The Protective Effect of Pumpkin Extract on Polyvinylchloride (PVC) Hepatotoxicity in Male Albino Rats

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Abstract

Polyvinylchloride PVC is a common source of microplastics found in everyday objects. It is the most dangerous type of plastic because of its persistence in marine organisms, accumulation in food chains, and exposure to humans. Microplastics can cause health issues, including reduced liver function. Pumpkin fruit has been traditionally used in diets, especially in rural areas. The present study aimed to investigate the potential therapeutic effects of pumpkin extract on PVC hepatotoxicity in albino rats. Male albino rats were assigned randomly into four groups, each with six rats. Group I, negative control rats were gavaged only corn oil. Group II (PVC), rats were gavaged 1000 mg/kg b.w. PVC was dissolved in corn oil. Group III, rats, were gavaged 200 mg/kg b.w. of pumpkin extract + PVC. Group IV, rats were gavaged 400 mg/kg b.w. of pumpkin extract + PVC. The doses were given via gastric tube daily for 6 (six) consecutive weeks. The study investigated the chemical composition and phenolic compounds of pumpkin extract. At the end of the experiment, biological data were analyzed, and liver and blood samples were taken for histopathological and biochemical analysis. The most abundant components of pumpkin were Protocatechuic acid, Cinnamic acid, Rutin, and Cathachin, respectively, followed by Rosmarinic acid, Chlorogenic acid, p-coumaric acid, p-hydroxybenzoic acid, and Caffeic acid. The study's results showed that the group who were given PVC had an increase in FI and BWG%, but a decrease in liver weight. In comparison, the group who received pumpkin extract had significantly higher activity of SOD, CAT, GPXs, and TNF-α in their liver tissues, as well as lower levels of MDA and NO. The group that was given a higher dose of pumpkin extract (400mg) showed significant improvement in liver function, with reduced levels of serum AST, ALT, ALP, and bilirubin, with an increase in total protein, albumin & globulin. Furthermore, pumpkin extract improved renal function by decreasing urea, uric acid, and creatinine. All the groups that received pumpkin extract showed improvement in the aforementioned parameters when compared to the PVC group. The study showed that PVC caused liver damage, which was reversed by treatment. Liver pathology was regressed, and ultrastructure morphology was restored. In conclusion, the consumption of pumpkin extract can reduce the side effects of PVC microplastic toxicants due to the presence of antioxidants and hepato-protective effects.

Keywords: PVC – pumpkin – hepatotoxicity – microplastic – oxidative stress – antioxidant.
Introduction

Vinyl chloride is used to manufacture polyvinyl chloride (PVC) plastic and vinyl products. PVC is commonly used in construction (pipes, cables, windows) and consumer goods (electronics, car parts, toys). However, there are concerns about the health risks associated with PVC. PVC microplastic is the most dangerous type of plastic. When metabolized by liver cytochrome P450 2E1, PVC breaks down into chloroethylene oxide and chloroacetaldehyde, which can potentially damage DNA and lead to liver cancer (Chen, et al., 2022). Exposure to vinyl chloride through ingestion, inhalation, and contaminated water is a major concern, while dermal contact is less significant (Kielhorn et al., 2000).

Acute exposure to high levels of vinyl chloride in the air can cause central nervous system effects such as dizziness, drowsiness, and headaches. Chronic exposure to vinyl chloride through inhalation and oral exposure can result in liver damage. Inhalation of vinyl chloride is also a significant risk factor for a rare form of liver cancer in humans. Microplastics, which contain carcinogenic materials, have been found in the digestive systems of marine species. They tend to accumulate in the liver, spleen, and kidney and can move to other organs and tissues, especially during inflammation. PVC can release toxic monomers into the environment, causing cytotoxicity, chronic inflammation, and oxidative stress in nearby organs and tissues (Wicaksono, et al., 2022).

Numerous studies have shown that microplastics (MP) can have adverse effects on the biochemistry, metabolism, and physiology of rodents, rabbits, and chickens. These effects can be particle size dependent and intergenerational, suggesting potential long-term risks associated with MP exposure (Banerjee et al., 2021). However, it is important to note that the mere ingestion of microplastics does not directly cause harm to animals (Wright and Kelly, 2017). The toxicity of microplastics largely depends on the duration, size, and concentration of the particles that enter the bodies of living organisms (Wright and Kelly, 2017; Wu et al., 2019; Campanale et al., 2020).

The liver is the most important organ in our body, as it plays a crucial role in regulating various physiological processes. It is responsible for many vital life functions, such as food digestion, glycogen storage, metabolism control, drug detoxification, and hormone production (Si-Tayeb et al., 2010). Any malfunction or failure of this organ can lead to high rates of morbidity and mortality, given its significant impact on our overall health (Negm, S. H. 2018).

(Haldar, et al., 2023) stated that the liver is the primary organ responsible for detoxifying the body in living organisms. When the liver is damaged, it can cause microplastics to release toxins that can attack other organs in the body, such as the heart (Li et al., 2021) skeletal muscles (Shengchen et al., 2021) the nervous system (Lei et al., 2018) kidneys (Deng et al., 2017; Chen, et al., 2023) the digestive system (Deng et al., 2017; Li et al., 2021), and lungs (Lu, et al., 2021).

Pumpkin is a member of the Cucurbits family and is an economical vegetable grown globally. Different pumpkin fruit parts (seeds, peels, and flesh) are rich sources of micro and macronutrients, including carbohydrates, fiber, amino acids, MUFA, PUFA, tocopherol, and carotenoids and terpenoids (Nakazibwe, et al., 2020).
Additionally, pumpkin contains Phyto-constituents like alkaloids, flavonoids, palmitic, oleic, and linoleic acids that have medicinal properties like anti-diabetic, antioxidant, anti-carcinogenic, anti-inflammatory and anti-ulcerative properties (Omer, et al., 2016 and Batool, et al., 2022). Pumpkins are a rich source of beta-carotene (>80%) which helps prevent vitamin A deficiency (Al-Barbary, et al., 2021).

Researchers have evaluated and utilized different pumpkin cultivars in Egypt (Al-Barbary et al., 2011) and studied the nutritional composition of pumpkin pulp in Uganda (Nakazibwe et al., 2020). This study aimed to investigate the hepatoprotective and antioxidant effect of pumpkin extract against polyvinyl chloride (PVC) induced hepatotoxicity.

**Materials and Methods**

**Plant material and animals**

Pumpkin fresh was obtained from local market in (Tanta, El Gharbiya, Egypt). Twenty four male albino rats (150±10 g) were obtained from the animal colony Helwan Farm, Vaccine and Immunity Organization, Cairo, Egypt. Animals were clinically healthy. They were acclimatized to the experimental conditions for one week before the start of the experiment. During this period, the rats were housed in plastic cages with galvanized iron filter tops and placed in a quiet room with natural ventilation and a 12:12-hour light-dark cycle. According to Reeves et al., (1993), the rats were fed on a basal diet and water ad-libitum throughout the experimental period.

**Chemicals, kits, and other required materials**

Casein, vitamins, minerals, cellulose, choline chloride, DL-methionine, and other required chemicals were purchase from El-Gomhoreya Company for Trading Drugs, Chemicals, and Medical Appliances, Cairo, Egypt. Kits used for biochemical determinations were bought from Gama Trade Company for Chemicals, Cairo, Egypt. Corn starch and corn oil were obtained from the local market, Tanta City, Al-Gharbia Governorate, Egypt. PVC was purchased from Sigma-Aldrich Company, Egypt.

**Extract preparation**

The pumpkins were thoroughly cleaned, washed with distilled water, peeled the seeds were removed, and sliced. These slices were then soaked in boiling water for 15 minutes, cooled with distilled water, and dried in an air oven dryer at 40-50°C for 24 hours. Once dried, the slices were milled and passed through a 30 mesh sieve as per the instructions of Mahagoub (2008).

To make the extract, 100 grams of the powdered pumpkin were added to 500 ml of ethanol in a beaker and homogenized for 15 minutes at 60°C using a magnetic stirrer. The mixture was then transferred to a dark glass bottle and left for 24 hours to ensure complete extraction. The extract supernatant was obtained by passing it through a 0.2 µm filter, and the ethanol was evaporated to give a pure extract.

**Determination of phenolic compounds**

The polyphenolic compounds present in pumpkin extract were separated and identified for phenolic and flavonoid compounds using a High-Performance Liquid Chromatography (HPLC) method described by Tarola et al. (2013). The phenolic acid standard was dissolved in a mobile phase and injected into HPLC. The concentration of phenolic compounds was calculated based on retention time and peak area.
Study design
(1000 mg/kg) from PVC dissolved in corn oil orally by using stomach tube for 6 weeks. (Sadeghi et al., 2020) to induce toxicity.

The rats were assigned randomly into four groups; each group had six rats. G I, Control rats received only corn oil and given by gastric tube. G II (PVC), rats were gavaged 1000 mg/kg b.w. PVC was dissolved in corn oil and given by gastric tube, according to Sadeghi et al., (2020). G III, rats, were gavaged 200 mg/kg b.w. of pumpkin extract according to Ghahremanloo et al., (2017) + PVC. G IV, Rats were gavaged 400 mg/kg b.w. of pumpkin extract according to Ghahremanloo et al., (2017) + PVC. The doses were given via gastric tube daily for six consecutive weeks.

Slaughter and biochemical analysis
After the experimental period, the rats were not given any food overnight before they were sacrificed. Blood samples were collected from each rat and then centrifuged for 10 minutes at 3000 revolutions per minute (r.p.m) to separate the serum. The serum was then carefully separated and transferred into clean and dry Eppendorf tubes and frozen at -20ºC for analysis, as described by Schermer (1967). The liver was removed from each rat through careful dissection, cleaned with a saline solution (0.9%) to remove any adhesive matter, dried with filter paper, and weighed. The liver was then divided into two parts: the first part was fixed in 10% formalin for histopathological examination, while the second part was homogenized for antioxidant analysis.

Growth-related parameters
The body weight gain (BWG%), feed intake (FI), feed efficiency ratio (FER) and also relative liver weight were calculated according to Chapman et al., (1959). Feed intake (FI) was recorded daily. Growth-related parameters, including the final body weight gain% (BWG%) and feed efficiency ratio (FER), were determined using the following equations:

\[
\text{BWG\%} = \frac{\text{Final body weight (g)} - \text{Initial body weight (g)}}{\text{Initial body weight (g)}} \times 100
\]

\[
\text{FER} = \frac{\text{FBWG (g)}}{\text{total FI (g)}}
\]

Relative organ weight (ROW%) = Liver weight/ Final body weight × 100

Preparation of liver homogenates and biochemical analysis
The liver was cleaned with an ice-cold saline solution. The liver parts were homogenized in a proportion of 1:10 (W/V) ice-cold KCL buffer (1, 15%; pH 7.2). The homogenate was centrifuged at 10,000×g for 10 minutes at 4°C to obtain post-mitochondrial supernatant (PMS), which was used to measure the levels of the lipid peroxidation marker malondialdehyde (MDA) and nitric oxide (NO). MDA was estimated according to Uchiyama & Mihara (1978), and NO was estimated according to Giustarini et al. (2008). The activity of the antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) were assessed as previously described (Oberley et al., 1985) and (Paoletti & Moccali, 1990), respectively, while the concentrations of GPx activity were determined as previously described by Hafeman et al. (1974). Tumor necrosis factor-α (TNF-α) was also measured on
incubated samples using ELISA kits and determined according to Bergmeyer et al. (1986). In addition, serum concentrations of aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) were performed according to the method described by Reitman & Frankel (1957). Serum alkaline phosphatase (ALP) determination was performed according to the colorimetric method of Roy (1970). Total bilirubin was measured according to Walter & Gerade (1970), and the total protein concentration and albumin were determined as stated by Jarett & Sonnenwirth (1980) and Drupt (1974), respectively. Globulin was calculated based on the report of Chary & Sharma (2004). Serum levels of uric acid, urea, and creatinine were determined according to the methods described by Fossati et al. (1980), Patton & Crouch (1977), and Bartels et al. (1972), respectively.

Histopathological examination

Liver samples were taken from various groups of rats and fixed in a 10% formalin solution. They were then washed under tap water and dehydrated using a series of graduated alcohol dilutions (including methyl, ethyl, and absolute ethyl). After that, the samples were cleared in xylene and embedded in liquid paraffin at a temperature of 56°C. Finally, sections of 4μm thickness were cut, deparaffinized, and stained with Hematoxylin/Eosin stains to carry out histopathological examination under a light microscope.

Statistical Analysis

The statistical analysis was performed using SPSS software (Version 20; Untitled – SPSS Data Editor). The results were presented as mean ± standard deviation (mean ± SD). One-way classification and analysis of variance (ANOVA) test were used to analyze the data. The significance of differences between means was determined by employing the Duncan test at p<0.05 (Garth, 2008).

Results

The polyphenolic compounds of pumpkin extract

HPLC identified the phenolic compounds of pumpkin; the results are listed in Table 1. The most abundant components of pumpkin were Protocatechuic acid, Cinnamic acid, Rutin and Catachin “187.68, 65.97, 37.99 and 37.05µg/ml”, respectively, followed by Rosmarinic acid, Chlorogenic acid, p-coumaric acid, p-hydroxybenzoic acid, and Caffeic acid.

<table>
<thead>
<tr>
<th>Table 1. Phenolic compounds of pumpkin extract (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic Compounds</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
</tr>
<tr>
<td>p-hydroxybenzoic acid</td>
</tr>
<tr>
<td>Catachin</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
</tr>
<tr>
<td>Caffeic acid</td>
</tr>
<tr>
<td>Syringic acid</td>
</tr>
<tr>
<td>Ferulic acid</td>
</tr>
<tr>
<td>Sinapic acid</td>
</tr>
<tr>
<td>Rutin</td>
</tr>
<tr>
<td>p-coumaric acid</td>
</tr>
<tr>
<td>Apigenin-7-glucoside</td>
</tr>
<tr>
<td>Rosmarinic acid</td>
</tr>
<tr>
<td>Cinnamic acid</td>
</tr>
</tbody>
</table>
Body and Liver Weights
Feed intake (FI), (BWG%), and FER exhibited a significant reduction (p < 0.05) in PVC treated group than in negative control. Administration of pumpkin extract with PVC resulted in a significant (p < 0.05) increase in these parameters than PVC treated group. Also, PVC treated group showed significantly (p < 0.05) increase in liver weight % compared to the control group. On the other hand, treated groups with the two doses of pumpkin extract showed a significant decrease in liver weight compared to PVC treated group (Table 2).

Table 2.
Protective effect of Pumpkin extract on FI(g/d), BWG%, FER and Liver weight % levels in PVC -treated male rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>FI(g/d)</th>
<th>BWG%</th>
<th>FER</th>
<th>Liver %</th>
</tr>
</thead>
<tbody>
<tr>
<td>control (-ve)</td>
<td></td>
<td>16.8 ± 0.100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.55±3.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.185±0.0053&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.611 ±0.041&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(PVC)</td>
<td></td>
<td>12.53 ± 0.433&lt;sup&gt;d&lt;/sup&gt;</td>
<td>17.72±0.52&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.055±0.0016&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.99 ±0.105&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pumpkin(200mg/kg)+ PVC</td>
<td></td>
<td>14.26 ±0.145&lt;sup&gt;c&lt;/sup&gt;</td>
<td>58.54±2.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.164±0.0087&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.875 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pumpkin(400mg/kg)+ PVC</td>
<td></td>
<td>15.43 ±0.176&lt;sup&gt;b&lt;/sup&gt;</td>
<td>65.54±0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.167±0.0025&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.927 ±0.087&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td>0.815</td>
<td>6.58</td>
<td>0.017</td>
<td>0.245</td>
</tr>
</tbody>
</table>

Data were expressed as mean ±SD. The different superscripts significantly differed within the same row. * level of significant p-value < 0.05

Pumpkin extract to improve the altered antioxidant status due to PVC
Antioxidant enzymes such as SOD and CAT play an important role in eliminating reactive oxygen species and protecting against oxidative stress. To investigate oxidative stress damage in liver tissue, Lipid peroxidation levels After PVC exposure, the enzymatic activity of Superoxide dismutase (SOD), Catalase(CAT) and Glutathione peroxidase (GPx) decreased significantly and reverse effects were shown by Pumpkin extract in a dose-dependent manner. PVC group significantly increased the levels of MDA ,NO and tumor necrosis factor-alpha (TNF−α) (p<0.05), which were remarkably subsided by pumpkin extract with two doses., as shown in Table 3.
## Table 3.

Protective effect of Pumpkin extract on SOD, CAT, GPX, MDA, NO, and TNF−α (Pg/ml) levels in PVC-treated male rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>SOD (U/mg)</th>
<th>CAT (U/mg)</th>
<th>GPX (mg/gm)</th>
<th>MDA (nmol/gm)</th>
<th>NO (umol/l)</th>
<th>TNF−α (Pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control (-ve)</td>
<td>0.99±0.011a</td>
<td>3.935±0.014a</td>
<td>4.275±0.037a</td>
<td>0.87±0.011a</td>
<td>1.085±0.043a</td>
<td>44.54±4.46d</td>
</tr>
<tr>
<td></td>
<td>(PVC) control</td>
<td>0.87±0.011d</td>
<td>0.935±0.008d</td>
<td>1.085±0.054d</td>
<td>0.73±0.086d</td>
<td>0.9±0.138a</td>
<td>240.53 ± 22.84d</td>
</tr>
<tr>
<td></td>
<td>Pumpkin (200mg/kg) + PVC</td>
<td>2.06±0.46d</td>
<td>1.975±0.020d</td>
<td>2.745±0.0606d</td>
<td>2.8±0.144d</td>
<td>3.645±0.054b</td>
<td>157.25 ± 14.56b</td>
</tr>
<tr>
<td></td>
<td>Pumpkin (400mg/kg) + PVC</td>
<td>2.885±0.05b</td>
<td>2.92±0.023b</td>
<td>3.515±0.0548b</td>
<td>2.06±0.008c</td>
<td>2.83±0.034c</td>
<td>94.83 ± 10.99c</td>
</tr>
<tr>
<td>LSD</td>
<td>0.119</td>
<td>0.057</td>
<td>0.171</td>
<td>0.27</td>
<td>0.25</td>
<td>48.22</td>
<td></td>
</tr>
</tbody>
</table>

Data were expressed as mean ±SD. The different superscripts significantly differed within the same row. * Levels of significant difference (p-value < 0.05).

Data presented in Table (4) showed the effects of Pumpkin extract on the activities of serum liver enzymes for PVC-intoxicated rats. It could be noticed that the activities of all studied liver enzymes, namely AST, ALT, ALP, and total bilirubin in serum of the (+ve) control group showed significant increase as compared to the (-ve) group. The rats received pumpkin extract with two doses showed significant decrease in all levels of above mentioned parameters (p<0.05). When compared with PVC group. Also, data presented in Table (4) showed the effect of pumpkin extract on serum total protein, albumin and globulin in PVC-intoxicated rats. The mean values of total protein and albumin in the (+ve) control group showed significant increase compared to the (-ve) control group. The rats that received pumpkin extract showed significant decrease (P<0.05) in total protein and albumin as compared to the (+ve) control group as shown in Table (4).

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Table 4.
Protective effect of Pumpkin extract on liver enzymes and serum proteins levels in PVC -treated male rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>BIL (IU/L)</th>
<th>Total Protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control (-ve)</td>
<td>35.59±0.207a</td>
<td>24.83±0.74a</td>
<td>2.815±0.049d</td>
<td>3.53±0.161a</td>
<td>13.83±0.164a</td>
<td>10.91±0.167a</td>
<td>2.918±0.0629a</td>
</tr>
<tr>
<td>Polyvinyl chloride (+ve)</td>
<td>141.07±5.12b</td>
<td>128.745±1.61b</td>
<td>9.5±0.167b</td>
<td>12.82±0.092b</td>
<td>6.54±0.304b</td>
<td>5.561±0.297b</td>
<td>0.98±0.010b</td>
</tr>
<tr>
<td>Pumpkin (200mg/kg) + Polyvinyl chloride</td>
<td>95.33±2.68b</td>
<td>77.6±1.102b</td>
<td>5.31±0.115b</td>
<td>9.52±0.0057b</td>
<td>7.98±0.070b</td>
<td>6.468±0.331b</td>
<td>1.511±0.357b</td>
</tr>
<tr>
<td>Pumpkin(400mg/kg)+ Polyvinyl chloride</td>
<td>68.2±1.54C</td>
<td>53.26±1.07C</td>
<td>3.3±0.167C</td>
<td>6.75±0.075C</td>
<td>11.3±0.17b</td>
<td>9.755±0.205b</td>
<td>1.545±0.035b</td>
</tr>
<tr>
<td>LSD</td>
<td>9.77</td>
<td>3.833</td>
<td>0.436</td>
<td>0.327</td>
<td>0.368</td>
<td>0.487</td>
<td>0.343</td>
</tr>
</tbody>
</table>

Data were expressed as mean ±SD. The different superscripts significantly differed within the same row. * Level of significant difference p < 0.05.

Pumpkin Extract Improve Kidney Functions due to PVC

Data presented in Table (5) showed the effects of Pumpkin extract on the activities of kidney functions in serum PVC - intoxicated rats. It could be noticed that ; all Kidney functions , namely serum uric acid, urea and creatinine of the (+ve) control group showed significant increase as compared to the (-ve) group. The rats received pumpkin extract with two doses showed significant decreas in the levels of uric acid, urea and creatinine (p<0.05), when compared with PVC group.

Table 5.
Protective effect of Pumpkin extract on uric acid, urea and creatinine levels in PVC -treated male rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>UricAcid (mg/dL)</th>
<th>Urea (mg/dL)</th>
<th>Creatinine(mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control (-ve)</td>
<td>2.925±0.02d</td>
<td>2.01±0.011d</td>
<td>2.835±0.066d</td>
</tr>
<tr>
<td>Polyvinyl chloride control (+ve)</td>
<td>11.795±0.095a</td>
<td>8.99±0.057a</td>
<td>8.93 ±0.028a</td>
</tr>
<tr>
<td>Pumpkin(200mg/kg)+ Polyvinyl chloride</td>
<td>7.93 ± 0.046b</td>
<td>6.215±0.02b</td>
<td>6.15± 0.057b</td>
</tr>
<tr>
<td>Pumpkin(400mg/kg)+ Polyvinyl chloride</td>
<td>5.955 ± 0.037c</td>
<td>3.585±0.02c</td>
<td>5.12 ± 0.051c</td>
</tr>
<tr>
<td>LSD</td>
<td>0.186</td>
<td>0.106</td>
<td>0.173</td>
</tr>
</tbody>
</table>

Data were expressed as mean ±SD. The different superscripts significantly differed within the same row. * Level of significance p-value < 0.05.

Histopathological results

Microscopic examination of liver tissue sections from the control group (Fig. 1,2) revealed normal histological structure of both centrilobular and periportal areas. Positive control group (Fig. 3,4) exhibited marked hepatocellular vacuolation in the centrilobular hepatocytes. The portal areas showed intense mononuclear inflammatory cells infiltration. Pumpkin 200 group (Fig. 5,6) showed diffuse
hepatocellular vacuolation in both centrilobular and periportal hepatocytes with subsided inflammatory cells infiltration. Marked improvement was detected in the examined liver sections from Pumpkin 400 group (Fig. 7,8) as they exhibited apparently normal hepatic parenchyma in both centrilobular and periportal areas.

Fig. (1,2) Photomicrograph of liver, control group higher magnification showing normal liver parenchyma in the periportal area (H&E). Fig. (3,4) Photomicrograph of liver, PC group higher magnification showing hepatocellular vacuolation in the centrilobular hepatocytes (arrow) and showing portal infiltration with mononuclear inflammatory cells (arrow) (H&E). Fig. (5,6) Photomicrograph of liver, Pumpkin 200 group higher magnification showing diffuse hepatocellular vacuolation (H&E). Also, fig. (6) showing diffuse hepatocellular vacuolation in the periportal areas (H&E). Fig. (7,8) Photomicrograph of liver, Pumpkin 400 group higher magnification showing apparently normal hepatocytes in the periportal area and normal centrilobular hepatocytes (H&E).
Discussion

Although human bodies produce antioxidant enzymes, additional dietary antioxidants are needed to boost immunity and fight harmful free radicals. Pumpkin has antioxidants and hepatoprotective effects, but yet no evidence of its immunomodulatory actions.

The polyphenolic compounds of pumpkin extract

The most abundant components of pumpkin were Protocatechuic acid, Cinnamic acid, Rutin, and Catachin, respectively, followed by Rosmarinic acid, Chlorogenic acid, p-coumaric acid, p-hydroxybenzoic acid, and Caffeic acid. The phytochemical components observed in this study were similar to those reported by Negm (2018), who recorded that fresh pulp pumpkin contains β-carotene, L-ascorbic acid, total phenols, total flavonoids, and antioxidant activity.

Pumpkin flour is a nutritious ingredient that contains a variety of nutrients such as moisture, protein, fiber, carbohydrates, and minerals like calcium, phosphorus, iron, zinc, potassium, magnesium, and sodium. The flour is rich in beta-carotene and yellow color supplements, making it a suitable ingredient in bakery products. Additionally, pumpkin is a good source of minerals due to its high levels of calcium, magnesium, and phosphorus. (Al-Barbary, 2011; Negm, 2018, and Fernández-López et al., 2020). Zdunić, et al., (2016) found eight phenolic compounds in pumpkin fruit, with phenolic acids predominating. Among flavonoids, flavanone glycoside hesperidin was identified. Batool, et al., (2022) reported that pumpkin pulp besides phenolic compounds contains rich nutrients that have the potential to confer health benefits.

The technology used, particularly heat treatments, has a significant impact on the total carotenoid content of pumpkin products (Assous et al., 2014). When pumpkin was cooked almost entirely without the use of water, the highest retention of carotenoids was obtained, while the lowest retention of carotenoids was linked to the use of a substantial amount of water during cooking. The temperature, as well as interaction with air and light, are the main elements that cause this loss (Provesi & Amante, 2015).

Pumpkin seed oil is rich in powerful antioxidants and beneficial nutritional supplements like linoleic acid, oleic acid, palmitic acid, omega 3, 6, and 9, carotenes, lutein, gamma and P-tocopherols, phytosterols, chlorophyll, selenium, and zinc. These findings were reported in studies conducted by Mohammadi et al. (2014) and Shaban et al. (2017).

Body and Liver Weights

Treated groups with an extract of pumpkin, showed a significant decrease in liver weight, this could be due to the extract’s antioxidant or lipid-reducing properties, possibly minimizing liver damage or fat storage. On the other hand, a significant increase in BWG compared with the PVC-treated group. This might be caused by improved nutrient absorption, increased appetite, or changes in energy metabolism.

Contrary to our findings, Monica et al. (2022) reported weight reductions in pumpkin seed consumers. However, these changes did not reach statistical significance in their study. Additionally, Song and Sun (2017) found that a meal high in pumpkin natural powder decreased weight gain as well as lipid profiles, liver organ glycogen, and plasma insulin levels. Mohamed, et al.,(2021) concluded that bread fortified by pumpkin for diabetic rats did not significantly affect their feed intake compared to control groups.
In a recent study, Sincihu, et al., (2022) found that the PVC group’s liver weight tended to be heavier compared to the control group, but not statistically significant. On the other hand, the present study found that treated groups with extract of pumpkin showed a significant decrease in liver weight compared to the PVC group.

According to research by Koziorzebska et al. (2018), incorporating a diet rich in dried pumpkin can boost the body's antioxidant potential and lower oxidative damage. In addition, pumpkin supplementation has been found to reduce NO levels, which can potentially alleviate oxidative stress (AboSeda et al., 2019). (Haro et al. 2023) have identified elevated MDA levels as a sign of increased oxidative stress and potential cellular harm. Furthermore, the PVC group was found to have significantly higher MDA levels than the negative control group, as reported by Gonenc et al. (2001).

In this study, when compared to the PVC group, pumpkin significantly increased SOD, CAT, GPXs, and TNF-α activity in the liver tissues while significantly reduced MDA and NO levels. This finding suggested that pumpkins had a protective effect against PVC-induced tissue oxidative damage by increasing antioxidant enzyme activity and lowering lipid peroxidation levels. These results are similar to previous studies about Cyclophosphamide side effects on the liver (Ran et al., 2020). Cytokines released by Kupffer cells in the liver initiate a chain reaction that results in the production of TNF-α by other macrophages. This, in turn, causes oxidative damage and the formation of harmful radicals that interfere with protein synthesis in hepatocytes, ultimately resulting in cell death (Lu et al., 2018 and Elmeligy, et al., 2019).

Pumpkin extract and Liver functions

This study revealed that pumpkin extract at both doses significantly decreased the activities of AST, ALT, ALP, and total bilirubin in PVC-intoxicated rats compared to the control group. This suggests a protective effect of pumpkin extract against PVC-induced liver damage. Also, protein levels (total protein, albumin, and globulin) significantly increased compared to the control group. This indicates that pumpkin extract may promote protein synthesis and improve overall protein metabolism. Omer, et al., (2016) revealed that pumpkin has precious components among all beta-carotene which have been proven to be a powerful antioxidant and profound protective action against oxidative stress. Protocatechuic acid (PCA) is a naturally occurring phenolic acid found in many plants (Kakkar and Bais, 2014). In this study, it was found that the compound most abundant in pumpkin is protocatechuic acid, which was found to have a major role in improving liver function levels. Administering this compound to diabetic rats as a supplement has been found to reduce weight gain, improve dyslipidemia and liver function, oral glucose utilization, and sensitivity to insulin, and mitigate oxidative and hepatic damage. It also ameliorates serum lipid levels, hepatic function parameters, and hepatosteatosis in type 2 diabetes rats. PCA mitigates hepatic lipid peroxidation and restores GSH and SOD to near-normal levels (Abdelmageed et al., 2021).

Elmeligy, et al., (2019) stated that pretreatment of rats with pumpkin seed oil improved liver and decreased hepatocellular disturbance induced by CCl4. Pumpkin seed oil pre-treatment shielded rats from liver damage caused by CCl4. It significantly reduced hepatic enzymes ALT and AST and total bilirubin levels compared to those given CCl4 alone. These findings matches with previous studies.
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(Seif, 2014). This protective effect likely stems from the antioxidant properties of pumpkin seed oil's phenolics and flavonoids, potentially inhibiting cytochrome P-450 aromatase activity. Interestingly, free polyphenols of pumpkin are known to have the highest hepatoprotective properties as compared to bound polyphenols.

Pumpkin extract and Kidney functions

In the current finding, the rats that received pumpkin extract with two doses showed significantly decreased levels of uric acid, urea, and creatinine (p<0.05), when compared with the PVC group. Pumpkin extract's extend its effect to the kidneys. Both serum urea and creatinine significantly dropped in rats receiving the extract, mirroring previous findings (K Omer et al., 2016). Improved antioxidant activity in the kidneys likely explains this boost in renal function.

Histopathology

Rats exposed to PVC developed hepatocyte vacuolation and inflammatory cell infiltration. Severe hepatic necrosis was also observed. Similar to Sincihu et al. (2022), our study found hepatocellular damage in rats after oral administration of polyvinyl chloride microplastics. The rats showed lobular inflammation, portal blood vessel congestion and fibroblastic cell proliferation. This is consistent with previous research by Mahli et al. (2015) and Elmeligy et al. (2019). However, rats that were given pumpkin extract especially high dose (400 mg) at the same time as PVC were largely protected against the usual degenerative effects of PVC. The pumpkin group showed mild focal lesions of coagulative necrosis, marked decrease of hepatic vacuolation, necrosis, and inflammatory cells infiltration. These results are consistent with the research conducted by Abou-Zeid et al. (2018), Fawzy et al. (2018), and Elmeligy et al. (2019).

Conclusion

Pumpkin may improve liver function and protect against PVC-induced oxidative stress. Promoting pumpkin's nutritional benefits and collaboration between researchers and industrialists can lead to useful pumpkin-based products. Overall, the study concludes that pumpkin has hepatoprotective and antioxidant effects. Clinical studies are required before generalization.
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التأثير الوقائي لمستخلص القرع على التسمم الكبدي المحدث بكلوريد البولي فينيل في ذكور الجرذان البيضاء

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الملخص العربي

 يعد كلوريد البولي فينيل مصدرًا شائعًا للمواد البلاستيكية الدقيقة الموجودة في الأشياء اليومية. وهو أخطر أنواع البلاستيك بسبب ثباته في الكائنات البحرية، وتراكمه في السلسلة الغذائية، وتعرضه للإنسان. يمكن أن تسبب المواد البلاستيكية الدقيقة مشاكل صحية، بما في ذلك انخفاض وظائف الكبد، وانخفاض كفاءة النفط الدقيق في الوجوه الغذائية، وخاصة في المناطق الريفية. هدف الدراسة الحالي إلى دراسة الأثر الواعدي المحتمل لمستخلص البصل على السمية الكبدية لكلوريد البولي فينيل في الجرذان البيضاء. المواد والطرق: تم تقسيم ذكور الجرذان البيضاء بشكل عشوائي إلى أربع مجموعات، كل مجموعة تحتوي على ستة فئران. المجموعة الأولى: تلقى الفئران كلوريد البولي فينيل بجرعات 1000 ملغم/كغم من وزن الجسم خلال 6 أسابيع متتالية. المجموعة الثانية: تناولت الفئران مستخلص البصل مع كلوريد البولي فينيل بجرعات 400 ملغم/كغم من وزن الجسم. المجموعة الثالثة: تناولت الفئران مستخلص البصل بجرعات 400 ملغم/كغم من وزن الجسم مع كلوريد البولي فينيل. المجموعة الرابعة: تناولت الفئران مستخلص البصل بجرعات 400 ملغم/كغم من وزن الجسم مع كلوريد البولي فينيل. المجموعة الأولى، المجموعة الثانية، والمجموعة الثالثة، والمجموعة الرابعة: تناولت الفئران مستخلص البصل بجرعات 400 ملغم/كغم من وزن الجسم مع كلوريد البولي فينيل. المجموعة الأولى، المجموعة الثانية، والمجموعة الثالثة، والمجموعة الرابعة: تناولت الفئران مستخلص البصل بجرعات 400 ملغم/كغم من وزن الجسم مع كلوريد البولي فينيل. المجموعة الأولى، المجموعة الثانية، والمجموعة الثالثة، والمجموعة الرابعة: تناولت الفئران مستخلص البصل بجرعات 400 ملغم/كغم من وزن الجسم مع كلوريد البولي فينيل. المجموعة الأولى، المجموعة الثانية، والمجموعة الثالثة، والمجموعة الرابعة: تناولت الفئران مستخلص البصل بجرعات 400 ملغم/كغم من وزن الجسم مع كلوريد البولي فينيل. المجموعة الأولى، المجموعة الثانية، والمجموعة الثالثة، والمجموعة الرابعة: تناولت الفئران مستخلص البصل بجرعات 400 ملغم/كغم من وزن الجسم مع كلوريد البولي فينيل. المجموعة الأولى، المجموعة الثانية، والمجموعة الثالثة، والمجموعة الرابعة: تناولت الفئران مستخلص البصل بجرعات 400 ملغم/كغم من وزن الجسم مع كلوريد البولي فينيل. المجموعة الأولى، المجموعة الثانية، والمجموعة الثالثة، والمجموعة الرابعة: تناولت الفئران مستخلص البصل بجرعات 400 ملغم/كغم من وزن الجسم مع كلوريد البولي فينيل. المجموعة الأولى، المجموعة الثانية، والمجموعة الثالثة، والمجموعة الرابعة: تناولت الفئران مستخلص البصل بجرعات 400 ملغم/كغم من وزن الجسم مع كلوريد البولي فينيل. المجموعة الأولى، المجموعة الثانية، والمجموعة الثالثة، المقاسم والكلمات المفتاحية: