50}), defined as the essential oil concentration necessary to cause 50% scavenging. Tests were carried out in triplicate.

GC/MS analysis program: The preparation of the grape leaves ethanol extract for GC/MS analysis was mentioned above. The carrier gas was helium with the linear velocity of 1 mL^{-1} min. The oven temperature was set at 55°C for 3 min and then programmed until 280°C at a rate of 11°C min. The injector and detector temperatures were 220°C. Injection mode, splitless, volume injected 1 µL. The MS operating parameters were as follows: ionization potential 70 eV, interface temperature 280°C. Selected ion monitoring (Scan) mode was applied used m/z at start mass 35 and end mass 600. The identification of components was based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature (Santana *et al*., 2013).

Statistical analysis: Statistical analysis (Standard Deviation "SD" and Standard Error "SE") was carried out according to Fisher (1970). Least Significant Difference (LSD) test was used to compare the significant differences between means of treatment (Waller and Duncan, 1969). The Costat program was used for all analysis.

RESULTS AND DISCUSSION

Biological and biochemical effects of acrylamide

Body weight: The effect of feeding acrylamide in fried rice on rats was studied. Results in Table 1 summarize the mean values of initial and final body weight, body weight gain and daily body weight gain. A significant decrease was observed in body weight gain and daily body weight gain for experiment when feeding acrylamide in rice (30% fried rice) to rats compared to control rats feeding non fried rice. While, group 3 which received 30% fried rice and 5% dried grape leaves showed significant increase compared to group 2 or respective control group. These results indicate that grape leaves might contain bioactive compound (s) which reduce the effect of acrylamide. This parameter effect of grape leaves led to the study of reducing capacity of leaves extract as well as its chemical compounds as preventive natural bioactive compounds.

The decrease in body weight due to acrylamide administration is probably due to the pathological changes of central and peripheral nerves including neurotransmitter metabolism and NO signaling pathway (Kim, 2005; LoPachin *et al*., 2008).

In coincidence with the reports of Sharma and Jain (2008), Park *et al*. (2010), Khan *et al*. (2013) and Raju *et al*. (2011), our results of the present study clearly demonstrated that oral administration of acrylamide to male rats induced significant reduction of body weight. These results are in covenant with the results reported by Wang *et al*. (2010), who suggested that acrylamide exerts detrimental effect on growth and development of immature male rats. While, increasing in group 3 may revealed the protective effect of grape leaves which reduced the effect of acrylamide in fried rice.

Organs weight: Table 2 shows the effects of acrylamide on rats relative organs weights. The results of organs weights showed significant decrease in liver, brain and testes relative weight in group 2 compared with respective controls group ($p<0.05$). On another hand, heart and lung were non-significant relative weight but liver and testes were a significant increase relative weight in group 3 compare with group 2. Our results confirm with Khalil and Abd El Aziem (2005), who found acrylamide reduced liver, brain and kidney relative weight but the relative weight of spleen increased.

Table 1: Body weight as affected by feeding rats 30% fried rice or 30% fried rice+5% grape leaves for 28 days

Group/dose	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Daily body weight gain (g)
Group 1 (Control 30% rice)	85.33±1.45[a]	98.72±1.49[ab]	12.93±1.06[b]	0.46±0.04[c]
Group 2 (30% fried rice)	85.67±3.18[a]	94.21±1.36[b]	6.55±0.33[c]	0.23±0.01[a]
Group 3 (30% fried rice+5% grape leaves)	83.00±0.87[a]	100.54±1.25[a]	17.54±0.42[a]	0.63±0.02[b]

Each value represents the Mean±SE of five replicates, values in the same column with the same letter are not significant at $p \leq 0.05$

Table 2: Organs weights of rats affected by feeding rats 30% fried rice or 30% fried rice+5% grape leaves for 28 days

	Organ weight (g)					
Group/dose	Heart and lung	Liver	Kidney	Brain	Testes	Spleen
Group 1 (Control 30% rice)	1.27±0.07[a]	3.78±0.04[a]	1.07±0.04[ab]	1.65±0.05[a]	2.18±0.06[a]	0.24±0.03[ab]
Group 2 (30% fried rice)	1.20±0.08[a]	3.21±0.15[b]	0.98±0.01[b]	1.33±0.06[b]	1.90±0.09[b]	0.30±0.03[a]
Group 3 (30% fried rice+5% grape leaves)	1.30±0.04[a]	3.70±0.11[a]	1.11±0.02[a]	1.38±0.09[b]	2.29±0.04[a]	0.17±0.02[b]

Each value represents the Mean±SE of five replicates, values in the same column with the same letter are not significant at $p \leq 0.05$

Table 3: Mean hematological parameters of rats affected by feeding rats 30% fried rice or 30% fried rice+5% grape leaves for 28 days

Groups/dose	HB (g dL^{-1})	RBC count (10^6 uL^{-1})	WBC count (10^3 mm^{-3})	HCT (%)	Platelet (cmm)
Group 1 (Control 30% rice)	15.28±0.19[a]	5.10±0.10[a]	16.87±0.62[a]	45.84±0.60[a]	363.33±4.40[b]
Group 2 (30 % fried rice)	14.17±0.12[b]	4.75±0.11[b]	11.75±0.28[c]	42.52±0.35[b]	313.33±2.73[c]
Group 3 (30 % fried rice+5% grape leaves)	14.53±0.10[b]	4.84±0.09[b]	14.71±0.44[b]	43.60±0.19[b]	393.33±4.41[a]

Each value represents the Mean±SE of five replicates, values in the same column with the same letter are not significant at $p \leq 0.05$

Al-Sowayan (2014) found acrylamide effect on weights of testes and accessory glands (seminal vesicle and prostate glands) revealed that there was a significant decrease in their weights.

Effect of acrylamide on some hematological parameters: The effect of feeding acrylamide in fried rice were studied. Results in Table 3 summarize the values of hematological parameters like hemoglobin content, red blood cells count, the white blood cells count, haematocrit and platelet. The results showed significant decrease in hemoglobin (Hb), Red Blood Cells (RBCs), White Blood Cells (WBCs), haematocrit (HCT) and platelet in group 2 compared with respective control group (p<0.05), while the current results shown platelet significant increase in group 3 which received fried rice and 5% grape leaves compared with these received 30% fried rice only.

Arihan *et al.* (2011) stated that acrylamide is not only neurotoxic and carcinogenic, but it also damages erythrocyte membrane and generates micronucleated erythrocytes as well as alters blood viscosity parameters. Tarskikh (2006) observed that changes in physiochemical characteristics of biological membranes decrease in erythrocyte acid resistance and activation of lipid peroxidation at the early stage after acrylamide administration. Moreover, it was noted that these changes are accompanied by a decrease in erythrocytes count. Bergmark *et al.* (1993) and Barber *et al.* (2001) in their study revealed that Hb decreased significantly to low levels of Hb might be either due to the retarded synthesis or destruction hemoglobin. Acrylamide is electrophilic and covalently binds to the cysteine residues and forms adducts with sulfhydryl groups on hemoglobin resulting in the loss of heme part of hemoglobin molecules thereby reducing the amount of Hb in the blood, which in turn may also be responsible for the anaemic conditions as evident by the low levels of RBCs observed in the present investigation. The present results are consistent with Sharma and Jain (2008) and Rawi *et al.* (2012) as they concluded that acrylamide completely disturbed the equilibrium of hematological and thyroid hormonal status. The haematocrit (HCT) depends on the erythrocyte mass, mean corpuscular volume and plasmatic volume. Generally, when the erythrocytes have a normal size, the modifications of HCT follow the red cell distribution width changes (Perkins, 2004).

The present study indicated that HCT values were decreased significantly subsequent to acrylamide administration. This reduction could be due to their diminished production and redistribution from peripheral blood into the tissues or rapid destruction of WBC similarly, some previous investigators indicated that, HCT value and WBCs were found to be decrease (Sharma and Jain, 2008; Rawi *et al.*, 2012).

Effect of acrylamide on liver and kidney functions: Table 4 showed the effects of rats feeding 30% fried rice on liver and kidney functions. The results revealed significant increase in level of AST and ALT in group 2 compared with respective control group (p<0.05). While, group 3 which received acrylamide in fried rice and 5% grape leaves as treatment showed no significant change compared with respective control group in AST.

Table 4: Mean liver and kidney function of rats affected by feeding rats 30% fried rice or 30% fried rice+5% grape leaves for 28 days

Groups/dose	AST activity (U L^{-1})	ALT activity (U L^{-1})	Urea (mg dL^{-1})	Creatinine (mg dL^{-1})
Group 1 (Control 30% rice)	36.12±0.53[b]	32.10±0.11[b]	31.00±1.15[b]	1.11±0.07[b]
Group 2 (30% fried rice)	40.16±0.13[a]	40.82±0.64[a]	41.71±1.21[a]	1.96±0.05[a]
Group 3 (30% fried rice+5% grape leaves)	37.47±0.84[b]	39.39±1.03[a]	35.35±1.22[b]	1.25±0.08[b]

Each value represents the Mean±SE of five replicates, values in the same column with the same letter are not significant at $p \leq 0.05$

Table 5: Mean lipid profile of rats affected by feeding rats 30% fried rice or 30% fried rice+5% grape leaves for 28 days

Groups/dose	Total cholesterol (mg dL^{-1})	Triglyceride (mg dL^{-1})	HDL-cholesterol (mg dL^{-1})	LDL-cholesterol (mg dL^{-1})	VLDL-cholesterol (mg dL^{-1})
Group 1 (Control 30% rice)	89.40±2.96[b]	60.80±5.75[b]	69.84±0.55[a]	6.11±2.52[b]	14.42±1.39[b]
Group 2 (30% fried rice)	121.10±4.87[a]	141.06±5.74[a]	38.00±0.95[c]	54.88±5.29[a]	28.21±1.09[a]
Group 3 (30% fried rice+5% grape leaves)	85.27±2.55[b]	143.60±3.63[a]	45.19±1.29[b]	11.61±4.48[b]	28.72±0.38[a]

Each value represents the Mean±SE of five replicates, values in the same column with the same letter are not significant at $p \leq 0.05$

Table 6: Mean thyroid hormone levels of rats affected by feeding rats 30% fried rice or 30% fried rice+5% grape leaves for 28 days

Groups	T_3 (ng dL^{-1})	T_4 (µg dL^{-1})	TSH (µIU mL^{-1})
Group 1 (Control 30% rice)	161.10±1.58[a]	10.20±0.15[a]	0.11±0.05[b]
Group 2 (30% fried rice)	155.77±1.34[b]	8.77±0.55[b]	0.31±0.06[a]
Group 3 (30% fried rice+5% grape leaves)	163.87±0.91[a]	10.90±0.35[a]	0.12±0.04[b]

Each value represents the Mean±SE of five replicates, values in the same column with the same letter are not significant at $p \leq 0.05$

Serum AST and ALT are the most sensitive biomarkers used in the diagnosis of liver diseases (Pari and Kumar, 2002). The presence of high concentration of these enzymes in serum of acrylamide treated having a strictly cytosolic localization in normal physiological conditions is translated by the occurrence of an advanced permeabilisation of the hepatic membrane that allowed the flowing of the enzyme into the serum (Burlacu and Prisacaru, 2008). In coincidence with previous studies (Sharma and Jain, 2008; Allam *et al*., 2010; El-Bohi *et al*., 2011; Rawi *et al*., 2012; Teodor *et al*., 2011) the results showed increment in serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities following acrylamide treatment in male rats as compared to their corresponding controls.

The results in Table 4 indicated significant increase in urea and creatinine in group 2 compared with respective control group ($p<0.05$). While, the supplementation of fried rice in group 3 with 5% grape leaves normalized the values of AST, urea and creatinine, but not ALT activity which still high compared to respective control.

The kidney functions were investigated in this study to determine if acrylamide induces kidney damage. In correspond with the reports of Khalil and Abd El Aziem (2005); the present study showed that, administration of acrylamide induced some alterations in the serum creatinine and urea to untreated group. This agrees with the results of Shelly (1996), who observed a transient impairment in renal function following acute ingestion of acrylamide and confirm with Teodor *et al*. (2011) and Rawi *et al*. (2012), who noted an increase in the levels of urea and creatinine.

Effect of acrylamide on lipid profile: Table 5 summarize the mean values±SD of Lipid profile. The values were significantly Increased in serum for total cholesterol, LDL-cholesterol, VLDL-cholesterol and triglycerides in feeding fried rice group compared with respective controls ($p<0.05$). Besides, HDL results decreased significantly in group 2, HDL in group 3 which resaved 5% grape leaves was significantly increased compare with group 2 feeding fried rice only. While, total cholesterol and LDL-cholesterol decreased.

Lipids have structural and function important roles in different body organs and cells. The lipid is an important part of healthy body, because it is used to form cell membranes, several hormones and is necessary for other cellular function. The observed increase in total cholesterol level may result from the decline in HDL-cholesterol level or from increased liver fatty acid synthesis (Gans, 1973).

Therefore, high serum cholesterol level can be due to hepatic dysfunction. The obtained data regarding the influence of acrylamide on serum total cholesterol, triglycerides, HDL-cholesterol LDL-cholesterol and VLDL levels significantly increased may be due to the high levels of trans-fat in most fried food and many common bakeries like bread. Trans fats clearly raise the LDL cholesterol and that agree with Allam *et al*. (2010) and Rawi *et al*. (2012).

Effect of acrylamide on T3, T4 and TSH: Data in Table 6 indicated that there were significant decreasing in T3 and T4 in group 2 compared to respective control group ($p<0.05$), while group 3 which fed 5% grape leaves with fried rice were non significant with respective control result and that could be strongly because of the grape leave treatment. The results was also revealed significant increasing in TSH in group 2 compared with respective control group ($p<0.05$), while group 3 showed no significant change as compared with the control group.

The present study exhibited changes in the levels of the thyroid hormones and this consistent with Sharma and Jain (2008), who reported that there was increasing in the levels of TSH and consequent reduced levels of T3 and T4. Supplementation of rats with grape leave at group 3 in Table 6 showed improvement in the level of thyroid hormones as compared with control group. This could be explained by the protective effect of grape leaves (5%) against acrylamide formed in fried rice (30%) used in feeding group 3.

Prevention of acrylamide effects using grape leaves: From the study of biological and biochemical effects of feeding fried rice+5% grape leaves it was obvious that most of the adverse effects of acrylamide were improved compared to control. This finding led to a trail to fined on explanation to the protective effect of grape leaves in which antioxidant activity and chemical constituents were studied.

Antioxidant activity (%): Grape leaves were studied for its preventive effects against acrylamide formed in fried rice when fed to rats. The preventive effect of feeding 5% grape leaves found in this study directed or attention to analysis antioxidant activity and chemical composition of phenolic compound in leaves. $DPPH^{\cdot}$ can provide advantages, if the antioxidants tested are more soluble in organic solvents. The ethanol extract of grape leaves was tested for determination of total antioxidant activity using $DPPH^{\cdot}$ compared to vitamin c (as a standard antioxidant compound) and results are given in Table 7. The quantity of grape leaves extract required for $DPPH^{\cdot}$ inhibition was about 4.33 fold when compared with the standard antioxidant ascorbic acid.

Disturbances in the normal redox state by acrylamide can cause toxic effects through the production of cell damaging peroxides and free radicals. It is suggested that mechanism of action of grape leaves are probably through scavenging of free radicals.

In this regard, Middleton *et al*. (2000) and Basu *et al*. (2001) stated that flavonoids are uniquely suited as a support or single therapy in the treatment of inflammatory due to their combination of antioxidants and anti-inflammatory properties of Flavonoids have been well established. The reason behind flavonoids inhibition of ROS production is the activity of the ROS generating enzymes MPO and NADPH oxidase and several signal transducing enzymes involved in the cell activation in neutrophils (Middleton *et al*., 2000).

The co-administration of acrylamide together with grape leaves reduces the effect of acrylamide on liver and kidney enzymes, lipid profile and thyroid hormones and histological injury by enhanced antioxidative abilities in these organs against acrylamide action.

Identification of phenolic compounds in grape leaves: The high antioxidant activity of grape leaves leads to examine the quantification and qualification of ethanolic extract of grape leaves by GC/MS analysis of ethanolic extracts given in Table 8. The results showed 34 identified compounds. The most two abundant phenolic components (49.53%) of grape leaves were hydroquinone (25.43%) and pyrocatechol (24.10%), followed by two compounds (17.64%), sinapic acid (9.13%) and quinacetophenone (8.51%). Then eight compounds comprise (16.90%) i.e., gallic acid, propyl ester, scopoletin, caffeic acid phenethyl ester, 5 flavonoid components and finally the antioxidant ascorbic acid (3.23%). The rest of identified compounds were 22 compounds (15.90%) with relative percentage of each of less than 1%.

GC/MS analysis results revealed that grape leaves were found to be richer in polyphenols and flavonoids (e.g., gentisic acid, pyrocatechol, sinapic acid and quercetin). Similarly, Orhan *et al*. (2010) found that the rich and the varied chemical composition of *V. vinifera* leaves appear to contribute to their biological potential. The authors investigated the presence of several organic acids, phenolic acids, flavonols, tannins, procyanidins, anthocyanins, lipids, enzymes, vitamins, carotenoids and terpenes, which may explain the preventive effect of grape leaves components against acrylamide adverse effects. Sarhan *et al*. (2014) indicated that phytochemical investigates on beet and brocli demonstrated the presence of polyphenolic compounds.

These phenolic compounds have been reported to protect liquids, blood and body fluids against the attack of reactive oxygen species like superoxide, peroxide and hydroxyl radicals. The presence of polyphenolic compounds might be responsible for the protective effect of grape leaves against acrylamide. El-Shawaf *et al*. (2014) revealed that most of samples (fresh and parboiled) treated with antioxidants had lower acrylamide content comparing to the control regardless of oil type. By means of sensory evaluation of these chips, potatoes samples treated with rosemary extract recorded the highest scores in taste, texture and overall acceptability, followed by cloves and then BHT treated samples. Babaei and Qagharbeigi (2011) investigated the efficiency of antioxidant of thyme and extract of cumin on the reduction of acrylamide in potato chips and summarized the optimal levels of two additives. Results indicated that both thyme and cumin could significantly reduce the acrylamide content generated in potato chips and keep original flavor and crispness of potato

Table 7: Total antioxidant activity of grape leaves ethanolic extract and ascorbic acid

Treatment	Concentration of essential oil (μg mL^{-1})	Inhibition of DPPH•%	IC_{50} (μg mL^{-1})
Grape leaves extract	100	40.76	149.76
	200	59.22	
	400	65.90	
	600	85.10	
	800	89.00	
Ascorbic acid	25	34.20	34.61
	50	61.66	
	75	81.58	
	100	91.70	
	200	96.58	

The values are Means±SE, the mean values with different small letters within a column indicate significant differences (p<0.05)

Table 8: Chemical components of ethanolic extract of grape leaves

R.t.	Identified constituent	Relative concentration (%)
3.204	Hydroquinone	25.43
3.27	Pyrocatechol	24.10
3.96	Myricetin	0.624
3.98	Quercetin	1.09
4.32	Caffeic acid phenethyl ester	1.43
4.58	Enterodiol	0.26
4.7	3-hydroxymandelic acid	0.26
4.901	Salicylic acid	0.35
5.851	β-resorcylic acid	0.56
6.18	Gentisic acid	0.92
6.41	Orcinol	0.51
6.743	Syringic acid	0.67
7.3	5-Hydroxy-7-methoxy-2-(3-methoxyphenyl)-4h-chromen-4-one	0.69
7.98	Vanillic acid	0.69
8.16	4',7-dimethoxyisoflavone	1.57
9.62	Scopoletin	2.71
10.41	Ferulic acid	0.67
10.63	Flavone, 3,3',4',7-tetramethoxy	1.30
10.81	Phloroglucinol	0.82
11.204	3-hydroxy-4-methoxycinnamic acid	0.45
12.224	Quercetin 3-β-D-glucoside	3.22
12.353	Flavone, 3',5,6-triethoxy-3,4',7-trimethoxy	2.55
13.511	Quinacetophenone	8.51
13.742	Plectaniaxanthin	0.68
13.93	Shikimic acid	0.53
14.249	Retinyl propionate	0.31
14.54	β-cryptoxanthin	0.34
14.724	Sinapic acid	9.13
15.317	Gallic acid, propyl ester	3.78
15.688	β-carotene	0.57
16.018	24,25-dihydroxyvitamin D3	0.42
16.864	Flavone, 4',5,7-triethoxy-3,3',6-trimethoxy	1.10
17.796	Ascorbic acid	3.23
18.697	Hexestrol	0.53

The value of concentration of compounds is 100%

chips. Cheng *et al*. (2015) stated that flavonoids could effectively reduce acrylamide formation, which may be closely related to the number of phenolic hydroxyl groups of flavonoids.

Histopathological studies

Liver: The control rats revealed normal appearance of hepatocytes and normal Kupffer cells (Fig. 1). The Microscopical examination of liver indicated that it was congested with yellowish discoloration and kidney has blood accumulation. Rats fed on 30% fried rice and 5% grape leave showed congestion of central vein and mild degeneration in hepatocytes (Fig. 1). While, rats fed on 30% fried rice showed dilatation of center vein with congestion (Fig. 1).

The present study revealed variable pathological changes in livers, the severity of the lesions increases with the dose of

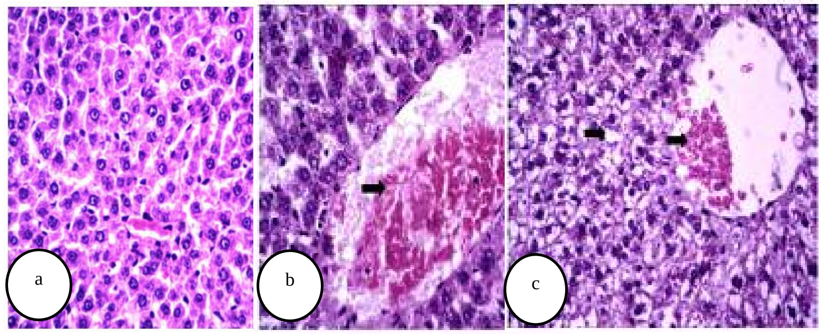

Fig. 1(a-c): (a) Control, rat showing normal histological picture photomicrographs of liver, (b) Rats fed 30% fried rice and (c) Rats fed 30% fried rice and 5% grape leaves. (400 x) H and E stain

acrylamide, which were in agreement with the reports of Donovan and Pearson (1987), who reported hepatoxicity following acute ingestion of acrylamide. The cytoplasmic vacuolization of the hepatic cells may be caused by progressive ischemia, Hypoxia and lipid accumulation in the cells (Abdul-Hamid and Moustafa, 2005; Moustafa and Abdul-Hamid, 2007). Another suggested by Rice-Evans and Burdon (1993) that the acrylamide could be generating Reactive Oxygen Species (ROS) which enhanced lipid peroxidase production; cellular fatty acids are readily oxidized by ROS to produce lipid peroxyl radicals and lipid hydroxides. The current findings agree with Khan *et al*. (2013), who found that animals treated with acrylamide, the liver showed a disrupted pattern of hepatic cords and the incidence of necrosis, congestion and disruption of the central canal wall. The current study is also consistent with Eman and Amany (2008) and Allam *et al*. (2010), who found that liver of rat treated by low dose of acrylamide revealed vacuolar degenerative changes of hepatocytes as well as congestion of the blood vessels. The significant increasing in liver enzymes level in serum was due to the pathological change in liver.

Kidney: The histopathological examination of kidney revealed normal histological appearance of control group 1 (Fig. 2) while, group 2 which rats fed on 30% fried rice showed degeneration of some renal tubules (Fig. 2) and rats group 3 which fed on 30% fried rice and 5% grape leave elucidate normal improvement in microscopical changes of kidneys (Fig. 2).

The current results agree with Eman and Amany (2008), who found that kidney treated with acrylamide showed periglomerular edema, infiltration of few mononuclear inflammatory cells as well as vacuolar degenerative changes of some cells lining the renal tubules and other cells showed necrosis. These findings may be due to the fact that kidneys are the way of excretion of acrylamide and its metabolites. These results were similar to that reported by Totani *et al*. (2007) and Mansour *et al*. (2008). Moreover, Hashimoto and Sakamoto (1982) noted degenerative changes in renal convoluted tubular epithelium in addition to degeneration and necrosis of hepatic parenchyma in monkeys received large dose of acrylamide. The significant increasing in kidney enzymes level in serum was due to the pathological change in kidney.

Brain: Transverse section of brain of control group 1 rat showed normal histological appearance (Fig. 3a) and normal nerve cells (Fig. 3b). Transverse section of brain of rats group 2, which fed on 30% fried rice showed congestion of meningeal blood vessels and blood capillaries in cerebrum (Fig. 3b) and areas of hemorrhages in between brain tissues (Fig. 3b) while, rats group 3, which fed on 30% fried rice and 5% grape leave elucidated improvement in the histological structure brain (Fig. 3c).

The present results are consistent with Raju *et al*. (2011), who found when treated chick embryo with 0.3 and 0.4 mg of acrylamide caused mild hemorrhages, increase degenerative changes and necrotic damages in brain and he suggested acrylamide induced free radical production in neurons has been suggested to be responsible for the oxidative brain damage. This fact proves the intensification of lipid peroxidation in rats with central nervous system. Levchenko and Demchuk (1999) obtained similar results in patients operated on for a brain tumor glioblastoma multiforme and meningioma. While, Eman and Amany (2008) when administered the low dose of acrylamide in the drinking water, it revealed focal gliosis and high dose of acrylamide showed lesions, severe diffuse gliosis as well as loss of the purkinje cells of cerebellum. Acrylamide has been reported to be neurotoxic (Lehning *et al*., 2003; Seale *et al*., 2012). Acrylamide

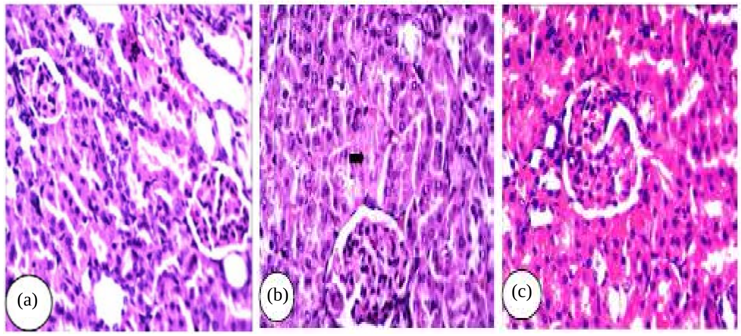

Fig. 2(a-c): Photomicrographs of kidney: (a) Control, rat showing normal histological picture photomicrographs of kidney, (b) Rats fed 30% fried rice and (c) Rats fed 30% fried rice and 5% grape leaves (400 x). H and E stain

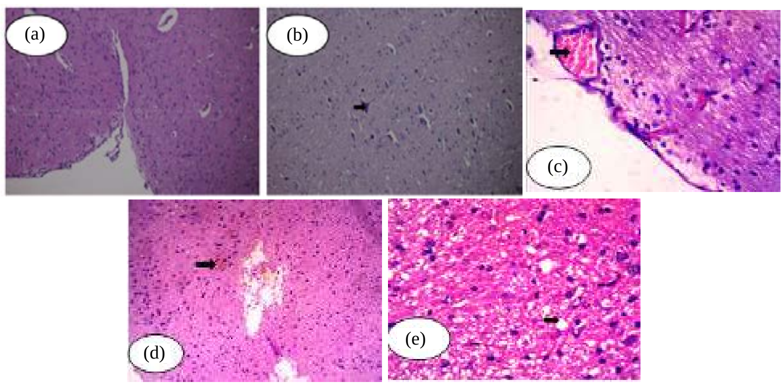

Fig. 3(a-e): Photomicrographs of brain: (a, b) Control, rat (200 x) (c, d) Rats fed 30% fried rice (400 x, 100 x) and (e) Rats fed 30% fried rice and 5% grape leaves (400 x) H and E stain

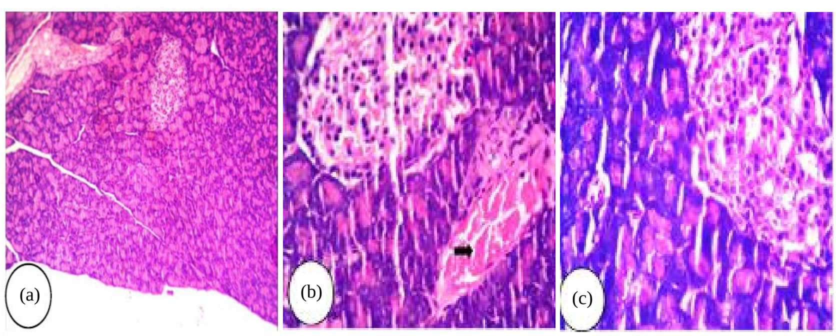

Fig. 4(a-c): Photomicrographs of pancreases: (a) Control, rat showing normal histological structure (100 x), (b) Rats fed 30% fried rice (400 x) and (c) Rats fed 30% fried rice and 5% grape leaves revealed normal histological structure (400 x) H and E stain

hydrolyses the neurotransmitter acetylcholine in synapses of the brain, automatic and neuromuscular junction of voluntary nervous system. Acrylamide inhibition leads to accumulation of acetylcholine at cholinergic sites causing disturbances in normal nervous system function (Gupta *et al*., 1991; Tusarova *et al*., 1999). While, Lopachin *et al*. (2002) and Sickles *et al*. (2002) suggested that nerve terminals were the primary site of acrylamide action and that synaptic dysfunction and subsequent degeneration.

Pancreases: The pancreas of control group 1 showed normal histological structure; note the normal islets of Langerhans

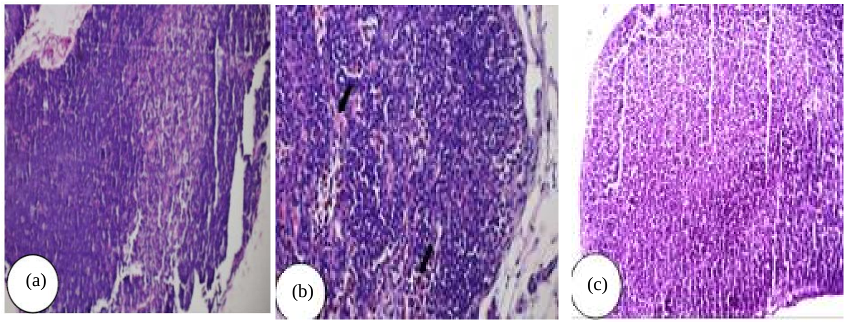

Fig. 5(a-c): (a) Control, rat showing normal histological appearance (100x), (b) Rats fed 30% fried rice (400x) and (c) Rats fed 30% fried rice and 5% grape leaves (100x) H and E stain

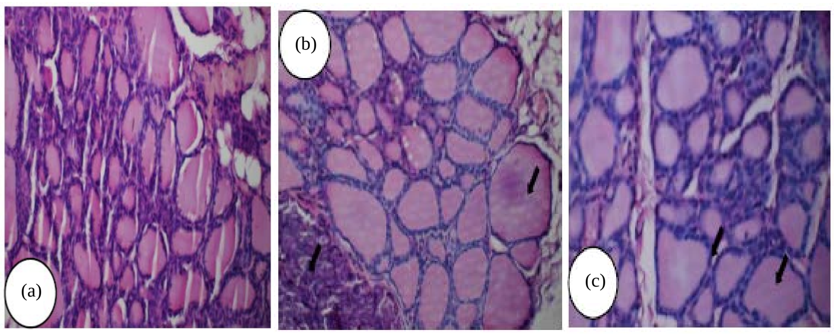

Fig. 6(a-c): Photomicrographs of thyroid gland: Representative sections of thyroid gland. Histopathology of thyroid gland section of male rat administered acrylamide for 28 days. (A) Control, rat showing normal histological appearance (400x), (B) Rats fed 30% fried rice (600x) and (C) Rats fed 30% fried rice and 5% grape leaves (100x) H and E stain

and acinar cells (Fig. 4a). The pancreas of rats group 2 showed congestion of blood vessels and degeneration of some cells of the islet of langerhans (Fig. 4b). Rats group 3, which fed on 30% fried rice and 5% grape leaves elucidated improvement in histological structure of pancreas (Fig. 4c).

Khalil and Abd El Aziem (2005) reported that acrylamide increasing serum glucose, which may indicate an effect on glucose utilization secretion. Larguinho *e al.* (2014) noted that when they treated fish with acrylamide 50 mg L^{-1} the pancreatic acini were considerably affected, consistent with chronic pancreatitis, exhibiting acinar cell necrosis and diffuse intrusion of macrophages containing irregular-sized bodies of lipofuscin-like pigments, which should indicate a response to increased cell death probably apoptosis. Sobel *et al.* (1986) and Schulz *et al.* (2001) found in a retrospective cohort study on occupational acrylamide exposure, a positive association was observed between cumulative acrylamide exposure and pancreatic cancer risk. Larguinho *et al.* (2014) showed that a freshwater fish, although able to deploy biochemical defenses towards a waterborne acrylamide induced challenge (namely boosting CYP1A biosynthesis and GST activity), the substance causes severe deleterious effects in the liver and moreover, in pancreatic acini.

Lymph nodes: Examination of the lymph nodes of the control group revealed normal histological appearance (Fig. 5a). Also, examination of the lymph nodes of the rats fed on 30% fried rice showed few hemorrhage with hemosiderin pigment (Fig. 5c). While, rats fed on 30% fried rice and 5% grape leave elucidated normal appearance (Fig. 5c).

Presence depletion in lymphoid follicles evidence that acrylamide in high dose has adverse on the immune system of the body. Hemorrhage with hemosiderin pigment due to hemolysis of red blood cells. These findings are similar to Arihan *et al.* (2011), who found that acrylamide damage the erythrocytes membrane and generate micronucleated erythrocytes.

Thyroid gland: Microscopic examination of the male rat thyroid glands of control group 1 revealed most of the lining epithelium of thyroid acini was cuboidal with normal secreting colloidal material in their lumen (Fig. 6a). Rats fed on 30% fried rice group 2 showed some follicles were dilated and lined with flattened epithelium with colloidal material in their lumen and other follicles were reduced in size with few colloidal material (Fig. 6b). This might explain the decrease of T3 and T4 and the increase in TSH in acrylamide treated rats. While, rats fed on 30% fried rice and 5% grape leave group 3 elucidated improvement in the thyroid follicles with normal histoarchitecture (Fig. 6c).

Thus, the observed histopathological change considered with biochemical changes in liver and kidney function as well as lipid profile. The results of organ weight, hematology and thyroid hormones T3, T4, TSH are in line and could explain each other.

CONCLUSION

The present study showed that grape leaves have a good antioxidant activity and have a role in treatment of biological damage caused by acrylamide treatment. Grape leaves administration led to modulation of all histological injury observed by acrylamide toxicity. These findings have indicated that grape leaves were promising source of compounds with nutritional properties and biological potential.

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REFERENCES

Abdul-Hamid, M. and N.A. Moustafa, 2005. Effect of ethanol administration on the liver and kidney of rat newborns at different conditions. J. Egypt. German Soc. Zool., 24: 267-294.

Al-Sowayan, N.S., 2014. Effects of acrylamide and children snack food on sex hormones nucleic acid and chromosomes of mature male Wister rats. World J. Pharma. Sci., 2: 1425-1434.

Allain, C.C., L.S. Poon, C.S.G. Chan, W. Richmond and P.C. Fu, 1974. Enzymatic determination of total serum cholesterol. Clin. Chem., 20: 470-475.

Allam, A.A., A.W. El-Ghareeb, M. Abdul-Hamid, A. El Bakery, M. Gad and M. Sabri, 2010. Effect of prenatal and perinatal acrylamide on the biochemical and morphological changes in liver of developing albino rat. Arch. Toxicol., 84: 129-141.

Arihan, O., N.B. Seringec, E.I. Gurel and N.H. Dikmenoglu, 2011. Effects of oral acrylamide intake on blood viscosity parameters in rats. Clin. Hemorheol. Microcircul., 47: 45-52.

Babaei, A.H.H. and P. Qagharbeigi, 2011. The inhibitory role of plant extracts on acrylamide formation in potato chips. J. Food Sci. Eng., 1: 214-220.

Barber, D.S., J.R. Hunt, M.F. Ehrich, E.J. Lehning and R.M. LoPachin, 2001. Metabolism, toxicokinetics and hemoglobin adduct formation in rats following subacute and subchronic acrylamide dosing. Neurotoxicology, 22: 341-353.

Basu, S., M. Whiteman, D.L. Mattey and B. Halliwell, 2001. Raised levels of F2-isoprostanes and prostaglandin F2α in different rheumatic diseases. Ann. Rheum. Dis., 60: 627-631.

Baytop, T., 1999. Turkiyede Bitkiler ile Tedavi (Gecmiste ve Bugun). Nobel Tıp Kitabevleri, Istanbul, pp: 357-358.

Bergmark, E., C.J. Calleman, F.S. He and L.G. Costa, 1993. Determination of hemoglobin adducts in humans occupationally exposed to acrylamide. Toxicol. Applied Pharmacol., 120: 45-54.

Brand-Williams, W., M.E. Cuvelier and C. Berset, 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol., 28: 25-30.

Brecher, G. and E.P. Cronkite, 1950. Morphology and enumeration of human blood platelets. J. Applied Physiol., 3: 365-377.

Burlacu, A.I. and C. Prisacaru, 2008. Evaluation of the antitoxic effect of some phthalides containing vegetal products. Proceedings of the 45th Congress of the European Societies of Toxicology, October 5-8, 2008, Rhodes, Greece.

Burstein, M., H.R. Scholnick and R. Morfin, 1970. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J. Lipid Res., 11: 583-595.

Carleton, M.A., R.A. Drury, E.A. Cuallington and H. Carmeror, 1967. Carleton`s Histological Technique. 4th Edn., Oxford University Press, New York, Toronto..

Cheng, J., X. Chen, S. Zhao and Y. Zhang, 2015. Antioxidant-capacity-based models for the prediction of acrylamide reduction by flavonoids. Food Chem., 168: 90-99.

Chopra, I.J., D.H. Solomon and R.S. Ho, 1971. A radioimmunoassay of thyroxine. J. Clin. Endocrinol. Metab., 33: 865-868.

Chopra, I.J., R.S. Ho and R. Lam, 1972. An improved radioimmunoassay of triiodothyronine in serum: Its application to clinical and physiological studies. J. Lab. Clin. Med., 80: 729-739.

Donovan, J.W. and T. Pearson, 1987. Ingestion of acrylamide with severe encephalopathy neurotoxicity and hepatotoxicity. Vet. Hum. Toxicol., 29: 462-462.

El-Bohi, K.M., G.G. Moustafa, N.I. El Sharkawi and L.M.E. Sabik, 2011. Genotoxic effects of acrylamide in adult male albino rats liver. J. Am. Sci., 7: 1097-1108.

El-Shawaf, A.E.G.M., F.M. El-Zamzamy and T.M. Mekky, 2014. Reduction of acrylamide formation in potato chips using natural antioxidants sources from plant extracts. Middle East J. Agric. Res., 3: 89-99.

Eman, M.A.E. and Y.M. Amany, 2008. Some studies on acrylamide intoxication in male albino rats. Egypt. J. Comparat. Pathol. Clin. Pathol., 21: 222-245.

Faulkner, N.R. and J.W. King, 1976. Fundamental of Clinical Chemistry. 2nd Edn., Sannders, Philadelphia, pp: 994-998.

First, M.R., 2003. Renal Function. In: Clinical Chemistry: Theory, Analysis, Correlation, Kaplan, L.A., A.J. Pesce and S.C. Kazmierczak (Eds.). 4th Edn., Mosby Inc., St. Louis USA., pp: 477-491.

Fisher, R.A., 1970. Statistical Methods for Research Workers. 14th Edn., Oliver and Boyed, USA., ISBN-13: 978-0050021705, pp: 140.

Fossati, P. and L. Prencipe, 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem., 28: 2077-2080.

Friedman, M.A., L.H. Dulak and M.A. Stedham, 1995. A lifetime oncogenicity study in rats with acrylamide. Fundam. Applied Toxicol., 27: 95-105.

Gans, J.H., 1973. The effect of carbon tetrachloride administration on cholesterol metabolism in mice. Biochem. Biophys. Acta-Lipids Lipid Metabolism, 326: 116-126.

Gupta, R.C., J.T. Goad and W.L. Kadel, 1991. *In vivo* alterations in lactate dehydrogenase (LDH) and LDH isoenzymes patterns by acute carbofuran intoxication. Arch. Environ. Contam. Toxicol., 21: 263-269.

Hashimoto, K. and J. Sakamoto, 1982. Anemia and porphyria caused by N,N'-methylene-bis-acrylamide (MBA) in mice and rats. Arch. Toxicol., 50: 47-55.

Henry, R.J., D.C. Cannon and J.W. Winkelman, 1974. Clinical Chemistry, Principle and Techniques. 2nd Edn., Harper and Row, New York.

Hogervorst, J.G.F., B.J. Baars, L.J. Schouten, E.J.M. Konings, R.A. Goldbohm and P.A. van den Brandt, 2010. The carcinogenicity of dietary acrylamide intake: A comparative discussion of epidemiological and experimental animal research. Crit. Rev. Toxicol., 40: 485-512.

Khalil, F.A. and B.H. Abd El Aziem, 2005. Effect of dietary acrylamide formed in potato crisps and toasted bread on rats. Egypt. J. Nat. Toxins, 2: 57-70.

Khan, M.R., M. Afzaal, N. Saeed and M. Shabbir, 2013. Protective potential of methanol extract of *Digera muricata* on acrylamide induced hepatotoxicity in rats. Afr. J. Biotechnol., 10: 8456-8464.

Kim, K., 2005. Effect of subchronic acrylamide exposure on the expression of neuronal and inducible nitric oxide synthase in rat brain. J. Biochem. Mol. Toxicol., 19: 162-168.

Kosar, M., E. Kupeli, H. Malyer, V. Uylaser, C. Turkben and K.H.C. Baser, 2007. Effect of brining on biological activity of leaves of *Vitis vinifera* L. (cv. Sultani Cekirdeksiz) from Turkey. J. Agric. Food Chem., 55: 4596-4603.

Larguinho, M., P.M. Costa, G. Sousa, M.H. Costa, M.S. Diniz and P.V. Baptista, 2014. Histopathological findings on *Carassius auratus* hepatopancreas upon exposure to acrylamide: Correlation with genotoxicity and metabolic alterations. J. Applied Toxicol., 34: 1293-1302.

Lee, R. and D. Niemann, 1996. Nutritional Assessment. 2nd Edn., Mosby Inc., St. Louis, MO., USA.

Lehning, E.J., C.D. Balaban, J.F. Ross and R.M. LoPachin, 2003. Acrylamide neuropathy: III. Spatiotemporal characteristics of nerve cell damage in forebrain. NeuroToxicology, 24: 125-136.

Levchenko, L.I. and M.L. Demchuk, 1999. [Lipid peroxidation and antioxidative activity in the tumor tissue and blood of patients with neuro-oncological diseases]. Zhurnal Voprosy Neirokhirurgii Imeni N. N. Burdenko, 4: 23-25.

Levy, A.L., 1981. Cholesterol, lipoproteins, apoproteins and heart disease: present status and future prospects. Clin. Chem., 27: 653-662.

LoPachin, R.M., J.F. Ross and E.J. Lehning, 2002. Nerve terminals as the primary site of acrylamide action: A hypothesis. NeuroToxicology, 23: 43-59.

LoPachin, R.M., D.S. Barber and T. Gavin, 2008. Molecular mechanisms of the conjugated α, β-unsaturated carbonyl derivatives: Relevance to neurotoxicity and neurodegenerative diseases. Toxicol. Sci., 104: 235-249.

Ma, Y., J. Shi, M. Zheng, J. Liu and S. Tian *et al*., 2011. Toxicological effects of acrylamide on the reproductive system of weaning male rats. Toxicol. Ind. Health, 27: 617-627.

Mansour, M.K., E.M. Ibrahim, M.M. El-Kholy and S.A. El-Madawy, 2008. Antioxidant and histopathological effect of catechin and neem leaves extract in acrylamide toxicity of rats. Egypt. J. Comp. Pathol. Clin. Pathol., 21: 290-313.

Middleton, Jr. E., C. Kandaswami and T.C. Theoharides, 2000. The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease and cancer. Pharmacol. Rev., 52: 673-751.

Moustafa, N.A. and M. Abdul-Hamid, 2007. Protective effect of phytic acid against the microscopical changes induced by aflatoxin B1 in the liver and kidney of white rats. J. Egypt. Ger. Soc. Zool., 53: 1-27.

Orhan, D.D., N. Orhan, B. Ozcelik and F. Ergun, 2009. Biological activities of *Vitis vinifera* L. leaves. Turk. J. Biol., 33: 341-348.

Osman, M.A., R.M. Romeilah, M.H. Elgammal and R.S. Hasan, 2015. Acrylamide formation in some Egyptian foods as affected by food processing conditions and pre-frying treatments. Biochem. Biotechnol. Res., 3: 11-18.

Pari, L. and N.A. Kumar, 2002. Hepatoprotective activity of *Moringa oleifera* on antitubercular drug-induced liver damage in rats. J. Med. Food, 5: 171-177.

Park, H.R., M.S. Kim, S.J. Kim, M. Park and K.H. Kong *et al*., 2010. Acrylamide induces cell death in neural progenitor cells and impairs hippocampal neurogenesis. Toxicol. Lett., 193: 86-93.

Perkins, S., 2004. Examination of the Blood and Bone Marrow. In: Wintrobe's Clinical Hematology, Wintrobe, M.M. and J.P. Greer (Eds.). Lippincott Williams and Wilkins, Philadelphia, ISBN-13: 9780781736503, pp: 21.

Raju, J., C. Sondagar, J. Roberts, S.A. Aziz, D. Caldwell, E. Vavasour and R. Mehta, 2011. Dietary acrylamide does not increase colon aberrant crypt foci formation in male F344 rats. Food. Chem. Toxicol., 49: 1373-1380.

Rawi, S.M., M.A.S. Marie, S.R. Fahmy and S.A. El-Abied, 2012. Hazardous effects of acrylamide on immature male and female rats. Afr. J. Pharm. Pharmacol., 6: 1367-1386.

Reeves, P.G., F.H. Nielsen and G.C. Fahey Jr., 1993. AIN-93 purified diets for laboratory rodents: Final report of the American institute of nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr., 123: 1939-1951.

Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 56-63.

Rice-Evans, C. and R. Burdon, 1993. Free radical-lipid interactions and their pathological consequences. Progr. Lipid Res., 32: 71-110.

Santana, P.M., M. Miranda, J.A. Payrol, M. Silva, V. Hernandez and E. Peralta, 2013. Gas chromatography-mass spectrometry study from the leaves fractions obtained of *Vernonanthura patens* (Kunth) H. Rob. Int. J. Org. Chem., 3: 105-109.

Sarhan, M.A.A., A.A. Shati and F.G. Elsaid, 2014. Biochemical and molecular studies on the possible influence of the *Brassica oleracea* and *Beta vulgaris* extracts to mitigate the effect of food preservatives and food chemical colorants on albino rats. Saudi J. Biol. Sci., 21: 342-354.

Schalm, O.W., 1965. Veterinary Haematology. 4th Edn., Lea and Febiger, Philadelphia, USA pp: 171-176.

Schalm, O.W., N.C. Jain and E.J. Carrol 1975. Veterinary Haematology. 3rd edn. Lea and Fibiger, Philadelphia, pp: 45-46.

Schulz, M.R., I. Hertz-Picciotto, E. van Wijngaarden, J.C. Hernandez and L.M. Ball, 2001. Dose-response relation between acrylamide and pancreatic cancer. Occup. Environ. Med., 10.1136/oem.58.9.609.

Seale, S.M., Q. Feng, A.K. Agarwal and A.T. El-Alfy, 2012. Neurobehavioral and transcriptional effects of acrylamide in juvenile rats. Pharmacol. Biochem. Behav., 101: 77-84.

Sharma, A. and J. Jain, 2008. Effects of oral exposure of acrylamide on plasma levels of thyroid hormones and hematological parameters in the Swiss albino mice. Asian J. Exp. Sci., 22: 317-324.

Shelly, 1996. Regina vs. Calder. Transcript records of New Zealand High Court, March 1996, Christchurch, New Zealand.

Sickles, D.W., J.D. Stone and M.A. Friedman, 2002. Fast axonal transport: A site of acrylamide neurotoxicity? Neurotoxicology, 23: 223-251.

Sobel, W., G.G. Bond, T.W. Parsons and F.E. Brenner, 1986. Acrylamide cohort mortality study. Br. J. Ind. Med., 43: 785-788.

Sumner, S.C.J., L. Selvaraj, S.K. Nauhaus and T.R. Fennell, 1997. Urinary metabolites from F344 rats and B6C3F1 mice coadministered acrylamide and acrylonitrile for 1 or 5 days. Chem. Res. Toxicol., 10: 1152-1160.

Surdyk, N., J. Rosen, R. Andersson and P. Aman, 2004. Effects of asparagine, fructose and baking conditions on acrylamide content in yeast-leavened wheat bread. J. Agric. Food Chem., 52: 2047-2051.

Tareke, E., P. Rydberg, P. Karlsson, S. Eriksson and M. Tornqvist, 2002. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. J. Agric. Food Chem., 50: 4998-5006.

Tarskikh, M.M., 2006. Damage to erythrocyte membranes as the mechanism for acrylate toxicity. Bull. Exp. Biol. Med., 142: 690-692.

Taubert, D., S. Harlfinger, L. Henkes, R. Berkels and E. Schomig, 2004. Influence of processing parameters on acrylamide formation during frying of potatoes. J. Agric. Food Chem., 52: 2735-2739.

Teodor, V., M. Cuciureanu, B.G. Slencu, N. Zamosteanu and R. Cuciureanu, 2011. Potential protective role of selenium in acrylamide intoxication. A biochemical study. Studia Universitatis 21: 263-268.

Totani, N., M. Yawata and Y. Ojiri and Y. Fujioka, 2007. Effects of trace acrylamide intake in Wistar rats. J. Oleo Sci., 56: 501-506.

Tusarova, I., E. Halamek and Z. Kobliha, 1999. Study on reactivation of enzyme-inhibitor complexes by oximes using acetylcholine esterase inhibited by organophosphate chemical warfare agents. Enzyme Microb. Technol., 25: 400-403.

Waller, R.A. and D.B. Duncan, 1969. Aboys rule for symmetric multiple comparison problem. An. State Assoc. J., 65: 1485-1503.

Wang, H., P. Huang, T. Lie, J. Li, R.J. Hutz, K. Li and F. Shi, 2010. Reproductive toxicity of acrylamide-treated male rats. Reprod. Toxicol., 29: 225-230.

Xia, E.Q., G.F. Deng, Y.J. Guo and H.B. Li, 2010. Biological activities of polyphenols from grapes. Int. J. Mol. Sci., 11: 622-646.

Yen, G.C. and P.D. Duh, 1994. Scavenging effect of methanolic extracts of peanut hulls on free-radical and active-oxygen species. J. Agric. Food Chem., 42: 629-632.

Young, D.S., L.C. Pestaner and V. Gibberman, 1975. Effects of drugs on clinical laboratory tests. Clin. Chem., 21: 1D-432D.