*Available online*www.ijpras.com

International Journal of Pharmaceutical Research & Allied Sciences, 2017, 6(2):22-36

Research Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Test of the efficiency of desert rose (AdeniumarabicumForssk.) seeds as a hypolipidemic and an antioxidant in male albino rats

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ABSTRACT

The seed powder of desert rose was orally supplemented in the diet to hypercholesterolemic male rats for 8 weeks to test its effect as an antioxidant and hypolipidemic. Eighteen rats were divided into three groups (n=6); the first group was the negative untreated control group, the second group was fed 2% cholesterol in the diet to induce hypercholesterolemia (positive control) and the third group was fed 2% cholesterol and cotreated with 100 mg/kg body weight desert rose seed. The positive control group showed a significant increase in lipid peroxide, lipid profile parameters, liver enzymes, and kidney function parameters and a significant decrease in antioxidant enzymes; glutathione reduced (GSH), serum glutathione reductase (GR) and serum superoxide dismutase (SOD) activity in the serum and kidney and liver tissue homogenate. Furthermore, liver, kidney, heart and testes tissues showed pathological changes compared with that of the negative control group. Treating the hypercholesterolemic rats with desert rose seeds for 8 weeks ameliorated the antioxidant enzyme activity, liver and kidney functions, and decreased lipid peroxidation. It was also restored the histology of the studied organs to their normal state. The desert rose seeds powder has hypolipidemic and antioxidant activity on hypercholesterolemic male rats. Desert rose seeds have also improved the tissues of the target organs in hypercholesterolemic rats under study.
Keywords: *antioxidant, hypercholesterolemic, rats, desert rose, seed, histopathology.*

INTRODUCTION

AdeniumarabicumForssk is poisonous and toxic succulent shrub belonging to the family Apocyanaceae. It has some medicinal properties; the sap and bark are used in bones dislocations, painful joints, wounds and skin infections [1,2,3]. Desert rose leaves have hypolipidemic and antioxidant effect [4,5].

Hypercholesterolemia causes coronary heart diseases and atherosclerosis resulting in the appearance of over atherosclerotic changes in the vascular wall and induces vascular functional changes that may lead to local ischemia and vascular remodeling [6,7]. Lwering lipids reduce the morbidity and mortality due to cardiovascular complication [8].

Oxidation of lipids is controlled by a variety of enzymatic and non-enzymatic antioxidants such as glutathione reductase, glutathione peroxides (GSHPx), superoxide dismutase (SOD), and vitamin E [9]. Vitamins C, vitamin E, carotenoids and phenolic compounds are non-enzymatic antioxidants important in the pathogenesis of oxidative stress related disorders [9,10]. Palé et al. [11] reported that Adeniumobesum flowers crude extract has antioxidant activity. Nitric oxide (NO) has an intracellular vasorelaxation effect, endothelial regeneration, reduction of oxidative

mechanism and inhibits leukocyte chemotaxis and platelet adhesion [12]. In addition, NO is a crucial modulator of vascular damage [13].

The objective of the current study was testing the efficiency of desert rose seed powder as an antioxidant and hypolipidemic in hypercholesterolemic male rats.

MATERIALS AND METHODS

The desert rose seeds

Desert rose seeds were collected from a local desert rose tree and was identified by a botanist as AdeniumarabicumForssk. Seeds were milled, and then mixed with lipid rich diet in a ratio of 100 mg/kg body weight.

Basal lipid rich diet

The following constituents represents the lipid rich diet: 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 [4].

Animals and housing conditions

Eighteen male albino rats (Rattusnorvegicus) weighing 180-200 g were purchased from Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia. The experiments were conducted under a protocol approved by the Institutional Animal House of the University of King Abdulaziz at Jeddah, Saudi Arabia. Rats were housed six per cage.

Design of the experiment

The animals were fed a standard basal diet and kept under observation for 2-weeks before the start of the experiment to exclude any undercurrent infection. The test animals were divided randomly into three groups as follows: the first group is untreated control group (G1) were fed lipid rich diet. The second group (G2) was the positive control group. Rats of G2 were fed 2% cholesterol in the diet to induce hypercholesterolemia according to Onody et al. [13]. Rats of the third group (G3) were fed 2% cholesterol and cotreated with 100 mg/kg body weight desert rose seed powder for 8 weeks.

The experiment was conducted for 8 weeks [15]. At the end of the experiment, rats were fasted 14-16 hours and blood samples were collected from the heart of Dimethyl-ether pre-anaesthetized rats. Blood serum was prepared by centrifugation at 1000 rpm for 10 min at room temperature, and then stored at -20oC for further analysis.

Animal dissection and blood collection

Anaesthetized animals were sacrificed, the abdomen was opened and liver, heart, testes and kidneys were rapidly dissected out and weighed. One testis, a kidney, a piece the heart and liver were saved in saline for histopathological investigations.

Biochemical tests

Blood samples were collected from ether anaesthetized animals. The abdomen was dissected and the target organs were rapidly excised and weighed. The left kidney and apiece of the liver were saved in ice-cold for tissue homogenate preparation.

Preparation of kidney and liver tissue homogenate

A piece of the kidney or liver tissue was dissected out, rinsed in ice-cold saline solution, and then homogenized in 0.1 M Tris–HCl buffer (pH 7.4) with a Teflon homogenizer at 4◦C. The mixture was centrifuged at 13000×g and the supernatant was used for estimation of lipid peroxide and antioxidant enzymes.

Biochemical analyses

Lipids of serum

Lipids were evaluated I serum as follows: serum total cholesterol (S.TC), serum high density lipoprotein cholesterol, serum triglyceride (S.TG) were estimated using Spinreact kit (Spain), and then serum low density lipoprotein cholesterol (S.LDLc) and serum very low density lipoproteins cholesterol (VLDLc) were calculated as follows:

S.LDL-C =S.TC – (HDL-C +S.TG\5).

S. VLDL=S.TC-(S.LDL+S.HDL)

Liver function

Serum aspartate transaminase (AST), serum alanine aminotransferase (ALT), and serum alkaline phosphatase (ALP) were estimated using Human Kit (Germany).

Kidney function

Serum uric acid was estimated using spinreact kit (Spain) according to the instruction of the supplier. Urea and creatinine were estimated in serum using biomerieux Kit (France).

Antioxidants enzymes estimation

Glutathione reductase (GR), superoxide dismutase (SOD) and glutathione reduced (GSH) were estimated in the serum, and in liver and kidney homogenate using Biodiagnostic Chemical Company kit (Egypt) according to the instructions of the supplier.

Lipid peroxide

Lipid peroxide was estimated in serum, and in liver and kidney homogenate using Biodiagnostic Chemical Company kit (Egypt).

Physiological evaluation

Water consumption: water consumption was measured for every 7 days.

Food Intake: daily food intake was calculated.

Total body weight: Rats were weighed every week.

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

Daily body weight gain (BWG), food efficiency ratio (FER) and percentage of food efficiency ratio (FER%) were calculated.

Histopathological investigations

Heart, liver, the right kidney and one testis were washed in sterile saline, and then fixed in 10% neutral formalin for histopathological studies. The target organs were dehydrated (in gradual ethanol; 50-99%), cleared in xylene, and embedded in paraffin. Organ sections were prepared and stained with hematoxylin and eosin (H&S) dye for microscopic preparation and photographing [16].

Statistical analysis

Data were analyzed using the SPSS program, Version 17.0 to calculate the mean values, the standard errors (SE) and test of significance using t-test.

RESULTS

Lipid profile

The effect of desert rose seeds treatment on lipid profile in hyperlipidemic male rats for 8 weeks is shown in Table (1). As shown, the serum TC, TG, LDL and VLDL of the positive control group were very high significantly at higher (at P<0.001) than that of the negative control. Whereas, the concurrent treatment of hypercholesterolemic rats with desert rose seed for 8 weeks has high significantly (at P<0.001) decreased TC, TG, LDL and S. VLDL compared with that of the positive control group.

The mean value of S.HDL was non significantly increased as a result of cholesterol feeding, whereas the concurrent treatment with desert rose seeds has significantly (at P<0.05) increased the S.HDL compared with the value of the positive control group.

Table (1): Effect of treatinghypercholesterolemic rats withdesert rose seedspowder for 8 weeks on serumlipids.

Treatments	Statistics	G1 -ve Control	G2 +ve Control	G3 Desert rose seeds
S.TC (mg %)	Mean±SE	158.00±4.05	276.33±5.23	184.50±3.62
	T-test		-15.31***	11.96***
S.T.G (mg/dl)	Mean±SE	126.17±4.73	203.17±3.65	158.50±3.35
	T-test		-9.37***	7.03***
S.HDLc (mg/dl)	Mean±SE	39.33±1.58	40.00±1.54	46.66±2.30
	T-test		-.24 (NS)	-2.11*
S.LDLc (mg/dl)	Mean±SE	93.47±5.06	195.97±5.82	106.14±2.95
	T-test		-10.96***	10.72***
S.VLDLc (mg/dl)	Mean±SE	25.43±0.87	40.56±0.62	31.20±3.62
	T-test		-14.32***	1.96***

Significantdifferenceswithcontrolscalculated by pairedsample t-test ; NS: Nonsignificant, *P<0.05*** P<0.001.

Liver enzymes and kidney function parameters

The effect of treating hypercholesterolemic rats with desert rose seeds for 8 weeks on liver enzymes and kidney functions parameters is illustrated in Table (2). The mean value of S.ALT and S.AST of the positive control group was high significantly (at P<0.001) higher than that of the negative control group, whereas the concurrent treatment with desert rose seeds has significantly (at P<0.001) decreased them compared with that of the positive control group.

On the other hand, the mean value of S.ALP was non significantly increased with cholesterol treatment (in the positive control group), and also non significantly increased with the concurrent desert rose seeds treatment for 8 weeks.

Table (2) shows also that, the mean value of serum uric acid in the positive control group was non significantly affected with hypercholesterolemia, and when treated with desert rose seeds it was also non significantly affected. The mean value of serum creatinine was significantly (at P<0.05) increased with hypercholesterolemia compared with that of the negative control group, and non-significantly decreased as a result of the concurrent treatment with desert rose seeds for 28 days compared with the positive control group. Similarly, the mean value of serum urea was

significantly (at P<0.01) increased with hypercholesterolemia compared with that of the negative control group, and non-significantly decreased as a result of the concurrent treatment with desert rose seeds for 28 days compared with the positive control group.

Table (2): Effect of treating hypercholesterolemic rats with desert rose seeds powder for 8 weeks on serumliver enzymes and kidney function parameters.

Treatments	Parameters	Statistics	G1 -ve Control	G2 +ve Control	G3 Desert rose seeds
Liver Enzymes	ALT (U/l)	Mean±SE	34.58±3.24	64.96±2.35	32.85±1.56
		T-test		-6.56***	11.98***
	AST (U/l)	Mean±SE	33.13±2.09	81.26±3.81	27.81±1.69
		T-test		-11.21***	10.44***
	ALP (U/l)	Mean±SE	83.66±3.56	89.83±6.14	86.50±3.55
		T-test		-0.86(NS)	0.45(NS)
Kidney function parameters	Uric acid mg/dl	Mean±SE	2.85±0.25	2.50±0.32	2.13±0.10
		T-Test		0.74 (NS)	1.17(NS)
	Creatinine umol/l	Mean±SE	0.50±0.02	0.58±0.01	0.58±.02
		T-Test		-2.30*	-0.22(NS)
	Urea umol/l	Mean±SE	20.33±0.48	24.48±0.82	25.35±1.46
		T-Test		-3.39**	-0.40(NS)

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

Antioxidants enzymes in serum, and kidney and liver homogenate

Table (3) shows the effect of desert rose seeds treatment on antioxidants enzymes in hypercholesterolemic rats for 8 weeks. As shown, the mean value of glutathione reduced (GSH) in the serum and liver homogenate of the positive control group was high significantly (at P<0.01 and P<0.001, respectively) lower than that of the negative control group as a result of cholesterol feeding, whereas the concurrent treatment of the hypercholesterolemic rats with desert rose seeds has significantly (at P<0.01 and P<0.001, respectively) increased the GSH value compared with that of the positive control group. However, the GSH in the kidney homogenate of the positive control group was non significantly affected, either by cholesterol feeding or with the concurrent treatment with desert rose seeds.

The mean value of glutathione reductase (GR) in the serum and liver homogenate of the positive control group was high significantly (at P<0.001) lower than that of the negative control group as a result of cholesterol feeding, whereas the concurrent treatment of the hypercholesterolemic rats with desert rose seeds has significantly (at P<0.001) increased the GR value compared with that of the positive control group. Similar to GSH, the mean value of GR in the kidney homogenate of the positive control group was non significantly affected, either by cholesterol feeding or with the concurrent treatment with desert rose seeds.

Similar to GR, the mean value of SOD in the serum and liver homogenate of the positive control group was high significantly (at P<0.001) lower than that of the negative control group as a result of cholesterol feeding, whereas the concurrent treatment of the hypercholesterolemic rats with desert rose seeds has significantly (at P<0.001) increased the GR value compared with that of the positive control group. Similar to GSH and GR, the mean value of SOD in the kidney homogenate of the positive control group was non significantly affected, either by cholesterol feeding or with the concurrent treatment with desert rose seeds.

Table (3): Effect of treating hypercholesterolemic rats with desert rose seeds powder for 8 weeks on antioxidants enzymes in the serum, and kidney and liver homogenate.

Blood/Tissue	Treatments	Statistics	G1 -ve Control	G2 +ve Control	G3 Desert rose seeds
Serum	(GSH) U/ml	Mean±SE	16.73±0.18	15.69±0.14	16.78±0.17
		T-test		3.56**	-3.46**
	(GR) U/ml	Mean±SE	8.52±0.28	2.83±0.14	8.20±0.21
		T-test		15.58***	-15.59***
	(SOD) U/ml	Mean±SE	606.85±12.13	437.98±12.99	567.68±4.65
		T-test		7.71***	-7.54***
Liver	(GSH) U/g. tissue	Mean±SE	17.82±0.19	9.21±0.21	13.81±0.14
		T-test		30.21***	-22.08***
	(GR) U/g. tissue	Mean±SE	16.76±0.49	3.35±0.22	12.35±0.55
		T-test		27.23***	-16.78***
	(SOD) U/g. tissue	Mean±SE	823.28±6.82	433.34±11.61	731.48±14.54
		T-test		34.27***	-13.71***
Kidney	(GSH) U/g. tissue	Mean±SE	10.96±0.25	11.02±0.17	11.22±0.24
		T-test		-0.149 (NS)	-0.55(NS)
	(GR) U/g. tissue	Mean±SE	6.26±0.20	5.98±0.17	6.23±0.16
		T-test		0.82 (NS)	-1.11
	(SOD) U/g. tissue	Mean±SE	666.07±11.75	654.09±9.27	652.78±8.72
		T-test		0.76 (NS)	0.08

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

Lipid peroxide

Table (4) shows the effect of desert rose seeds treatment on lipid peroxide in hypercholesterolemic rats for 8 weeks. The mean value of lipid peroxide in the serum and liver homogenate of the positive control group was very high significantly (at P<0.001) higher than that of the negative control as a result of cholesterol feeding. Treating the hypercholesterolemic rats with desert rose seeds has significantly (at P<0.001) decreased SOD compared with that of the positive control group in the serum and liver homogenate.

The mean value of lipid peroxide in the kidney homogenate of the positive control group was high significantly (at P<0.01) higher than that of the negative control as a result of cholesterol feeding. Treating the hypercholesterolemic rats with desert rose seeds has significantly (at P<0.001) decreased the SOD in the kidney homogenate compared with that of the positive control group.

Table (4): Effect of treating hypercholesterolemic rats with desert rose seeds powder for 8 weeks on lipid peroxide.

Serum/Tissue	Treatments	Statistics	G1 -ve Control	G2 +ve Control	G3 Desert rose seeds
Serum	MDA (nmol/ml)	Mean±SE	2.79±0.07	4.79±0.23	2.74±0.24
		T-test		-8.17***	4.79***
Liver	MDA (nmol/ g. tissue)	Mean±SE	5.22±0.21	10.56±0.29	7.09±0.10
		T-test		-12.89***	10.12***
Kidney	MDA (nmol/ g. tissue)	Mean±SE	3.34±0.11	3.71±0.04	3.25±0.10
		T-test		-2.64**	4.41***

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

Water consumption

Data recorded in Table (5) illustrates the effect of desert rose treatment on the consumed water in hypercholesterolemic rats for 8 weeks. As shown, the mean value of consumed water in the positive control group and the desert rose treated group in all weeks was non significantly affected compared with that of the negative control group.

Table (5): Effect of treating hypercholesterolemic rats with desert rose seeds powder for 8 weeks on water consumption.

water consumption ml	Statistics	G1 -ve Control	G2 +ve Control	G3 Desert rose seeds
1st week	Mean±SE	30.83±2.00	29.16±1.53	30.83±2.00
	T-test		0.67 (NS)	-1.00 (NS)
2nd week	Mean±SE	28.33±1.66	29.16±1.53	30.83±1.53
	T-test		-1.00 (NS)	-0.79 (NS)
3rd week	Mean±SE	30.83±1.53	29.16±1.53	29.16±2.00
	T-test		1.00 (NS)	0.00 (NS)
4th week	Mean±SE	29.16±2.00	30.00±1.82	29.16±1.53
	T-test		-0.27 (NS)	0.54 (NS)
5th week	Mean±SE	23.33±1.05	27.50±2.14	25.83±1.53
	T-test		-1.53 (NS)	0.67 (NS)
6th week	Mean±SE	27.50±1.70	27.50±1.11	28.33±1.66
	T-test		0.00 (NS)	-0.30 (NS)
7th week	Mean±SE	29.16±.83	27.50±1.70	28.33±1.66
	T-test		1.00 (NS)	-0.27 (NS)
8th week	Mean±SE	27.50±1.70	27.50±1.70	28.33±1.66
	T-test		0.00 (NS)	-0.27 (NS)

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

Food intake

Table (6) illustrates the effect of desert rose seeds treatment on food intake in the hypercholesterolemic rats for 8 weeks. As shown, except for the positive control group in the 7th week, that was significantly (at P<0.05) higher than that of the negative control all other mean values of food intake were non significantly affected with hypercholesterolemia compared with the negative control group.

Table (6): Effect of treating hypercholesterolemic rats with desert rose seeds powder for 8 weeks on food intake.

Food intake g	Statistics	G1 -ve Control	G2 +ve Control	G3 Desert rose seeds
1st week	Mean±SE	16.83±0.16	16.50±0.22	17.00±0.25
	T- test		1.00 (NS)	-1.46 (NS)
2nd week	Mean±SE	19.33±.42	18.66±.61	19.00±0.44
	T-test		0.93 (NS)	-0.59 (NS)
3rd week	Mean±SE	18.83±0.54	18.33±0.55	18.33±0.55
	T-test		0.65 (NS)	0.00 (NS)
4th week	Mean±SE	19.33±0.42	18.66±0.61	19.00±0.44
	T-test		0.93 (NS)	-0.59 (NS)
5th week	Mean±SE	19.33±0.42	19.33±0.42	19.00±0.44
	T-test		0.00 (NS)	0.54
6th week	Mean±SE	19.00±0.44	18.83±0.54	19.00±0.44
	T-test		0.25 (NS)	-0.30 (NS)
7th week	Mean±SE	19.00±.44	19.83±.16	19.00±0.44
	T-test		*-2.07	1.53 (NS)
8th week	Mean±SE	19.50±0.34	19.66±0.33	19.66±0.21
	T-test		-0.30 (NS)	0.00 (NS)

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

Total body weight

Data recorded in Table (7) illustrates the effect of desert rose seeds treatment for 8 weeks in hyperchlesterolemic rats on total body weight. The mean body weight value of the positive control group after the first, second, third and fourth weeks was high significantly (at P<0.01) higher than that of the negative control group. The mean body weight value of the positive control group after the other four weeks (the 5th, 6th, 7th, 8th) was significantly higher (at P<0.05) than that of the positive control. Treating the hypercholesterolemic rats with desert rose seeds has significantly reduced the body weight in all weeks compared with that of the positive control group.

Table (7): Effect of treating hypercholesterolemic rats with desert rose seeds powder for 8 weeks on total body weight.

Total body weight (g)	Statistics	G1 -ve Control	G2 +ve Control	G3 Desert rose seeds
1st week	Mean±SE	193.67±6.13	211.83±4.59	190.50±3.81
	T-test		**-2.57	3.23**
2nd week	Mean±SE	197.33±5.94	215.33±4.56	194.17±3.80
	T-test		-2.69**	3.150**
3rd week	Mean±SE	201.83±5.44	218.33±4.41	198.00±3.51
	T-test		-2.68**	3.18**
4th week	Mean±SE	205.50±5.51	221.83±4.29	202.50±3.43
	T-test		**-2.59	3.11**
5th week	Mean±SE	210.50±5.50	226.00±4.35	206.83±3.01
	T-test		*-2.42	3.22**
6th week	Mean±SE	214.17±5.43	230.33±4.16	210.67±2.27
	T-test		*-2.46	3.63**
7th week	Mean±SE	218.17±5.54	234.00±3.88	215.00±1.71
	T-test		-2.30*	3.82**
8th week	Mean±SE	222.33±5.78	237.8±4.04	219.50±1.02
	T-test		*-2.02	3.72**

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

Organs weight

Data recorded in Table (8) illustrate the effect of desert rose seeds powder treatment for 8 weeks on organs (liver, right kidney and left kidney, right testis and left testis) weight in hypercholesterolemic male rats.

The mean value of liver weight in the positive control group was significantly (at P<0.05) higher than that of the negative control group, whereas the mean value of liver weight of the desert rose seeds treated group was very high significantly (at P<0.001) lower than that of the positive control. On the other hand, the mean values of weight of other organ (heart, right kidney, left kidney, and right and left testis) in the positive control group and the desert rose seeds treated group were non significantly affected compared with that of the negative control group.

Relative organ weight

Table (9) illustrates the effect of desert rose supplementation for 8 weeks on relative organ (liver, right kidney and the left kidney) weight in hypercholesterolemic rats. As shown, the mean value of relative weight for all organs in the positive control and the desert rose treated group were non significantly higher than that of the negative control group.

Daily food intake (FI), body weight gain (BWG) and food efficiency ratio (FER)

Table (10) illustrates the effect of desert rose seeds treatment for 8 weeks on daily food intake (FI), body weight gain (BWG) and food efficiency ratio (FER) in hypercholesterolemic rats. As shown, the mean value of daily food

intake, body weight gain and food efficiency ratio of the positive control group was non significantly affected compared with that of the negative control group. Treating the hypercholesterolemic rats with desert rose seed powder for 8 weeks has non significantly affected the above mentioned parameters compared with that of the positive control group.

Table (8): Effect of treating hypercholesterolemic rats with desert rose seeds powder for 8 weeks on organs weight.

Organ weight (g)	Treatments Statistics	G1 -ve Control	G2 +ve Control	G3 Desert rose seeds
Heart	Mean±SE	0.58±.06	0.63±0.07	0.68±0.06
	T-test		-0.50 (NS)	-0.56 (NS)
Liver	Mean±SE	7.48±0.26	8.58±0.33	6.95±0.21
	T-test		-2.54*	5.55***
Right kidney	Mean±SE	0.61±0.05	0.71±0.01	0.65±0.04
	T-test		-1.93 (NS)	1.58 (NS)
Left kidney	Mean±SE	0.70±0.08	0.68±0.01	0.63±0.03
	T-test		0.18 (NS)	1.16 (NS)
Right testis	Mean±SE	1.13±0.07	1.36±0.04	1.16±0.05
	T-test		-2.44 (NS)	3.87 (NS)
Left testis	Mean±SE	1.16±0.05	1.30±0.03	1.15±0.07
	T-test		-1.86 (NS)	0.66 (NS)

Significant differences with controls calculated by paired sample t-test; NS: Non-significant, *P<0.05

Table (9): Effect of treating hypercholesterolemic rats with desert rose seeds powder for 8 weeks on relative organ weight.

Relative organ weight %	Statistics	G1 -ve Control	G2 +ve Control	G3 Desert rose seeds
Heart %	Mean±SE	0.24±0.02	0.27±0.02	0.31±0.03
	T-test		-0.63 (NS)	-1.04 (NS)
Liver %	Mean±SE	3.38±0.15	3.49±0.12	3.22±0.15
	T-test		-0.49 (NS)	1.09 (NS)
Right kidney %	Mean±SE	0.26±0.02	0.29±0.00	0.30±0.02
	T-test		-0.96 (NS)	-0.17 (NS)
Left kidney %	Mean±SE	0.29±0.04	0.27±0.00	0.29±0.02
	T-test		0.28 (NS)	-0.53 (NS)
Right testis %	Mean±SE	0.48±0.04	0.57±0.02	0.53±0.04
	T-test		-1.70 (NS)	0.78 (NS)
Left testis %	Mean±SE	0.48±0.04	0.53±0.01	0.53±0.04
	T-test		-0.97 (NS)	0.00 (NS)

Significant differences with controls calculated by paired sample t-test; NS: Non-significant.

Table (10): Effect of treating hypercholesterolemic rats with desert rose seeds powder for 8 weeks on daily food intake (FI) body weight gain (BWG) and food efficiency ratio (FER).

Parameter	Statistics	G1 -ve Control	G2 +ve Control	G3 Desert rose seeds
FI g/day	Mean±SE	18.76±0.16	18.63±0.18	18.60±0.16
	T-test		0.74 (NS)	0.19(NS)
BWG g/8 week	Mean±SE	28.00±4.12	29.83±2.66	31.66±5.45
	T-test		-0.29 (NS)	-0.29(NS)
BWG %	Mean±SE	14.34±2.06	14.45±1.44	17.16±3.53
	T-test		-0.03 (NS)	-0.71(NS)
FER g/day	Mean±SE	0.024±0.003	0.026±0.002	0.028±0.004
	T-test		-0.42 (NS)	-0.29(NS)
FER %	Mean±SE	2.40±0.36	2.63±0.23	2.80±0.48
	T-test		-0.42 (NS)	-0.29(NS)

Significant differences with controls calculated by paired sample t-test; NS: Non-significant.

The histopathology of liver, heart, kidney and testis

Histopathology of liver

Figure (1) shows the histopathology of liver tissues of normal, hypercholesterolemic and desert rose seeds treated rats for 8 weeks. Histology of liver of the negative control group fed basal diet shows liver tissue with preserved normal architecture, portal vein and hepatic artery (Figure 1A). Liver of rat from the positive control group fed fat rich diet with 2% cholesterol for 8 weeks shows disturbed liver architectures with swollen hepatocytes with moderate cytoplasmic vacuolization and sinusoidal congestion as shown in Figure (1B). Liver of a rat from the group that fed 2% cholesterol diet and treated with desert rose seeds for 8 weeks shows restored normal liver tissues as shown in Figure (1C).

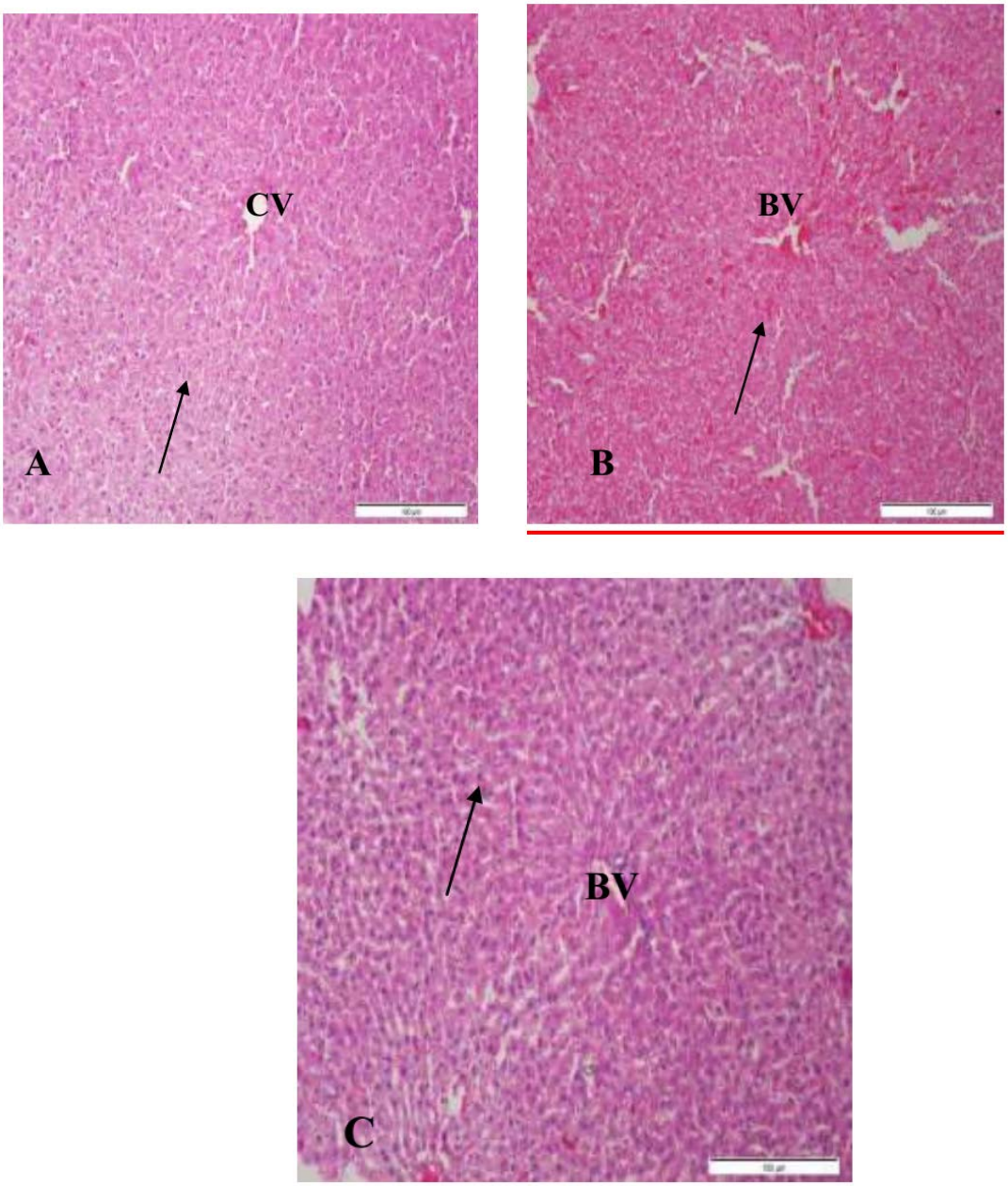

Figure (1): Histopathology of liver A; Liver of normal rats of the negative control group fed basal diet. B; Liver of a rat from the positive control group fed 2% cholesterol for 8 weeks. C; Liver of a rat from the third group thatfed 2% cholesteroldiet and cotreatedwithdesert rose seeds for 8 weeks. Arrow: hepaticcells, CV: central vein, BV: bloodvessel. (h &e . x 100).

Histopathology of the heart

The cardiac tissues of the negative control group that was fed basal fat rich diet for 8 weeks reveals normal cardiac tissues with no histopathological changes Figure (2A). In the positive control group that was fed fat rich diet with 2% cholesterol for 8 weeks, cardiac tissues show moderate myocardial atrophy and interstitial edema Figure (2B). The desert rose seed treated group that was fed 2% cholesterol diet for 8 weeks shows nearly normal myocardial muscles with no histopathological changes (Figure 2C).

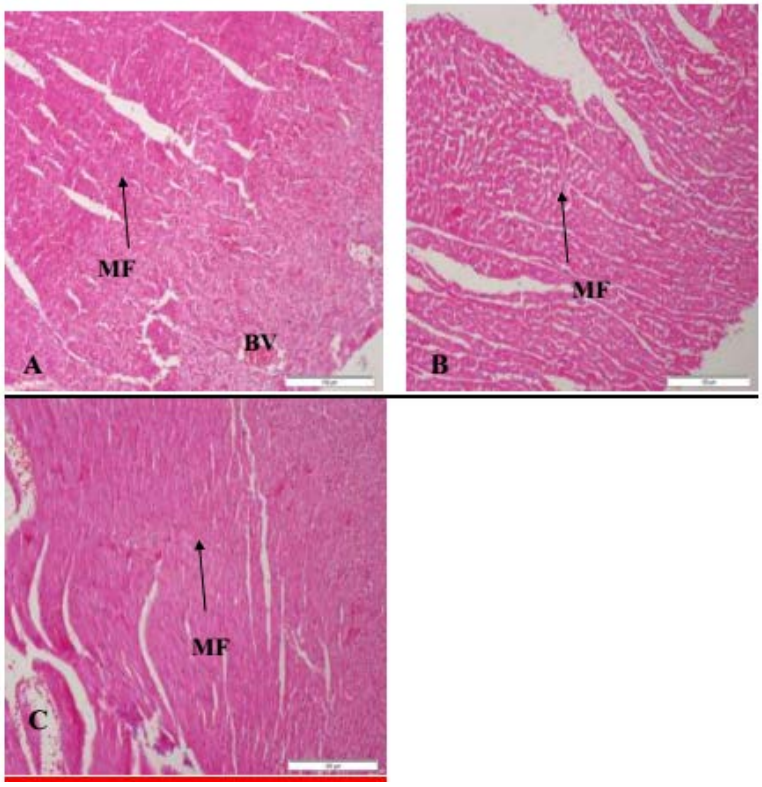

Figure (2): Histopathology of heart. A; Normal heart of a rat from negative control group fed basal diet for 8 weeks. B; Heart of a rat from the control positive group fed fat rich diet with 2% cholesterol for 8 weeks. C; Heart of a rat from the third group that fed 2% cholesterol diet and treated with desert rose seeds for 8 weeks. BV: blood vessel, MF: muscle fibers. (h & e x 100).

Histopathology of the kidney

Histopathology of kidney of the negative control group that was fed basal diet reveals the normal histological structure of renal parenchyma, blood vessels and interstitium with no histopathological changes, normal glomeruli with normal structure and pattern, normal renal tubuli in living epithelium and normal interstitial tissue with normal in composition and normal blood vessels as shown in Figure (3A). The kidney of the positive control group that was fed fat rich diet with 2% cholesterol for 8 weeks shows shrunkage of glomerular tuft, congestion of glomerular capillaries, mild tubular atrophy and interstitial edema with formation of scattered intratubular granules as shown in Figure (3B). Kidney of the third group that fed 2% cholesterol diet and cotreated with desert rose seeds for 8 weeks shows restored kidney tissue with normal architecture and normal glomeruli with normal structure and pattern and no histopathological changes were noticed (Figure 3C).

Histopathology of testis

The testicular tissues of the negative control group are shown in Figure (4A), revealing normal seminiferous tubules with no histopathological changes. The testicular tissues of the positive control group (Figure 4B) that was fed on fat rich diet with 2% cholesterol for 8 weeks reveals abnormal seminiferous tubules and distorted testicular tissue.

Whereas the testicular tissues of the desert rose treated group that was fed 2% cholesterol diet for 8 weeks (Figure 4C) reveals nearly normal tissues with no histopathological changes, normal seminiferous tubules, spermatocytes.

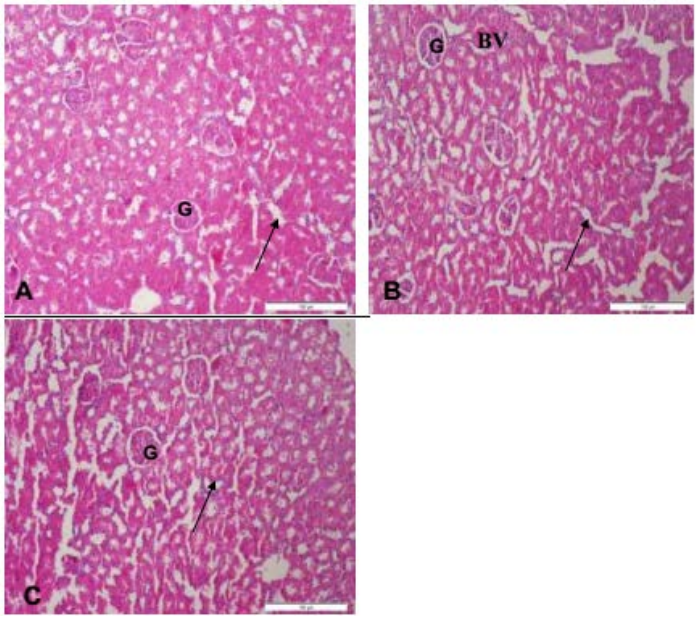

Figure (3): Histopathology of Kidney A; Normal kidney of the negative control group fed basal diet. B; kidney of the control positive group fed fat rich diet with 2% cholesterol for 8 weeks. C; kidney of a rat fed 2% cholesterol diet and cotreated with desert rose seeds for 8 weeks. G: glomeruli, arrows: collecting tubules, BV: blood vessel. (h&e x 100).

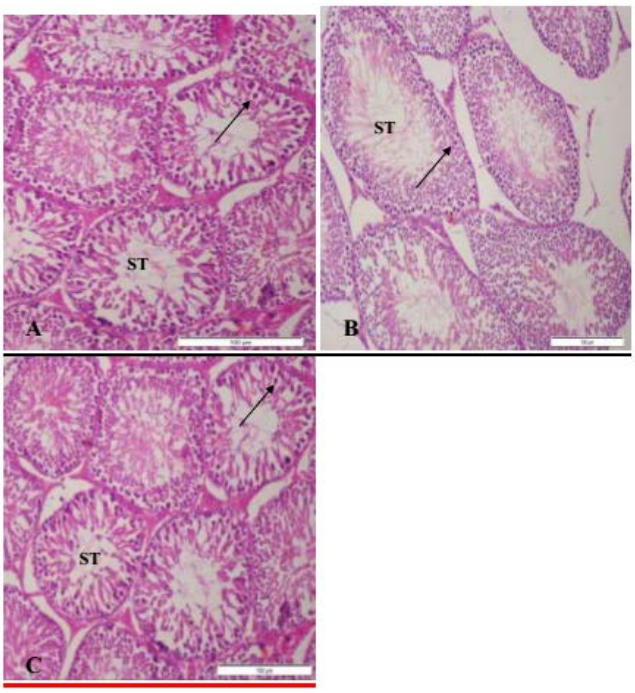

Figure (4): Histology of testes. A; Testis of of the negative control group fed basal. B; Testis of the positive control group fed fat rich diet with 2% cholesterol for 8 weeks. C; Testis of a rat from the third group that fed 2% cholesterol diet and cotreated with desert rose seeds for 8 weeks. Spermatocytes (arrow), ST: seminiferous tubules. (h& e x 100).

DISCUSSION

Hypercholesterolemia occurs in conjunction with other metabolic risk factors such as diabetes, metabolic syndromes, glucose intolerance, obesity, and oxidation of low-density lipoproteins that leads to a change in the lipoprotein conformation [13]. This study focused on testing the efficiency of desert rose seeds as a hypolipidemic and as an antioxidant and reducing lipid peroxidation in male albino rats.

In a recent study, desert rose leaves supplementation ameliorated antioxidant enzymes activity, lipid profile and reduced lipid peroxidation in male albino rats [4,5].

Feeding rats on 2% cholesterol in the diet for 8 weeks increased the serum total cholesterol and induced hypercholesterolemia in the positive control group [4,5,14,17]. Treating these hypercholesterolemic rats with desert rose seeds (100 mg/kg body weight in the diet for 8 weeks) has significantly improved the serum lipid profile parameters. It decreased the TC, TG, LDL and VLDL and increased the HDL levels. This result is in concordant with that of Al-Sieni [4].

Furthermore, liver function parameters (ALT, AST and ALP) were also significantly increased under induced hyper lipidemic condition in male rats. The current result is consistent with that of Mahfouz and Kummerow [18] and El Rabey et al. [17]. In addition, treating hypercholesterolemic rats with desert rose seeds has greatly decreased the liver function enzymes under study [4,5].

Uric acid was not affected with hypercholesterolemia, whereas creatinine and urea were slightly increased in the positive control group. This result is consistent with other studies demonstrated a relationship between kidney disease and increased cholesterol in the diet [4,5,17,19,20]. On the other hand, treating hypercholesterolemic rats with desert rose seeds for 8 weeks greatly improved all kidney function parameters [4,5].

In the current study, the activity of three antioxidants enzymes; glutathione reduced, serum glutathione reductase and serum superoxide dismutase was estimated in the serum, and kidney and liver homogenate. The activity of the three enzymes (GR, GSH and SOD) were significantly decreased as a result of cholesterol supplementation for 8 weeks in the positive control group, in both serum and liver homogenate, while non significantly affected in the kidney homogenate. This result is consistent with the kidney function parameters that were not highly affected with hypercholesterolemia and with previous studies [4,5,17,21].

Lipid peroxide (MDA) was significantly increased as a result of induced hypercholesterolemia. This result is consistent with previous studies [17,21]. Treating hypercholesterolemic rats with desert rose seeds has significantly reduced the lipid peroxide and increased the antioxidant enzymes [4,5,11]. This means that the desert rose seeds powder has a powerful antioxidant effect resisting the oxidant systems of free radicals, molecules or molecular fragments containing one or more unpaired electron [9]. Oxidative stress resulted by the reactive oxygen species (ROS) and the free radicals cause several diseases such as cancer, neurodegenerative diseases, cardiovascular and ageing [22].

Water consumption and food intake were not affected by hypercholesterolemia or treating with desert rose seed powder, whereas body weight was significantly increased by hypercholesterolemia and decreased by desert seed treatment for 8 weeks.

Organ weight (liver, heart, right kidney, left kidney, right testis and left testis) and relative organ weight were almost non significantly affected either by cholesterol supplementation or desert rose seeds treatment. Similarly, the daily food intake (FI), body weight gain (BWG) and food efficiency ratio (FER) were non significantly affected either by hypercholesterolemic or desert rose treatment.

The histopathological investigations of liver and kidney showed histopathological alteration as a result of the 2% cholesterol supplementation in the positive control group. This result is consistent with other studies showed a correlation between hypercholesterolemia and pathological changes in the organs under study [4,5,17,23,24], whereas an improvement in microscopic examination of tissues of the desert rose seeds fed group was scored [4,5].

CONCLUSIONS

Desert rose seeds succeeded in treating hyperlipidemia induced by cholesterol feeding for 8 weeks in male rats. The use of desert rose seeds improved lipid profile, increased antioxidant activity, decreased lipid peroxidation, improved liver and kidney function, and restored tissues of the target organs to their normal structure.

Abbreviations:ALT: Serum alanine aminotransferase; AST: serum aspartate transaminase; ALP: serum alkaline phosphatase ; CHD: Coronary heart diseases, G: Glomerulus; G1: Fed normal diet; G2: Fed 2.0% w/w cholesterol in the diet; G3: Fed 2.0% w/w cholesterol and 100 mg/kg body weight desert rose seeds powder, GR: Glutathione reductase; GSH: Glutathione reduced ; H&S: Hematoxylin and eosin; LDL: Low density lipoproteins; LDL-C: Low density lipoproteins cholesterol; s.LDL-C: Serum low density lipoproteins cholesterol; s.TC: Serum total cholesterol; s.TG: Serum triglyceride; s.VLDL-C: Serum very low density lipoprotein-cholesterol; SE: Standard errors; SOD: Super oxide dismutase; SPSS: Statistical Program for Sociology Scientists; TC: Total cholesterol.

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