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The Protective Effects of Carob Seeds in Hepatotoxic Rats with Liver Dysfunction

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Abstract:

The second-largest organ in the human body is the liver. Numerous causes might harm the liver, including genetic conditions and environmental toxins. Because of its numerous pharmacological advantages, carob is a plant with significant relevance in traditional medicine. The current investigation aimed to determine that carob seeds (Ceratonia silique, L.) in powder and aqueous extract affected CCl4-induced liver damage in rats. In this investigation, thirty mature male albino rats weighing (150±10g) were divided into six groups with five rats each. The (+ve) group and the other four groups were fed the food plus 0.2 ml/100 g body weight of 40 ml/l CCl4 for 14 days to induce liver fibrosis, while one of them was kept as a control (-ve) group. Carob seeds (powder and aqueous extract) were introduced to the main diet at 5 and 10%, respectively. Body weight gain, food intake, feed efficiency ratio, glucose level, renal function tests (creatinine, uric acid, and urea levels), serum liver enzymes (ALT et al.), and lipid profile (TG, TC, HDL-c, LDL-c, and VLDL-c) have all been determined. According to the results, eating carob seeds (powder and aqueous extract) increased HDL-c levels significantly (P≤0.05) but also significantly decreased body weight gain compared to the control (+ve) group, improved liver and kidney functions, and returned serum glucose and blood lipid levels to normal, reflecting the potent nutraceutical therapeutic effect for eating carob seeds (powder and aqueous extract).

***Keywords**: Carob seeds, Fibrosis, Rats, Biochemical analysis*

Introduction

Liver is the second largest organ in human body, more than 5,000 separate bodily functions including helping blood to clot, cleansing the blood of toxins to converting food into nutrients to control hormone levels, fighting infections and illness, regenerating back after injury and metabolizing cholesterol, glucose, iron and controlling their levels. Most people never give their liver a thought until something goes wrong, yet liver diseases on rise, affecting one in ten. Liver diseases can be inherited or caused by a variety of factors that damage the liver.

In fact, there are many types of liver diseases that can be caused by a virus, damage from drugs or chemicals, obesity, diabetes or an attack from own immune system, when the condition is left untreated, it can become life threatening and can permanently damage the liver or the bile duct (1).

Liver disease accounts for approximately 2 million deaths per year worldwide, 1 million due to complications of cirrhosis and 1 million due to viral hepatitis and hepatocellular carcinoma (HCC) (2).

Previous studies proved that carbon tetrachloride (CCl_4) has some toxic effects on the liver, as CCl_4 creates free radicals that cause liver damage (3).

Carob as a plant bears a great significance in the traditional medicine because of its various pharmacological benefits (4).

Carob tree (*Ceratonia siliqua L*.) belongs to legume family, and it is native to the Mediterranean region (5). Carob fruit is a non-cracking pod, long and flattened, straight or curved. It is composed of two major parts, pulp (90%) and seed (10%). They are widely used as raw material in food, pharmaceutical and cosmetic industries (6).

Carob pulp contains numerous bioactive compounds such as sugars, cyclitols, polyphenols, amino acids, fibers and minerals, while the composition of carob seeds includes gum, polyphenols and proteins (7). Meanwhile, the seed consists of three main components: gum, polyphenols and protein (8). Also Dakia *et al.* reported that The composition of the carob seed includes approximately 9% moisture, 1% ash, 1% protein, 1.1% fat, 0.4% sucrose, 0.1% D-glucose, fructose, 0.1% starch, and a total phenols content of 0.661 mg/g(9).

Seeds and pods are a particularly rich source of flavonoids such as proanthocyanidin, ellagitannin and gallotannin. These phytochemicals exhibited scavenging activities against numerous diseases caused because of free radical attack (10). Due to the chemical composition, carob exhibits a powerful antioxidant activity and possesses many valuable therapeutic functions, such as lipid-lowering, anti-cardiovascular and nephroprotective properties (11).

Furthermore, it was suggested that carob pods extract exhibited a potential gastro-protective effect and ameliorative effects against oxidative damage in different tissues induced by alcohol or carbon tetrachloride in rats (12).

carob seeds have a hepatoprotective effect and antioxidant activity in rats with ethanol toxicity (13).

This study aimed to study the effect of carob seeds as aqueous extract and powder on liver damage in rats injected by CCl_4.

Materials and methods

Materials:

The used of carob seeds (*Ceratonia siliqua L.*):

Carob seeds **(*Ceratonia siliqua L.*)** were obtained from the local market at Menoufia Governorate in March 2021.

Rats:

A total of 30 adult normal male albino rats Sprague Dawley strain weighting 150±10g were obtained from Vaccine and Immunity Organization, Ministry of Health, Helwan Farm, Cairo, Egypt.

Basal diet components:

Casein, cellulose, choline chloride and DL methionine powder were obtained from Morgan Co. Cairo, Egypt.

Chemicals and kits:

Carbon Tetra Chloride (CCL_4) and all chemical kits used in this study were obtained from El-Gomhoryia company for Trading Chemicals, Drugs and Medical Instruments, Cairo, Egypt. CCL_4 was used as a toxic chemical for liver poisoning according to **(14).**

Methods:

Preparation of carob seeds powder:

The dried carob seeds were ground in a grinder (Braun Biotech International GMBH. D.34212 Melsungen, Germany) to pass through a 1.6 mm sieves and stored at - 12°C until used.

Preparation of el carob seeds extract:

Exactly 200g of the fine powder was soaked in 1000 ml (IL) of distilled water in a conical flask, the mixture allowed to stand the laboratory bench for 30 minutes, there after shaken and boiled four 1 hour. It was then cooled and filtered **(15).**

The induction of liver experimental:

Rats were injected subcutaneously at a dose of 0.2 ml/100 g body weight of 40 ml/l CCl_4 (Morgan Chemical Factory, Egypt) dissolved in paraffin oil (Morgan Chemical Factory, Egypt)**.** Carbon tetrachloride was injected two times per week for 2 consecutive weeks. Liver fibrosis was determined at the end of experimental by killing rats **(16)**.

Animals:

Thirty adult normal male albino rats weighting 150±10g. Rats were housed in wire cages under the normal laboratory condition and were fed on basal diet for a week as an adaptation period. Diet was offered to rats in special food cups to avoid loses conditions of feed, water was provided to the rats by glass tubes supported to one side of the cage, feed and water provided ad-labium and checked daily.

Biological experiments:

Basal diet composition (standard diet)

The standard diet was formulated according to AIN guidelines **(17, 18)**. Minerals mixture and vitamins mixture were prepared according to **(19, 20).**

Experimental design:

The experiment was done in the Faculty of Home Economics, Menoufia University, Shebin El-Kom. Rats were housed in wire cages at a room temperature of 25°C and kept under normal healthy condition.

All rats were fed on standard diet according to American Institute of Nutrition (AIN) for 7 days for adaptation **(17).** Rats were randomly divided into two main groups.

The first group, negative control group (n = 5), fed standard diet only.

The second main group hepatic rats (n=25). In this group rats were injected by CCL_4 by 2 mg per kg of rat's body weight.

Hepatic rats were divided into 5 sub-groups (5 rats each) according to the following:

Sub-group 1: Hepatic rats fed on standard diet only, used as positive control.

Sub-group 2: Hepatic rats fed on standard diet replaced with 5% of el carob seeds extract.

Sub-group 3: Hepatic rats fed on standard diet replaced with 10% of el carob seeds extract.

Sub-group 4: Hepatic rats fed on standard diet replaced with 5% of el carob seeds powder.

Sub-group 5: Hepatic rats fed on standard diet replaced with 10% of el carob seeds powder.

During the experimental period, the body weight gain and feed intake were estimated weekly, and the general behavior of rats was observed.

Blood sampling:

At the end of experimental period (28 days) each rat weight separately then, rats are slaughtered and collect blood samples after 12 hours of fasting. Blood samples were centrifuged at (4000 rpm) for ten minutes to separate blood serum, then kept in deep freezer till using.

Biological evaluation:

Biological Evaluation of the different diets were carried out by determination of body weight gain % (BWG), feed efficiency ratio (FER) according to **(22),** using the following formulas:

$$\textbf{Body weight gain (g)} = \textbf{Final weight} - \textbf{Intinal weight}$$

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

$$\textbf{FER} = \frac{\textbf{Gain in body weight (g)}}{\textbf{Food intake (g)}}$$

Biochemical analysis:

Alanine amino transferase (ALT), aspartate amino transferase (AST) enzymes and alkaline phosphatase (ALP) were measured according to the methods described by **(23, 24, 25)**, respectively. Serum glucose was estimated according to **(26).** Urea, uric acid, and creatinine levels were determined in serum according to the method described by **(27).** Total cholesterol (TC), Triglycerides (TG) and High density lipoprotein (HDL-c) lipid were determined according to **(28, 29, 30, 29),** respectively**.** Low density lipoprotein (LDL-c) and very low density lipoprotein (VLDL-c) were calculated according to the methods of **(31)**, as follows:

VLDL=TG/5.

LDL=Total cholesterol (HDL+VLDL).

Statistical analysis:

Statistical analysis was performed by using computer program (Costate) one-way ANOVA when a significant main effect was detected, the means were separated with the student new man-keuls test. Differences bet ween treatments ($P \leq 0.05$) were considered significant **(33).**

Results and Discussion

Data presented in Table (1) showed the effect of carob aqueous seeds extract and powder on body weight gain, feed intake and feed efficiency ratio of CCl_4 injected rats. In case of body weight gain the highest value recorded for negative control group, while positive control group recorded the lowest value with significant differences(P≤0.05). The mean values were 3.81 and 2.45 g⁄28 days, respectively. For treated groups the highest body weight gain value recorded for 5% carob seeds powder, while 10% carob aqueous seeds extract group recorded the lowest value with significant differences(P≤0.05). The mean values were 3.02 and 2.63 g⁄28 days, respectively. These results agree with, **(34)** who found that the soluble fiber in carob powder had shown a potential benefit for enhancing weight loss

As for feed intake there were no significant differences (P≤0.05) between negative control, positive control and all treated groups.

In case of feed efficiency ratio, the highest value recorded for control group, while control group recorded the lowest value with significant (P≤0.05) differences. The mean values were 0.208 and 0.146, respectively. For treated groups the highest feed efficiency ratio value recorded for 5% carob seeds powder, while 10% carob aqueous seeds extract group recorded the lowest value with significant differences (P≤0.05). The mean values were 0.167 and 0., respectively.

Table (1): The effect of carob aqueous seeds extract and powder on body weight gain, feed intake and feed efficiency ratio of CCl4 injected rats.

Parameters Groups	Body weight gain (g⁄28 day)	Feed intake (g⁄day)	Feed efficiency ratio %
G_1: Control positive	2.45^{b}±0.19	16.80^{a}±0.32	0.146^{b}±0.009
G_2: Control negative	3.81^{a}±0.76	18.20^{a}±0.75	0.208^{a}±0.033
G_3:(5%) carob aqueous seeds extract	2.98^{ab}±0.21	17.90^{a}±0.80	0.166^{ab}±0.004
G_4: (10%) carob aqueous seeds extract	2.63^{b}±0.49	17.10^{a}±0.36	0.154^{b}±0.026
G_5:(5%) carob seeds powder	3.02^{ab}±0.28	18.00^{a}±0.50	0.167^{ab}±0.011
G_6:(10%) carob seeds powder	2.80^{ab}±0.35	17.50^{a}±0.16	0.16^{ab}±0.02
LSD (P ≤ 0.05)	0.76	0.95	0.035

Each value represents mean ± standard deviation. Mean under the same column bearing different superscript letters are different significantly (P ≤ 0.05).

Data presented in Table (2) showed the effect of carob seeds as aqueous extract and powder on serum liver functions levels (ALP, AST, and ALT) of hepatic rats. The obtained results indicated that the highest value of serum ALP levels recorded for positive control group, while negative control group recorded the lowest value with significant (P≤0.05) differences. The mean values were 127.50 and 65.20 U/L, respectively. For treated groups the highest value of serum ALP levels recorded for 5% carob seeds powder, while 10% carob aqueous seeds extract group recorded the lowest value with significant (P≤0.05) differences. The mean values were 102.60 and 69.33U/L, respectively.

In case of serum AST, it could be concluded that the highest value of serum AST levels recorded for positive control group, while negative control group recorded the lowest value with significant (P≤0.05) differences. The mean values were 173.50 and 98.46 U/L, respectively. For treated groups the highest value of serum AST levels recorded for 5% carob seeds powder, while 10% carob aqueous seeds extract group recorded the lowest value with significant (P≤0.05) differences. The mean values were 161.20 and 115.08 U/L, respectively. Data also indicated that the highest value of serum ALT levels recorded for positive control group, while negative control group recorded the lowest value with significant (P≤0.05) differences. The mean values were 95.62 and 50.13 U/L, respectively. For treated groups the highest value of serum ALT levels recorded for 5% carob seeds powder, while 10% carob aqueous seeds extract group recorded the lowest value with significant (P≤0.05) differences. The mean values were 80.91 and 53.02 U/L, respectively. These results agree with **(35)** who reported that liver function parameters (serum aspartate aminotransferase, serum alanine aminotransferase, and serum alkaline phosphatase) were significantly increased in the positive control group due to induced hypercholesterolemia, compared with the negative control, treatment this rat with carob legume methanol extract for 8 weeks significantly improved the liver functions by decreasing the liver enzymes activity.

Also, **(36)** reported that CCl_4 caused a significant increase in the AST and ALT levels in comparison to those of control rats whereas carob supplementation caused a significant decrease in these serum enzymes in comparison to those of CCl_4 treated rats, the reasons for such effect of the carob addition may be due to antioxidant activity.

(37) reported that carob powder improved liver functions by reducing serum AST, ALT and ALP levels.

Table (2): The effect of carob seeds as aqueous extract and powder on serum liver functions levels (ALP, AST, and ALT) of hepatic rats.

Parameters Groups	ALP U/L	AST U/L	ALT U/L
G_1: Control negative	65.20^{f}±0.21	98.46^{f}±0.36	50.13^{f}±0.84
G_2: Control positive	127.50^{a}±0.69	173.50^{a}±0.08	95.62^{a}±0.30
G_3:(5%) carob aqueous seeds extra	90.15^{c}±0.30	150.16^{c}±0.99	72.48^{c}±0.77
G_4: (10%) carob aqueous seeds extr	69.33^{e}±0.71	115.08^{e}±0.56	53.02^{e}±0.15
G_5:(5%) carob seeds powder	102.60^{b}±0.58	161.20^{b}±0.28	80.91^{b}±0.43
G_6:(10%) carob seeds powder	75.42^{d}±0.11	133.70^{d}±0.40	60.20^{d}±0.60
LSD(P≤0.05)	0.88	0.94	1.02

Each value represents mean ± standard deviation. Mean under the same column bearing different superscript letters are different significantly (P≤0.05). ALP: Alkaline phosphatase. AST: Aspartate transaminase. ALT: Alanine aminotransferase.

Data presented in Table (3) showed the effect of carob aqueous seeds extract and powder on serum glucose levels of hepatic rats. The obtained results indicated that the highest value of glucose levels recorded for positive control group, while negative control group recorded the lowest value with significant (P≤0.05) differences. The mean values were 195.60 [a] and 104.23 mg/dl, respectively. For treated groups the highest value of glucose levels recorded

for 5% carob seeds powder, while 10% carob aqueous seeds extract group recorded the lowest value with significant differences(P≤0.05). The mean values were 161.44 and 109.12 mg/dl, respectively. These results agreed with. **(38)** who reported that methanolic extract of dry carob pods was active against STZ-nicotinamide-induced hyperglycemia in rats by inhibiting α-amylase and α-glucosidase **(38)**.

Table (3): The effect of carob aqueous seeds extract and powder on serum glucose levels of hepatic rats.

Parameters Groups	Glucose mg/dl
G_1: Control negative	104.23^{f}±0.92
G_2: Control positive	195.60^{a}±0.70
G_3:(5%) carob aqueous seeds extract	150.70^{c}±0.18
G_4: (10%) carob aqueous seeds extract	109.12^{e}±0.25
G_5:(5%) carob seeds powder	161.44^{b}±0.63
G_6:(10%) carob seeds powder	132.60^{d}±0.41
LSD	1.030

Each value represents mean ± standard deviation. Mean under the same column bearing different superscript letters are different significantly (P≤ 0.05).

Data presented in Table (4) showed the effect of carob aqueous seeds extract and powder on serum total cholesterol levels (TC) and triglycerides (TG) hepatic rats. The obtained results indicated that the highest value of serum cholesterol levels recorded for positive control group, while negative control group recorded the lowest value with significant differences(P≤0.05). The mean values were 165.20 and 98.05 mg/dl, respectively. For treated groups the highest value of serum cholesterol levels recorded for 5% carob seeds powder, while 10% carob aqueous seeds extract group recorded the lowest value with significant differences(P≤0.05). The mean values were 140.35 and 105.60 mg/dl, respectively.

In case of serum triglycerides, it could be concluded that the highest value of serum triglycerides levels recorded for positive control group, while negative control group recorded the lowest value with significant differences(P≤0.05). The mean values were 170.30and 104.00 mg/dl, respectively. For treated groups the highest value of serum triglycerides levels recorded for 5% carob seeds powder, while 10% carob aqueous seeds extract group recorded the lowest value with significant differences(P≤0.05). The mean values were 143.10 and 122.25^{e} mg/dl, respectively. These results agreed with **(39)** reported that aqueous carob extract had the ability to reduce total cholesterol and T.G. This might be due to presence of an insoluble dietary fiber which comprised of 80% insoluble polyphenol in the carob pod extract.

Also, (40) examined the effects of carob fiber on fat digestion and postprandial lipemia in healthy rats. The result proved that carob fiber could lower triglycerides, total cholesterol, because carob contains a large amount of insoluble dietary fiber and polyphenols.

Table (4): The effect of carob aqueous seeds extract and powder on serum total cholesterol levels (TC) and triglycerides (TG) hepatic rats.

Parameters Groups	Total cholesterol mg/dl	Triglycerides mg/dl
G_1: Control negative	98.05 ^{f} ± 0.62	104.00 ^{f} ± 0.18
G_2: Control positive	165.20 ^{a} ± 0.80	170.30 ^{a} ± 0.98
G_3:(5%) carob aqueous seeds extract	136.81 ^{c} ± 0.75	134.56 ^{c} ± 0.72
G_4: (10%) carob aqueous seeds extract	105.60 ^{e} ± 0.33	122.25 ^{e} ± 0.64
G_5:(5%) carob seeds powder	140.35 ^{b} ± 0.28	143.10 ^{b} ± 0.53
G_6:(10%) carob seeds powder	123.98 ^{d} ± 0.67	125.90 ^{d} ± 0.88
LSD (P ≤ 0.05)	1.081	1.250

Each value represents mean ± standard deviation. Mean under the same column bearing different superscript letters are different significantly (P ≤ 0.05).

Data presented in Table (5) showed the effect of carob aqueous seeds extract and powder on serum lipid profile levels; high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL–c) and very low-density lipoprotein cholesterol (VLDL–c) of Hepatic rats. It's clear to notice that the highest high density lipoprotein cholesterol levels recorded for negative control group, while positive control group recorded the lowest value with significant differences(P≤0.05). The mean values were 67.20 and 47.34 mg/dl, respectively. On the other hand, the highest high density lipoprotein cholesterol levels of treated groups recorded for 10% carob aqueous seeds extract group, while 5% carob seeds powder group recorded the lowest value with significant differences(P≤0.05). The mean values were 65.12 mg/dl and 57.30 mg/dl, respectively.

Data also indicated that the highest low density lipoprotein cholesterol levels recorded for positive control group, while negative control group recorded the lowest value with significant differences(P≤0.05). The mean values were 83.80 mg/dl and 10.05 mg/dl, respectively. For treated groups the highest value of serum low density lipoprotein cholesterol levels recorded for 5% carob seeds powder, while 10% carob aqueous seeds extract group recorded the lowest value with significant differences (P≤0.05). The mean values were 54.43 and 16.03 mg/dl, respectively.

In case of very low-density lipoprotein cholesterol the highest value recorded for positive control group, while negative control group recorded the lowest value with significant (P≤0.05) differences. The mean values were 34.06 and 20.80 mg/dl, respectively.

For treated groups the highest value of very low-density lipoprotein cholesterol levels recorded for 5% carob seeds powder, while 10% carob aqueous seeds extract group recorded the lowest value with significant differences (P≤0.05). The mean values were 28.62 and 24.45 mg/dl, respectively. These results agreed with **(41)** who reported that consumption of hydro-alcoholic seed extract of *Ceratonia siliqua, L.* can reduce blood lipids levels in diabetic male rats, because of its high of fibre, phytosterols and tocopherol in the extract, these compounds could prevent the absorption of lipids and increase the concentration of bile acids, there by declining the blood lipids levels **(41)**.

Also**, (42)** reported that feeding rats with 10 and 20% carob powder improved lipid profile parameters by reducing LDL-c and VLDL-c and increasing HDL-c levels.

Table (5): The effect of carob aqueous seeds extract and powder onserum lipid profile levels (HDL-c, LDL-c and VLDL-c) of Hepatic rats.

Parameter: Groups	HDL-c mg/dl	LDL-c mg/dl	VLDL-c mg/dl
G_1: Control negative	67.20^{a}±0.05	10.05^{f}±0.53	20.80^{f}±0.04
G_2: Control positive	47.34^{f}±0.18	83.8^{a}±0.42	34.06^{a}±0.20
G_3:(5%) carob aqueous seeds extract	60.82^{d}±0.31	49.08^{c}±0.30	26.90^{c}±0.15
G_4: (10%) carob aqueous seeds extract	65.12^{b}±0.44	16.03^{e}±0.24	24.45^{e}±0.13
G_5:(5%) carob seeds powder	57.30^{e}±0.21	54.43^{b}±0.04	28.62^{b}±0.11
G_6:(10%) carob seeds powder	62.50^{c}±0.69	36.30^{d}±0.2	25.18^{d}±0.18
LSD(P≤0.05)	0.671	0.580	0.260

Each value represents mean ± standard deviation. Mean under the same column bearing different superscript letters are different significantly (P≤0.05). HDL-c= High-density lipoprotein cholesterol. LDL-c = Low-density lipoprotein cholesterol. VLDL -c = Very low-density lipoprotein cholesterol.

Data presented in Table (6) showed the effect of carob aqueous seeds extract and powder on kidney functions levels (urea, uric acid and creatinine) of hepatic rats. It's clear to notice that the highest serum urea levels recorded for positive control group, while negative control group recorded the lowest value with significant differences (P≤0.05). The mean values were 49.27 and 22.50 mg/dl, respectively. For treated groups the highest value of serum urea levels recorded for 5% carob seeds powder, while 10% carob aqueous seeds extract group recorded the lowest value with significant differences(P≤0.05). The mean values were 38.78 and 25.41 mg/dl, respectively.

Data also showed that the highest serum uric acid levels recorded for positive control group, while negative control group recorded the lowest value with significant differences(P≤0.05). The mean values were 5.32 mg/dl and 2.10 mg/dl, respectively. On the other hand, for treated groups the highest value of serum uric acid levels recorded for 5% carob seeds powder, while 10% carob aqueous seeds extract group recorded the lowest value with significant differences(P≤0.05). The mean values were 3.65 and 2.50 mg/dl, respectively.

In case of serum creatinine, it could be concluded that the highest value recorded for positive control group, while negative control group recorded the lowest value with significant differences(P≤0.05). The mean values were 1.72 and 0.60 mg/dl, respectively. On the other hand, for treated groups the highest value of serum creatinine levels recorded for 5% carob seeds powder, while 10% carob aqueous seeds extract group recorded the lowest value with significant (P≤0.05) differences. The mean values were 1.25 and 0.71 mg/dl, respectively. Our results agreed with (43) who reported that carob play an important role in improving the renal dysfunction induced in rats by the cyclosporine, because of its anti-inflammatory effect. (44) reported that carob honey had a protective effect against lead-induced kidney damage in rats; it ameliorated the elevation of serum creatinine and blood urea induced by lead.

Table (6): The effect of carob aqueous seeds extract and powder on kidney functions levels (urea, uric acid and creatinine) of hepatic rats.

Parameters / Groups	Urea mg/dl	Uric acid mg/dl	Creatinine mg/dl
G_1: Control negative	22.50^{f}±0.10	2.10^{c}±0.05	0.60^{c}±0.03
G_2: Control positive	49.27^{a}±0.48	5.32^{a}±0.71	1.72^{a}±0.36
G_3:(5%) carob aqueous seeds extract	31.08^{c}±0.36	3.02^{bc}±0.65	1.06^{bc}±0.27
G_4: (10%) carob aqueous seeds extract	25.41^{e}±0.70	2.50^{c}±0.27	0.71^{bc}±0.08
G_5:(5%) carob seeds powder	38.78^{b}±0.59	3.65^{b}±0.40	1.25^{b}±0.33
G_6:(10%) carob seeds powder	29.62^{d}±0.82	2.88^{bc}±0.13	0.83^{bc}±0.11
LSD (P ≤ 0.05)	0.990	0.791	0.420

Each value represents mean ± standard deviation. Mean under the same column bearing different superscript letters are different significantly (P ≤ 0.05).

Conclusion

In conclusion, a strong therapeutic effect of feeding carob seeds as powder and aqueous extract for the treatment of hepatic rats induced by CCl_4, revealed that feeding experimental animals with carob seeds as powder and aqueous extract significantly increased (P≤0.05) HDL-c levels and decreased liver and kidney functions, glucose level, and serum lipid profile compared to the positive control group (+ve).

References

1. Sivakrishnan, S. and Pharm, M. Liver diseases-An overview. *World J. Pharm. Pharm. Sci.,* 2019;8, 1385-1395.
2. Mokdad, A.A.; Lopez, A.D.; Shahraz, S.; Lozano, R. et al. Liver cirrhosis mortality in 187 countries between 1980 and 2010: A systematic analysis. *BMC medicine,* (2014); 12(1): 1-24.
3. Weber, L. W.; Boll, M. and Stampfl, A. Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. *Critical reviews in toxicology,* (2003); 33(2): 105-136.
4. Rtibi, K.; Selmi, S.; Grami, D.; Saidani, K.; Sebai, H.; Amri, M. and Marzouki, L. *Ceratonia siliqua*, L.(immature carob bean) inhibits intestinal glucose absorption, improves glucose tolerance, and protects against alloxan-induced diabetes in rat. *Journal of the Science of Food and Agriculture,*(2017); 97(8): 2664-2670.
5. Benković, M.; Srečec, S.; Bauman, I.; Ježek, D.; Karlović, S.; Kremer, D. and Erhatić, R. Assessment of drying characteristics and texture in relation with micromorphological traits of carob (Ceratonia silliqua L.) pods and seeds. *Food Technology and Biotechnology,* 2016; 54(4): 432-440.
6. Fidan, H.; Stankov, S.; Petkova, N.; Petkova, Z.; Iliev, A.; Stoyanova, M. and Ercisli, S. Evaluation of chemical composition, antioxidant potential and functional properties of carob (*Ceratonia siliqua,*L.) seeds. *Journal of Food Science and Technology,* 2020; 57(7), 2404-2413.
7. Zhu, B. J.; Zayed, M. Z.; Zhu, H. X.; Zhao, J. and Li, S. P. Functional polysaccharides of carob fruit: a review. *Chinese Medicine,* 2019; 14(1), 1-10.

8. Ahmed, J.; Almusallam, A.S.; Al-Salman, F.; AbdulRahman, M.H. and Al-Salem, E. Rheological properties of water insoluble date fiber incorporated wheat flour dough. *LWT-Food Science and Technology*, 2013; 51(2), 409-416.

9. Dakia, P. A.; Wathelet, B. and Paquot, M. Isolation and chemical evaluation of carob (Ceratonia siliqua L.) seed germ. *Food Chemistry,* 2007; 102(4), 1368-1374.

10. Luthria, D. L. Significance of sample preparation in developing analytical methodologies for accurate estimation of bioactive compounds in functional foods. *Journal of the Science of Food and Agriculture,* 2006; 86 (14), 2266-2272.

11. Ahmed, M. M. Biochemical studies on nephroprotective effect of carob (Ceratonia siliqua L.) growing in Egypt. *Nature and Science,* 2010; 8(3), 41-47.

12. Souli, A.; Sebai, H.; Chehimi, L.; Rtibi, K.; Tounsi, H.; Boubaker, S. and Amri, M. Hepatoprotective effect of carob against acute ethanol-induced oxidative stress in rat. *Toxicology and industrial health,* 2015; 31(9), 802-810.

13. Temiz, M. A.; Temur, A. and Çelik, I. Antioxidant role and hepatoprotective effects of carob (*Ceratonia siliqua,* L.) seeds against ethanol-induced oxidative stress in rats. *Journal of Food and Nutrition Research,* 2015; 3(1), 57-61.

14. Passmore, R. and Eastwood, M. A. Human Nutrition and Dietetics. Eight Editions. *Longman Group UK LTD*. Churchill Livingstone. (1986).

15. Garba, H. A.; Mohammed, A.; Ibrahim, M. A. and Shuaibu, M. N. Effect of lemongrass (*Cymbopogon citratus* Stapf) tea in a type 2 diabetes rat model. *Clinical Phytoscience,* 2020; 6(1), 1-10.

16. Diao, Y.; Zhao, X. F.; Lin, J. S.; Wang, Q. Z. and Xu, R. A. Protection of the liver against CCl4-induced injury by intramuscular electrotransfer of a kallistatin-encoding plasmid. *World J. Gastroenterol,* 2011; 17: 111–117.

17. AIN. American institute of nutrition purified diet for laboratory Rodent, Final Report. J. Nutrition, 123: 1939-1951 and *O.Compactum* Benth.*J. Essential Oil Res.,* 1993; 8 (6): 657-664.

18. Reeves, P. G.; Nielsen, F. H. and Fahey, G. C., Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition adhoc writing committee on the reformulation of the AIN- 76 A rodent diet. *J. Nutr.,*1993; 123: 1939–1951.

19. Hegested, D.M.; Mills, C.; Elvehjem, C.A. and Hart, E.B. Choline in the nutrition of chicks. *J. Biol. Chem.,* 1941; 138: 459-470.

20. Campbell, A. Sensitive mutants of bacteriophage λ. *Virology,* 1961; 14(1), 22-32.

21. Drury, R. A. B. and Wallington, E. A. Carleton's histological technique 5th ed. New York: *Churchill Livingstone.* 1980.

22. Chapman, D.G.; Castilla, R. and Campbell, J.A. Evaluation of protein in food. LA. Method for the determination of protein efficiency ratio.*Can. J. Biochem. Physiol.,* 1959; 37: 679 – 686.

23. Bergmeyer, H. U. and Harder, M. A colorimetric method of the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase.*Clin. Biochem.,* 1986; 24: 481-486.

24. Kachmar, J. F. and Moss, D. W. Enzymes, in Tietz, N. W., "Fundamentals of Clinical Chemistry "Philadelphia, USA. : W. B. *Sanders Company,* 1976; 653-654.
25. Moss, D. W. Alkaline phosphatase isoenzymes. *Clinical chemistry,* 1982; 28(10), 2007-2016.
26. Rojas, E.; Lopez, M.C .and Valverde, M. Single cell gel electro phoresis assay: methodology and applications. *J Chromatogr Biomed Sci Appl.,* 1999; 722: 225–254.
27. Houot, O.; Bednawska, M.W.; Zhiri, A. and Slest, G. Simultaneous Determination of Uric Acid and Creatinine in Plasma by Reversed-Phase Liquid Chromatography.*Journal of Clinical Chemistry,* 1985; 31(1):109.
28. Schmidt-Sommerfeld,E.; Penn, D. andWolf, H. The influence of maternal fat metabolism on fetal carnitine levels. *Early human development,* 1981; 5(3), 233-242.
29. Allain, C.C. Cholesterol enzymatic colorimetric method. *J. of Clin. Chem.,* 1974; 20; 470.
30. Fossati, P.and Prencipe, L. Determination of serum triglycerides: *Clin. Chem.,* 1982; 28.2077.
31. Lee, R.and Nieman, D. Nutrition Assessment. *2nd Ed. Mosby, Missouri, USA.,* 1996; 591 – 594.
32. Bancroft, D.; Steven, A. and Turner, R. Theory and practice of Histological Techniques, 4th Ed. Churchill Livingstone, Edinburg, *London, Melbourne,*1996;
33. Sendcor, G. and Cochran, W. Statistical methods (6th ed.). USA: *Lowa State Collage,* 1979; 841.
34. Brennan, C. S. Dietary fibre, glycaemic response, and diabetes. *Molecular nutrition & food research,* 2005; 49(6), 560-570.
35. El Rabey, H. A.; Al-Seeni, M. N. and Al-Ghamdi, H. B. Comparison between the hypolipidemic activity of parsley and carob in hypercholesterolemic male rats. *BioMed research international,* 2017;2017.
36. Süzek, H.; Celik, I. and Dogan, A. Nephroprotective hepatoprotective potential and antioxidant role of carob pods (*Cerotonia siliqua,* L.) against carbon tetrachloride-induced toxicity in rats. *Indian J. Pharm Educ. Res.,* 2017; 51(2), 312-320.
37. Khader, S. A. and KHadrah, N. M. Effect of tamarind and carob in treatment of kidney functions induced gentamicin in rats. *Journal of Home Economics,* 2019; 29(4): 24-31.
38. Qasem, M. A.; Noordin, M. I.; Arya, A.; Alsalahi, A. and Jayash, S. N. Evaluation of the glycemic effect of *Ceratonia siliqua* pods (Carob) on a streptozotocin-nicotinamide induced diabetic rat model. *PeerJ.,* 2018; 6, e4788.
39. Abulyazid, I.; Abd Elhalim, S. A.; Sharada, H. M.; Aboulthana, W. M. and Abd Elhalim, S. T. Hepatoprotective effect of carob pods extract (Ceratonia siliqua L.) against cyclophosphamide induced alterations in rats. *International Journal of Current Pharmaceutical Review and Research,* 2017; 8(2), 149-162.
40. Macho-González, A.; Garcimartín, A.; Naes, F.; López-Oliva, M. E.; Amores-Arrojo, A.; González-Muñoz, M. J. and Sánchez-Muniz, F. J. Effects of fiber purified extract of carob fruit on fat digestion and postprandial lipemia in healthy rats. *Journal of agricultural and food chemistry,* 2018; 66(26), 6734-6741.

41. Mokhtari, M.; Sharifi, S. and Shahamir, T. M. The effect of hydro–alcoholic seeds extract of *Ceratonia siliqua* on the blood glucose and lipids concentration in diabetic male rats. *Yafte,* 2011; 13(2), 74-82.

42. Hassanein, K. M.; Ali, H. M. and El-Manfaloty, M. M. The influence of carob powder on lipid profile and histopathology of some organs in rats. *Comparative Clinical Pathology,* 2015; 24(6), 1509-1513.

43. Shalby, A. B.;Hamza, A. H. and Ahmed,H. H. New insight on the anti-inflammatory effect of some Egyptian plants against renal dysfunction induced by cyclosporine. *Eur. Rev. Med Pharmacol. Sci.,* 2012; 16(4), 455-461.

44. Fihri, A. F.; Al-Waili, N. S.; El-Haskoury, R.; Bakour, M.; Amarti, A.; Ansari, M. J. and Lyoussi, B. Protective effect of morocco carob honey against lead-induced anemia and hepato-renal toxicity. Cellular Physiology and Biochemistry, 2016; 39(1), 115-122.

الآثار الوقائية لبذور الخروب في علاج الاختلال الوظيفي في الكبد في الفئران المصابة بالتسمم الكبدي

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الملخص العربي:

صممت الدراسة الحالية لدراسة تأثير بذور الخروب في صورة مسحوق ومستخلص مائي على الفئران المصابة بتسمم الكبد بواسطة رابع كلوريد الكربون. تم استخدام 30 فأر من ذكور الألبينو في هذه الدراسة يتراوح أوزانها (150±10) وتم تقسيمها الى 6 مجموعات 5 فئران في كل مجموعة، تركت مجموعتان إحداها كمجموعة ضابطة سالبة والأخرى ضابطة موجبة، أما المجموعات الأربع مجموعات الأخرى بالإضافة إلى المجموعة الضابطة الموجبة تم تغذيتهم على الوجبة الاساسية بالإضافة إلى 0.2% مل لكل 100 جم من وزن الجسم 40مل/لتر من رابع كلوريد الكربون لمدة 14 يوم لإحداث التليف. تم إضافة بذور الخروب (مسحوق - مستخلص مائي) بنسبة 5%، 10% من الوجبة الاساسية. تم تقدير الوزن المكتسب، المأخوذ من الغذاء، معدل الاستفادة الغذائية، إنزيمات الكبد (أسبارتاتأمينوترانسفيريز، ألانينأمينوترانسفيريز وألكالين فوسفاتيز)، بروتينات الدم (الألبيومين والجلوبيولين)، دهون الدم (الدهون الثلاثية، الكوليسترول الكلي، البروتين الدهني مرتفع الكثافة، البروتين الدهني منخفض الكثافة والبروتين الدهني منخفض الكثافة جدا)، سكر الدم، وظائف الكلى (مستوى الكرياتينين، حمض اليوريك واليوريا) والتغيرات النسيجية في الكبد. من النتائج التي تم الحصول عليها يمكن استنتاج أن التغذية على بذور الخروب (مسحوق و مستخلص مائي) تسببت في زيادة معنوية ($P \leq 0.05$) في البروتين الدهني عالي الكثافة، الألبيومين والجلوبيولين. على النقيض انخفاض ملحوظ في الوزن المكتسب مقارنة بالمجموعة الضابطة الموجبة، أيضا تعزيز وظائف الكبد والكلي واستعادة المستوي الطبيعي لسكر الدم ودهون الدم، مما يعكس التأثير التغذوي العلاجي لبذور الخروب (مسحوق ومستخلص مائي) على الفئران المصابة بتسمم الكبد بواسطة رابع كلوريد الكربون.

الكلمات الأفتتاحية: بذور الخروب، تليف الكبد، الفئران، التحاليل الكيموحيوية