The hypolipidemic and antioxidant activity of Christ's thorn (*Ziziphus spina- Christi*) leaves powder in hypercholesterolemic male rats

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Abstract: Christ's thorn (*Ziziphus spina- Christi*) leaves powder was orally administered in the diet (500 mg/kg body weight) to hypercholesterolemic male rats (fed 2% cholesterol) for 8 weeks to test their effect on lipid profile, antioxidants enzymes and lipid peroxide. Eighteen male rats weighing 155-170 gm were divided into three groups. The first group is untreated control group fed basal diet, the second group was fed 2% cholesterol in diet to induce hypercholesterolemia (positive control group), the third group was fed 2% cholesterol and co-supplemented with 500 mg/kg body weight Christ's thorn leaves for 8 weeks. The positive control group showed a significant increase in lipid profile, liver enzyme, lipid peroxide and kidney function parameters, and decrease in antioxidant enzymes activity. In addition, heart, liver, kidney and testes showed pathological changes compared with the negative control. Treating the hypercholesterolemic rats with Christ's thorn leaves improved the biochemical blood tests and the histology of the studied organs tissues. In conclusion, Christ's thorn leaves has an anti-oxidant activity and ameliorated the hyperlipidemia, improved liver and kidney functions and decreased lipid peroxide in hypercholesterolemic male rats. The antihyperlipidemic activity of Christ's thorn could be attributed to inhibiting oxidative stress due to its contentment of phenols compounds.
[Haddad A. El Rabey, Eman S. Attia, Madeha N. Al- Seeni, Abdulbasit I. Al-Sieni, Ibrahim H. Ibrahim, Mohamed F. Meerasahib, Abdulkader M. Shaikh-Omer and Abuelgassim O. Abuelgassim. **The hypolipidemic and antioxidant activity of Christ's thorn (*Ziziphus spina- Christi*) leaves powder in hypercholesterolemic male rats.** *Life Sci J* 2014;11(10):1010-1021]. (ISSN:1097-8135). http://www.lifesciencesite.com. 155

Key words: Christ's thorn, leaves, antioxidant, hypercholesterolemic, rats.

1.Introduction

Zizyphus spina-christi (Christ's thorn) is a wild tree belongs to the family *Rhamnaceae*. Christ's thorn species are used in folk medicine for the treatment of different diseases such as liver complaints, obesity, urinary troubles, fever, diarrhea, diabetes, digestive disorders, weakness, insomnia and skin infections (Han *et al.,* 1990; Abdel-Zaher *et al.,* 2005 and Al-Sieni, 2014).

The aqueous extract of *Z. spina-christi* root bark might relieve pain via central and peripheral mechanisms, provide some justification for the folkloric use of *Z spina-christi* in the treatment of stomach pains in mice and rats (Adzu *et al.,*2001). The antimicrobial activity of different extracts of fruits and seeds of *Z. spina-christi* L. revealed the highest activity of fatty acid fraction of lipids of seeds of *Z. spina-christi* L. against *Bacillus subtilis*, *E. coli* and *Streptococcus pyogenes* (Nazif, 2002).

Leaves of Christ's thorn are reported to possess anti-hyperglycemic activity and triterpenoidal saponin glycosides, christinin-A, B, C and D, were isolated from the butanol extract of them (Mahran *et al.,* 1996). *Zizyphus spina-christi* leaves decrease the serum glucose level in control and type-II diabetic rats. This effect is mediated by releasing insulin and the insulinotropic effect of *Zizyphus spina-christi* leaves, may be due to blockade of KAT P channels in pancreatic beta-cell membranes. *Zizyphus spina-christi* leaves may potentially be safe for use as an antidiabetic agent (Abdel-Zaher *et al.,* 2005). Christ's thorn leaf extract, plain or formulated, improved glucose utilization in diabetic rats by increasing insulin secretion which may be due to both saponin and polyphenol content and controlling hyperglycemia through attenuation of meal-derived glucose absorption that might be attributed to the total polyphenol content (Michel *et al.,* 2011).

The seeds of *Zizyphus jujuba* are effective in the improvement of the blood glucose, lipid compositions in serum of dietary hyperlipi-demic rats and have anti-inflammatory properties (Al-Reza *et al.,* 2010). Fruits

and vegetables are rich source of antioxidants such as vitamin C, tocopherol, phenolics and b-carotene which contribute to their antioxidant or free radical or scavenging effects. Amongst these, phenolics serve as powerful antioxidants by virtue of the hydrogen-donating properties of their phenolic hydroxyl groups, as well as by donating electrons to stop free radical chain reactions emerging from oxidative stress (John and Shahidi, 2010). The derived hydrolysates and the purified peptides from *Z. jujuba* proteins can prevent oxidative reactions and might be under utilized for food preservation and medicinal purposes (Memarpoor-Yazdi *et al.,* 2013).

Hyperlipidemia has been shown to be a strong risk factor for coronary heart diseases indication and risk factor for early atherosclerosis prior to the appearance of over atherosclerotic changes in the vascular wall (Bentley *et al.,* 2002 and Makni *et al.,* 2008). Clinical trials lane show that lowering lipids reduces the morbidity and mortality associated with cardiovascular complication (Amundsen *et al.,* 2002). Lipid profile could be lowered using food supplementation in the diet (El Rabey *et al.,* 2013).

The aim of this study was testing the effect of treating hypercholesterolemic male rats with Christ's thorn leaves for 8 weeks to test their effect on lipid profile, antioxidants enzymes and lipid peroxide.

2.Materials and Methods

Christ's thorn leaves were collected from wild Christ's thorn trees, dried at room temperature and milled by mixer then mixed to the diet in a ratio of 500 mg/kg body weight.

Basal lipid rich diet:

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

Animals and housing

All the animal experiments were carried out under protocols approved by the Institutional Animal House of the University of King Abdulaziz at Jeddah, Saudi Arabia. Twenty four male rats "*Rattas rattas*" weighing 150-170g were obtained from King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. Rats were housed six per polycarbonate cages. Cages, bedding, and glass water bottles (equipped with stainless steel sipper tubes) were replaced twice per week. The stainless steel feed containers were changed once per week.

Experiment design

The animals were kept at room temperature (25 ± 5ºC) with a natural lighting cycle (12 hours), fed a standard basal diet and kept under observation for 2-weeks before the experiment starts to exclude any undercurrent infection. The test animals were then divided randomly into four groups as follows: first group is untreated control group was fed normal diet, the second group was fed 2% cholesterol (Onody *et al.,* 2003 and Rathod *et al.,* 2011) in diet to induce hypercholesterolemia (positive control group), the third and the fourth groups were fed 2% cholesterol (to induce hypercholesterolemia) and treated with 500 mg/kg body weight Christ's thorn leaves powder for 8 weeks.

The current study was continued for 8 weeks to induce hypercholesterolemia (Ahmed, 2001 and El Rabey *et al.,* 2013). At the end of the experiment, animals were fasted 14-16 hours after their last feeding and blood samples were collected from the heart of pre-anaesthetized rats (anaesthetized by Dimethyl-ether). Blood was collected in plain tubes for chemistry analyses. Blood serum was obtained by centrifugation at 1000 rpm for 10 min at room temperature, and then stored at -20^{o}C until analysis was performed.

Biochemical tests

After collection of blood, anaesthetized animals were scarified by cervical dislocation. The abdomen was opened and the organs were rapidly dissected out and weighed. A piece of liver was saved in ice-cold for antioxidant enzyme.

All biochemical analyses were achieved using the specified kits from Human (Germany) according to the instructions of the suppliers. The following parameters were estimated:

i- **Serum lipids:** serum total cholesterol (S.TC), serum triglyceride (S.TG), serum high density lipoprotein cholesterol, low density lipoprotein cholesterol (S.LDLc) and serum very low density lipoproteins cholesterol (VLDLc) were estimated using Human kit (Germany).

ii- Liver enzymes: serum alanine aminotransferase (ALT), serum Gamma-glutamyl transferase (γ-GT) and serum alkaline phosphatase (ALP) were estimated using Human Kit (Germany).

iii- Antioxidants enzymes: catalase, glutathione reductase (GR) and superoxide dismutase (SOD) were estimated in liver homogenate using the specified kits from Biodiagnostic Chemical Company (Egypt) according to the instructions of the suppliers.

iv- Lipid peroxide: malondialdehyde (MDA) was estimated in liver homogenate using the specified kits from Biodiagnostic Chemical Company (Egypt) according to the instructions of the suppliers.

v- Kidney functions: serum uric acid, serum creatinine and serum urea were estimated using Human Kit (Germany).

vi- Serum electrolytes: calcium, sodium, phosphorus and potassium ions were estimated in serum using the specified kit from Human (Germany) according to the instructions of the suppliers.

Biological evaluations

The following biological parameters were estimated:

i- Total body weight: Rats were weighed every week.

ii- Organ weight and relative organ weight: heart, liver, right kidney, left kidney and testes were weighed after dissection and the relative organ weight was calculated by dividing the organ weight on the total body weight of each rat and then multiplied by 100.

Histopathological examination

Target organs; liver, heart, right kidney and testes were washed in sterile saline and fixed in 10% neutral formalin for histopathological studies. Organs were dehydrated in gradual ethanol (50-99%), cleared in xylene, and embedded in paraffin. Sections were prepared and then stained with hematoxylin and eosin (H&E) dye for microscopic investigation (Drury *et al.,* 1976). The stained sections were examined and photographed under a light microscope.

Statistical analysis

All data were analyzed using the SPSS (Statistical Program for Sociology Scientists) Statistics Version 17.0 for computing the mean values, the standard errors (SE) and test of significance (T-test).

3.Results

Lipid profile

Table (1) shows the effect of Christ's thorn leaves on serum lipids in hypercholesterolemic rats for 8 weeks. Feeding the rats on 2% cholesterol increased most of lipid parameters. As shown, the mean value of serum TC of the negative control group and the Christ's thorn leaves treated group was very high significantly (at $P<0.001$) lower than that of the positive control (100.27±4.99, 68.08±2.38 and 128.35±1.22 mg%, respectively).

Table (1): Effect of treating hypercholesterolemic rats with Christ's thorn leaves powder for 8 weeks on serum lipids.

Parameters	Treatments / Statistics	G1 -ve Control	G2 +ve Control	G3 Christ's thorn leaves
S.TC (mg %)	Mean±SE	100.27±4.99	128.35±1.22	68.08±2.38
	T-test		-5.36^{***}	30.36^{***}
S.T.G mg/dl	Mean±SE	81.41±1.60	117.07±6.56	36.71±2.58
	T-test		-5.09^{***}	11.18^{***}
S.HDlc mg/dl	Mean±SE	42.70±3.13	40.16±1.49	49.18±0.64
	T-test		0.66^{NS}	-6.11^{***}
S.LDlc mg/dl	Mean±SE	43.66±2.47	46.35±1.00	16.07±1.58
	T-test		-0.79^{NS}	20.14^{***}
S.VLDlc mg/dl	Mean±SE	16.28±0.32	23.32±1.30	7.34±0.51
	T-test		-5.06^{***}	11.19^{***}

Significant differences with controls calculated by paired sample t-test; ^{NS}: Nonsignificant $^{*}P<0.05$ $^{***}P<0.001$.

The mean value of serum TG of the negative control group and the Christ's thorn leaves treated group was very high significantly (at $P<0.001$) lower than that of the positive control (81.41±1.60, 36.71±2.58 and 117.07±6.56 mg/dl, respectively). As shown in the same Table, feeding the rats with cholesterol had decreased the HDLc compared with the negative control. Treating with Christ's thorn leaves had very high significantly (at $P<0.001$) increased the mean value of HDLc high compared with the positive control (49.18±0.64 and 40.16±1.49 mg/dl, respectively). The mean values of serum LDLc of Christ's thorn leaves treated group was very high significantly (at $P<0.001$) lower than that of the positive control (16.07±1.58 and 46.35±1.00 mg/dl, respectively). The mean value of serum VLDLc of the negative control and Christ's thorn leaves group was very high significantly (at $P<0.001$) lower than that of the positive control (16.28±0.32, 7.34±0.51 and 23.32±1.30 mg/dl, respectively).

Serum liver enzyme

Table (2) shows the effect of Christ's thorn leaves on serum liver enzymes in hypercholesterolemic rats for 8 weeks. As shown, all the mean values of S. ALT of negative control and Christ's thorn leaves treated group was high significantly (at $P<0.01$) lower than that of the positive control group(23.61±1.59, 26.65±1.93 and 41.50±5.24 U/L, respectively).

The mean value of S.γ-GT in the positive control group was non-significantly higher than that of the negative control. Treating the hyperlepidemic rats with Christ's thorn leaves had non-significantly reduced the mean value of S.γ-GT. The mean value of serum ALP of the positive control group was significantly (at $P<0.05$) higher than that of the negative control group (91.66±3.98 and 61.78±13.36 U/L, respectively), the mean value of serum ALP of the thorn leaves treated group was also was significantly (at $P<0.05$) higher than that of the negative control group (88.20±12.29 and 61.78±13.36 U/L, respectively).

Antioxidants enzymes

Table (3) shows the effect of Christ's thorn leaves on serum antioxidants enzymes in hypercholesterolemic rats for 8 weeks. Feeding rats on 2% cholesterol decreased all studied antioxidants enzymes in the liver tissue homogenate.

Table (2): Effect of treating hypercholesterolemic rats with Christ's thorn leaves powder for 8 weeks on serum liver enzymes.

Parameters	Treatments / Statistics	G1 -ve Control	G2 +ve Control	G3 Christ's thorn leaves
S.ALT U/l))	Mean±SE	23.61±1.59	41.50±5.24	26.65±1.93
	T-test		-3.49**	2.68**
S. γ-GT U/l))	Mean±SE	3.75±0.18	4.28±0.47	3.18±0.13
	T-test		-0.94NS	1.91NS
S.ALP U/l))	Mean±SE	61.78±13.36	91.66±3.98	88.20±12.29
	T-test		-2.16*	0.25*

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

Table (3): Effect of treating hypercholesterolemic rats with Christ's thorn leaves powder for 8 weeks on antioxidant enzymes of the liver tissue homogenate.

Parameters	Treatments / Statistics	G1 -ve Control	G2 +ve Control	G3 Christ's thorn leaves
Catalase U/I	Mean±SE	258.50±15.65	113.00±2.768	173.50±4.91
	T-test		10.855***	-11.47***
Glutathione reductase mmol/g.tissue	Mean±SE	16.98±0.69	14.78±0.39	17.78±0.54
	T-test		2.78**	16.68***
Superoxide dismutase U/ml	Mean±SE	262.63±16.58	245.50±29.01	265.85±13.61
	T-test		0.53NS	-0.57NS

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

As shown, all the mean values of catalase in the liver tissue homogenate of negative control and Christ's thorn leaves treated groups were very high significantly (at P<0.001) higher than that of the positive control group (258.50±15.65, 173.50±4.91 and 113.00±2.768 U/L, respectively). Similarly, the mean value of glutathione reductase in the liver tissue homogenate of the negative control group was high significantly (at P<0.01) higher than that of the positive control (16.98±0.69 and 14.78±0.39 mmol/g. tissue, respectively). Also, the mean value glutathione reductase in the liver tissue homogenate of the Christ's thorn leaves treated group was very high significantly (at P<0.001) higher than that of the positive control (17.78±0.54 and 14.78±0.39 mmol/g. tissue, respectively). The superoxide dismutase in the liver tissue homogenate was non-significantly affected. It was non-significantly decreased as a result of cholesterol feeding and non-significantly increased as a result of Christ's thorn leaves treatment.

Lipid peroxide

Table (4) shows the effect of Christ's thorn leaves on lipid peroxide in hypercholesterolemic rats for 8 weeks. Feeding rats on 2% cholesterol had very high significantly (at P<0.001) increased the lipid peroxide in the liver tissue homogenate compared with that of the negative control group (792.33±31.28 and 626.77±28.05 nmol/g tissue, respectively).

Table (4): Effect of treating hypercholesterolemic rats with Christ's thorn leaves powder for 8 weeks on lipid peroxide.

Parameters	Treatments / Statistics	G1 -ve Control	G2 +ve Control	G3 Christ's thorn leaves
Lipid peroxide nmol/g.tissue	Mean±SE	626.77±28.05	792.33±31.28	745.67±36.43
	T-test		-4.23***	15.25***

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

Treating these hypercholesterolemic rats with Christ's thorn leaves powder had very high significantly (at P<0.001) reduced the lipid peroxide in the liver tissue homogenate compared with that of the positive control (745.67±36.43 and 792.33±31.28 nmol/g. tissue, respectively).

Renal functions

Table (5) shows the effect of Christ's thorn leaves on renal functions in hypercholesterolemic rats for 8 weeks. As shown, the mean value of uric acid of Christ's thorn leaves treated group was significantly (at P<0.05) lower than that of the positive control (1.43±0.14 and 2.70±0.46 mg/dl, respectively).

Table (5): Effect of treating hypercholesterolemic rats with Christ's thorn leaves powder for 8 weeks on renal functions.

Parameters	Treatments / Statistics	G1 -ve Control	G2 +ve Control	G3 Christ's thorn leaves
Uric acid mg/dl	Mean±SE	2.63±0.11	2.70±0.46	1.43±0.14
	T-test		-0.15^{NS}	2.28^{*}
Creatinine mg/dl	Mean±SE	0.74±0.02	0.76±0.02	0.53±0.01
	T-test		-0.60^{NS}	7.62^{***}
Urea mg/dl	Mean±SE	31.86±5.174	40.65±2.86	38.00±1.74
	T-test		-1.625^{NS}	0.73^{NS}

Significant differences with controls calculated by paired sample t-test; ^{NS}: Nonsignificant $^{*}P<0.05$ $^{**}P<0.01$ $^{***}P<0.001$.

The mean value of serum creatinine in the Christ's thorn leaves treated group was very high significantly (at $P<0.001$) lower than that of the positive control (0.53±0.01, 0.25±0.01 and 0.76±0.02 mg/dl, respectively). On the other hand, the mean value of urea in the Christ's thorn leaves treated group was non-significantly lower than that of the positive control group.

Serum electrolytes

Table (6) shows the effect of Christ's thorn leaves on serum electrolytes in hypercholesterolemic rats for 8 weeks. As shown, the mean values of serum calcium of the negative control group was significantly (at $P<0.05$) higher than that of the positive control group (9.83±0.21 and 8.61±0.37 mg/dl, respectively), whereas the mean value of Christ's thorn leaves treated group was very high significantly (at $P<0.001$) higher than that of the positive control (13.06±0.86 and 8.61±0.37 mg/dl, respectively). The mean value of serum sodium in the positive control and Christ's thorn leaves treated group was non-significantly more than that of the negative control group.

Table (6): Effect of treating hypercholesterolemic rats with Christ's thorn leaves powder for 8 weeks on serum electrolytes; calcium, sodium, phosphorus and potassium.

Parameters	Treatments / Statistics	G1 -ve Control	G2 +ve Control	G3 Christ's thorn leaves
Calcium mg/dl	Mean±SE	9.83±0.21	8.61±0.37	13.06±0.86
	T-test		2.37^{*}	-7.66^{***}
Sodium mmol/l	Mean±SE	136.53±7.93	156.78±6.10	165.70±9.02
	T-test		-1.75^{NS}	-0.67^{NS}
Phosphorus mg/dl	Mean±SE	4.60±0.48	4.98±0.67	6.11±0.31
	T-test		-0.47^{NS}	-2.05^{*}
Potassium mmol/l	Mean±SE	4.78±0.46	5.01±0.27	6.13±0.36
	T-test		-0.37^{NS}	-2.54^{*}

Significant differences with controls calculated by paired sample t-test; ^{NS}: Nonsignificant $^{*}P<0.05$ $^{***}P<0.001$.

The mean value of serum phosphorus of the positive control group was non significantly higher than that of the negative control group. Whereas, the mean value of serum phosphorus in the Christ's thorn leaves treated group was significantly (at $P<0.05$) higher than that of the positive control group (6.11±0.31 and 4.98±0.67 mg/dl, respectively). Also, mean value of serum potassium of the positive control group was non significantly higher than that of the negative control group. On the other hand, the mean value of the serum potassium of Christ's thorn leaves treated group was significantly (at $P<0.05$) higher than that of the positive control group (6.13±0.36 and 5.01±0.27 mmol/l, respectively).

Total body weight

Table (7) shows the effect of Christ's thorn leaves on total body weight in hypercholesterolemic rats for 8 weeks. After the first week of the experiment, the mean value of Christ's thorn leaves group was significantly (at $P<0.05$) higher than that of the positive control (161.67±3.07 and 175.00±5.62 g, respectively). The mean value of body weight after the second week of the experiment in the negative control and Christ's thorn leaves group were very high significantly (at $P<0.001$) lower than that of the positive control (181.67±1.66, 181.67±1.66 and 206.67±3.33 g, respectively).

Table (7): Effect of treating hypercholesterolemic rats with Christ's thorn leaves powder for 8 weeks on total body weight.

Total body weight (g)	Treatments / Statistics	G1 -ve Control	G2 +ve Control	G3 Christ's thorn leaves
1st week	Mean±SE	161.67±3.07	175.00±5.62	161.67±3.07
	T-test		-2.00 NS	2.16*
2nd week	Mean±SE	181.67±1.66	206.67±3.33	181.67±1.66
	T-test		-7.31***	5.83***
3rd week	Mean±SE	201.67±1.66	215.00±2.23	201.67±1.66
	T-test		-4.00**	6.32***
4th week	Mean±SE	211.67±1.66	215.00±2.23	211.67±1.66
	T-test		-1.58 NS	1.00 NS
5th week	Mean±SE	218.33±1.66	231.67±4.01	218.33±1.66
	T-test		-2.69**	2.69**
6th week	Mean±SE	235.00±4.28	246.67±3.33	202.00±36.12
	T-test		-3.79**	1.28 NS
7th week	Mean±SE	243.33±8.81	265.00±7.18	243.33±8.81
	T-test		-1.45 NS	1.45 NS
8th week	Mean±SE	261.67±14.24	280.00±16.93	248.33±12.22
	T-test		-2.10*	1.21 NS

Significant differences with controls calculated by paired sample t-test; NS: Nonsignificant *P<0.05 **P<0.01 ***P<0.001.

The mean value for the negative control group after the third week was high significantly (at P <0.01) lower than that of the positive control (201.67±1.66 and 215.00±2.23 g, respectively), while that of the Christ's thorn leaves was very high significantly (at P <0.001) lower than that of the positive control (201.67±1.66, 201.67±1.66 and 215.00±2.23 g, respectively). The mean body weight after the fourth week of the experiment, in the negative control and Christ's thorn leaves treated groups was nonsignificantly lower than that of the positive control group. The mean values after the fifth week of the experiment for the negative control and Christ's thorn leaves were high significantly less (at P <0.01) lower than positive control (218.33±1.66, 218.33±1.66 and 231.67±4.01 g, respectively). The mean values after the sixth week of the experiment for positive control was high significantly less (at P <0.01) higher than that of the negative control (246.67±3.33 and 235.00±4.28 g, respectively). The Christ's thorn leaves treatment nonsignificantly decreased the body weight. The mean values of the body weight after the seventh week of the experiment, the mean value of the positive control group was nonsignificantly higher than that of the negative control. Treating with Christ's thorn leaves powder had nonsignificantly reduced the mean value of the body weight compared with the negative control group. After the eighth week of the experiment, the mean body weight of the positive control group was significantly (at P <0.05) higher than that of the negative control group (280.00±16.93 and 261.67±14.24 g, respectively). Treating with Christ's thorn leaves powder had nonsignificantly reduced the mean value of the body weight compared with the negative control group.

Organs weight

Data recorded in table (8) illustrate the effect of Christ's thorn leaves treatment for 8 weeks on organs weight in hypercholesterolemic rats. As shown, except for Christ's thorn leaves treated group in heart weight, that was high significantly (at P <0.01) lower than that of the positive control (0.90±0.36 and 1.09±0.03 g, respectively), all other mean values of heart, liver, right kidney, left kidney and testes weight were non significantly less than that of the positive control group.

Table (8): Effect of treating hypercholesterolemic rats with Christ's thorn leaves powder for 8 weeks on organ weight.

Organ weight g	Treatments / Statistics	G1 -ve Control	G2 +ve Control	G3 Christ's thorn leaves
Heart	Mean±SE	1.06±0.04	1.09±0.03	0.90±0.36
	T-test		-0.55 NS	3.89**
Liver	Mean±SE	7.51±0.65	8.94±0.62	8.73±0.57
	T-test		-1.80 NS	0.31 NS
Right kidney	Mean±SE	1.01±0.05	1.14±0.09	1.04±0.06
	T-test		-1.10 NS	1.04 NS
Left kidney	Mean±SE	0.93±0.06	1.10±0.09	1.01±0.05
	T-test		-1.62 NS	0.76 NS
Testes	Mean±SE	4.09±0.37	3.34±0.20	3.16±0.15
	T-test		1.77 NS	0.69 NS

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

Relative organs weight:

Data recorded in Table (9) illustrate the effect of Christ's thorn on the relative organs weight of heart, liver, right kidney, left kidney and testes in hypercholesterolemic rats for 8 weeks.

Table (9): Effect of treating hypercholesterolemic rats with Christ's thorn leaves powder for 8 weeks on relative organ weight.

Relative organs weight%	Treatments / Statistics	G1 -ve Control	G2 +ve Control	G3 Christ's thorn leaves
Heart	Mean±SE	0.41±0.02	.39±0.010	0.35±0.00
	T-test		0.64^{NS}	1.74^{NS}
Liver	Mean±SE	2.88±0.24	3.18±0.10	3.50±0.13
	T-test		-1.20^{NS}	-2.35^{*}
Right kidney	Mean±SE	0.38±0.02	0.40±0.01	0.41±0.02
	T-test		-0.61^{NS}	-0.64^{NS}
Left kidney	Mean±SE	0.35±0.02	0.47±0.09	0.40±0.02
	T-test		-1.26^{NS}	0.65^{NS}
Testes	Mean±SE	1.54±0.08	1.19±0.03	1.27±0.06
	T-test		3.81^{**}	-1.23^{NS}

Significant differences with controls calculated by paired sample t-test; ^{NS}: Nonsignificant $^{*}P<0.05$ ** $P<0.01$ *** $P<0.001$.

As shown, except the mean value of the relative liver weight in the Christ's thorn leaves treated group that was high significantly (at $P<0.05$) higher than the positive control (3.50±0.13 and 3.18±0.10 %, respectively), and the mean value of testes weight of the positive control group that was high significantly (at $P<0.01$) lower than that of the negative control group (1.19±0.03and 1.54±0.08 %, respectively), all other mean values of relative heart, liver, right kidney, left kidney and testes weight were non significantly more or less than that of the positive control group.

Histopathology

Liver

Figure (1) shows the histopathology of liver in the studied animals. The hepatic tissues of the negative control group rats show normal hepatic strands of cells and blood sinusoids (Figure 1A). Figure (1B) shows the hepatic tissue of hypercholesterolemic rats of the positive control group fed 2% cholesterol for 8 weeks with fatty liver tissue with disrupted cells and hepatic strands, and vacuolated cytoplasm and necrosis. Figure (1C) shows the hepatic tissues of hypercholesterolemic rats of the third group cosupplemented with Christ's thorn leaves for 8 weeks showing restored normal appearance of the hepatic strands with well defined hepatic cords containing polyhedral hepatocytes and normal appearing round nuclei.

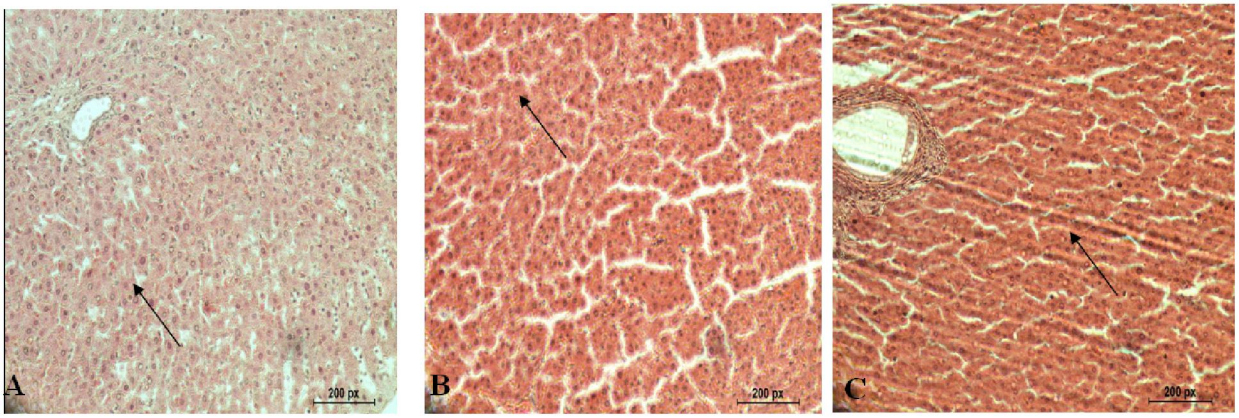

Figure (1): Histopathology of Liver. A; Normal hepatic tissues of negative control group rats fed basal diet (arrow). **B;** Hepatic tissues of positive control group fed 2% cholesterol for 8 weeks showing pathological changes (arrow). **C;** Hepatic tissues of Christ's thorn leaves treated group rats fed 2% cholesterol and cosupplemented with 500 mg/kg body weight Christ's thorn leaves powder for 8 weeks showing restored normal configuration (arrow). (H&E).

Heart

Figure (2) shows histology of heart of the animals under study. Figure (2A) shows cardiac tissues of the negative control group rats with normal structure of cardiac muscles. Figure (2B) shows cardiac tissues of hypercholesterolemic rats fed 2% cholesterol of the second group fed for 8 weeks showing increased hyalinization with cardiac muscles damage and necrosis of muscle fibers. Figure (2C) shows cardiac tissues of hypercholesterolemic rats of the third group cosupplemented with Christ's thorn leaves for 8 weeks showing minimal cardiac muscles damage and nearly restored normal tissues appearance.

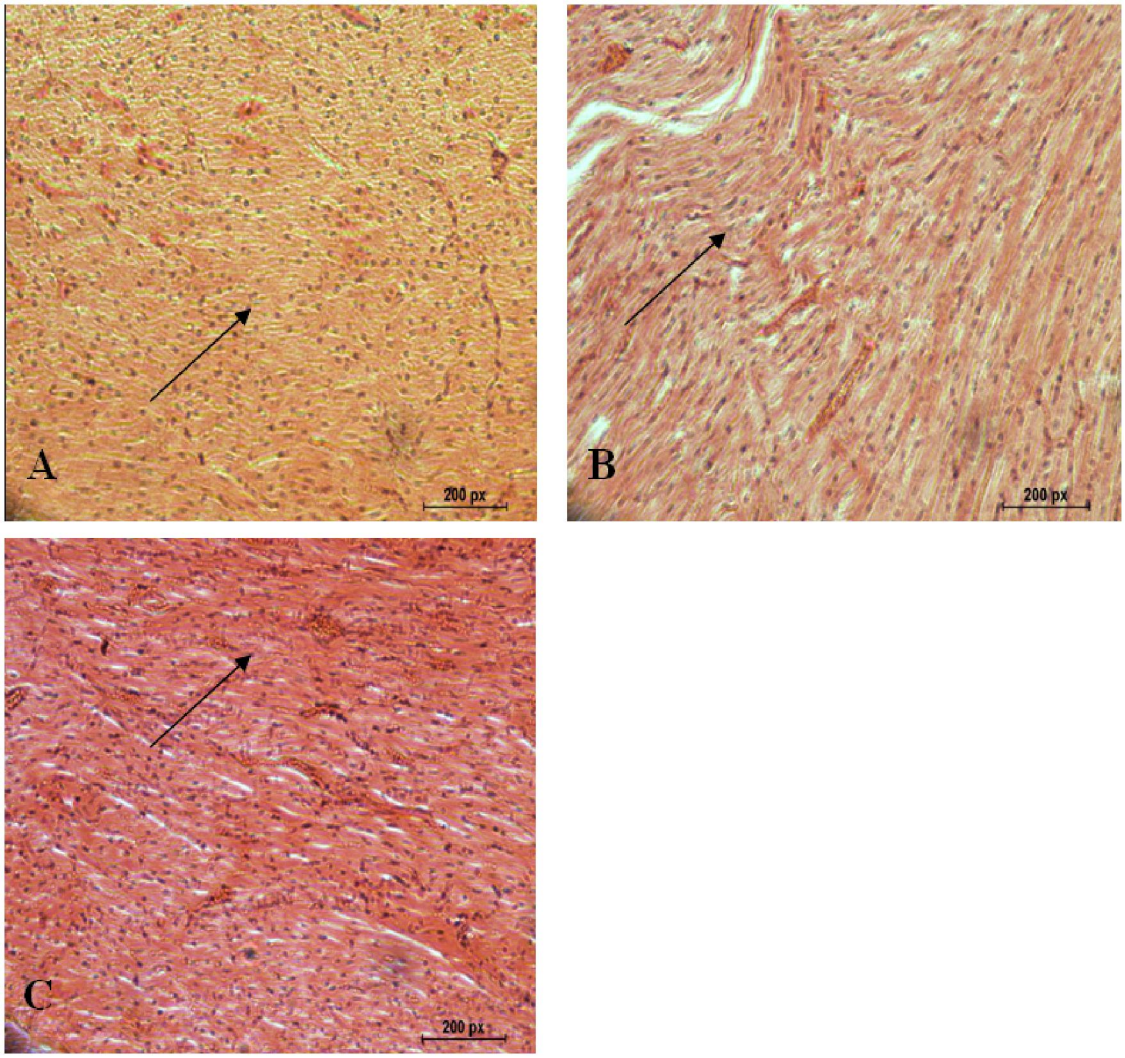

Figure (2): Histopathology of Heart. A; Normal cardiac tissues of negative control group fed basal diet. **B;** Cardiac tissues of positive control group fed 2% cholesterol for 8 weeks showing pathological changes. **C;** Cardiac tissues of Christ's thorn leaves treated group fed 2% cholesterol and cosupplemented with 500 mg/kg body weight Christ's thorn leaves powder for 8 weeks showing restored normal configuration. Arrows: muscle fibers (H&E).

Kidney

Figure (3) shows the histopathology of Kidney in rats under study. Figure (3A) shows the negative control group rats with normal renal structure, regulated nuclear arrangement of uriniferous tubules and collecting tubules with glomerulus. Figure (3B) shows the renal tissues of hypercholesterolemic rats fed 2% cholesterol for 8 weeks showing disrupted small uriniferous tubule and dilated and shrinkaged glomeruli led to a dilated urinary and collecting tubular space. Figure (3C) shows renal tissues hypercholesterolemic rats fed 2% cholesterol cosupplemented with Christ's thorn leaves treated group for 8 weeks with nearly restored normal renal tissues.

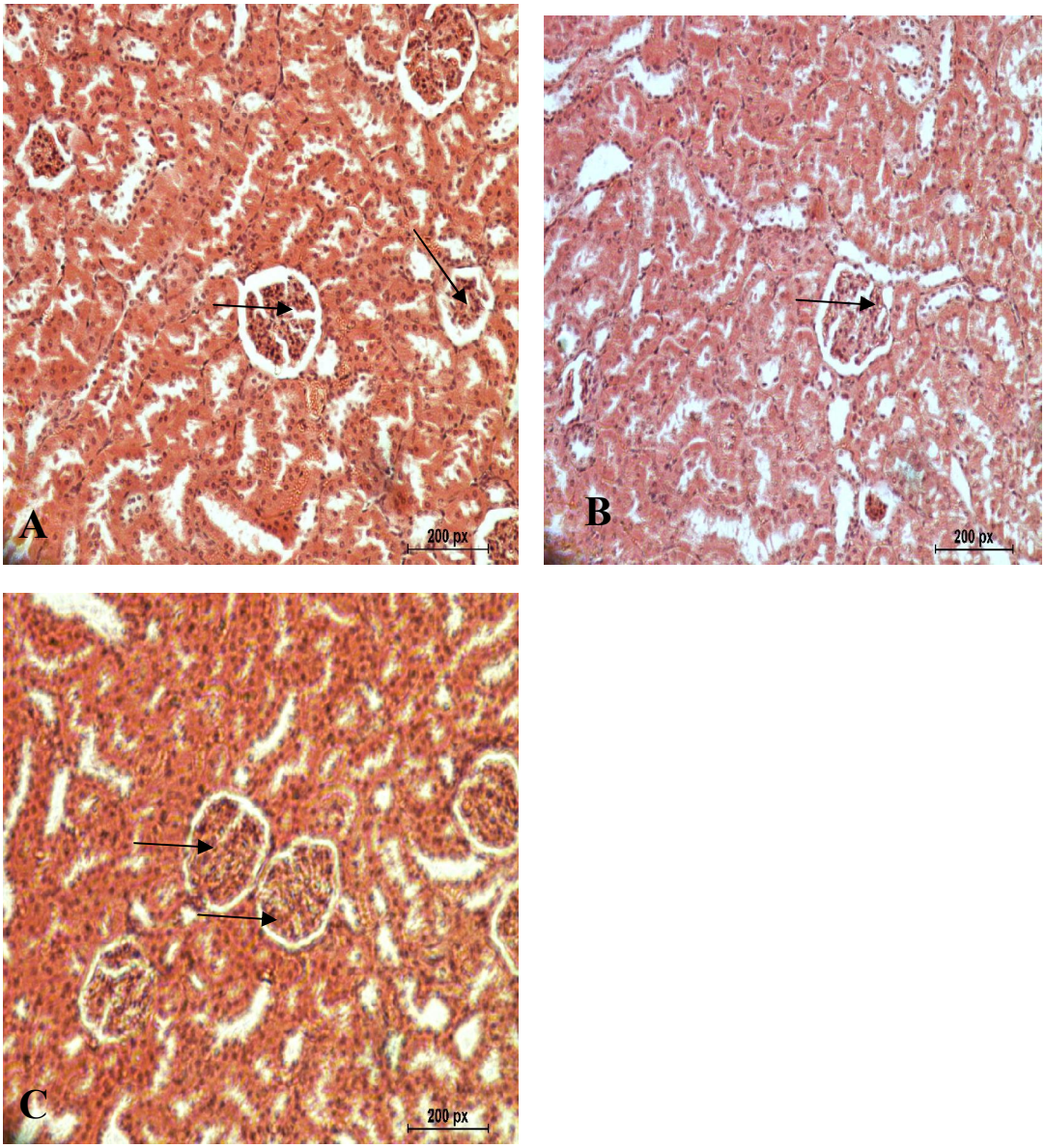

Figure (3): Histology of kidney. A; Normal renal tissues of negative control group fed basal diet. **B;** Renal tissues of positive control group fed 2% cholesterol for 8 weeks showing pathological changes. **C;** Renal tissues of Christ's thorn leaves treated group fed 2% cholesterol and co-supplemented with 500 mg/kg body weight Christ's thorn leaves powder for 8 weeks showing restored normal configuration. Arrows: glomeruli. (H&E).

Testes

The testicular structure of the testis of rats under study is shown in Figure (4). Figure (4A) shows the testicular tissues of the negative control group rats with normal and regular seminiferous tubules. Figure (4B) shows the testicular structure of the testis of hypercholesterolemic group rats fed 2% cholesterol for 8 weeks suffering pathologic effects with severely damaged seminiferous tubules showing thickened tubular walls and decreased germinal cells. Figure (4C) shows testicular structure of hypercholesterolemic rats of the third group fed 2% cholesterol and cosupplemented with Christ's thorn leaves for 8 weeks with restored normal structure and regular seminiferous tubules.

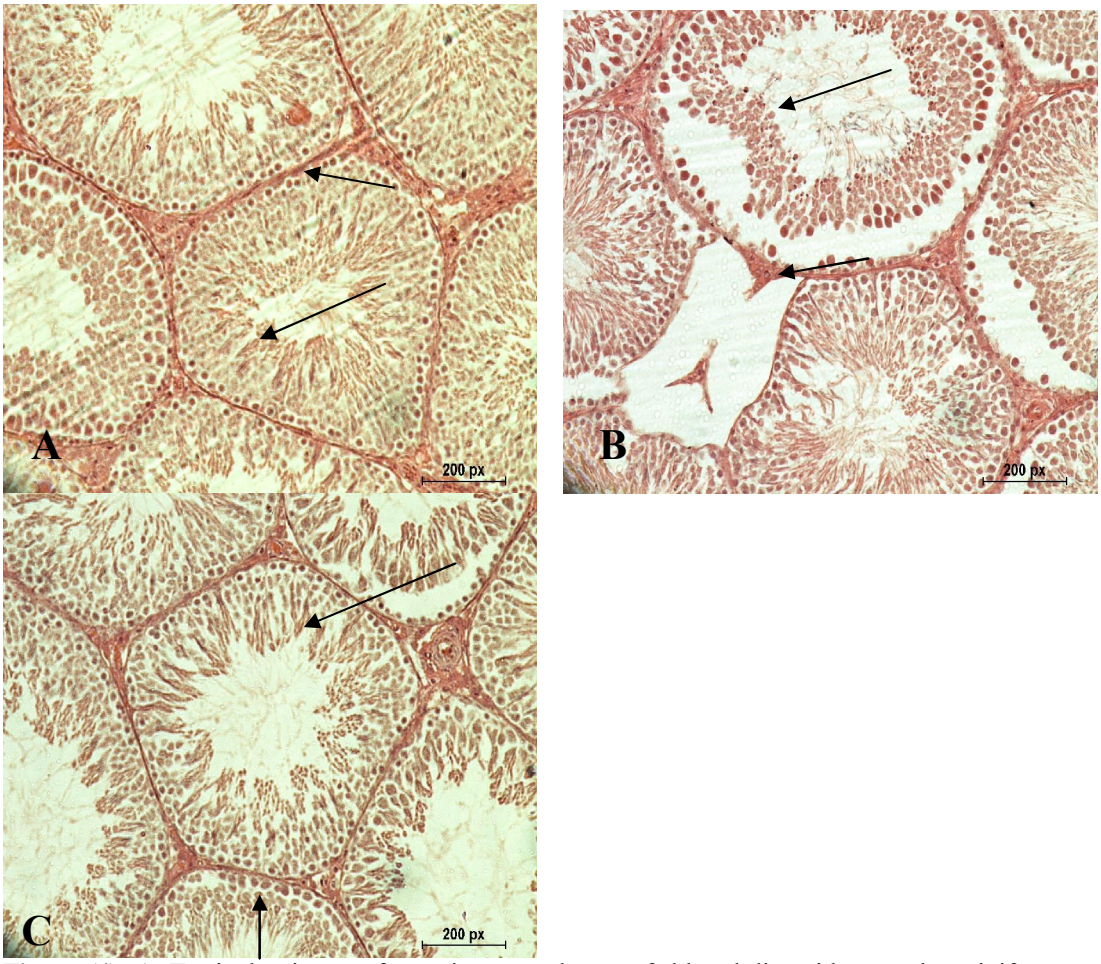

Figure (4): A; Testicular tissues of negative control group fed basal diet with normal seminiferous tubules (arrow). **B;** Testicular tissues of positive control group fed 2% cholesterol for 8 weeks showing pathological changes showing thickened tubular walls. **C;** Testicular tissues of Christ's thorn leaves treated group fed 2% cholesterol and cosupplemented with 500 mg/kg body weight Christ's thorn leaves powder for 8 weeks showing restored normal configuration. Long arrows: seminiferous tubules, short arrow: Tubular walls. (H&E).

4.Discussion

Hypercholesterolemia often occurs in conjunction with other metabolic risk factors including glucose intolerance, obesity, diabetes and metabolic syndromes and oxidation of the lipid core of low-density lipoproteins leads to a change in the lipoprotein conformation (Rathod *et al.,* 2011). In the present study, feeding rats on 2% cholesterol increased the serum total cholesterol in the positive control group (Onody et al., 2003 and El Rabey *et al.,* 2013). Treating hypercholesterolemic rats with Christ's thorn leaves (500 mg/kg body weight in the diet for 8 weeks) has significantly reduced the serum lipid profile parameters.

Feeding male rats on 2% cholesterol has significantly increased blood lipid parameters specially, serum total cholesterol, serum triglycerides, low and very low density lipoprotein and decreased the high density lipoproteins (Ono dy *et al.,* 2003, Rathod *et al.,* 2011 and El Rabey *et al.,* 2013).

Meanwhile, treating these hypercholeterolemic male rats with Christ's thorn leaves had significantly ameliorated lipid profile by lowering serum total cholesterol, serum triglycerides, low and very low density lipoprotein and increasing the high density lipoproteins. This result is consistent with that of Bentley *et al.* (2002) and Makni *et al.* (2008).

Induced hypercholeterolemic as a result of feeding male rats on 2% cholesterol has significantly increased liver enzymes activity (S.ALT, S.γ-GT and S.ALP) (El Rabey *et al.,* 2013 and Al-Sieni, 2014), whereas treating the hupercholeterolemic male rats with Christ's thorn leaves had significantly ameliorated the liver enzymes under study.

The antioxidant enzymes (catalase, SOD and Glutathione reductase) were significantly decreased as a result of induced hyperlipidemia, whereas lipid peroxide was increased (El Rabey *et al.,* 2013 and Al-Sieni, 2014).

Treating these rats with Christ's thorn leaves has significantly ameliorated these parameters by increasing the levels of antioxidant enzymes and reducing the lipid peroxide.

Renal parameters were also affected by hyperlipidemia as revealed by the increased levels of creatinine, uric acid and urea (El Rabey *et al.,* 2013 and Al-Sieni, 2014). Treating these rats with Christ's thorn leaves had significantly ameliorated these parameters by lowering these renal parameters in the treated groups. This result is consistent with previous investigations (Ghule *et al.,* 2012).

In addition, the present study showed nonsignificantly disturbed serum electrolytes as a result of hypercholesterolemia. This might be due to affecting glomerular Filtration rate or disorders in membrane permeability in the kidney (Ganong 1999 and El-Missiry *et al.,* 2001). Treating these rats with Christ's thorn leaves had restored the electrolytes to the normal levels in the treated groups. This result is consistent with previous investigations.

The histopathological investigations, showed pathological changes in the target organs (liver, heart, kidney and testes) in the 2% cholesterol fed group (G2). This result supported with previous studies suggesting a correlation between hypercholesterolemia and histological changes in the organs (Altunkaynak *et al.,* 2008; Dimitrova-Shumcovska *et al.,* 2010, Ouvrier *et al.,* 2010 and El Rabey *et al.,* 2013). An improvement in tissues in groups fed on Christ's thorn leaves showed a protective role against these pathologic changes and restored the normal histology of the target organs due to their higher content of antioxidant that help to prevent cell damage that work in association with enzymes and reduces the effect of dietary cholesterol resulting in restoring the normal histology of the target organs (El Rabey *et al.,* 2013 and Al-Sieni, 2014).

It could be concluded that treating the hypercholesterolemic rats with Christ's thorn leaves had improved the biochemical blood tests and restored the normal histology of the studied organs tissues. This is might be to the fact that Christ's thorn leaves had an anti-oxidant activity that ameliorated the hyperlipidemia, improved liver and kidney functions and decreased lipid peroxide in hypercholesterolemic male rats. The antihyperlipidemic activity of Christ's thorn could be attributed to inhibiting oxidative stress by its phenol constituent.

References

1. Abdel-Zaher AO, Salim SY, Assaf MH and Abdel-Hady RH (2005). Antidiabetic activity and toxicity of *Zizyphus spina-christi* leaves. Journal of Ethnopharmacology 101: 129-138.
2. Adzu B, Amos S, Wambebe and Gamaniel K (2001). Antinociceptive activity of *Zizyphus spina-christi* root bark extract. Fitoterapia 72: 344-350.
3. Ahmed O.M. (2001). Histopathological and biochemical evaluation of Liver and kidney lesions in streptozotocin diabetic rats treated with glimepiride and various plant extracts. J. Union Arab Biol. 164: 585-625.
4. Al-Reza SM, Yoon JI, Kim HJ, Kim, JS, Kang SC (2010). Anti-inflammatory activity of seed essential oil from *Zizyphus jujube*. Food Chem. Toxicol. 48: 639-643.
5. Al-Sieni AI (2014). The antioxidant activity of desert rose (*Adenium arabicum*) leaves on hyper cholesterolemic male rats. Life Sci J 11(9):149-158.
6. Altunkaynak ME, Ozbek E, Altunkaynak BZ, Can I, Unal D and Unal B (2008). The effects of high fat diet on the renal structure and morphometric parametric of kidneys in rats. Journal of anatomy 212: 845-852.
7. Amundsen AL, Ose L, Nenseter MS and Natnios FY (2002). Plant sterol ester-enriched spread lowers plasma total and LDL cholesterol in children with familial hypercholesterolemia. Am. J. Clin. Nutr. 76: 338-344.
8. Bentley MD, Rodriguez-Porcel M, Lerman A, Hershman-Sarafov M and Romero JC, *et al.* (2002). Enhanced renal cortical vascularization in experimental hypercholesterolemia. Kidney Int. 61: 1056-1063.
9. Dimitrova-Shumcovska J, Veenman L, Ristoski T, Leschiner S and Gavish M (2010). Chronic high fat, high cholesterol supplementation decreases 18kDa Translocator Protein binding capacity in association with increased oxidative stress in rat liver and aorta. Food and Chemical Toxicology 48: 910-921.
10. Drury RA, Wallington EA and Cancerson R (1976). Carlton's histopathological techniques, 4th edn. Oxford University Press, Oxford, 1976.
11. El Rabey HA, Al-Seeni MN and Amer HM (2013). Efficiency of Barley Bran and Oat Bran in Ameliorating Blood Lipid Profile and the Adverse Histological Changes in Hypercholesterolemic Male Rats. BioMed Research International, Article ID 263594, 10 pages.
12. El-Missiry M, Othman A, Amer M and Abd El-Aziz M (2001). Attenuation of the acute adriamycin-induced cardiac and hepatic oxidative toxicity by N-(2-mercaptopropionyl) Glycine in rats, Free Radical Res., 35: 575-581.
13. Ganong WF (1999). Review and Medical Physiology, Appelton, Lange, editors. Medical Publications, Calfornia, 19th Ed, 1999.
14. Ghule AE, Jadhav SS and Bodhankar SL (2012). Trigonelline ameliorates diabetic hypertensive

nephropathy by suppression of oxidative stress in kidney and reduction in renal cell apoptosis and fibrosis in streptozotocin induced neonatal diabetic (nSTZ) rats. International Immunopharmacology 14 (2012) 740-748.
15. Han BH, Park MH and Han YN (1990). Cyclic peptide and peptide alkaloids from seeds of *Zizyphus vulgaris*. Phytochemistry 29: 3315-3319.
16. John JA and Shahidi F (2010). Phenolic compounds and antioxidant activity of Brazil nut (*Bertholletia excelsa*). J. Func. Foods 2: 196-209.
17. Mahran GH, Glombitza KW, Mirhom YW, Hartmann R and Michel CG (1996). Novel saponins of *Zizyphus spina-christi* (L.) Willd cultivated in Egypt. Planta Medica 62: 163-165.
18. Makni M, Fetoui H, Gargouri NK, Garoui EM, Jaber H, Makni J, Boudawara T and Zeghal N (2008). Hypolipidemic and hepatoprotective effects of flax and pumpkin seed mixture rich in 3% Omega and 6% Omega fatty acids in hypercholesterolemic rats. Food and Chemical Toxicology 46: 3714-3720.
19. Memarpoor-Yazdi M, Mahaki H and Zare-Zardini H (2013). Antioxidant activity of protein hydrolysates and purified peptides from *Zizyphus jujuba* fruits. Journal of functional foods 5: 62–70.
20. Michel CG, Nesseem DI and Ismail MF (2011). Anti-diabetic activity and stability study of the formulated leaf extract of *Zizyphus spina-christi* (L.) Willd with the influence of seasonal variation. Journal of Ethnopharmacology 133: 53–62.
21. Nazif NM (2002). Phytoconstituents of *Zizyphus spina-christi* L. fruits and their antimicrobial activity. Food Chemistry 76: 77–81.
22. Onody A, Csonka C, Giricz Z and Ferdinandy P (2003). Hyperlipidemia induced by a cholesterol-rich diet leads to enhanced peroxynitrite formation in rat hearts. Cardiovasc Res. 1 58(3): 663-70.
23. Ouvrier A, Alves G, Damon-Soubeyrand C, Marceau G, Cadet R, Janny L, Brugnon F, Kocer A, Pommier A and Lobaccaro JMA (2011). Dietary cholesterol-induced post-testicular infertility. PloS one 6 (11): 26966.
24. Rathod D, Dodiya H and Goswami S (2011). Effect of Nicorandil: A Potassium Channel Opener against Experimentally-induced Hyperlipidemia. International Journal of Pharmacology 7 (6): 690-696.

9/23/2014