Original Article

The Therapeutic Effects of Pumpkin Seeds on Rats Injected with Carbon Tetrachloride (CCl_4)

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Abstract

Interest in medicinal plants has burgeoned due to increased efficiency of new plant-derived drugs and the growing interest in natural products. Because of the concerns about the side effects of conventional medicine, the use of natural products as an alternative to conventional treatment in healing and treatment of various diseases has been on the rise in the last few decades. The use of plants as medicines dates from the earliest years of man's evolution. Medicinal plants serve as therapeutic alternatives, safer choices. A larger number of these plants and their isolated constituents have shown beneficial therapeutic effects so we worked. This study to determine the effect of different levels of pumpkin seeds on rats injected with carbon tetrachloride (CCl_4). The experiment was performed in animal house. All rats were fed for one week on basal diet before starting the experiment, then divided into two main groups, the first group (n= 6 rats) was fed on the basal diet only as a control negative (C –ve) normal rats for 28 days. The rats of second main group (n= 24 rats) were injected with carbon tetrachloride (CCl_4), The second main group is divided into four sub-groups, including 3 groups fed with different concentrations of (5%, 10%, and 15%) pumpkin seeds and one group control positive infected with the disease do not feed on the experimental diet. the results showed that there is no significant difference between the values of ALP enzyme of group 3 and control positive group. Also, there is no significant difference between groups 4, and 5 showed similar (P>0.05. There is no significant difference between group 5 and control positive group. Also,. Groups 3, and 4 showed similar (P>0.05) mean values of ALT. Also there is no significant difference between group 3 and control positive group. Also, group 4 was similar (P>0.05) to normal group. Group 5 showed lower (P<0.05) urea nitrogen than both control groups.

Keywords: Pumpkin seeds; Hepatic; Therapeutic nutrition

Introduction

Pumpkin is a herbaceous, vineyard, creeping or climbing plant ,which its origins are belongs to the Cucurbitaceous family ,it's considered one of the pumpkin plants which means that its lives for one year or one agricultural season or it may be short lived, And the stems of the pumpkin plant are 10 meters long. [1] This plant produces fruits that mainly consist of pulp and seeds, it's consider a source of many nutrients, the most important of it are the carotene compounds; which are responsible for giving the pumpkin an orange color, and these compounds are transformed inside the body into vitamin A, which is important for healthy vision, growth, and other Important functions. Lutein, which gives the pumpkin a bright yellow color. These fruits also contain a good amount of essential and non-essential amino acids. Among the most important essential amino acids available in pumpkin fruits is lysine. It should be noted that pumpkin seeds were usually classified as industrial and agricultural trash, therefore it's always discarded, but they are consumed in some parts of the world, whether in the form of fresh, roasted, or cooked, and it has been recently discovered that these seeds are rich with many nutrients beneficial to health, such as proteins, fiber, minerals, and polyunsaturated fatty acids, in addition to vegetable sterols, so that it's began to be used in some food industries. The effective benefit of pumpkin seeds according to it does contain of antioxidants nutrients. [2] The liver is the largest internal organ in the body, accounting for approximately 2% to 3% of the total body weight of an adult. Liver is multiple vital functions and its regenerative abilities. [3] Carbon tetrachloride (CCl_4) has been widely used to experimentally induce liver injury in rodents. A single dose of CCl_4 leads to centrizonal necrosis and steatosis. Toxicity experienced by the liver during poisoning results from the production of a metabolite, CCl_4 which is a direct hepatotoxin responsible for change in cell permeability and it inhibits mitochondrial activity followed by cell death. [4] It has also been reported that chronic CCl_4 exposure produced cirrhosis in rats. [5] Many plants were suggested to ameliorate or care the liver diseases, among them were Pumpkin seeds. [6]

Aim of the Study

This work aims to show the probable benefit of pumpkin seeds to correct liver function in rats.

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Materials and Methods

Materials

Preparation of pumpkin seeds: Pumpkin seeds cleaned thoroughly by washing, and cut into small slice and dried in drying oven at temperature 50°C for 3 days, then crushed and milled as fine powder.

Experiraental animals: Thirty male albino rats, Sprague Dawley strain, weighing 150 ± 10 g were used in the study.

Used chemicals: Carbon tetrachloride (CCl_4) was obtained from El-Gomhoryia Company for Chemical Industries, Cairo, Egypt as 10% liquid solution. It was dispensed in white plastic bottles each containing one liter as a toxic chemical material for liver poisoning according to Passmore and Eastwood.[7] In the same time, it is mixed with paraffin oil which obtained from the pharmacy for dilution during the induction.

Methods

Biological experiments

Basal diet composition of tested rats: The basal diet in the experiment consisted of casein (10%) , com oil (10%), vitamin mixture (1%), salt mixture (4%), choline chloride (0.2%), methionine (0.3%), cellulose (5%) and the remained is corn starch (69.5%) according to Campbell[8] as seen in Table 1.

Induction of liver intoxication in rats: Twenty four male albino rats were treated subcutaneous injection of carbon tetrachloride (CCl_4) in paraffin oil 50% V/V (2 ml/kg b. wt.) twice a week for two weeks to induce chronic damage of the liver according to the method described by Jayasekhar et al.[9] After the injection of CCl_4, blood samples were obtained by retro orbital method to ensure occurrence of liver injury and to estimate liver function.

Experimental design and animal groups: Thirty Sprague Dawley white male albino rats, weighing about 150 ± 10 g were used in the study. Rats were housed in wire cages under the normal laboratory condition and fed on basal diet for a week as adaptation period. Diet was given in non-scattering feeding cups to avoid loss or contamination of food, water was provided to the rats by means of glass tubes projecting through the wire cage from an inverted bottle supported to one side of the cage.

The rats were divided into 5 groups each of 6 rats. The groups of rats were as follows:

- **Group (1):** These were kept without any treatment as a control positive (C +ve group) and fed on basal diet for 28 days.
- **Group (2):** These were kept without any treatment as a control positive (C +ve group) and fed on basal diet for 28 days.
- **Group (3):** These were fed on basal diet plus 5% of pumpkin seeds.
- **Group (4):** These were fed on basal diet plus 10% of pumpkin seeds.
- **Group (5):** These were fed on basal diet plus 15% of pumpkin seeds.

Table 1: Composition of basal diet.

Compound	Amount
Protein (Casein)	10%
Corn oil	10%
Mineral mixture	4%
Vitamin mixture	1%
Cellulose	5%
Choline chloride	0.2%
Methionine	0.3%
Corn starch	Up to 100%

*12.3 g casein gives 10 g protein

Biological evaluation

During the experimental period (28 days), the consumed feed was recorded every day, and body weight recorded weekly. The body weight gain (B.W. G.%), food efficiency ratio (F.E.R) and also organs weight were determined according to Chapman et al.[10]

Blood sampling

Blood samples were collected after 12 hour fasting at the end of the experiment. Using the retro - orbital method by means of a micro capillary glass tubes, blood was collected into a dry clean centrifuge tube and left to clot in a water bath (37ºC) at room temperature for half an hour. The blood was centrifuged for 10 minutes at 3000 RPM. to separate the serum a part of was subjected to glucose determination and the reminder was carefully aspirated and transferred into clean quit fit plastic tubes and kept frozen at (-20ºC) until analysis. The organs (liver, kidney, heart, and spleen) were removed and washed in saline solution, weighted and kept in formalin solution (10%) according to methods described by Drury and Wallington.[11]

Biological evaluation

Food intake (consumption), body weight gain% (BWG %), feed efficiency ratio (FER) according to Chapman et al.[10] Using the following equation.

$$BWG\% = \frac{Final\ weight - Initial\ weight}{Initial\ weight} \times 100$$

$$FER = \frac{Gain\ in\ body\ weight\ (g/day)}{Food\ Intake\ (g/day)}$$

$$\text{Relative weight of organs} = \frac{\text{Organs weight}}{\text{Animal body weight}} \times 100$$

Biochemical analysis

- **Determination of the activity of liver enzymes estimation of the activity of aspartate aminotransferase (AST):** Determination of AST enzyme was carried out by spectrophotometer using specific kits (BioMerieux) according to Reitman and Frankel.[12]

- **Determination of the activity of serum alanine aminotransferase (ALT):** The activity of ALT enzyme was measured using the colorimetric procedure described by Reitman and Frankel. [12]
- **Determination of the activity of serum alkaline phosphatase:** Alkaline phosphatase (ALP) determination procedure based on colorimetric determination of ALP was performed according to the method of Roy. [13]
- **Determination of serum total bilirubin:** Serum total bilirubin was determined colorimetrically as described by Doumas et al. [14] using spectrophotometer adjusted at 578 nm.
- **Determination of total cholesterol in serum:** Total cholesterol was determined according to Ratliff and Hall. [15]
- **Determination of triglycerides:** Enzymatic colorimetric determination of triglycerides was carried out according to Jacobs and Van Denmark. [16]
- **Determination of HDL:** Determination of HDL was carried out according to the method of Jacobs and Van Denmark. [16]
- **Determination of VLDL and LDL:** The determination of VLDL and LDL was carried out according to the method of Lee and Nieman [17] as follows:

Statistical analysis

The obtained data were statistically analyzed using computerized SPSS (Statistic Program Sigmastat, statistical soft-ware, SAS Institute, Cary, NC). Effects of different treatments were analyzed by one way ANOVA (Analysis of variance) test using Duncan's multiple range test and p<0.05 was used to indicate significance between different groups, the following formulas were used. [18]

Results and Discussion

This work aims to show the probable benefit of pumpkin seeds to correct liver function in rats.

Biological results

Effect of feeding CCl_4- intoxicated rats with different levels of pumpkin seeds on feed intake (FI), feed efficiency ratio (FER) and body weight gain (BWG).

Data presented in Table 2 showed the effect of feeding CCl_4-intoxicated rats with tested materials on feed intake (FI).

It could be observed that rats were injected with CCl_4 (control positive group) had feed intake value 6.74 ± 0.88 g/d. In normal rats (control negative group), the mean value of FI was 14.87 ± 0.62 g/d. These finding denote that there was a significant decrease in FI of rats poisoned by CCl_4 as compared to normal rats. The mean values of FI on diets of G3 (5% of pumpkin seeds), G4 (10% of pumpkin seeds), G5 (15% of pumpkin seeds), were significantly higher than positive control group which were 10.84 ± 0.21, 9.85 ± 0.1, and 10.06 ± 1.5 g/d, respectively. It could be noticed that there is no significant difference between the values of FI on groups 3, 5. Also, there is no significant difference between groups 4 and 5. Groups 5 similar (P>0.05) mean values of FI and recorded the best result of all treatment as compared to positive control group [Table 3].

Feed efficiency ratio (FER)

It is clear from Table 4 that control positive group had significantly higher mean value ± SD of FER than normal rats, it was being 0.16 ± 0.003 and 0.09 ± 0.002 g/day, respectively. In rats given CCl_4 then fed on all treatments, there were significant increases in FER as compared to normal group which were 0.15 ± 0.003, 0.16 ± 0.09 and 0.13 ± 0.07 g/day for groups 3, 4, 5, 6, 7, 8 and 9, respectively. There is no significant difference between group 3, 4, 5 and control positive group.

Body weight gain (BWG)

Data presented in the same Table 4 revealed that, treated rats with CCl_4-intoxicated diet control positive group (30.97 ± 6.1 g/d) led to a significant increase (P<0.05) in BWG when compared with control negative group (35.92 ± 6.0 g/d). The mean values of groups 3, 4 and 5 were significantly higher than control groups which were 47.35 ± 1.3, 44.78 ± 1.0 and 38.99 ± 1.6, respectively. The obtained results in Table 4 are in the same trend of Bakr [19] Shehata [20] Riad [21] and Elbanna [22] who found that injected rats by CCl_4 caused decrease in FI, FER and BWG.

Table 2: Composition of salt mixture%.

Compound	Amount
$CaCO_3$	600 mg
K_2HPO_4	645 mg
$CaHPO_4.2H_2O$	150 mg
$MgSO_4.2H_2O$	204 mg
NaCl	334 mg
$Fe (C_6H_5O_7)_2 6H_2O$	55 mg
KI	1.6 mg
$MnsO_4.4H_2O$	10 mg
$ZnCL_2$	0.5 mg
$CuSO_4.2H_2O$	0.06 mg
$CaCO_3$	600 mg

Table 3: Composition of vitamin mixture.

Vitamin	Amount
Vitamin E	10 Iu
Vitamin K	0.50 Iu
Vitamin A	200 Iu
Thiamin	0.50 mg
Riboflavin	1.0 mg
Pyridoxine	0.40 mg
Niacin	4.00 mg
Vitamin C	20.0 mg
Panathothenic acid	4.0 mg
Vitamin D	100 Iu
Choline chloride	200 mg
Folic acid	0.02 mg
Inositol	25 mg
Para- amino- benzoic acid	0.02 mg
Vitamin B12	2.00 mg
Biotin	0.02 mg
Corn starch	Up to 100 g

Effect of feeding CCl_4 intoxicated rats with different levels of pumpkin seeds on organs weight

Data presented in Table 5 showed the effect of feeding CCl_4-intoxicated rats with tested materials on organs weight (g). It could be noticed that for (negative control group) of normal rats, the weight of liver, spleen and kidneys were 2.52 ± 0.34, 0.48 ± 0.17 and 0.43 ± 0.21g, respectively. In CCl_4 intoxicated rats (positive control group), the weights of the previously mentioned organs were 3.82 ± 0.76, 0.68 ± 0.02 and 0.53 ± 0.01g, respectively. These results denote that there were significant increased in liver, spleen and kidneys weight of rats poisoned by CCl_4 as compared to the normal rats. For weight of liver, there is no significant difference among rats poisoned by CCl_4 then fed on diet of G3, G5 which were 3.51 ± 0.84, 3.42 ± 1.03 and 3.52 ± 0.66 g, respectively. Also, the mean values of liver weight for G4 was being 3.02 ± 0.14g indicated nonsignificant differences at ($P<0.05$). Regarding spleen weight, there is no significant change among rats injected with CCl_4 and fed on diet of G3, G4 and G5 it was being 0.63 ± 0.001, 0.60 ± 0.005 and 0.61 ± 0.07 g, respectively. Concerning kidneys weight, there is no significant difference among rats given CCl_4 then fed on diet of G3, G5, and control positive group it was being 0.51 ± 0.06, 0.50 ± 0.01 and 0.53 ± 0.01 g, respectively, as well as G4 was 0.48 ± 0.07 g. The obtained results in Table 6 are in agreement with those found by Bakr [19] Shehata [20] Riad [21] and Elbanna [22] who found that injected rats by CCl_4 caused an increase in all organs weight, while treatment with tested plants reversed such a change.

Liver Enzymes

Data presented in Table 6 show the different levels of pumpkin seeds on liver enzymes (AST, ALT, and ALP) of hypercholesterolmic rats.

Relative AST enzyme

The obtained data Table 6 it could be noticed that rats intoxicated with CCl_4 without treatment (positive control group), the mean value of AST enzyme was 37.25 ± 5.82 while in normal rats (C -ve) was 22.06 ± 1.07 U/L. These results denote that there was a significant increase in the mean value of AST enzyme of rats poisoned by CCl_4 as compared to normal rats. Rats intoxicated by CCl_4 then fed on treatment groups 3, and 4 was significantly lower than control positive group in AST enzyme level which

Table 4: Effect of feeding different level of pumpkin seeds on FI, FER and BWG of CCl_4-intoxicated rats.

Parameters / Groups	B. W. G. (g)	F. I. (g)	F. E. R.
Control (-ve)	14.87 ± 0.62^{a}	0.09 ± 0.002^{B}	35.92 ± 6.0°
Control (+ve)	6.74 ± 0.88^{e}	0.16 ± 0.003^{A}	30.97 ± 6.1^{E}
5% pumpkin seeds	10.84 ± 0.21^{c}	0.15 ± 0.003^{a}	47.35 ± 1.3^{a}
10% pumpkin seeds	9.85 ± 0.1°	0.16 ± 0.09^{a}	44.78 ± 1.0^{B}
15% pumpkin seeds	10.06 ± 1.5^{C}	0.13 ± 0.07^{a}	38.99 ± 1.6^{C}

Values are expressed as mean ± SD; Significant at $P>0.05$; Values which don't share the same letter in each column are significantly different.

Table 5: Effect of feeding different levels of pumpkin seeds on organs weight of CCl_4-intoxicated rats.

Parameters / Groups	Liver (%)	Spleen (%)	idney (%)
Control (-ve)	2.52° ± 0.34	0.48^{D} ± 0.17	0.43^{c} ± 0.21
Control (+ve)	3.82^{A} ± 0.76	0.68^{a} ± 0.02	0.53^{A} ± 0.01
5% pumpkin seeds	3.51^{B} ± 0.84	0.63^{B} ± 0.001	0.51^{A} ± 0.06
10% pumpkin seeds	3.02^{c} ± 0.14	0.60^{B} ± 0.005	0.48^{B} ± 0.07
15% pumpkin seeds	3.42^{B} ± 1.03	0.61^{B} ± 0.07	0.50^{A} ± 0.01

Values are expressed as mean ± SD; Significant at $P>0.05$; Values which don't share the same letter in each column are significantly different.

Table 6: Effect of feeding different levels of pumpkin seeds on AST, ALT and ALP of CCl_4-intoxicated rats.

Parameters / Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Control (-ve)	22.06 ± 1.07^{D}	9.51 ± 0.94°	99.3 ± 1.32^{E}
Control (+ve)	37.25 ± 5.82^{A}	16.10 ± 1.10^{A}	230.7 ± 3.47^{A}
5% pumpkin seeds	28.66 ± 1.76^{B}	12.29 ± 0.26^{c}	227.3 ± 5.06^{A}
10% pumpkin seeds	24.87 ± 0.42^{c}	11.79 ± 2.28^{c}	222.4 ± 3.13^{B}
15% pumpkin seeds	37.52 ± 7.22^{A}	15.93 ± 1.25^{A}	222.3 ± 7.5^{B}

Values are expressed as mean ± SD; Significant at $P>0.05$; Values which don't share the same letter in each column are significantly different.

were 28.66 ± 1.76 and 24.87 ± 0.42, U/L, respectively. There is no significant difference between groups 5and control positive group. Also,

Relative ALT enzyme

Data presented in same Table 6 revealed that CCl_4- intoxicated rats without treatment (C +ve), the serum level of ALT enzyme activity was 16.10 ± 1.10 U/L while negative group was 9.51 ± 0.94 U/L, showing a significant increase in serum level of ALT in rats poisoned by CCl_4 as compared to normal rats. In rats given CCl_4 then fed on all treatments G3, G4 and G5 it could be noticed that the mean value of serum ALT was 12.29 ± 0.26, 11.79=1=2.28, and 15.93=1=1.25, 10U/L, respectively which was significantly higher than control negative group. There is no significant difference between group 5 and control positive group. Also,. Groups 3, and 4 showed similar (P>0.05) mean values of ALT.

Relative ALP enzyme

Data presented in the same Table 6 indicated that mean value of ALP enzyme, rats injected with CCl_4 (Control +ve group) was 230.7 ± 3.47 U/L while in normal rats (control -ve group) was 99.3 ± 1.32 U\L. These results denote that there was a significant increase in the mean value of ALP enzyme of rats poisoned by CCl_4 as compared to normal rats. The mean values of ALP enzyme of G3, G4, and G5 were significantly higher than control negative group which were 227.3 ± 5.06, 222.4 ± 3.13, and 222.3 ± 7.5,U\L, respectively except rats fed on diet contained 5% pumpkin seeds. It could be noticed that there is no significant difference between the values of ALP enzyme of group 3 and control positive group. Also, there is no significant difference between groups 4, and 5 showed similar (P>0.05) mean values of ALP. The obtained results of Table 6 are agree with those found by Bakr [19] Shehata [20] Riad [21] and Elbanna [22] who indicated that injected rats by CCl_4 caused an increase of AST, ALT and ALP enzymes. The reactive electrons species from CCl_4 induces rat liver cirrhosis that resembles the human disease, and it can serve as a suitable animal model for studying human liver cirrhosis. [23] Toxicity experienced by the liver during CCl_4 poisoning results from the production of a metabolite; CCl_4 which is a direct hepatotoxin responsible for change in cell permeability and it inhibits mitochondrial activity followed by cell death. [4] It has also been reported that chronic CCl_4 exposure produced cirrhosis in rats. [5] On the other hand, the study demonstrated that the treatment with Cactus pear extract caused marked ameliorations of transaminase enzymes activity (ALT and AST). The results are in accordance with Tapiero et al. [24] who showed the effect of extracts from carrot on carbon tetrachloride-induced hepatotoxicity in rats. The results are agreement with the results obtained by Katikova et al. [25] who found that the juices of beet-root and carrot decoction of these plants was improved the liver function of acute toxic hepatitis for laboratory rats. In addition to Poudyal et al. [26] who reported that anthocyanins, phenolic acids and carotenoids present in purple carrot were improving hepatic structure function. Also,

Effect of feeding different levels of pumpkin seeds on lipid profile of CCl_4- intoxicated rats.

Table 7 illustrated the effect of tested materials on the serum cholesterol and triglycerides level of CCl_4- intoxicated rats. Date presented in Table 8 showed that injection of CCl_4 led to significant (P<0.05) increase in serum total cholesterol level in hepatotoxic rats. The mean value ± SD of serum cholesterol in hepatotoxic control +ve group was 172.55 ± 12.38 mg/dl compared to 89.78 ± 5.25 mg/dl in control -ve group. The mean

Table 7: Effect of feeding different levels of pumpkin seeds on total cholesterol and triglyceride of CCl_4-intoxicated rats.

Parameters / Groups	Total cholesterol (mg\dl)	Triglyceride (mg\dl)
Control (-ve)	89.78 ± 5.25^F	39.40 ± 0.96^g
Control (+ve)	172.55 ± 12.38^a	110.70 ± 3.11^a
5% pumpkin seeds	151.29 ± 6.92^c	91.30 ± 1.44^c
10% pumpkin seeds	165.78 ± 1.72^B	103.40 ± 2.04^b
15% pumpkin seeds	124. 12 ± 4.71^e	70.00 ± 3.82^d

Values are expressed as mean ± SD; Significant at P>0.05; Values which don't share the same letter in each column are significantly different.

Table 8: Effect of feeding different levels of c pumpkin seeds on HDL-c, LDL-c, VLDL-c and LDL-c/HDL-c on CCl_4-intoxicated rats.

Parameters / Groups	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	LDL-C/HDL-c (mg/dl)
Control (-ve)	60.58 ± 3.62^A	20.86 ± 2.74^H	7.92 ± 0.19^E	0.34 ± 0.04^e
Control (+ve)	28.38 ± 5.33^E	104.03 ± 8.07^A	22.20 ± 0.64^A	3.67 ± 1.03^A
5% pumpkin seeds	50.05 ± 4.08^c	62.97 ± 8.48^D	11 ,42 ± 0.94^D	1.26 ± 0.22^c
10% pumpkin seeds	43.38 ± 4.36^D	74.85 ± 6.59^c	15.00 ± 0.76^B	1.73 ± 0.19^B
15% pumpkin seeds	49.55 ± 1.64^c	83.30 ± 3.71^B	13.16 ± 0.76^c	1.68 ± 0.13^B

Values are expressed as mean ± SD; Significant at P>0.05; Values which don't share the same letter in each column are significantly different.

values of total cholesterol in rats given CCl_4 then fed on all diets of groups 3, 4, and 5, which were 151.29 ± 6.92, 165.78 ± 1.72, and 124.12 ± 4.71, mg/dl, respectively were significantly lower than positive control group. Concerning triglycerides, the data in the same Table 8 revealed that rats injected with CCl_4 (positive control group) had higher ($P<0.05$) value of serum levels triglycerides compared to normal rats (control negative group) which were 110.70 ± 3.11 and 39.40 ± 0.96 mg/dl, respectively. The mean values of serum triglycerides in rats given CCl_4 then fed on all diets of groups 3, 4, and 5, which were 91.30 ± 1.44, 103.40 ± 2.04 and 70.00 ± 3.82, mg/dl, respectively were significantly lower than positive control group.

Effect of feeding different levels of pumpkin seeds on cholesterol fractions of CCl_4-intoxicated rats

Results in Table 8 exhibited the effects of feeding CCl_4-intoxicated rats with tested materials on cholesterol fractions, high density lipoprotein (HDL-c), low density lipoprotein (LDL-c), very low density lipoprotein (VLDL-c) and the ratio between LDL-c/HDL-c.

High density lipoprotein- cholesterol (HDL-c)

Data presented in Table 8 showed the effect of feeding CCl_4-intoxicated rats with tested materials on the serum levels of (HDL-c). It is obvious that rats injected with CCl_4 in (control +ve group) had serum levels HDL-c value 28.38 ± 5.33 mg/dl. In normal rats (control -ve group), the mean value of serum levels HDL-c was 60.58 ± 3.62 mg/dl. These finding denote that there was a significant decrease in HDL-c in the serum of rats poisoned by CCl_4 as compared to normal rats. The mean values of serum level HDL-c in rats given CCl_4 then fed on all diets of groups 3, 4 and 5, which were 50.05 ± 4.08, 43.38 ± 4.36, and 49.55 ± 1.64, mg/dl, respectively were significantly higher than positive control group. There is no significant difference among rats given CCl_4 and fed with diet of groups 3, and groups 5.

Low density lipoprotein - cholesterol (LDL-c)

Data presented in the same Table 8 evidenced that the serum (LDL-c) levels were significantly elevated in control positive group to 104.03 ± 8.07 from 20.86 ± 2.74 mg/dl in control negative group. All rats intoxicated with CCl_4 then fed on all tested plant materials showed significant decrease in LDL-c as compared to control positive group. All rats intoxicated with CCl_4 then fed on all tested plant materials of groups 3, 4 and 5 showed significant decrease in LDL-c as compared to control positive group, which were begin 62.97 ± 8.48, 74.85 ± 6.59, and 83.30 ± 3.71, mg/dl, respectively.

Very low density lipoprotein-cholesterol (VLDL-c)

Data presented in Table 8 indicated that injected of CCl_4 led to significant ($P<0.05$) increase in serum VLDL-c level in hepatotoxic rats. The mean value ± SD of serum VLDL-c in hepatotoxic control +ve group was 22.20 ± 0.64 mg/dl compared to 7.92 ± 0.19 mg/dl in control -ve group. The mean values of serum VLDL-c in rats given CCl_4 then fed on all diets of groups 3, 4, and 5 which were 11.42 ± 0.94, 15.00 ± 0.76, 13.16 ± 0.76, mg/dl, respectively were significantly lower than positive control group.

The ratio between LDL-c/HDL-c

Regarding rats injected with CCl_4 without treatment (control positive group), the serum level of LDL-c/HDL-c increase dramatically from 0.34 ± 0.04 for control negative group to 3.67 ± 1.03 for positive control group in Table 9. The mean values of serum LDL-c/HDL-c in rats given CCl_4 then fed on all diets of groups 3, 4, and 5 which were 1.26 ± 0.22, 1.73 ± 0.19, and 1.68 ± 0.13, mg/dl, respectively were significantly lower than positive control group. There is no significant difference between G4 and G5. The obtained results in Table 7 and Table 8 are in agreement with those obtained by Bakr [19] Shehata [20] Riad [21] and Elbanna [22] who found that hepatointoxication raised total cholesterol, triglycerides, LDL-c and VLDL-c while decrease of HDL-c in serum. The same aurthors corrected the above changes by feeding rats on diets containing some plants other that used in present work. The present results are also in agreement with Nicolle et al. [27] who concluded that pumpkin seeds consumption modifies cholesterol absorption and bile acids excretion and increases antioxidant status and these effects could be interesting for cardiovascular protection. In addition to Nicolle et al. who showed that feeding the pumpkin seeds diet resulted in a decrease of cholesterol and triglycerides in plasma and in the liver in animals fed on cholesterol-supplemented diets. Chou et al. investigated feeding the micronized insoluble fibers, particularly the micronized carrot IFF, significantly ($p<0.05$) improved their abilities in lowering the concentrations of serum triglyceride, serum total cholesterol, and liver lipids to different extents by means of enhancing ($p<0.05$) the excretion of lipids, cholesterol, and bile acids in feces.

Table 9: Effect of feeding different levels of pumpkin seeds on uric acid and urea nitrogen levels (mg/dl) CCl_4-intoxicated rats.

Groups \ Parameters	Uric acid (mg/dl)	Urea Nitrogen (mg/dl)
Control (-ve)	1.05 ± 0.1 l^{c}	14.7 ± 0.9^{B}
Control (+ve)	1.14 ± 0.12^{A}	15.6 ± 2.2^{A}
5% pumpkin seeds	1.13 ± 0.11^{A}	15.6 ± 1.4^{A}
10% pumpkin seeds	1.14 ± 0.09^{A}	14.9 ± 0.6^{B}
15% pumpkin seeds	1.12 ± 0.05^{B}	13.6 ± 1.9^{C}

Values are expressed as mean ± SD; Significant at P>0.05; Values which don't share the same letter in each column are significantly different.

Effect of feeding CCl_4- intoxicated rats with different levels of pumpkin seeds on kidney functions

Results of serum uric acid and urea nitrogen (kidney function) are presented in Table 9.

Uric acid

Data presented in Table 9 showed the effect of feeding CCl_4-intoxicated rats with tested materials on serum uric acid. The mean value of serum uric acid for experimental group that fed on basal diet only (normal rats) was 1.05 ± 0.11 mg/dl, while uric acid of CCl_4-intoxicated rats (control positive group) was 1.14 ± 0.12 mg/dl. Results revealed that, rats treated with CCl_4-intoxicated diet (positive control group) led to a significant decrease ($P<0.05$) in serum uric acid when compared with control positive group. The mean value of uric acid in rats given CCl_4 then fed on all diets of groups 3, 4, and 5 which were 1.13 ± 0.11, 1.14 ± 0.09, and 1.12 ± 0.05, mg/dl, respectively were significantly lower than positive control group, except G3 and G4.

Non-significant differences

Urea nitrogen

Data presented in the same Table 9 indicated that the mean value of serum urea nitrogen of CCl_4-intoxicated rats (positive control group) was 15.6 ± 2.2 mg/dl, while urea nitrogen of normal rats (control negative group) was 14.7 ± 0.9 mg/dl. Results revealed that, treated rats with CCl_4-intoxicated diet (positive control group) led to a significant decrease ($P<0.05$) in serum urea nitrogen when compared with control positive group. The mean value of urea nitrogen in rats given CCl_4 then fed on all diets of groups 3, 4, and 5 which were 15.6 ± 1.4, 14.9 ± 0.6, and 13.6 ± 1.9, mg/dl, respectively showing a significant difference decreases as compared to positive group, except G3. There is no significant difference between group 3 and control positive group. Also, group 4 was similar ($P>0.05$) to normal group. Group 5 showed lower ($P<0.05$) urea nitrogen than both control groups. The obtained results in Table 9 were agreement with the results obtained by Polunin and Robbins, [28] who studied that pumpkin seeds increased urine production (diuretic), prevents the formation of kidney stones,

Effect of feeding CCl_4- intoxicated rats with different levels of pumpkin seeds on glucose levels.

Table 10: Effect of feeding different levels of pumpkin seeds on glucose levels (mg/dl) of CCl_4-intoxicated rats.

Parameters / Groups	Glucose (mg/dl)
Control (-ve)	1.05 ± 0.1 l^c
Control (+ve)	1.14 ± 0.12^A
5% pumpkin seeds	1.13 ± 0.11^A
10% pumpkin seeds	1.14 ± 0.09^A
15% pumpkin seeds	1.12 ± 0.05^B

Values are expressed as mean ± SD; Significant at $P>0.05$; Values which don't share the same letter in each column are significantly different.

Data presented in Table 10 showed the effect of feeding CCl_4-intoxicated rats with tested materials on glucose level. Positive control group had significantly higher mean value ± SD of glucose than normal rats; it was being 140.14 ± 0.02 and 80.05 ± 2.11 mg/dl, respectively. In rats given CCl_4 then fed on all treatments, there were significant increases in the glucose levels as compared to normal group which were 117.14 ± 0.59, 130.13 ± 0.21, 130.12 ± 0.85, mg/dl for groups 3, 4 and 5 respectively. Blood glucose concentration is known to depend on the ability of the liver to absorb or produce glucose. The liver performs its glucostatic function owing to its ability to synthesize or degrade glycogen according to the needs of the organism, as well as via gluconeogenesis. [23]

Conclusion and Recommendations

- It is suggested to use different levels of pumpkin seeds for hepatic patients.
- Different levels of pumpkin seeds especially that of 15% of pumpkin seeds may be used for remedy of diabetic.
- Different levels of pumpkin seeds may be suggested for lowering LDL and atherogenig index levels.
- Future studies may be suggested to evaluate the efficacy and advantage of using pumpkin seeds as extracts versus dried powder.

Competing Interests

The authors declare that they have no competing interests.

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