



Protective effect of pumpkin seed oil against hepatotoxicity and nephrotoxicity in rats administered high doses of aspartame

Reem Alazragi

Department of Biochemistry, Faculty of Science, University of Jeddah, Jeddah, Kingdom of Saudi Arabia
E-mail address: rsazraqi@hotmail.com

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General Note

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ABSTRACT

Aspartame, an artificial sweetener, is widely used in food and beverage production. However, high doses of aspartame may induce various organotoxic effects. Pumpkin seed oil (PSO) possesses antioxidant properties owing to high biologically active compound content. This work aimed at evaluating the toxic effects of supplementation of high doses of aspartame in rats and the possible protective effects of PSO against this toxicity. Rats were randomized into three groups: control, aspartame, and aspartame+PSO groups; administered a daily dose of 1000 mg/kg b.wt dissolved in water and pumpkin seed oil at a dose of 4 ml/kg b.wt for four weeks. Liver and kidney function tests as well as tissue oxidant/antioxidant biomarker levels, liver and kidney DNA content, percentage of DNA fragmentation, and histological examinations were performed. The administration of high doses of aspartame impaired liver and kidney function and also induced oxidative stress in liver and kidney. Treatment with PSO demonstrated the

ability to protect the liver and kidney against aspartame toxicity and indicated its role in ameliorating the toxic effects of aspartame in rats.

Keywords: Aspartame, pumpkin seed oil, hepatotoxicity, nephrotoxicity

1. INTRODUCTION

Liver is the primary site for metabolism and essential for several biochemical reactions in the body. It primarily functions to detoxify toxic compounds and synthesizes several biomolecules. Consequently, liver damage leads to serious consequences (Yakubu et al., 2003).

Kidneys maintain blood homeostasis by filtering toxins and metabolic waste. Different environmental agents, including certain drugs, can affect kidney function. Some drugs and chemical substances interfere with tissue functions and cause side effects in the liver and kidneys (Ustuner et al., 2017).

Aspartame, also known as N-(L- α Aspartyl)-L-phenylalanine, 1-methyl ester, is a dipeptide alpha methyl ester consisting of phenylalanine and aspartic acid and is a non-nutrient sweetener about 200 times more intense/sweet than sucrose (Fawzy et al., 2018). It is stable under dry conditions but not when subjected to prolonged heating. Aspartame is used as a sweetener in a variety of foods and beverages in over 90 countries (Wu et al., 2017). It is added in more than 6,000 products, such as powdered and carbonated soft drinks, candied hot chocolate, tabletop sweeteners, desserts, yogurt, chewing gum, and pharmaceutical products, such as vitamins and sugar-free drops (Soffritti et al., 2010). Upon consumption, aspartame is hydrolyzed to its essential ingredients, i.e. aspartic acid (40%), amino acid phenylalanine (Phy) (50%), and methanol (10%). Phenylalanine is specifically transformed into tyrosine after absorption (Choudhary and Lee, 2018).

Aspartame has been found to induce toxicity at different levels. Various studies proved the multi-potential carcinogenic properties of aspartame in elevating the risk of lymphoma, urinary tract tumors, leukemia, and neurological tumors (Soffritti et al., 2010). Moreover, an association was observed between consumption of aspartame and type 2 diabetes, premature delivery, nephrotoxicity, hepatotoxicity, and initiation of histopathological alterations in salivary glands (Okasha, 2016). Researchers have recently focused on investigations on dietary plants and herbal preparations for finding a substitute for aspartame with clinical remedies (Caili et al., 2006). Pumpkin (*Cucurbita spp.*) is traditionally used in Arabic countries, its seeds are salted and roasted and used as snacks for human consumption. Pumpkin seeds are rich sources of proteins (25.2–37%), vitamins and oil (37.8–45.4%), particularly, omega 6 fatty acids. They contain substantial quantities of fatty palm, stearic, oleic, and linoleic acids and also tocopherol and vitamin E in large quantities (Barbara and Murkovic, 2004). PSO was demonstrated efficient in the treatment of benign prostatic hyperplasia (Hong et al., 2009). In addition to anti-inflammatory and hypolipidemic effects, it showed decrease in lipid peroxidation and oxidative damage caused by aflatoxins (Eraslan et al., 2013). Pumpkin seed oil (PSO) is a rich natural source of triterpenes, phenolic antioxidants, phytosterols, tocopherol, polyunsaturated fatty acids, and carotenoids (Suresh and Das, 2003). Animal studies showed that PSO could be used for treating hypertension, arthritis, hypercholesterolemia, and diabetes. Pumpkin-rich diets were correlated with reduced incidence of lung, stomach, breast, and colorectal cancers (Ali and Abdelzaher, 2017). PSO has been used in some African and North-Eastern countries as cooking oil and also for preparing salad oil and margarine (Jiao et al., 2014).

This study aimed at exploring the protective potential of PSO against liver and kidney toxicity caused by long-term aspartame consumption.

2. MATERIALS AND METHODS

Materials

Aspartame was purchased from Sigma, USA. PSO was purchased from local markets in Saudi Arabia.

Methods

Animals

Thirty male Albino rats were housed at King Fahd Medical Research Center Animal Facility Breeding Colony and at constant temperature (25 °C) under controlled conditions of light/dark cycle. During acclimatization period, rats were given free access to water and standard laboratory diet. Experiments were approved by the Ethical Committee of King Fahd Medical Research Center. Jeddah, KSA. Approval number (163-19).

Animals were grouped into the following three groups (10 rats/group):

Group 1: Control: healthy control rats

Group 2: Asp rat group: rats were administered a daily dose of aspartame at 1000 mg/kg b.wt (dissolved in water according to Abhilash et al. [2011]) for four weeks.

Group 3: Asp +PSO group; rats were administered a daily dose of 1000 mg/kg b.wt (dissolved in water and PSO) at a dose of 4 ml/kg body weight for four weeks according to AbouSeif (2014).

Sample collection

After experimental endpoint, rats were anesthetized using diethyl ether and blood samples were collected from the retro-orbital vein plexus. Serum was separated by centrifugation at 3000 r.p.m for 20 min. Rat organs (liver and kidney) were removed, rinsed with ice cold saline, weighed, and stored for further biochemical and histological determinations.

Biochemical analysis in serum

Liver function tests including alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) activities were determined using commercial kits (Bio-Diagnostic, ARE). Total bilirubin was determined using commercial kits purchased from (Bio-Diagnostic, ARE). Total protein, albumin, and globulin vales were examined using commercial kits obtained from (Biovision, Milpitate, CA, USA).

Kidney function tests

Tests including serum urea, creatinine, and uric acid were determined by commercial kits purchased from (Bio-Diagnostic, ARE).Serum electrolytes (Na⁺, K⁺) were determined using commercial kits purchased from (Bio-Diagnostic, ARE).

Biochemical analysis in liver and kidney homogenates

Glutathione reductase (GSH) and malondialdehyde (MDA) were determined by commercial kits from (Bio-Diagnostic, ARE). Catalase (CAT) and nitric oxide (NO) were determined by commercial kits purchased from (Bio-Diagnostic, ARE).

Determination of nucleic acid (DNA and RNA) content

Total DNA content in the liver and kidney was determined according to Pears (1985). DNA fragmentation was quantified by the di-phenyl Amine I method (Gibb et al., 1997). About 0.2 g of rat tissue was homogenized in saline solution (5 ml), centrifuged at 1500 r.p.m for 10 min at 4 °C. Cells were resuspended and cold lysis buffer (5 mMTris + 20 mM EDTA + 0.5% Triton X-100, pH 8.0) were added to the suspension for DNA isolation and centrifuged. The supernatant containing the fragmented DNA was transferred to a new tube. Trichloroacetic Acid (TCA) were added to the fragmented and intact DNA and incubated further for 10 min at 25 °C. Blank sample was obtained by adding 1 mL of 5% TCA to 2 mL of diphenylamine. The amounts of both fragmented and intact DNA were measured spectrophotometrically at 600 nm.

Histological examination

Rat liver and kidneys were removed and fixed in 10% neutral formalin, embedded in paraffin wax and subjected to sectioning. Slides were stained with hematoxylin and eosin.

Statistical analysis

Results were expressed as mean + significant differences. Results among groups were analyzed using analysis of variance one-way ANOVA coupled with statistical package for the social science program, p <0.05 was considered significant.

3. RESULTS

Results presented in Table 1 revealed that administration of aspartame results in an increase (p<0.05) in values of Alanine aminotransferase ALT, Aspartate aminotransferase AST (Figure 1), Alkaline phosphatase ALP (Figure2), and total bilirubin compared with untreated control rats (Figure 3). The administration of PSO reduced these levels significantly (p <0.05) and reduced them to nearly control levels. Additionally, administration of aspartame significantly reduced the levels of total protein, albumin, and globulin (p <0.05) compared with those of untreated rats (Figure 4).

Table 1 Effect of supplementation of pumpkin seeds on serum liver functions levels in rats supplemented with aspartame for 5 weeks

Group Parameters	G1 Control	G2 ASP	G3 ASP+PSO
ALT U/L	30.54 ±1.4	55.65±2.5 ^a	41.55±1.9 ^b
AST U/L	34.64±2.1	62.54±3.2 ^a	52.26±3.6 ^b
ALP U/L	172.65±7.4	230.68±8.5 ^a	190.54±8.8 ^b
Total bilirubin mg/dl	0.62±0.02	0.95±0.09 ^a	0.71±0.03 ^b
Total protein g/dl	8.32±1.2	5.21±0.98 ^a	6.53±0.94 ^b
Albumin g/dl	5.43±0.95	2.76±0.57 ^a	4.54±0.55 ^b
Globulin g/l	3.55±0.42	2.63±0.64 ^a	3.14±0.68 ^b

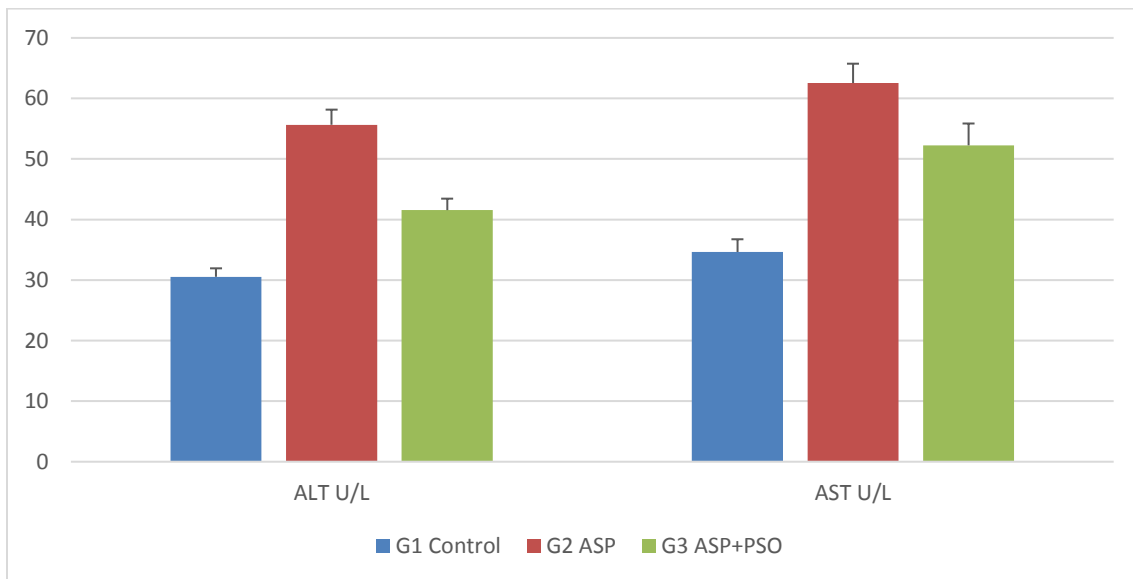


Figure 1 Effect of PSO on the levels of ALT and AST

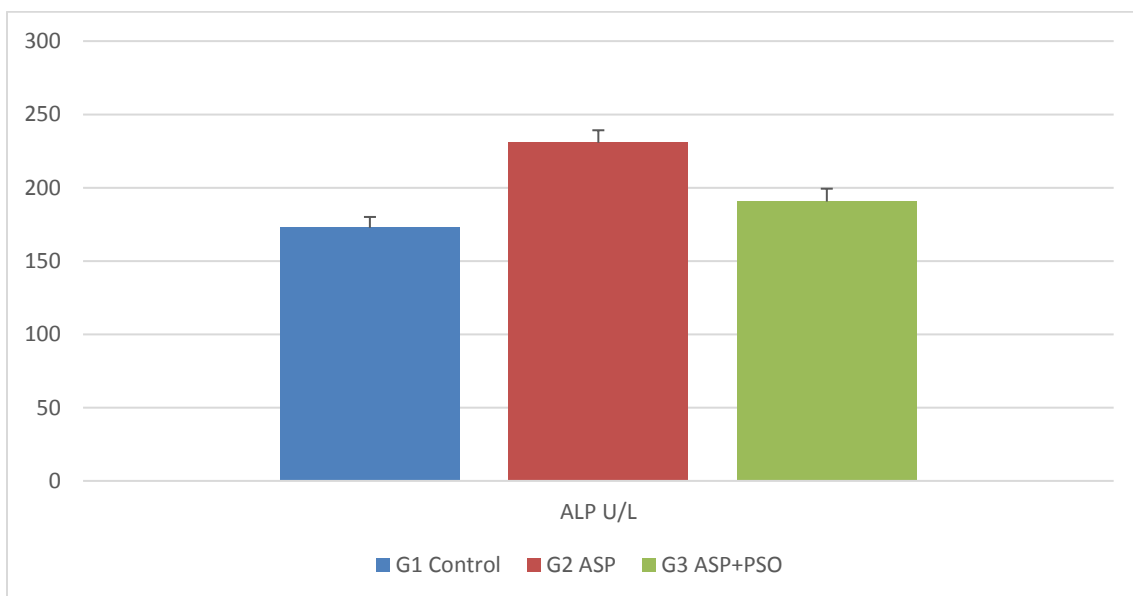


Figure 2 Effect of PSO on the levels of ALP

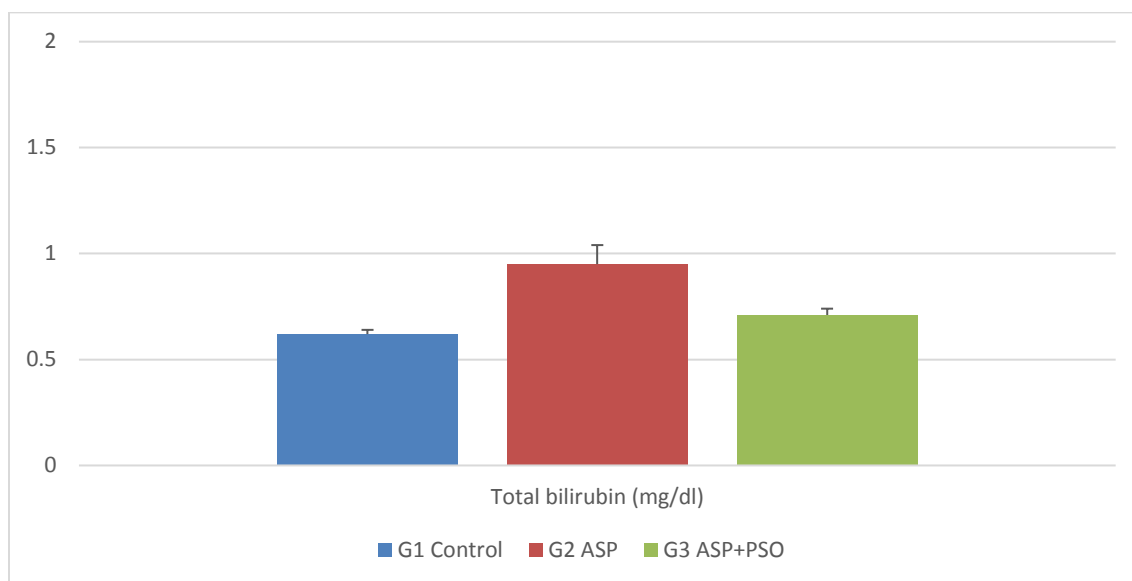


Figure 3 Effect of PSO on bilirubin level

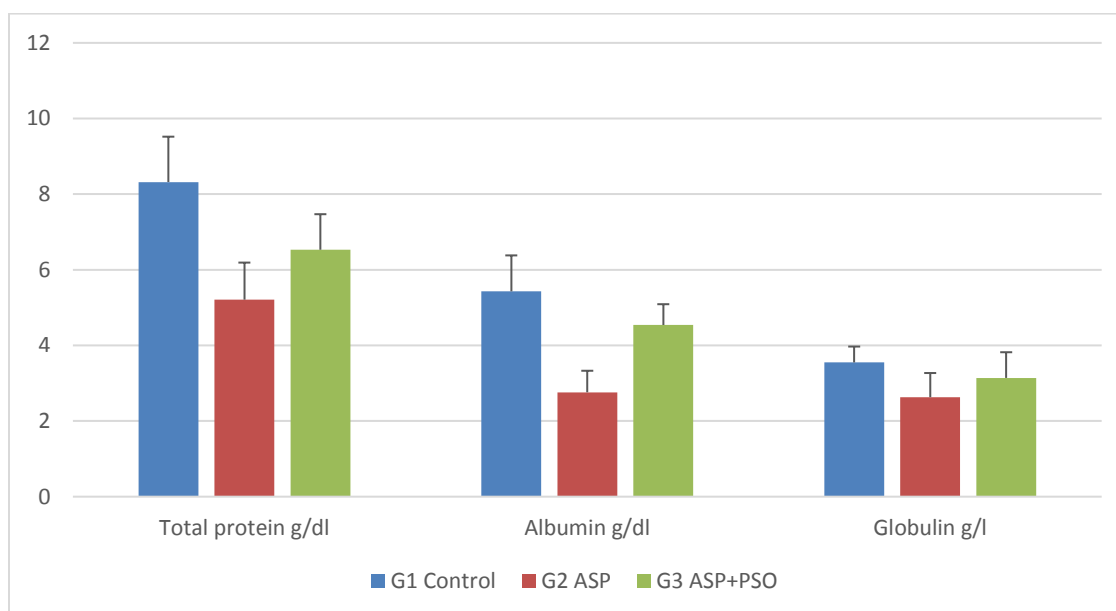


Figure 4 Effect of PSO on the levels of total protein, albumin, and globulin

Results presented in Table 2 revealed that the administration of aspartame induced a significant ($p < 0.05$) elevation in kidney function parameters (urea, creatinine, uric acid, Na^+ , and K^+ levels) when compared with those of the control untreated rat group. All the parameters that showed changed values were encountered after treatment with PSO (Figure 5 and 6).

Table 2 Effect of supplementation of pumpkin seeds on serum kidney function in rats supplemented with aspartame for 5 weeks

Group parameters	G1 Control	G2 ASP	G3 PSO
Urea mg/dl	25.54±2.3	54.86±4.2 ^a	46.88±2.9 ^b
Creatinine mg/dl	0.78±0.76	1.97±0.17 ^a	1.23±0.58 ^b
Uric acid mg/dl	3.89±0.77	7.23±1.1 ^a	5.65±0.47 ^b
Na^+ mmol/l	140.43±6.2	142.65±8.4 ^a	139.6±5.3 ^b
K^+ mmol/l	4.96±0.94	5.12±0.55 ^a	4.90±0.83 ^b

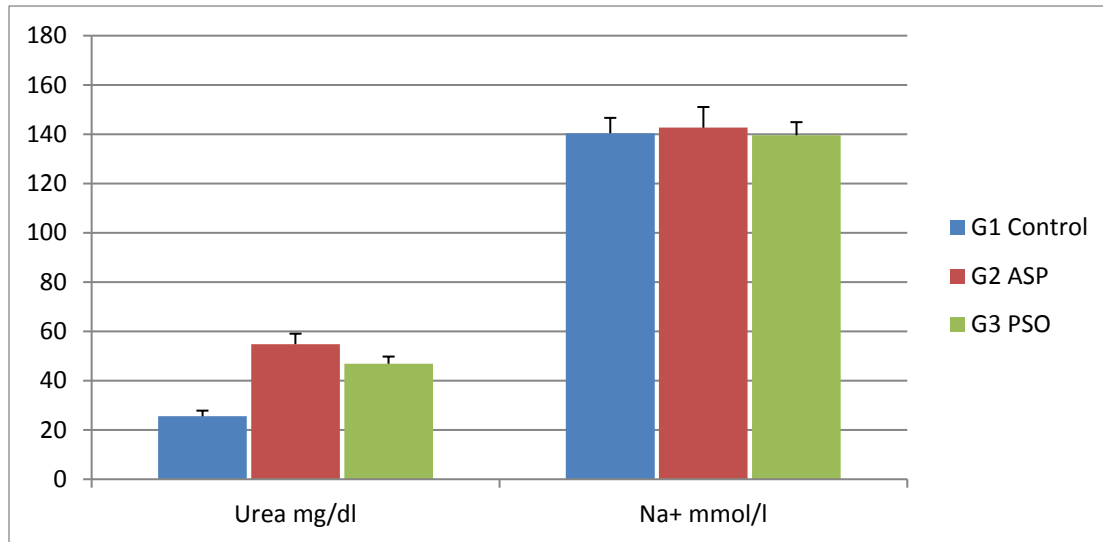


Figure 5 Effect of POS on the levels of urea and Na+

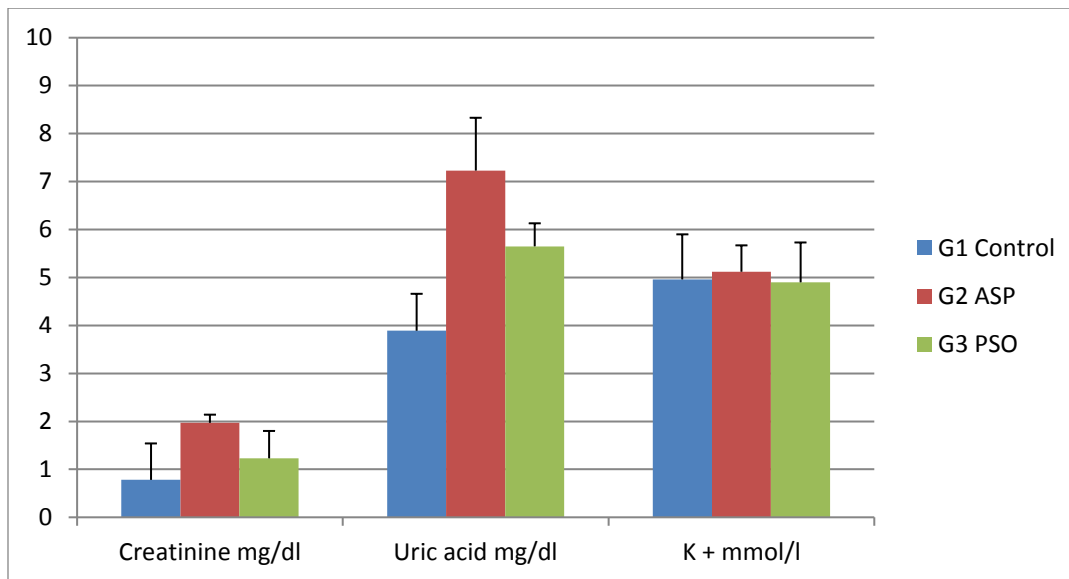


Figure 6 Effect of POS on the levels of creatinine, uric acid and K+

Table 3 presents results obtained after supplementation of pumpkin seeds on oxidative status, total DNA and total RNA in tissue homogenates of rats supplemented with aspartame for 5 weeks. Aspartame led to a noticeable reduction in kidney and liver tissue homogenate levels of glutathione (GSH) and catalase (CAT) accompanied by a significant ($p < 0.05$) increase in malondialdehyde (MDA) levels, which reflects the occurrence of oxidative stress following aspartame administration, (Figure 7 and Figure 8). DNA fragmentation indicated that administration of aspartame led to a slight increase in both kidney and liver tissues as compared with the control group, (Figure 9). Meanwhile, administration of pumpkins seed oil restored these levels to near normal.

Table 3 Effect of supplementation of pumpkin seed oil on oxidative status, total DNA, and total RNA in tissue homogenates of rats supplemented with aspartame for 5 weeks

Group Parameters	G1 Control		G2 ASP		G3 PSO	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
GSH mg/g protein	5.69±1.65	4.12±1.32	3.21±0.93	2.11±0.86	4.54±0.33	4.32±1.4
MDA nmol/mg protein	2.64±1.33	2.4±0.94	6.21±1.22	5.8±1.43	5.41±1.76	3.1±0.93
CAT u/mg protein	59.4±5.32	44.6±4.43	26.3±2.64	28.4±2.83	40.3±3.73	36.8±1.7
DNA content (mg/d tissue weight)	0.498	0.259	0.35	0.18	0.42	0.26
%DNA fragmentation	0.77%	0.14%	1.8%	1.2%	0.91%	0.13%

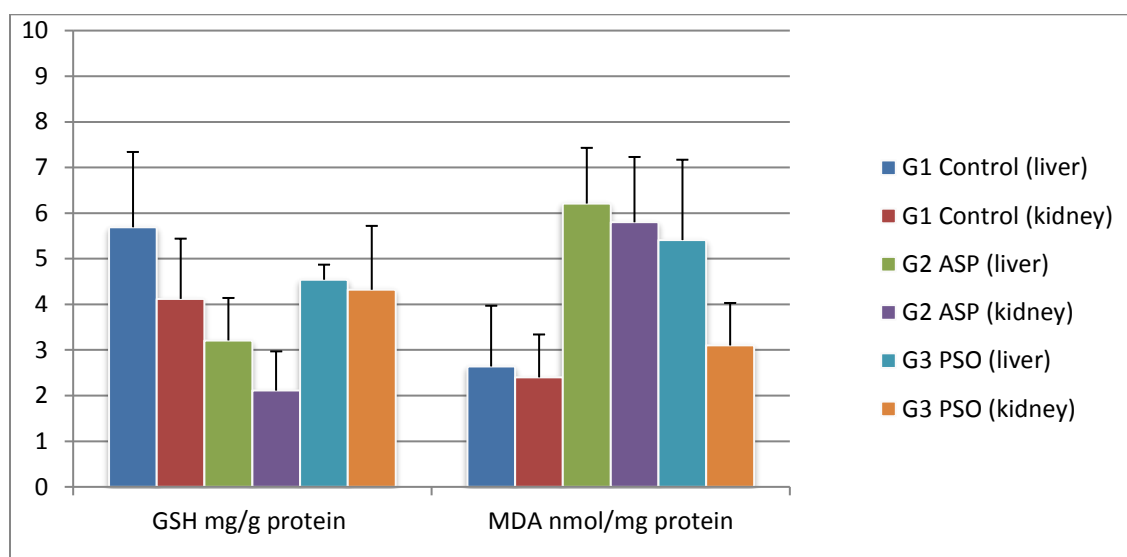


Figure 7 Effect of POS on the levels of GSH and MDA

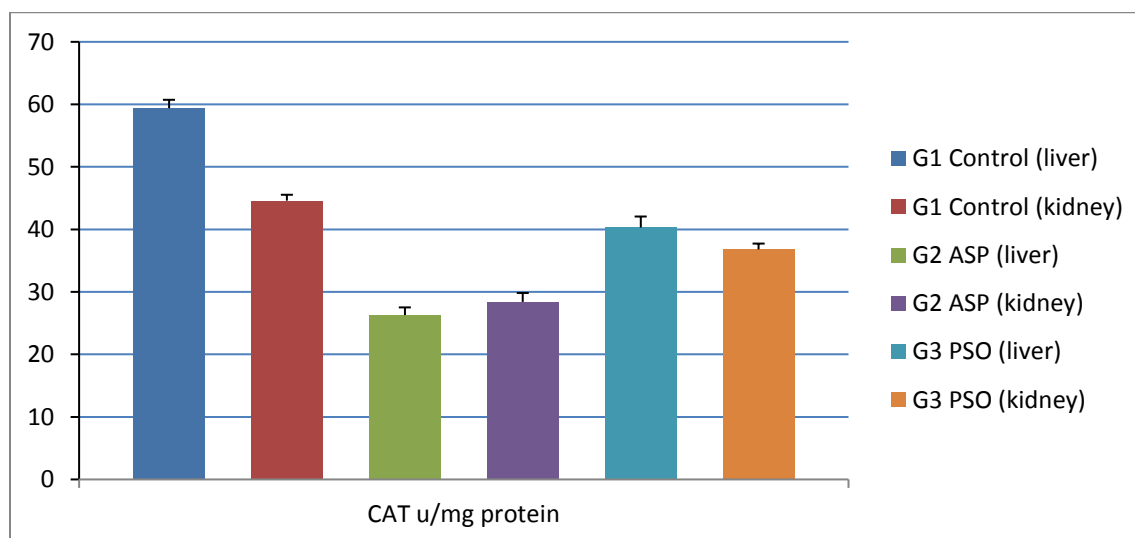


Figure 8 Effect of POS on the levels of CAT

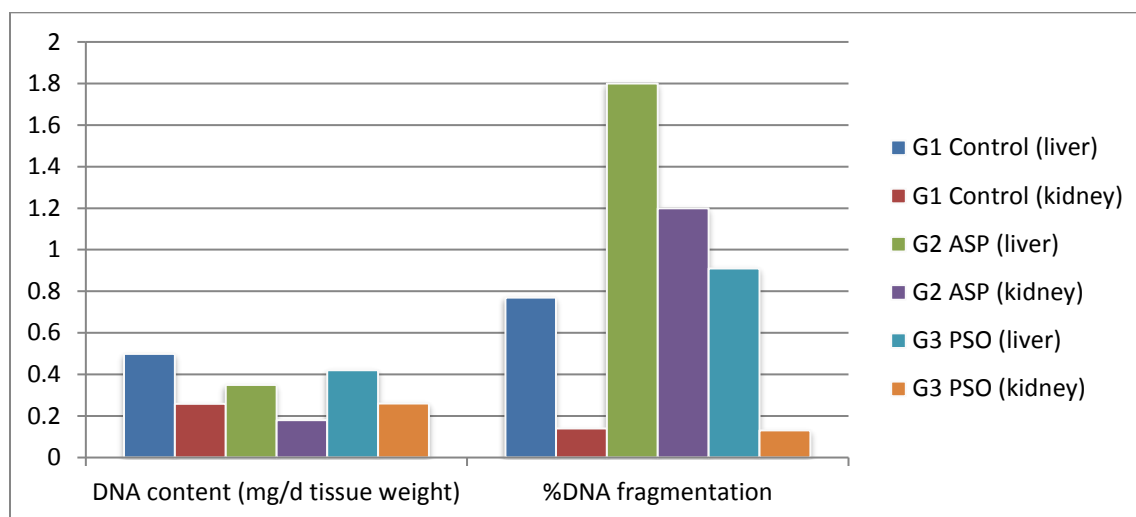
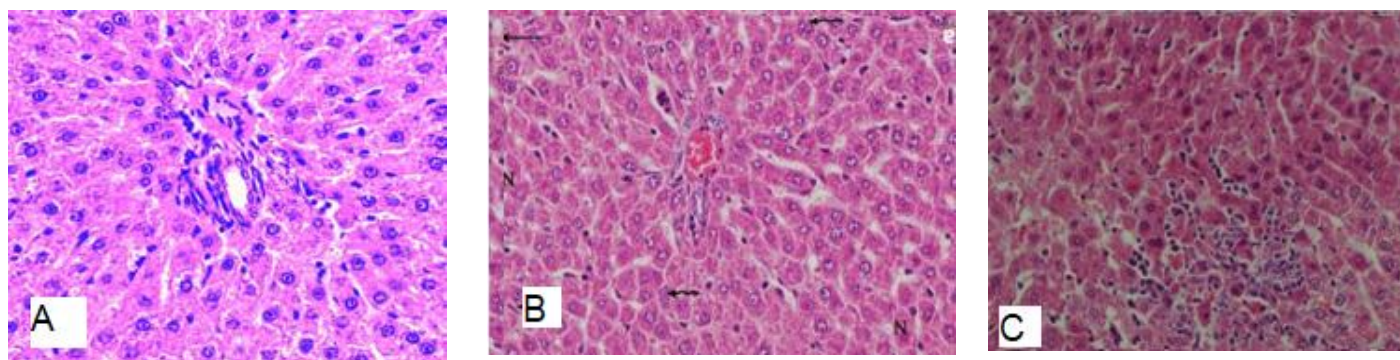


Figure 9 Effect of POS on DNA fragmentation

Histological examinations were performed to determine if there has been a protection effect of PSO on liver and kidney, (Figure 10 and Figure 11).



(A) Control group showed normal histological appearance of the liver; (B) Asp-treated rats showed congested central veins and some necrosis accompanied by increased hypertrophied Kupffer cells; (C) ASP+PSO-treated rat group showed a relatively normal appearance of kidneys as compared with the ASP group along with slight necrosis.

Figure 10 Histopathological examination of the liver in rat groups

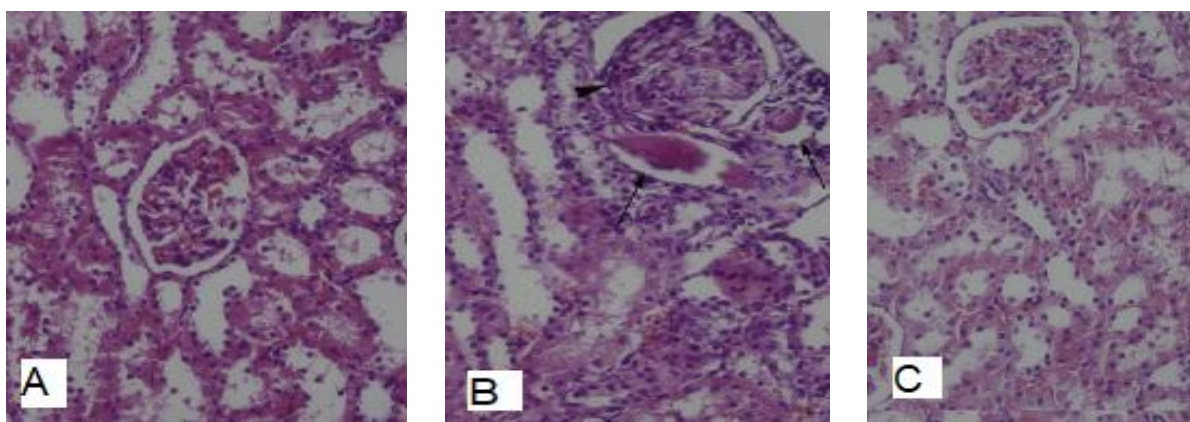
4. DISCUSSION

Due to their high nutritional value, the constituents of pumpkin, particularly PSO, have received significant attention in recent years. Pumpkin seeds have traditionally been salted and roasted for human consumption as snacks in Arab countries.

Results of the current study revealed that aspartame affects liver function by increasing liver enzyme levels accompanied with a decrease in total protein, albumin, and globulin levels. Giannini et al. (2005) showed that ALT, AST, ALP are usually found in the cytoplasm of hepatic cells and released into blood circulation if they are affected or damaged. The elevated enzyme levels detected in this study were attributed mainly to methanol, which results in oxidant/antioxidant imbalance and surface load density, thereby resulting in leakage of liver enzymes (Parthasarathy et al., 2006). An increased production of bilirubin, increased hepatic conjugation, and pigment biliary excretion in aspartame-administered animals, results from decreased uptake and increased production of bilirubin. The decrease in albumin and globulin in rats treated with aspartame has been attributed to increased free radical production by the aspartame metabolite methanol (Ashok et al., 2014).

Oboh (2005) observed that the levels of ALT, AST, and ALP were significantly reduced after intoxication with CCl₄. Pumpkin oil can play a major role in protecting the liver against alcohol induced hepatotoxicity and oxidative stress. Pre-treatment with PSO showed hepatoprotective effects, including antioxidant protection and enhanced detoxification (AbouSeif, 2014). The kidney

maintains blood homeostasis and electrolyte balance by excreting urea and creatinine. Kidney function can be evaluated from the serum electrolyte levels (Na^+ and K^+) and metabolites (creatinine and urea) (Uboh et al., 2009). The current study revealed aspartame-induced impairment of kidney function. Lower serum sodium values showed the inability of the kidney to retain sodium under aspartame overdose. Increased K^+ levels due to its reduced excretion were related to intracellular potassium leakage into the bloodstream resulting from aspartame-induced metabolite lesions in the renal tubular epithelium (Choudhary and Devi, 2014). Results of the study agreed with Al-Zuhair et al. (2000) who showed that the treatment of spontaneously hypertensive rats with felodipine combined with PSO improved free radical scavengers in the heart and kidney. In addition, PSO has been implicated as a potential agent in reducing kidney bladder stone disease due to its high phosphorus content (Fruhworth and Hermetter, 2007).



(A) The control group showed normal kidney histology; (B) Asp-treated rats showed a marked glomerulosclerosis with the appearance of proteinous material; (C) the ASP+PSO treated rat group showed a relatively normal appearance of kidney compared with the ASP group.

Figure 11 Histopathological examinations of kidneys in all rat groups

Aspartame-induced oxidative stresses in both the liver and the kidney. After its ingestion, aspartame is reportedly metabolized to triple toxins in the gastrointestinal tract namely, phenylalanine, aspartic acid, and methanol and also additional products such as formaldehyde and formic acid. Furthermore, the formation of superoxide anions and hydrogen peroxide accompany these processes (Parthasarathy et al., 2006). These metabolites of aspartame can lead to protein denaturation, fragmentation, and alteration of physicochemical properties (Sogut et al., 2004).

Glutathione is a major non-enzymatic antioxidant with a critical defensive function against exogenous and endogenous toxic chemicals in the cell defense system. Depletion of cellular GSH increases the vulnerability of cells to oxidative damage (Oyama et al., 2002). Following aspartame ingestion, a reduction in the levels of GSH levels after methanol intoxication are relayed on GSH. A decrease in glutathione levels increases the toxicity of formaldehyde metabolism. The antioxidant potential involving cellular GSH content and related enzyme activity was reportedly reduced in the liver upon methanol poisoning (Pandanaoboina et al., 2012).

In addition, the declined CAT activity observed in this study may be attributed to the formation of methanol formaldehyde. This is in line with Gulec et al. (2006), where exposure to formaldehyde in the liver tissue resulted in a decrease in CAT activity compared to control. Treatment with PSO counteracts oxidative parameters. A study by Makni et al. (2010) revealed that a diet supplemented with a mixture of flax and pumpkin seeds improved antioxidant enzyme activity in diabetic rats and significantly reduced MDA levels. The strong antioxidant activity of constituents of pumpkin led to an effective protection of the mitochondrial membrane as underscored by a significant decline in MDA value. These were in line with Eraslan et al. (2013), where PSO led to production of physiological alterations in the antioxidant enzyme action due to its potential to eliminate reactive oxygen species produced under normal conditions. Another study by Xu (2000) found that polysaccharides from pumpkin may increase the activity of GSH-Px while decrease serum MDA content in mice tumors due to its high vitamin A and tannin content, particularly present in oil, which possess antioxidant activity. Vitamin E and tocopherols prevent damage caused by free radicals; suppressed lipid peroxidation enhances GSH activity and improves membrane integrity. In addition, linoleic acid present in PSO permits osmosis and increased membrane fluidity (Ebaid et al., 2013). Further, oleic acid, a monounsaturated fatty acid, reduces the lipid peroxidation susceptibility of testis (Rizk and Darwish, 2012). Similar protective effects have been reported against cyclophosphamide lead on rat testis (Al-Masri, 2015). Our

findings are in line with those of Eraslan et al. (2013) who demonstrated in mice that treatment with PSO decreased adverse effects caused by aflatoxin. This treatment restored MDA, CAT, and GPx values to near normal levels.

The current study showed that the histological changes induced by aspartame improved significantly in the PSO group. This was in agreement with Christian et al. (2004) who reported that recovery from aspartame-induced toxicity may be gradual and incomplete.

The results of the current study are also in agreement with the results of a study by Elfiky et al. (2012) where PSO treatment in azathioprine-treated mice showed a significant decrease in the DNA fragmentation percentage compared with that observed after subjecting to either 5 or 10 mg/kg of azathioprine.

5. CONCLUSION

This work showed that administration of aspartame results in kidney and liver dysfunction. It also disturbs the oxidative status and increases DNA fragmentation in both the liver and the kidney tissues. A concurrent PSO treatment leads to protective effects against aspartame toxicity on the liver and kidney.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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