The effect of dried bottle gourd and pumpkin as hepatoprotective agent from acrylamide toxicity in experimental rats

Mona Ebrahim Ahmed Serag, Eman E. Abd-Elhady and Amira M. ElMoslemany

ABSTRACT

Acrylamide (ACR) is found in foods containing carbohydrates and proteins, where it is formed during the thermal processing. It is classified as neurotoxic and probably carcinogenic to humans. The present study investigated the effect of dried pumpkin and bottle gourd versus acrylamide induced oxidative stress and hepatotoxicity in male albino rats. The rats were divided into equal six groups. The first group (6 rats) were fed on basal diet and served as negative control. The all other groups were injected intraperitoneal (i.p.) with acrylamide (50 mg/kg body weight) for 28 days. The second group fed on basal diet and served as a positive control group. The third and fourth groups fed on basal diet supplemented with bottle gourd fruits powder (5%) and (10%). The fifth and sixth groups fed on basal diet added with pumpkin fruits powder (5%) and (10%). The chemical composition and phenolic compounds of both fruits were done. At the end of the experiment biological data were calculated, blood samples were taken to biochemical analysis. In addition, liver tissues were analyzed for antioxidant markers, malondialdehyde (MDA), as well as histological examination was done. The results revealed that acrylamide group increased liver weight, liver functions, serum lipid profile, liver MDA and NO, decreased in serum HDL-C, Liver GPx, SOD and CAT. All treated groups with two fruits showed improvement previously parameters compared with positive control group. In conclusion, the consumption of pumpkin and bottle gourd fruits powder can lower the side effects of acrylamide toxicant.

Key words: acrylamide, bottle gourd, pumpkin, hepatotoxicity
INTRODUCTION

Acrylamide (ACR) has been shown to be a carcinogen, reproductive toxicant and neurotoxicant in animals (El-Assouli, 2009). ACR is a toxic material that induces oxidative stress and is related to the accumulation of excessive reactive oxygen species (Acaroz et al., 2018). ACR is an alpha, beta-unsaturated vinyl monomer of a reacted molecule of polyacrylamide (conjugated). ACR's co-polymers and polymers have a wide variety of uses; they are used as soil stabilizers, paper processing, wastewater treatment and used in biochemistry (EL-Bohe et al., 2011).

Dietary acrylamide is mainly produced during heat processing (baking and frying) of plant foods such as potato fries and cereals through Maillard reaction between the mainly amino group of free amino acid precursor asparagine and carbonyl groups of glucose and fructose. ACR is absorbed and transmitted to different organs after ingestion, where it can react with DNA, neurons, hemoglobin, and essential enzymes (Rayburn and Friedman, 2010).

Today, the use of natural antioxidants found in food and other biological materials have great attention due to their presumed protection, nutritional and therapeutic value. Bottle gourd (Lagenaria siceraria) has a good source of vitamin-B complex and choline along with fair source of vitamin-C and β-carotene. It is reported to contain cucurbitacins, fibers, and polyphenol. In addition, campesterol and sitosterol are two sterols have been isolated from the extract of bottle-gourd fruits, which is reported to have antitoxic activity (Amit et al., 2012). Bottle gourd ethanolic extract was tested against hepatotoxicity of CCl₄ in which they showed ameliorative effects (Upaganlawar, 2017). Barot et al., (2018) found that bottle gourd contains basic elements that are mandatory for human beings' steady and virtuous well-being.
In Egypt, pumpkin is a common food that is prepared using respective ways. Pumpkin the same as squash is a gourd of the genus *cucurbita* and the *cucurbitaceae* family (which also includes gourds). Caili *et al.*, (2006) showed that *cucurbitamoschota* is more tolerant to harsh environmental conditions than other species. Lin *et al.*, (2009) found that β-carotene, which is abundant in pumpkins, could prevent liver damage caused by ethanol. Undoubtedly, pumpkin diets enhanced liver function. In addition, Pumpkin polysaccharides have hypoglycemic and antioxidant effects (*Li et al.*, 2020). The antioxidant activity was reflected in its ability to increase GSH-Px, SOD activities, and reduce MDA levels *in vivo* (*Chen et al.*, 2020).

The purpose of this study was to evaluate the hepatoprotective activity of bottle gourd and Pumpkin fruits on acrylamide induced liver toxicity in experimental albino rats.

**Plant Material**

Bottle gourd (*Lagenaria siceraria*) and pumpkin (*cucurbita moschota*) fruits were obtained from Carrefour market, Tanta, Egypt.

**Preparation of Plant**

Bottle gourd and pumpkin fruits were washed and separated pulp from seeds and peel. Pulps were cut into small pieces and were dried in oven (at 40°C). The dried samples were milled and kept in dark glass containers in -20°C until using. Chemical composition (protein, fat, moisture, ash and carbohydrates calculated by difference) determined according to (*A.O.A.C, 2010*). Phenolic and flavonoids compounds of both powder evaluated by method of Tarola *et al.*, (2013).

**Animals**

Thirty six adult male albino rats *Sprague Dawley* strain weighing (150± 10g) were housed in well-aerated cages under hygienic condition and were fed on basal diet for one week to adapt.
Experimental design:

Rats were divided into equal six groups. The first group (6 rats) were fed on basal diet and served as a negative control. All other groups were injected intraperitoneal (i.p.) with acrylamide (50 mg/kg body weight) for 28 days. The second group fed on basal diet and served as a positive control group. The third and fourth groups fed on basal diet supplemented with bottle gourd fruits powder (5%) and (10%) respectively. The fifth and sixth groups fed on basal diet supplemented with pumpkin fruits powder (5%) and (10%) respectively. At the end of experiment (28) days, the animals were deprived from food and water overnight before being sacrificed. Blood samples were collected. The liver was removed, washed, dried, weighed, and taken two samples of liver. The first sample of liver was put in 10% formalin saline for histopathological examination. The second sample was kept at -80°C for determination the antioxidant parameters.

Biological evaluation:

During the experiment (28 days), feed intake was recorded every day and body weight was recorded every week. Biological evaluation of the different diets was carried out by calculating of body weight gain % (BWG %) and feed efficiency ratio (FER) according to Chapman et al., (1959).

Biochemical analysis of serum:

Serum was analyzed for various biochemical parameters like Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), γ glutamyl transferase (GGT) activities and total bilirubin, measured according to Bergmeyer et al., (1986); Roy, (1970); Shaw et al., (1983) and Walter and Gerade, (1970) respectively. The total protein concentration and albumin) determined as stated by Sonnenwirth, Jaret, (1980), Drupt, (1974), while globulin calculated on the report of Chary, and Sharma, (2004). Also, Total cholesterol, Triglycerides and HDL-C were evaluated on the authority of
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Allain et al., (1974); Trinder and Ann, (1969) and Lopes - Virella et al., (1977) but LDL-C and VLDL-C calculated according to Friedwald et al., (1972).

Assessment of Oxidant / Antioxidant Activity in liver tissue:
The supernatant of homogenate liver tissue was used for estimation of different antioxidant level. Lipid peroxidation as a malondialdehyde (MDA) and nitric oxide (NO) determined by procedure of Uchiyama and Mihara, (1978), and method developed by Green et al., (1982). Endogenous antioxidant systems such as Superoxide dismutase (SOD) estimated by steps progressing by Misra and Fridovich, (1972), Catalase (CAT) by colorimetric assay (Sinha, 1972) and GPx by the process advanced by Lawrence and Burk, (1976).

Statistical analysis:
Results are expressed as mean ± standard deviation (SD). Differences between means indifferent groups were tested for significance using a one-way analysis of variance (ANOVA) followed by Duncan's test and probability value of 0.05 or less was considered significant. Comparative of means were performed according to least significant differences test (LSD) according to (Snedecor, 1969) using SPSS (version 20).

RESULT AND DISCUSSION:
Table (1) showed the averages (g) of moisture, protein, fat, carbohydrate and ash per 100g pumpkin and bottle gourd fruits powder. The results of chemical compositions for pumpkin and bottle gourd powder revealed that carbohydrate recorded the highest average followed by protein, ash, moisture and fat respectively. These results agree with Modgil et al., (2004) ; Kaushik et al., (2008) who showed that the edible portion of L. siceraria contains carbohydrates, protein, fat, and minerals, including calcium and phosphorous. Sim et al., (2020) illustrated that the Cucurbita moschata have high

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carbohydrate and crude protein contents (53.32, 19.09 respectively), while crude ash and crude fat contents were relatively low (13.45, 0.60 respectively).

Table (2) showed that dried pumpkin recorded the higher content from pyrogallol, catechol, catechein, benzoic, p-OH-benzoic, vanillic, caffeic, caffeine, ferulic, salycillic, ellagic and coumarin than dried bottle gourd. In addition, it contains chlorogenic on the contrary to the other. This is accordance with Sello and Mostafa, (2017) who reported that the deep-colored vegetables and fruits are considered to be healthy sources of phenolic, including flavonoid and anthocyanin, and carotenoids. Analysis of the phenolic compound of pumpkin showed that it contains pyrogallol, gallic, 4-amino-benzoic, catechein, catechol, p-OH-benzoic, caffeine, chlorogenic, vanillic, caffeic, ferulic, benzoic, ellagic, coumarin and salycilcin, this is accordance with Sello and Mostafa, (2017). Analysis of the phenolic compound of bottle gourd in current study showed that it contains the previous phenolics in pumpkin except chlorogenic. Phytochemical screening on bottle gourd fruit has revealed the presence of fucosterol, compesterols, flavonoids, cucurbitacins, saponins, polyphenolics, triterpenoids, C-flavone glycosides and ellagittannins stimulating anti-inflammatory and hepatotoxic activity (Mohan et al., 2012).

In table (3) it was evident that the injection with ACR caused a significant reduction in FI, BWG % and FER compared to normal control group. AL-Mosaibih, (2013); Prasad and Muralidhara, (2013) and Ghorbel et al., (2017) reported that a metabolic disorder that triggers energy metabolism pathways which interfere with ACR could explain the decrease in body weight. Mahmood et al., (2015) clarified the decreased body weight in rats was possibly due to ACR-induced tissue and blood cell breakdown.

Feeding on diet supplemented with bottle gourd
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or pumpkin improved these results. Bottle gourd is considered a good source of ascorbic acid. Treatment with ascorbic acid elevated the body weight reduced in cisplatin treated rats (Abdel-Daim et al., 2019). In addition, treatment with bottle gourd reduced the inflammation and fibrosis process in animals exposed to heavy metal toxicity (Qureshi et al., 2019). In another study, there is an increase in body weight for rats administrated with total flavonoid (found in bottle gourd) extract due to its inflammatory activities (Xu et al., 2019). Pumpkin was found to possess components with antioxidant activities, including vitamin E, xanthophyll's, carotenes especially β carotene, and phenolic compounds which have the principle role in protecting against oxidative tissue injury (Chanwitheeuk et al., 2005).

Liver weight was significantly higher in the positive control group compared with negative control group. These results agree with histopathological examination showed diffuse severe hydropic degeneration. Khan et al., (2011) reported that relative liver weight was increased with the ACR treatment and released enhanced liver weight to elimination of fibrosis and reduction in the invasion of inflammatory cells. In the present study, bottle gourd and pumpkin improved liver weight partly towards normal values, which contains flavonoids. Plants which have flavonoids have an impact on arachidonic acid metabolism and are thought to have anti-inflammatory effect subsequently enhanced liver weight (Qureshi et al., 2019). Also, in another study, there is a decrease in liver weight for rats treated with total flavonoids extract due to its inflammatory activities and hepatoprotection from liver injury by CCl₄ (Xu et al., 2019). Pumpkin-based foodstuff is well recognized as a source of anti-inflammatory remedies causing improvement in liver weight (Salehi et al., 2019). Zhou et al., (2019) & Wang et al., (2019) reported that pumpkin contains polyphenols that found to
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decrease the liver weight in mice with liver injury.

The present study showed that feeding on pumpkin (10%) cause significant increase in FI, BWG% and FER and reduced liver weight when compared with other groups. Thus, it recorded the best result.

In Table (4) a significant increase (P <0.05) in ALT, AST, ALP, GGT and total bilirubin were seen in rats injected with ACR, when compared to (-ve) control group. These data agree with Onyema et al., (2006)& EL-Bohe et al., (2011) who reported that administration of ACR significantly increase the activities of the serum enzyme marker of hepatocellular injury (AST, ALT and GGT) in rats. In addition, in another study it was demonstrated that ACR induced hepatotoxicity by inducing inflammatory processes, increasing oxidative stresses, and decreasing antioxidant defenses (Gedik et al., 2017). Benziane et al., (2018) added that the macrophagic pigment deposits found in the livers of rats treated with three different doses of ACR are likely a sign of hepatocyte degeneration, congestion of blood vessels (portal veinlets) and infiltration of inflammatory mononuclear cells, particularly in the pores. Hammad et al., (2020) reported that ACR has induced inflammatory cells infiltration in hepatic sinusoids and coagulative necrosis areas infiltrated by inflammatory cells.

Treatment with both powder significantly decreased (P < 0.05) all above parameters when compared with the (+ve) control group. Pumpkin (10%) recorded the superior result. These results in according with (Mali et al., 2010& Saha et al., 2011b). Wang et al., (2019) reported that polyphenols, found in bottle gourd, could reduce the levels of AST, ALT caused by liver injury and alleviate liver injury caused by oxidative stress. Olaleye et al., (2014) reported that flavonoid extract had hepatoprotective effects against acetaminophen-induced hepatic necrosis. Xu et al., (2019) revealed that total flavonoids had anti-cytotoxic and anti-inflammatory
activities, which protect liver from injury-induced by CCl₄. Carotenoids are highly present in this fruit namely α-carotene, β-carotene, ζ-carotene, neoxanthin, violaxanthin, lutein, zeaxanthin, taraxanthin, luteoxanthin, auroxanthine, neurosporene, flavoxanthin, phytfluene, cryptoxanthin and β-cryptoxanthin (Salehi et al., 2019). The treatment with beta carotene significantly reduced the hepatotoxic effect of imidacloripid shown by the significant decrease in serum liver enzymes and bilirubin. Beta-carotene could reduce lipid peroxidation and had antioxidant defense system (Bashandy et al., 2017). Also, treatment with lutein (naturally in pumpkin) reduced serum levels of ALP, total bilirubin and GGT in rats (Sindhu et al., 2010).

It was seen in table (5), the positive control group showed a reduction in total protein, albumin and globulin when compared to negative control group. These data agree with Mahmood et al., (2015) who showed that hepatocellular dysfunction could have resulted in hypoproteinemia in rats with different ACR concentrations. A steady decrease in hepatic protein levels with higher doses of ACR was documented by Sharma et al., (2008) and attributed this to retarded protein synthesis, shift in protein metabolism, or leakage of protein reserves from hepatocytes. There are two reactive sites in an ACR molecule, the conjugated double bond and the amide group that can conjugate with a thiol group of sulfur containing amino acid and -NH₂ (Friedman, 2003). This may clarify how few amino acids are not available for protein synthesis. In addition, ACR can bind with proteins and make them undetectable, being an electrophilic compound. The specific oxidative damage of some sensitive protein amino acids at the site is known to be the key cause of metabolic dysfunction during pathogenesis (Babu et al., 2011).

Feeding on both fruits showed an improvement in all above parameters when compared with (+ve) control.
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group. Famurewa et al., (2019) reported that the beneficial health impact of natural product polyphenols in scavenging and preventing free radicals that cause membrane impairment and damage to intracellular proteins and structures increasing albumin and total protein that represent liver synthetic function. Akkara and Sabina, (2020) found that the decrease in levels of albumin and total protein induced by bromobenzene resulting from pre-treatment with beta-carotene inhibiting damage to liver by its protective action thereby facilitating anabolism of albumin and proteins. Saha et al., (2011b) reported that methanolic extract of bottle gourd supplementation decreased total protein content induced by CCl₄ to near normal value and improved the functional status of the hepatic cells. Khan et al., (2011) showed that the decrease in total protein and albumin in rats treated with acrylamide were significantly recovered by chemicals such as flavonoids, terpenoids, tannins and other chemicals. 10 % as a dose from pumpkin and bottle ground groups recorded the preferable results which showed no-significant difference with (-ve) control group.

In table (6), group induced by acrylamide showed an increase in total cholesterol, triglycerides, LDL and VLDL and a reduction in HDL when compared to normal rats, these in agreement with (Benziiane et al., 2018). Tarskikh, (2006) and Akram et al., (2016) explanted that the ACR injection leads to a reduction in resistance to erythrocyte membrane acid and activation of lipid peroxidation.

The pumpkin and bottle gourd groups showed a significant decrease in in total cholesterol, triglycerides, LDL and VLDL when compared with (+ve) control group but increase HDL and improving the damage caused by acrylamide. These results agree with (Ghule et al., 2009). Kumar, (2019) indicated that hyperlipidemia leads to an increase in oxygen free radical production, which exert there cytotoxic effect by
causing lipid peroxidation. Administration of various plant extracts leading to reduction in lipid peroxidation products in hyperlipidemic rats. **Sedigheh et al., (2011)** demonstrated the antioxidant function of pumpkin protect cell membranes and other components of an organism against damage caused by oxidants. These compounds work through collecting free radicals, transferring electrons to them and ultimately rendering them inactive (**Vaya and Aviram, 2002** & **Venkat et al., 2006**). Since cholesterol plays a crucial role in lipoprotein biosynthesis and the highest level of cholesterol is contained in LDL, LDL is likely to decrease after a decrease in cholesterol levels. LDL reduction, on the other hand, may be due to an increase in the catabolism of LDL. Flavonoids increase the number of LDL receptors on the surface of liver cells through the regulation of the LDL receptor gene. LDL is driven into the hepatocyte after recognition and attachment of LDL apoprotein to LDL receptors and removed from the blood stream (**Pal et al., 2003**). The pumpkin (10%) group revealed the best results in all above parameters.

Table (7) showed significantly (p<0.05) lesser activities for CAT, SOD and GPx in liver tissue of rats injected with ACR while value of lipid peroxide (MDA and NO) for (+ve) control group was significantly increased when compared to negative control group. These results in accordance with (**Ramadhan, 2018 and Kandemir et al., 2020**). **Uthra et al., (2017)** who reported that the ACR has been free radicals, impair the status of antioxidants and eventually contribute to carcinogenesis and oxidative stress. ACR distorts the oxidant/antioxidant balance in favor of oxidants such as MDA and NO rises oxidative injury that has a key role in ACR-induced toxicity (**Rahangadale et al., 2012a, b; Al-Qahtani et al., 2017 & Ghorbel et al., 2017**).

All treated groups with dried fruits showed a significant decrease (P<0.05) in liver tissue MDA and NO and a significant
increase in all antioxidant enzymes for liver tissue as compared to positive control group. **Mondal et al., 2008** and **Saha et al., (2011c)** showed that the methanolic extract of *L. Siceraria* 's (MELS) reductive potential means its ability to donate hydrogen atoms in a dose-dependent way. The extract's elevated phenolic and flavonoid content can contribute to its antioxidant activity. As a hydrogen donor, phenolic constituents can act because of the presence of the hydroxyl groups. The fascinating topic of several studies is the antioxidant capacity of flavonoids against oxidative stress. **Vardi et al., (2010)** suggested that the rise in oxidative damage in liver tissue was avoided by dosing beta-carotene to methotrexate-induced oxidative administration. Due to its properties of scavenging lipid and peroxyl radicals, it plays an important role in protecting the cell membrane from oxidative harm. Lutein could prevent the degenerative conditions of the liver by increasing antioxidant enzyme and glutathione levels in liver tissue, attenuate lipid peroxidation (decreased MDA one of the end products of the lipid peroxidation process and oxidative stress) and effectively demonstrating its protective effect (**Sindhu et al., 2010**& **Elvira-Torales et al., 2019**).

The best result recorded by the group treated with pumpkin (10%).

**Histopathological examination of liver tissue:**

Microscopic photos of H&E stained hepatic sections showing normal radially arranged hepatic cords around central veins (CV) with normal sinusoids (S) and portal areas in control group (C). Diffuse severe hydropic degeneration (arrowheads) appears in hepatocytes with obliterated sinusoids and congested central veins (CV) (red arrows) in diseased group (A); this is agree with (**Kandemir et al., 2020**). **Hammad et al., (2020)** reported that rats in control group showing normal histologic architecture of central vein and hepatic cords. While acrylamide injected group showing
congestion of central vein and hepatic sinusoids, hydropic degeneration of most hepatocytes.

Multifocal areas of intermingled micro- to macrovesicular steatosis (black arrows) appears in hepatocytes with obliterated sinusoids and congested central veins (CV) (red arrow) in group received 5% green bottle gourd. Fewer cytoplasmic vacuoles appear in hepatocytes with opened sinusoids in-group received 5% yellow pumpkin. Hepatic sections showing very few cytoplasmic vacuoles in individual hepatocytes with opened sinusoids in-group received 10% green bottle gourd. Hepatic sections retained normal picture of hepatic cords around central veins (CV) with normal sinusoids (S) and portal areas in-group received 10% yellow pumpkin. Rats injected with ACR followed flavonoids, terpenoids, tannins and other chemicals alone or combined action of phytoconstituents, showed much less damage to liver structure. Hepatocytes were polyhedral and hepatic cords were well defined. The hepatic cells in the middle zone were normal (Khan et al., 2011). Wang et al., (2019) showed that polyphenols can alleviate the liver tissue structural damage caused by CCl₄. Polyphenols has a strong inhibitory effect of oxidative stress. It can function as an active substance with antioxidant and liver protection potential. Histopathological results are in agreement with the biochemical results.

**CONCLUSION**

Injection of acrylamide causes oxidative stress induced hepatotoxicity in rats. Bottle gourd and Pumpkin fruits powder contain tocopherol, β-carotene, unsaturated fatty acids, sterols and other phenolic compounds, showed ameliorative potential against the toxic effects of acrylamide by restoring the serum and hepatic tissue biomarker levels. The results revealed that pumpkin and bottle gourd fruits powder had considerable hepatoprotective and oxidative stress lowering ability.
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Table 1: The averages of moisture, protein, fat, carbohydrate and ash (g/100g) in pumpkin and bottle gourd fruits powder

| Proximate composition (g/100g) | Pumpkin | bottle gourd |
|-------------------------------|---------|--------------|
| Moisture                      | 5.3     | 5.2          |
| Protein                       | 16.36   | 8.86         |
| Fat                           | 1.11    | 0.06         |
| Carbohydrates                 | 67.64   | 78.73        |
| Ash                           | 9.59    | 7.15         |

Table 2: The phenolic compounds for dried pumpkin and bottle gourd fruits (ppm)

| Phenolic compounds (ppm)       | Pumpkin | bottle gourd |
|--------------------------------|---------|--------------|
| Pyrogallol                     | 3850.46 | 565.22       |
| Gallic                         | 28.95   | 48.48        |
| Catechol                       | 17.44   | 3.92         |
| 4-Aminobenzoic                 | 12.87   | 15.16        |
| Catechine                      | 898.82  | 327.79       |
| Chlorogenic                    | 230.31  | -            |
| Benzoic                        | 102.94  | 26.12        |
| P-OH-Benzoic                   | 170.32  | 57.77        |
| Vanillic                       | 26.60   | 11.76        |
| Caffeic                        | 49.63   | 21.62        |
| Caffeine                       | 111.61  | 32.03        |
| Ferulic                        | 19.01   | 11.58        |
| Salycillic                     | 36.33   | 4.47         |
| Ellagic                        | 63.75   | 6.94         |
| Coumarin                       | 13.76   | 4.05         |
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Table 3: The effect of bottle gourd and pumpkin fruits powder on changes in feed intake, body weight gain %, feed efficiency ratio and liver weight of rats with hepatotoxicity induced by acrylamide

| Parameter groups | FI (g/day) | BWG % | FER | Liver weight (g) |
|------------------|-----------|-------|-----|-----------------|
| (-ve) control    | 19.15 ± 0.10<sup>a</sup> | 33.34 ± 2.57<sup>a</sup> | 0.10 ± 0.004<sup>a</sup> | 5.30 ± 0.20<sup>f</sup> |
| (+ve) control    | 13.88 ± 0.12<sup>e</sup> | 8.18 ± 0.60<sup>f</sup> | 0.03 ± 0.000<sup>f</sup> | 8.41 ± 0.13<sup>a</sup> |
| Bottle gourd (5%) | 16.75 ± 0.10<sup>d</sup> | 12.64 ± 1.19<sup>e</sup> | 0.04 ± 0.000<sup>c</sup> | 7.70 ± 0.06<sup>b</sup> |
| Bottle gourd (10%) | 17.48 ± 0.12<sup>c</sup> | 19.07 ± 0.89<sup>e</sup> | 0.06 ± 0.000<sup>c</sup> | 7.32 ± 0.07<sup>c</sup> |
| Pumpkin (5%)     | 17.40 ± 0.14<sup>c</sup> | 16.82 ± 0.91<sup>d</sup> | 0.05 ± 0.000<sup>d</sup> | 6.65 ± 0.06<sup>d</sup> |
| Pumpkin (10%)    | 18.35 ± 0.10<sup>b</sup> | 25.65 ± 1.79<sup>b</sup> | 0.08 ± 0.004<sup>b</sup> | 6.01 ± 0.07<sup>e</sup> |
| LSD              | 0.13645   | 1.75075 | 0.0037 | 0.13285 |

Values denote arithmetic means ± SD. Means with different letters (in the same column are significantly at (p ≤ 0.05) using one-way ANOVA test, while those with similar letters are non-significant.
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Table 4: The effect of bottle gourd and pumpkin fruits powder on changes in ALT, AST, ALP, GGT and total bilirubin of rats with hepatotoxicity induced by acrylamide

| Parameters groups | ALT (U/L) | AST (U/L) | ALP (U/L) | GGT (U/L) | Total bilirubin (mg/dl) |
|-------------------|-----------|-----------|-----------|-----------|------------------------|
| (-Ve) control     | 36.29 ± 1.01<sup>f</sup> | 97.74 ± 8.08<sup>f</sup> | 120.20 ± 18.70<sup>f</sup> | 1.08 ± 0.16<sup>f</sup> | 0.35 ± 0.06<sup>e</sup> |
| (+Ve) control     | 275.31 ± 8.32<sup>a</sup> | 362.70 ± 6.72<sup>a</sup> | 311.50 ± 4.92<sup>a</sup> | 5.06 ± 0.23<sup>a</sup> | 1.40 ± 0.13<sup>a</sup> |
| Bottle gourd (5%) | 199.29 ± 1.04<sup>b</sup> | 289.16 ± 2.08<sup>b</sup> | 254.70 ± 8.89<sup>b</sup> | 3.51 ± 0.27<sup>b</sup> | 1.05 ± 0.06<sup>b</sup> |
| Bottle gourd (10%) | 131.37 ± 3.62<sup>c</sup> | 173.93 ± 6.22<sup>d</sup> | 195.63 ± 5.57<sup>d</sup> | 1.88 ± 0.12<sup>d</sup> | 0.74 ± 0.07<sup>d</sup> |
| Pumpkin (5%)      | 106.92 ± 11.74<sup>d</sup> | 209.07 ± 2.38<sup>c</sup> | 223.97 ± 6.35<sup>c</sup> | 3.01 ± 0.12<sup>c</sup> | 0.88 ± 0.03<sup>c</sup> |
| Pumpkin (10%)     | 56.80 ± 3.57<sup>e</sup> | 138.59 ± 3.12<sup>e</sup> | 162.70 ± 6.49<sup>e</sup> | 1.44 ± 0.07<sup>e</sup> | 0.34 ± 0.07<sup>e</sup> |
| LSD               | 7.3184    | 6.25125   | 11.45695  | 0.20775   | 0.0904                 |

Values denote arithmetic means ± SD. Means with different letters (in the same column are significantly at (p ≤ 0.05) using one-way ANOVA test, while those with similar letters are non-significant.
Table 5: The effect of bottle gourd and pumpkin fruits powder on total protein, albumin, and globulin of rats with hepatotoxicity induced by acrylamide

| Parameters groups | Total protein (g/dl) | Albumin (g/dl) | Globulin (g/dl) |
|-------------------|----------------------|----------------|----------------|
| (-Ve) control     | 7.28 ± 0.28<sup>a</sup> | 4.62 ± 0.25<sup>a</sup> | 2.66 ± 0.14<sup>a</sup> |
| (+Ve) control     | 5.06 ± 0.22<sup>d</sup> | 3.31 ± 0.16<sup>c</sup> | 1.75 ± 0.06<sup>b</sup> |
| Bottle gourd (5%) | 5.71 ± 1.29<sup>d</sup> | 4.01 ± 0.08<sup>b</sup> | 1.70 ± 1.29<sup>b</sup> |
| Bottle gourd (10%)| 6.10 ± 0.17<sup>ab</sup> | 4.42 ± 0.17<sup>a</sup> | 2.55 ± 0.08<sup>a</sup> |
| Pumpkin (5%)      | 6.28 ± 0.39<sup>bc</sup> | 4.06 ± 0.30<sup>b</sup> | 2.22 ± 0.13<sup>ab</sup> |
| Pumpkin (10%)     | 6.93 ± 0.25<sup>ab</sup> | 4.46 ± 0.07<sup>a</sup> | 2.46 ± 0.25<sup>a</sup> |
| LSD               | 0.6859               | 0.2247          | 0.63865         |

Values denote arithmetic means ± SD. Means with different letters (in the same column) are significantly at (p ≤ 0.05) using one-way ANOVA test, while those with similar letters are non-significant.
Table 6: The effect of bottle gourd and pumpkin fruits powder on total cholesterol, triglycerides, HDL, LDL and VLDL of rats with hepatotoxicity induced by acrylamide

| Parameter groups | Total cholesterol (mg/dl) | Triglycerides (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | VLDL (mg/dl) |
|------------------|--------------------------|----------------------|-------------|-------------|--------------|
| (-Ve) Control    | 86.23 ± 7.31\(^e\)       | 77.56 ± 2.22\(^e\)   | 53.58 ± 1.14\(^a\) | 17.15 ± 1.92\(^e\) | 14.75 ± 1.27\(^e\) |
| (+Ve) control    | 289.26 ± 19.62\(^a\)     | 192.95 ± 4.87\(^a\)  | 41.88 ± 1.14\(^d\) | 207.18 ± 21.13\(^a\) | 40.19 ± 2.66\(^a\) |
| Bottle gourd (5%) | 208.67 ± 2.64\(^b\)     | 179.24 ± 10.91\(^b\) | 49.36 ± 0.60\(^b\) | 121.89 ± 1.35\(^b\) | 35.85 ± 2.18\(^b\) |
| Bottle gourd (10%) | 179.03 ± 1.23\(^c\)     | 151.17 ± 8.24\(^c\)  | 49.51 ± 0.33\(^b\) | 100.15 ± 0.23\(^c\) | 30.23 ± 1.65\(^c\) |
| Pumpkin (5%)     | 169.55 ± 0.94\(^c\)     | 151.97 ± 10.56\(^c\) | 47.45 ± 0.63\(^c\) | 91.34 ± 1.31\(^c\) | 30.39 ± 2.11\(^c\) |
| Pumpkin (10%)    | 134.41 ± 7.44\(^d\)     | 110.21 ± 0.36\(^d\)  | 49.27 ± 1.48\(^b\) | 62.34 ± 2.45\(^d\) | 22.80 ± 1.18\(^d\) |
| LSD              | 10.79375                 | 8.7086               | 1.1439      | 10.32175    | 2.2595       |

Values denote arithmetic means ± SD. Means with different letters (in the same column) are significantly at \((p ≤ 0.05)\) using one-way ANOVA test, while those with similar letters are non-significant.
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Table 7: The effect of bottle gourd and pumpkin fruits powder on antioxidants (CAT, GPX& SOD) and oxidant (NO and MDA) parameters of rats with hepatotoxicity induced by acrylamide

| Parameters groups | CAT (ng/mg) | GPX (ng/mg) | SOD (u/mg) | NO (umol/l) | MDA (nMol/mg) |
|-------------------|-------------|-------------|------------|-------------|---------------|
| (-Ve) control     | 0.40 ± 0.02<sup>a</sup> | 0.42 ± 0.01<sup>a</sup> | 0.40 ± 0.01<sup>a</sup> | 0.28 ± 0.02<sup>b</sup> | 0.11 ± 0.02<sup>f</sup> |
| (+Ve) control     | 0.10 ± 0.01<sup>f</sup> | 0.10 ± 0.02<sup>f</sup> | 0.11 ± 0.02<sup>e</sup> | 0.40 ± 0.02<sup>a</sup> | 0.41 ± 0.02<sup>a</sup> |
| Bottle gourd (5%) | 0.21 ± 0.01<sup>c</sup> | 0.21 ± 0.02<sup>e</sup> | 0.22 ± 0.01<sup>d</sup> | 0.08 ± 0.01<sup>f</sup> | 0.26 ± 0.01<sup>b</sup> |
| Bottle gourd (10%) | 0.25 ± 0.01<sup>d</sup> | 0.27 ± 0.01<sup>d</sup> | 0.27 ± 0.01<sup>c</sup> | 0.14 ± 0.01<sup>e</sup> | 0.23 ± 0.01<sup>c</sup> |
| Pumpkin (5%)      | 0.30 ± 0.01<sup>c</sup> | 0.31 ± 0.01<sup>c</sup> | 0.29 ± 0.01<sup>c</sup> | 0.20 ± 0.02<sup>d</sup> | 0.19 ± 0.01<sup>d</sup> |
| Pumpkin (10%)     | 0.34 ± 0.01<sup>b</sup> | 0.36 ± 0.01<sup>b</sup> | 0.34 ± 0.01<sup>b</sup> | 0.25 ± 0.01<sup>c</sup> | 0.15 ± 0.01<sup>e</sup> |
| LSD               | 0.0165      | 0.0172      | 0.0155     | 0.0184      | 0.0159        |

Values denote arithmetic means ± SD. Means with different letters (in the same column) are significantly at (p ≤ 0.05) using one-way ANOVA test, while those with similar letters are non-significant.
Photo 1: Histopathological examination of liver tissue of the rats in different groups.
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