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The aqueous extract of Christ's thorn (*Ziziphus spina-christi*) seed modulates hyperlipidemia in hypercholesterolemic male rat.

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Abstract

The hypolipidemic and antioxidant activity of the crude aqueous extract of Christ's thorn (*Ziziphus spina-christi*) seed was tested in hypercholesterolemic male rat. Eighteen albino rats were divided into 3 groups (n=6), the 1st group was the negative control fed fat rich diet, the 2ndwas the positive control fed 2% cholesterol in the fat rich diet and the 3rd was fed 2% cholesterol and concurrently treated with 200 mg/kg b.w. Christ's thorn seed aqueous extract. The induced hypercholesterolemia in G2 increased lipid profile, lipid peroxide, liver and kidney function parameters, and decreased antioxidants. The tissues of kidney, liver, heart and testes showed altered histopathological changes. The concurrent treatment with Christ's thorn seed aqueous extract in G3 restored the altered biochemical and histological features to normal. Phenolic constituents of Christ's thorn seed aqueous extract suppressed the oxidative stress and reduced the hypercholesterolemia, and restored the altered biochemical and histopathological features.

Keywords: Hypercholesterolemia, Christ's thorn, Seed, Antioxidant, Aqueous extract, Rats.

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Introduction

Globally, hyperlipidemia has become a major cause of most serious diseases that threaten human health and life such as diabetes, high blood pressure coronary heart disease, stroke, peripheral vascular disease [1-3]. Hyperlipidemia is also considered a strong risk factor for atherosclerotic changes in the vascular wall [4,5].

Blood lipids control reduces the morbidity and mortality resulted from cardiovascular complication [6]. Exercise and supplementing diet with natural antioxidant can modulate lipid profile [3,7].

Zizyphusspina-christi (Christ's thorn) of the family Rhamnaceae is commonly used in folk medicine in treating obesity, liver complaints, fever, urinary troubles, diabetes, diarrhea, digestive disorders, skin infections, weakness and insomnia [8,9]. The purified peptides from *Z. jujuba* proteins prevent oxidative reactions and can be used in medicinal purposes, and food preservation [10]. The leave extract of *Zizyphusspina-christi* revealed antidiabetic activity [9,11-13] by controlling meal-derived glucose absorption and has antioxidantand anti-inflammatory properties [14-17]. In addition, extracts of fruits and seeds of *Z. spina-christi L.* also revealed an antimicrobial activity against *Bacillus subtilis*, *E. coli* and Streptococcus pyogenes [18].

This study was focused in testing the effect of treating hypercholesterolemic male rats with Christ's thorn seeds for 8 weeks to test their effect on lipid profile and antioxidants activity.

Materials and Methods

The Christ's thorn seeds were collected from Christ's thorn fruits from Jeddah, Saudi Arabia. All chemicals used are of analytical grade. All commercial kits used in the analysis were of international reputation.

Fat rich diet

The fat rich diet consisted of the following constituents: 64.5% Corn starch, 10% corn oil, 16% casein, 4% Salt Mixture, 4% N.N cellulose, 1% Vitamin Mixture, 0.2% DL, methionine and 0.2% choline chloride.

Christ's thorn seed crude aqueous extract preparation

The Christ's thorn seeds were washed with distilled water, sun dried for 72 h, and then milled using mixer. A 500 g of the powder were soaked in 5 litre distilled water for 72 h under constant shaking with intervals of 30 min. The mixture was filtered using 250 mm filter papers, and then the filtrate was freeze-dried at (-52°C). A 200 g semisolid product was produced. To obtain the recommended concentration, the suitable weight of the semisolid product was dissolved in the suitable amount of distilled water.

Experimental animals and experiment design

Eighteen Sprague Dawley male rats of East Asian origin were obtained from King Fahad Center for Medical Research, King Abdulaziz University, KSA. The rats were allocated in 3 stainless cages (n=6) and kept two weeks under observation for acclimatization prior the start of the experiment. All experiments were conducted in King Fahad Center for Medical Research under a protocol licensed by the bioethical committee of King Abdulaziz University. The rats were randomly divided into three groups as follows: the 1st negative control group (G1): normal rats fed fat rich diet, the 2nd positive control group (G2): fed 2% cholesterol in the fat rich diet to induce hypercholesterolemia [3,19,20], the 3rd group (G3): fed 2% cholesterol in the fat rich diet and concurrently treated with a daily dose of 200 mg/kg b.w. Christ's thorn seed aqueous extract. The experiment was conducted for 8 weeks.

Blood collection

By the end of the experiment, animals were euthanized by cervical dislocation and blood samples were collected for serum preparation. The abdomen was dissected and the organs were rapidly dissected out. A piece of liver was kept cold in ice for liver tissue homogenate preparation.

Liver tissue homogenate

The liver tissue homogenate was prepared according to the method described in Al-Seeni et al.,[21].

The biochemical analyses

The biochemical analyses were carried out using the specified commercial kits from Human (Germany) according to the instructions of the suppliers.

The following tests were measured in serum: total cholesterol (TC), serum triglyceride (TG), serum high density lipoprotein (HDL). The serum low density lipoprotein (LDL) was calculated as follows: LDL (mg/dl)=total cholesterol – HDl – (triglycerides/5), whereas the very low density lipoprotein (VLDL)=triglycerides/5.

Liver enzymes; gamma-glutamyltransferase (γ-GT), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), kidney function parameters; uric acid, creatinine, urea, and electrolytes: sodium, potassium, phosphorus and calcium ions were estimated in serum using Human (Germany) according to the instructions of the suppliers.

Antioxidants and lipid peroxidation

The antioxidants enzymes; glutathione reductase (GR), catalase, and superoxide dismutase (SOD) were estimated in liver tissue homogenate using Biodiagnostic (Egypt) according to the instructions of the suppliers.

Lipid peroxidation was estimated by measuring the malondialdehyde (MDA)-the final product of the unsaturated fatty acids in the homogenate of the liver tissue using Biodiagnostic kit (Egypt) according to the instructions of the supplier.

Histopathology

A piece of the liver, a kidney, a testis and the heart were washed in sterile saline and fixed in 10% formalin, dehydrated in an ascending series of ethanol (50%-99%), cleared in xylene, and then embedded in paraffin. 5 microtomes sections were stained in hematoxylin and eosin (H&E) dye, air dried, and then mounted in Canada balsam. The prepared sections were examined and photographed under a light microscope with a digital camera.

Statistical analysis

The SPSS (Statistical Program for Sociology Scientists) software Version 23.0 was used in analyzing data for computing the mean, the standard errors (SE) and test of significance (T-test).

Results and Discussion

Lipid profile

Hypercholesterolemia in G2 significantly increased serum total cholesterol, triglycerides, low density lipoprotein and very low density lipoprotein and decreased the useful high density lipoprotein as shown in Table 1. The concurrent treatment with Christ's thorn seed aqueous extract significantly ameliorated all altered lipid parameters and nearly restored them to the normal levels as in G1.

Table 1. *Effect of Christ's thorn seed aqueous extract on serum lipids in hypercholesterolemic male rats.*

Parameters	Statistics	G1 Negative control	G2 Positive control	G3 Christ's thorn seeds
TC	Mean ± SE	100.27 ± 4.99	128.35 ± 1.22	71.86 ± 7.71
(mg %)	T.test		-5.36***	7.50***
TG	Mean ± SE	81.41 ± 1.60	117.07 ± 6.56	34.21 ± 3.40
mg/dl	T.test		-5.09***	11.36***
HDL	Mean ± SE	42.70 ± 3.13	40.16 ± 1.49	41.01 ± 0.88
mg/dl	T.test		0.66*	-0.52*
LDL	Mean ± SE	43.66 ± 2.47	46.35 ± 1.00	29.95 ± 7.98
mg/dl	T.test		-0.79**	2.04***
VLDL	Mean ± SE	16.28 ± 0.32	23.32 ± 1.30	16.84 ± 0.68
mg/dl	T.test		-5.06***	1.37***

Significant differences with controls calculated by paired sample t-test; *p<0.05, **p<0.01, ***p<0.001.

Antioxidant enzymes and lipid peroxidation

Table 2 shows that the antioxidant enzymes catalase, superoxide dismutase and glutathione reductase were increased in liver tissue homogenate as a result of induced hypercholesterolemia compared to the negative control, whereas lipid peroxidation was significantly increased. The concurrent administration of Christ's thorn seed aqueous extract in G3 significantly increased the antioxidant enzymes under study and decreased the lipid peroxidation to the normal levels in G1.

Table 2. *Effect of treating hypercholesterolemic rats with Christ's thorn seed aqueous extract for 8 weeks on antioxidants and lipid peroxidation in liver tissue homogenate.*

Parameters	Statistics	G1 Negative control	G2 Positive control	G3 Christ's thorn seeds
Catalase U/l	Mean ± SE	258.50 ± 15.65	113.00 ± 2.768	242.50 ± 3.20
	T.test		10.855***	-9.66***
Superoxide dismutase U/ml	Mean ± SE	262.63 ± 16.58	245.50 ± 29.01	295.22 ± 14.57
	T.test		0.53***	-1.53***
Glutathione reductase U/ml	Mean ± SE	16.98 ± 0.69	14.78 ± 0.39	6.95 ± 0.35
	T.test		2.78 **	14.25 ***
Lipid peroxidase nmol/g tissue	Mean ± SE	626.77 ± 28.05	792.33 ± 31.28	228.50 ± 21.11
	T.test		-4.23***	13.38***

Significant differences with controls calculated by paired sample t-test; *p<0.05, **p<0.01, ***p<0.001.

Liver function

Induction of hypercholesterolemia in G2 increased all liver function enzymes; ALT, AST and γ-GT in serum compared to the negative control as shown in Table 3. While the concurrent administration of the aqueous extract of Christ's thorn seed in G3 significantly restored the liver enzymes to their normal levels.

Table 3. *Effect of Christ's thorn seed aqueous extract on liver enzymes in hypercholesterolemic male rats.*

Parameters	Statistics	G1 Negative control	G2 Positive control	G3 Christ's thorn seeds
ALT U/l)) γ-GT U/l)	Mean ± SE	23.61 ± 1.59	41.50 ± 5.24	24.00 ± 0.68
	T.test		-3.49**	3.48**
	Mean ± SE	3.75 ± 0.18	4.28 ± 0.47	3.46 ± 0.20
	T.test		-0.94	1.78*
ALP U/l)	Mean ± SE	61.78 ± 13.36	91.66 ± 3.98	71.46 ± 4.43
	T.test		-2.16*	3.24**

Significant differences with controls calculated by paired sample t-test; NS: Nonsignificant*p<0.05 **p<0.01.

Kidney function and serum electrolytes

Kidney function parameters were nonsignificantly increased as a result of induced hypercholesterolemia in G2 compared to the negative control as shown in Table 4, whereas the concurrent treating of the hyepercholesterolemic male rats in G3 with Christ's thorn seed aqueous extract restored the studied kidney function parameters to the normal.

Table 4 shows also that in G2, the induced hypercholesterolemia nonsignificantly increased serum sodium, potassium and phosphorus ions, whereas significantly decreased calcium ions compared to G1. Treating hypercholesterolemia in G3 with Christ's thorn seed aqueous

extract, non-significantly affect serum sodium, potassium and phosphorus ions, whereas significantly increased calcium ions.

Table 4. *Effect of Christ's thorn seed aqueous extract on kidney function in hypercholesterolemic male rats.*

Parameters	Statistics	G1 Negative control	G2 Positive control	G3 Christ's thorn seeds
Uric acid mg/dl	Mean ± SE	2.63 ± 0.11	2.70 ± 0.46	1.36 ± 0.07
	T.test		-0.15 NS	3.06**
Creatinine mg/dl	Mean ± SE	0.74 ± 0.02	0.76 ± 0.02	0.25 ± 0.01
	T.test		-0.60 NS	19.27***
Urea mg/dl	Mean ± SE	31.86 ± 5.174	40.65 ± 2.86	27.23 ± 6.44
	T.test		-1.625 NS	2.63**
Sodium mmol/l	Mean ± SE	136.53 ± 7.93	156.78 ± 6.10	185.17 ± 12.51
	T.test		-1.75 NS	-1.75 NS
Potassium mmol/l	Mean ± SE	4.78 ± 0.46	5.01 ± 0.27	5.80 ± 0.42
	T.test		-0.37 NS	-1.37 NS
Phosphorus mg/dl	Mean ± SE	4.60 ± 0.48	4.98 ± 0.67	6.12 ± 0.21
	T.test		-0.47 NS	-1.61 NS
Calcium mg/dl	Mean ± SE	9.83 ± 0.21	8.61 ± 0.37	15.45 ± 0.31
	T.test		2.37*	-10.92***

Significant differences with controls calculated by paired sample. t-test; NS: non significant, *p<0.05, **p<0.01, ***p<0.001.

Histopathology of heart

Figure 1A shows the normal structure of the cardiac muscles of the negative control. Figure 1B shows damaged cardiac muscles and necrotic muscle fibres with increased hyalinization of the hypercholesterolemic rats of G2. Figure 1C shows nearly normal cardiac muscles of the hypercholesterolemic rats of G3 treated with Christ's thorn seed aqueous extract.

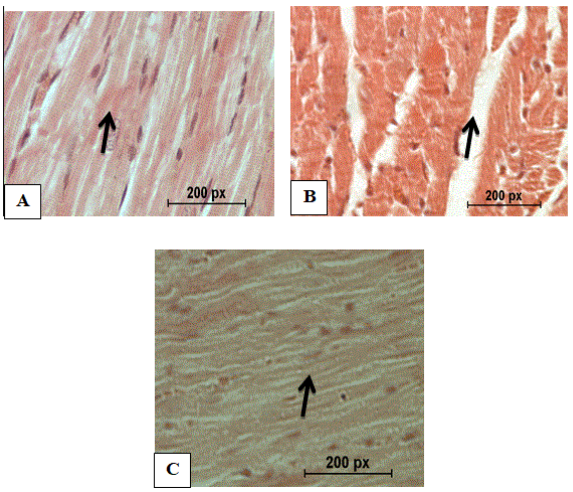

Figure 1. *(A) Normal cardiac tissues of negative control (G1), (B) Cardiac tissues of positive control group showing pathological changes (G2), (C) Cardiac tissues of Christ's thorn seed aqueous extract treated group (G3) showing restored normal configuration. Arrows: muscle fibers, (H&E × 400).*

Table 5. *Effect of Christ's thorn seed aqueous extract administration on total body weight of male rats under study.*

Parameters	Statistics	G1 Negative control	G2 Positive control	G3 Christ's thorn seeds
Initial weight	Mean ± SE	151.50 ± 2.58	151.67 ± 3.33	151.80 ± 1.59
	T.test		-3.95 NS	3.37 NS
1st week	Mean ± SE	161.67 ± 3.07	175.00 ± 5.62	162.66 ± 2.07
	T.test		-2.00 ***	2.69***
2nd week	Mean ± SE	181.67 ± 1.66	206.67 ± 3.33	183.61 ± 1.12
	T.test		-7.31***	5.83***
3rd week	Mean ± SE	201.67 ± 1.66	205.00 ± 2.23	204.67 ± 2.16
	T.test		-4.00**	6.32***
4th week	Mean ± SE	211.67 ± 1.66	215.00 ± 2.23	212.65 ± 1.61
	T.test		-1.58 **	1.00 NS
5th week	Mean ± SE	218.33 ± 1.66	231.67 ± 4.01	220.38 ± 1.16

	T.test		-2.69**	2.69**
6th week	Mean ± SE	235.00 ± 4.28	246.67 ± 3.33	239.08 ± 4.28
	T.test		-3.79**	3.79**
7th week	Mean ± SE	243.33 ± 8.81	265.00 ± 7.18	248.36 ± 8.81
	T.test		-1.45***	1.45***
8th week	Mean ± SE	261.67 ± 14.24	280.00 ± 16.93	251.77 ± 16.00
	T.test		-2.10***	1.47***

Significant differences with controls calculated by paired sample t-test; NS: Nonsignificant, **p<0.01, ***p<0.001.

Total body weight

Table 5 shows that induced hypercholesterolemia in G2 significantly increased total body weight, whereas treating the hypercholesterolemic male rats in G2 significantly reduced the weight approaching nearly the normal values of G1.

Histopathology of liver

Figure 2 shows the normal hepatic tissues of the negative control group G1 with normal cells and blood sinusoids. Figure 2 shows fatty liver of the positive control group (G2) with disrupted hepatic cell strands and vacuolated cytoplasm and necrosis. Figure 2 shows nearly normal hepatic tissues of hypercholesterolemic rats of G3 treated with Christ's thorn seed aqueous extract with normal cell strands and nuclei.

Histopathology of testes

Figure 3 shows the normal testicular tissues of the negative control group (G1) with regular seminiferous tubules. Figure 3 shows severely evacuated seminiferous tubules with thickened tubular walls and decreased germinal cells of the hypercholesterolemic positive control G. Figure 3 shows nearly normal testicular structure of hypercholesterolemic rats of the third group fed 2% cholesterol and co treated with Christ's thorn seed aqueous extract for 8 weeks with restored normal structure.

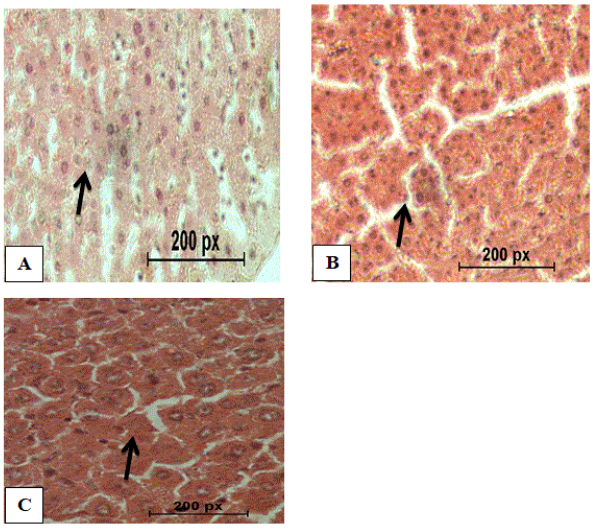

Figure 2. *Normal hepatic tissues of negative control (G1), B: Hepatic tissues of positive control group (G2) showing fatty hepatic tissues, C: Hepatic tissues of Christ's thorn seed aqueous extract treated group (G3) showing restored normal configuration (arrow: hepatic cells), (H&E × 400).*

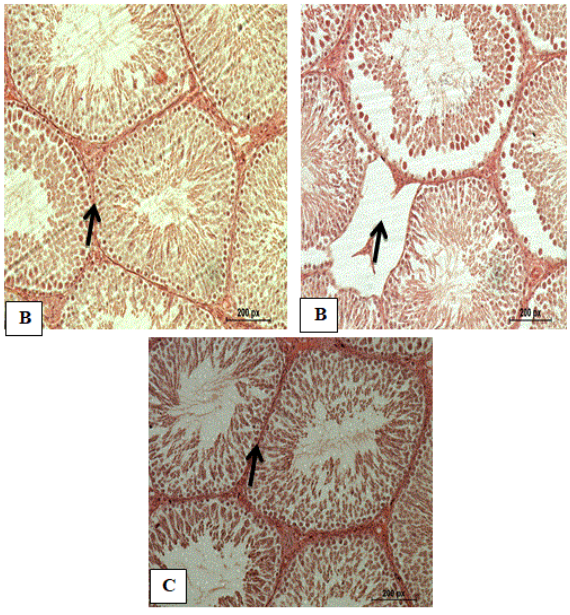

Figure 3. *Normal testicular tissues with normal seminiferous tubules of the negative control (G1). (B) Testicular tissues of positive control (G2) with evacuation and thickened tubular walls, (C) Testicular tissues of Christ's thorn seed aqueous extract treated group (G3) showing restored normal configuration. Long arrows: seminiferous tubules, arrow: tubular wall, (H&E × 400).*

Histopathology of kidney

Figure 4 shows the normal renal tissues of the negative control (G1) with normal renal pattern, uriniferous tubules with regulated nuclear arrangement and normal glomerulus. Figure 4 shows mildly altered renal tissues of the hypercholesterolemic rats of G2 with mildly dilated and shrinkage glomeruli. Figure 4 shows nearly normal renal tissues of G3 hypercholesterolemic rats that were concurrently treated with Christ's thorn seed aqueous extract for 8 weeks with nearly restored normal renal tissues.

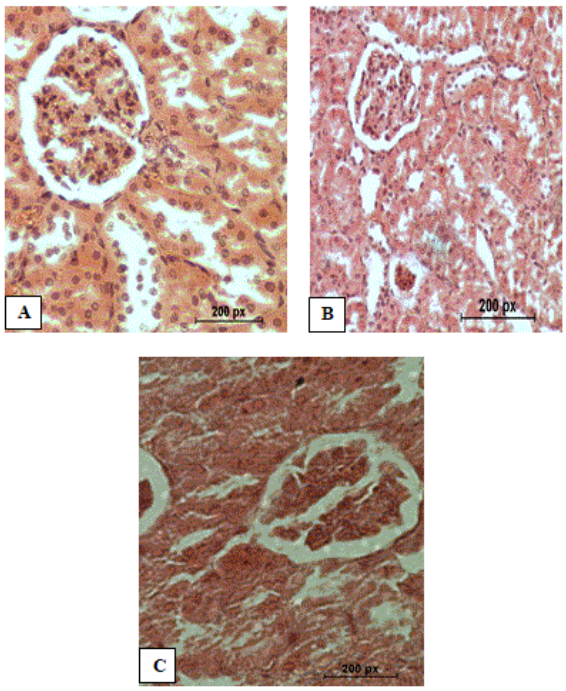

Figure 4. *(A) Normal renal tissues of negative control group (G1), (B) Renal tissues of the positive control group (G2) showing mild pathological changes, (C) Renal tissues of Christ's thorn seed aqueous extract treated group (G3) showing normal configuration. Arrows: glomerulus, (H&E × 400).*

In the present study, the hypolipidemic and antioxidant activity of Christ's thorn seed aqueous extract was investigated in hypercholesterolemic male rats. Induced hypercholesterolemia elevated lipid profile parameters [3,20,21]. While, treating hypercholesterolemic rats with Christ's thornseed aqueous extract ameliorated the lipid parameters by decreasing the total cholesterol, triglycerides LDL and VLDL, and increased the HDL compared to the positive control hypercholesterolemic group. The leaf powder and extract of Christ's thorn revealed a similar hypolipidemic effect [8]. This bioactive antioxidant and hypolipidemic activity of Christ's thorn crude aqueous extract may be due to its constituents; gallocatechin, epigallocatechin, 6'' sinapoylspinosin, spinosin, 6''' feruloylspinosin [22].

Similarly, the induced hypercholesterolemia increased lipid peroxidation as revealed by the increase in the malondialdehyde (MDA) content in the liver tissue homogenate, whereas the antioxidant enzymes; superoxide dismutase, catalase and Glutathione reductase were decreased. This result is in congruent with previous studies [3,21].

The concurrent administration of Christ's thorn crude seed aqueous extract also suppressed the oxidative stress by decreasing lipid peroxidation and increasing the antioxidant enzymes activity due to its active bio-constituents [22].

The activity of ALPAL and γ-GT representing liver function in this study was also increased in the hypercholesterolemic male rats of the positive group [3].These liver function enzymes restored their normal activities by administrating Christ's thorn crude seed aqueous extract which is consistent with Dkhil et al., [16] who stated that Christ's thorn methanolic leaf extract inhibited liver and heart injury. The kidney function parameters were nonsignificantly affected by the induced hyperlipidemiain the positive control [4,7,23]. On the line of the other studied parameters, administration of Christ's thorn seed aqueous extract ameliorated the levels of creatinine, uric acid and urea in the serum.

Similarly, serum electrolytes were also nonsignificantly affected by the induced hypercholesterolemiain in the positive control due to the altered membrane permeability or *glomerular filtration rate* [24]. Administration of Christ's thorn seed aqueous crude extractameliorated thelevels of all electrolytes and restored the normal levels. This therapeutic effect of the aqueous extract of Christ's thorn seeds may be due to its antioxidant and anti-inflammatory activity [13,16,22].

The histology of the studied organs reflected the altered biochemical investigations is in congruent with previous studies; the liver was severely affected [2,21]. Also the heart [3,21] and testes [8,25] were severely injured whereas the kidney was mildly affected [7] by the induced hypercholesterolemia in the positive control. However, Bentley et al., [4] stated that hypercholesterolemia causes renal morphological damage due to the increased microvascular density in the renal cortex that may lead to real disease progression.

This result is consistent with similar studies confirmed a correlation between hypercholesterolemia and alteration in organ's histology [21,24-26]. The concurrent administration of Christ's thorn crude seed aqueous extract alleviated these histological changes of the studied organs and restored their normal histology. This alleviating activity of Christ's thorn seed aqueous extract is due to its antioxidant and freed radical scavenging activity of the phenolic constituents of the seed aqueous extract which helped in suppressing oxidative stress that lead to cell damage and reduced the effect of hypercholesterolemia [13].

In addition, total body weight was significantly increased by hyperlipidemia [21] and restored its normal weight as in the negative control with the concurrent treatment with Christ's thorn seed aqueous extract.

Conclusion

In conclusion, induction of hypercholesterolemia severely altered lipid profile, liver function, oxidative stress, and histology of liver, heart, testis and kidney. While treating the hypercholesterolemic male rats with Christ's thorn seed aqueous extract restored all biochemical blood tests and the histology of the studied organs early to the normal. This may be attributed to the antioxidant and inhibition of oxidative stress by the phenolic constituents of the Christ's thorn seed.

Conflict of Interest

The authors declare that they have no conflict of interests.

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