The Antioxidant Activity of Desert Rose (*Adenium arabicum*) Leaves On Hyper Cholesterolemic Male Rats

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Abstract: The leaf powder of desert rose (*Adenium arabicum*) was supplemented orally in the diet to hypercholesterolemic male Albino rats for 8 weeks to test its antioxidant activity and its effect on lipid peroxidation. Eighteen male rats weighing 180-200 gm were divided into three groups. The first group is untreated control group fed normal diet, the second group was fed 2% cholesterol in diet to induce hypercholesterolemia (positive control group), and the third group was fed 2% cholesterol (to induce hypercholesterolemia) and treated with 500 mg/kg body weight rose leaf powder for 8 weeks. The positive control group showed a significant increase in lipid peroxide and a significant decrease in antioxidant enzymes; glutathione reduced (GSH), serum glutathione reductase (GR) and serum superoxide dismutase (SOD) activity in the serum and tissue homogenate. Moreover, histopathology of liver and kidney in the positive control group showed histopathological changes compared with the negative control ones. Treating the hypercholesterlemic rats with desert rose leaves increased the antioxidant enzyme activity and decreased lipid peroxide. In addition, the histopathology of the studied organs was also recovered and seemed normal after desert rose treatment. In conclusion, desert rose leaves have an important anti-oxidant activity, decreased lipid peroxidation and improved the tissues of the kidney and liver of hypercholesterolemic rats under study.

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1. Introduction

Adenium arabicum Forssk. (desert rose, giant desert rose, impala lily, Adnah) belongs to the family *Apocyanaceae. Adenium arabicum*; is an endemic, rare, on the mountains of southern region of Saudi Arabia (Rahman et al., 2004). It is poisonous and toxic and has some medicinal properties; the sap and bark are used in bones dislocations, painful joints, wounds and skin infections (Mossa et al., 1987 and Shahina, 1994).

The increase of lipid parameters has been shown to be a strong risk factor for coronary heart diseases in many populations (Makni et al., 2008). In addition, free radicals induced oxidation of lipids is controlled by a wide spectrum of enzymatic antioxidants and non-enzymatic antioxidants such as superoxide dismutase (SOD), glutathione reductase, and glutathione peroxides (GSHPx), vitamin E and (Valko et al., 2007). In addition, some non-enzymatic antioxidants such as vitamins C, vitamin E, carotenoids and phenolic compounds may be important in the pathogenesis of oxidative stress related disorders (Southom and Powis, 1988 and Valko et al., 2007).

It was also found that nitric oxide (NO) is a crucial modulator of vascular damage (Rathod et al., 2011). Napoli *et al.* (2001) reported that NO has intracellular effects that lead to vasorelaxation, endothelial regeneration, reduction of oxidative mechanism, inhibit leukocyte chemotaxis and platelet adhesion. In a study of the antioxidant activity of *Adenium obesum* flowers crude extract, Palé et al. (2004) reported that the antioxidative activities of the crude extract was similar to that of ascorbic acid used as reference.

The objective of the current study was testing the efficiency of desert rose leaves as an antioxidant and lowering lipid peroxidation in hypercholesterolemic in serum and tissue homogenate of liver and kidney in male wistar albino rats.

2. Materials and Methods

The desert rose leaves

The desert rose leaves were collected from a local desert rose tree identified as *Adenium arabicum* Forssk. These leaves were washed, air dried, milled by mixer and then mixed to the diet in a ratio of 500 mg/kg body weight.

Basal lipid rich diet: The basal diet consisted of the following: 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 conditions

Eighteen male albino wister rats "*Rattas rattas*" weighing 180-200g were obtained from Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia. Rats were housed six per polycarbonate cage. 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 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 then divided randomly into three groups as follows: first group is untreated control group, fed normal diet, the second group, fed 2% cholesterol (Onody et al., 2003) in diet to induce hypercholesterolemia (positive control group), and the third group fed 2% cholesterol (to induce hypercholesterolemia) and treated with 500 mg/kg body weight rose leaf powder for 8 weeks.

The period of the current experiment was 8 weeks because as an adequate period to induce hypercholesterolemia (Jain et al., 1997 and Ahmed, 2001). At the end of the experiment, animals 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 samples were 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

At the end of the experiment and after the collection of blood, anaesthetized animals were scarified by cervical dislocation. The abdomen was dissected and the organs were rapidly excised and weighed. The left kidney and the liver were saved in ice-cold for antioxidant enzymes and lipid peroxide estimation in tissue homogenate.

Preparation of kidney and liver tissue homogenate

A piece of the kidney or liver tissue was dissected out, rinsed with 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 homogenate was centrifuged at 13,000×g to remove the debris, and then the supernatant was used for estimation of the antioxidant enzymes and lipid peroxide.

Antioxidants enzymes estimation

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

Lipid peroxide:

Malondialdehyde (MDA) was estimated in serum and in liver and kidney homogenate using Biodiagnostic Chemical Company kit (Egypt) according to the instructions of the suppliers.

Biological evaluation

The following biological parameters were estimated:

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

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

iii- Organ weight and relative organ weight: liver, right kidney and left kidney 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.

iv- Food Intake: daily food intake was calculated.

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

Histopathological investigations

The liver and the right kidney were washed in sterile saline and fixed in 10% neutral formalin for histopathological studies. These organs were then dehydrated in gradual ethanol (50-99%), cleared in xylene, and embedded in paraffin. Sections were prepared and then stained with hematoxylin and eosin (H&S) 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

1. Antioxidants enzymes in serum and kidney and liver homogenate

As shown in Table (1), the effect of desert rose leaves treatment on antioxidants enzymes in hypercholesterolemic rats for 8 weeks. The mean value of glutathione reduced (GSH) in plasma of the positive control group was high significantly (at $P<0.01$) lower than that of the negative control group (15.69±0.14, and 16.73±0.18 U/ml, respectively), and the mean value of desert rose leaves treated group was high significantly (at $P<0.01$) higher than that of the positive control group (16.59±0.25 and 15.69±0.14 U/ml, respectively). The mean value of serum glutathione reductase (GR) in the positive control group was very high significantly (at $P<0.001$) lower than that of the negative control (2.83±0.14, and 8.52±0.28 U/ml, respectively), and the mean GR value of desert rose leaves treated group was very high significantly (at $P<0.001$) higher than that of the positive control (7.66±0.22 and 2.83±0.14 U/ml, respectively). The mean value of serum superoxide dismutase (SOD) in the positive control group was very high significantly (at $P<0.001$) lower than that of the negative control (437.98±12.99 and 606.85±12.13

U/ml, respectively), and the mean value of SOD in the desert rose leaves treated group was very high significantly (at P<0.001) higher than the positive control (506.70±12.60 and 437.98±12.99 U/ml, respectively).

Table (1): Effect of treating hypercholesterolemic rats with desert rose leaves powder for 8 weeks on antioxidants enzymes.

Blood/Tissue	Antioxidants enzymes	Treatments / Statistics	G1 -ve Control	G2 +ve Control	G3 Desert rose leaves
Plasma	(GSH) U/ml	Mean±SE	16.73±0.18	15.69±0.14	16.59±0.25
		T. test		3.56**	-3.59**
	(GSR) U/ml	Mean±SE	8.52±0.28	2.83±0.14	7.66±0.22
		T. test		15.58***	-15.77***
	(SOD) U/ml	Mean±SE	606.85±12.13	437.98±12.99	506.70±12.60
		T. test		7.71***	-4.54***
Liver	(GSH) U/g. tissue	Mean±SE	17.82±0.19	9.21±0.21	11.93±0.21
		T. test		30.21***	-6.50***
	(GR) U/g. tissue	Mean±SE	16.76±0.49	3.35±0.22	6.49±0.44
		T. test		27.23***	-6.83***
	(SOD) U/g. tissue	Mean±SE	823.28±6.82	433.34±11.61	637.73±8.14
		T. test		34.27***	-13.69***
Kidney	(GSH) U/g. tissue	Mean±SE	10.96±0.25	11.02±0.17	10.93±0.21
		T. test		-0.149 (NS)	0.37 (NS)
	(GR) U/g. tissue	Mean±SE	6.26±0.20	5.98±0.17	6.30±0.21
		T. test		0.82 (NS)	-1.01 (NS)
	(SOD) U/g. tissue	Mean±SE	666.07±11.75	654.09±9.27	654.03±9.46
		T. test		0.76 (NS)	0.00 (NS)

Significant differences with controls calculated by paired sample t-test; NS: Nonsignificant, **P<0.01*** P<0.001.
G1: Control-ve: Normal rats fed on basal diet.
G2: Control +ve: Rats supplemented with 2% cholesterol and fed on basal diet.
G3: Rats treated with 2% cholesterol and fed on desert rose leaves.

Table (1) shows also the mean value of antioxidant enzymes in the liver tissue homogenate of rats under study. The mean value of GSH in liver tissue homogenate of the positive control was very high significantly (at P<0.001) lower than that of the negative control group (9.21±0.21 and 17.82±0.19 U/g. tissue, respectively). On the other hand, the mean value of GSH in desert rose leaves treated group was very high significantly (at P<0.001) higher than that of the positive control group (11.93±0.21 and 9.21±0.21 U/g. tissue, respectively). The mean value of GR in liver tissue homogenate of positive control group was very high significantly (at P<0.001) lower than that of the negative control group (3.35±0.22 and 16.76±0.49 U/g. tissue, respectively), and the mean value of GR in desert rose leaves treated group was very high significantly (at P<0.001) higher than that of the positive control group (6.49±0.44 and 3.35±0.22 U/g. tissue, respectively). The mean value of SOD in liver tissue homogenate of positive control was very high significantly (at P<0.001) higher than that of the negative control group (433.34±11.61 and 823.28±6.82 U/g. tissue, respectively) and the mean value of SOD in the desert rose leaves treated group was very high significantly (at P<0.001) higher than that of the positive control group (637.73±8.14 and 433.34±11.61 U/g. tissue, respectively).

The mean values of antioxidant enzymes in the kidney tissue homogenate of rats under study are also shown in Table (1). The mean value of GSH in the kidney tissue homogenate of the positive control group was non significantly higher than that of the negative control group (11.02±0.17 and 10.96±0.25U/g. tissue, respectively), and the mean value of desert rose leaves treated group was non significantly lower than that of the positive control group (10.93±0.21 and 11.02±0.17 U/g. tissue, respectively). The mean value of GR in the kidney tissue homogenate of the positive control group was non significantly higher than that of the negative control group (5.98±0.17 and 6.26±0.20 U/g. tissue, respectively), and the mean value of desert rose leaves treated group was non significantly higher than that of the positive control group (6.30±0.21 and 5.98±0.17 U/g. tissue, respectively). The mean value of SOD in the kidney tissue homogenate of the positive control group was non significantly higher than that of the negative control group (654.09±9.27 and 666.07±11.75U/g. tissue, respectively), and the mean value of desert rose leaves treated group was non significantly lower than that of the positive

control group (654.03±9.46 and 654.09±9.27 U/g. tissue, respectively).

***2.* Lipid peroxide:**

As illustrated in table Table (2), the effect of desert rose leaves treatment on malondialdehyde (MDA) in hypercholesterolemic rats for 8 weeks. The mean value of MDA in plasma of the positive control group was very high significantly (at P<0.001) higher than that of the negative control (4.79±.23 and 2.79±.07 nmol/ml, respectively), and the mean MDA value of desert rose leaves treated group was very high significant (at P<0.001) lower than that of the positive control group (3.10±0.05 and 4.79±.23 nmol/ml, respectively).

Table (2): Effect of treating hypercholesterolemic rats with desert rose leaves powder for 8 weeks on lipid peroxide.

Blood/Tissue	Oxidants	Treatments / Statistics	G1 -ve Control	G2 +ve Control	G4 Desert rose leaves
Plasma	MDA (nmol/ml)	Mean±SE	2.79±0.07	4.79±0.23	3.10±0.05
		T.test		-8.17***	7.43***
Liver	MDA (nmol/ g.tissue)	Mean±SE	5.22±0.21	10.56±0.29	7.32±0.58
		T. test		-12.89***	4.14***
Kidney	MDA (nmol/ g.tissue)	Mean±SE	3.34±0.11	3.71±0.04	3.31±0.12
		T. test		-2.64**	3.65**

Significant differences with controls calculated by paired sample t-test; **P<0.01*** P<0.001.
G1: Control-ve: Normal rats fed on basal diet.
G2: Control +ve: Rats supplemented with 2% cholesterol and fed on basal diet.
G3: Rats treated with 2% cholesterol and fed on desert rose leaves.

The mean value of MDA in the liver tissue homogenate of the positive control group was very high significantly (at P<0.001) higher than that of the negative control group (10.56±0.29 and 5.22±0.21 nmol/ g. tissue, respectively), and the mean value of desert rose leaves treated group was very high significantly (at P<0.001) lower than that of the positive control group (7.32±0.58 and 10.56±0.29 nmol/ g. tissue, respectively).

The mean value of MDA in kidney tissue homogenate of the positive control group was high significantly (at P<0. 01) higher than that of the negative control group (3.71±0.04 and 3.34±0.11nmol/ g. tissue, respectively), and the mean value of desert rose leaves treated group was high significantly (at P<0. 01) lower than that of the positive control (3.31±0.12 and 3.71±0.04 nmol/ g. tissue, respectively).

***3.* Total body weight**

Data recorded in Table (3) illustrates the effect of supplementation of desert rose leaves for 8 weeks to hyperchlesterolemic rats on total body weight. As shown, the mean value of body weight of the positive control group after the first week was high significantly (at P<0.01) higher than that of the negative control group (211.83±4.59 and 193.67±6.13 g, respectively), and the mean body weight value of desert rose leaves treated group was non significantly lower than that of the positive control group (189.67±12.21 and 211.83±4.59 g, respectively).

Table (3): Effect of treating hypercholesterolemic rats with desert rose leaves powder for 8 weeks on total body weight.

Total body weight (g)	Treatments / Statistics	G1 -ve Control	G2 +ve Control	G3 Desert rose leaves
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.
G1: Control-ve: Normal rats fed on basal diet.
G2: Control +ve: Rats supplemented with 2% cholesterol and fed on basal diet.
G3: Rats treated with 2% cholesterol and fed on desert rose leaves.

After the second week, the mean body weight value of the positive control group was also high significantly (at P<0.01) higher than that of the negative control (215.33±4.56 and 197.33±5.94 g, respectively), whereas the mean value of body weight of the desert rose leaves treated group was non significantly lower than that of the positive control group (193.33±12.08 and 215.33±4.56 g, respectively).

After the third week, the mean body weight value of the positive control group was high significantly (at P<0.01) higher than that of the negative control group (218.33±4.41 and 201.83±5.44 g, respectively), and the mean body weight value of the desert rose leaves treated group was non significantly lower than that of the positive control group (197.50±11.85 and 218.33±4.41 g, respectively).

The body weight in the fourth week of the experiment showed that, the mean value of positive control was high significantly (at P<0.01) higher than that of the negative control group (221.83±4.29 and 205.50±5.51 g, respectively), and the mean value of desert rose leaves treated group was non significantly lower than that of the positive control group (201.17±12.10 and 221.83±4.29 g, respectively).

The same Table shows also the mean value of body weight in the fifth week of the experiment, the mean value of the positive control group was significantly (at P<0.05) higher than that of the negative control group (226.00±4.35 and 210.50±5.50 g, respectively), whereas the mean value of body weight in the desert rose leaves treated group was non significantly lower than that of the positive control group (204.67±11.87 and 226.00±4.35 g, respectively).

The mean value of body weight in sixth week of the experiment of the positive control group was significantly (at P<0.05) higher than the that of the negative control (230.33±4.16 and 214.17±5.43 g, respectively), and the mean value of desert rose leaves treated group was non significantly lower than that of the positive control group (208.33±11.86, and 230.33±4.16 g respectively).

The mean value of body weight in seventh week in the positive control was significantly (at P<0.05) higher than that of the negative control group (234.00±3.88 and 218.17±5.54 g, respectively), and the mean value of desert rose leaves treated group was non significantly lower than that of the positive control group (212.33±12.06 and 234.00±3.88 g, respectively).

The mean value of body weight in eighth week of the experiment in the positive control was significantly more at (P<0.05) when compared to negative control, which were 237.8 ±4.04, and 222.33±5.78 g, respectively), whereas the mean value of desert rose leaves treated group was non significantly lower than that of the positive control group (217.00±1.19, and 237.8 ±4.04 g respectively).

4. Organ weight

Data recorded in Table (4) illustrates the effect of desert rose supplementation for 8 weeks on organ weight (liver, right kidney and left kidney) 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 (8.58±0.33 and 7.48±0.26 g, respectively), and the mean value of liver weight of the desert rose leaves treated group was non-significantly lower than that of the positive control (7.56±0.52, and 8.58±0.33 g respectively). On the other hand, the mean values of weight of other organs (right kidney and left kidney) in the positive control group were non significantly higher than the negative control, whereas the mean values of these organs in the desert rose leaves treated group were non-significantly lower than that of the positive control group.

Table (4): Effect of treating hypercholesterolemic rats with desert rose leaves powder for 8 weeks on organ weights.

Organ Weight (g)	Treatments / Statistics	G1 -ve Control	G2 +ve Control	G4 Desert rose leaves
Liver	Mean±SE	7.48±0.26	8.58±0.33	7.56±0.52
	T. test		-2.54*	1.39 (NS)
Right kidney	Mean±SE	0.61±0.05	0.71±0.01	0.68±0.04
	T. test		-1.93 (NS)	0.67 (NS)
Left kidney	Mean±SE	0.70±0.08	0.68±0.01	0.66±0.06
	T. test		0.18 (NS)	0.27 (NS)

Significant differences with controls calculated by paired sample t-test; NS: Nonsignificant,
*P<0.05
G1: Control-ve: Normal rats fed on basal diet.
G2: Control +ve: Rats supplemented with 2% cholesterol and fed on basal diet.
G3: Rats treated with 2% cholesterol and fed on desert rose leaves.

Data recorded in Table (4) illustrates the effect of desert rose supplementation for 8 weeks on organ weight (liver, right kidney and left kidney) 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 (8.58±0.33 and 7.48±0.26 g, respectively), and the mean value of liver weight of the desert rose leaves treated group was non-significantly lower than that of the positive control (7.56±0.52, and 8.58±0.33 g respectively). On the other hand, the mean values of weight of other organs (right kidney and left kidney) in the positive control group were non significantly higher than the negative control, whereas the mean values of these organs in the desert rose leaves treated group were non-significantly lower than that of the positive control group.

5. Relative organ weight

Table (5) 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.

Table (5): Effect of treating hypercholesterolemic rats with desert rose leaves powder for 8 weeks on relative organ weight.

Relative Organ Weight %	Treatments / Statistics	G1 -ve Control	G2 +ve Control	G3 Desert rose leaves
Liver %	Mean±SE	3.38±0.15	3.49±0.12	3.57±0.21
	T. test		-0.49 (NS)	-0.26 (NS)
Right kidney %	Mean±SE	0.26±0.02	0.29±0.00	0.32±0.02
	T. test		-0.96 (NS)	-1.32 (NS)
Left kidney %	Mean±SE	0.29±0.04	0.27±0.00	0.32±0.03
	T. test		0.28 (NS)	-1.28 (NS)

Significant differences with controls calculated by paired sample t-test; NS: Non significant.
G1: Control-ve: Normal rats fed on basal diet.
G2: Control +ve: Rats treated with cholesterol and fed on basal diet.
G3: Rats treated with Cholesterol and fed on desert rose leaves.

6. Water Consumption

Data recorded in Table (6) illustrates the effect of desert rose treatment on water consumed 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 were non significantly more or less than that of the negative control. So, the water consumption did not reflect any significant differences as a result of treating rats under study with desert rose leaves in this experiment.

Table (6): Effect of treating hypercholesterolemic rats with desert rose leaves powder for 8 weeks on water consumption.

water consumed ml	Treatments / Statistics	G1 -ve Control	G2 +ve Control	G3 Desert rose leaves
Before experiment	Mean±SE	27.50±1.11	28.33±1.05	28.33±1.66
	T. test		-0.54 (NS)	0.00 (NS)
1st week	Mean±SE	30.83±2.00	29.16±1.53	30.00±1.82
	T. test		0.67 (NS)	-0.41 (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	30.00±1.82
	T. test		1.00 (NS)	-0.34 (NS)
4th week	Mean±SE	29.16±2.00	30.00±1.82	30.83±2.00
	T. test		-0.27 (NS)	-0.23 (NS)
5th week	Mean±SE	23.33±1.05	27.50±2.14	32.50±1.70
	T. test		-1.53 (NS)	-1.93 (NS)
6th week	Mean±SE	27.50±1.70	27.50±1.11	29.16±2.00
	T. test		0.00 (NS)	-0.79 (NS)
7th week	Mean±SE	29.16±.83	27.50±1.70	29.16±2.00
	T. test		1.00 (NS)	-0.59 (NS)
8th week	Mean±SE	27.50±1.70	27.50±1.70	30.00±1.82
	T. test		0.00 (NS)	-1.16 (NS)

Significant differences with controls calculated by paired sample t-test; NS: Nonsignificant.
.G1: Control -ve: Normal rats fed on basal diet.
G2: Control +ve: Rats supplemented with 2% cholesterol and fed on basal diet.
G3: Rats treated with 2% cholesterol and fed on desert rose leaves.

7. Food Intake

Data recorded in Table (7) illustrates the effect of desert rose leaves treatment on food intake in hypercholesterolemic rats for 8 weeks. As shown, except for the desert rose treated group in the 1st week and the positive control group in the 7th week, that were significantly (at P<0.05) higher than that of the negative control all other mean values of food intake were non significantly more or less than the negative control group.

Table (7): Effect of treating hypercholesterolemic rats with desert rose leaves powder for 8 weeks on food intake.

Food Intake g	Treatments / Statistics	G1 -ve Control	G2 +ve Control	G3 Desert rose leaves
Before experiment	Mean±SE	17.16±0.30	17.16±0.30	17.33±0.33
	T. test		0.00 (NS)	-0.34 (NS)
1st week	Mean±SE	16.83±0.16	16.50±0.22	17.00±0.25
	T. test		1.00 (NS)	-2.23*
2nd week	Mean±SE	19.33±.42	18.66±.61	18.50±0.50
	T. test		0.93 (NS)	0.19 (NS)
3rd week	Mean±SE	18.83±0.54	18.33±0.55	17.83±0.47
	T. test		0.65 (NS)	0.80 (NS)
4th week	Mean±SE	19.33±0.42	18.66±0.61	18.50±0.50
	T. test		0.93 (NS)	0.19 (NS)
5th week	Mean±SE	19.33±0.42	19.33±0.42	19.16±0.54
	T. test		0.00 (NS)	0.20 (NS)
6th week	Mean±SE	19.00±0.44	18.83±0.54	19.16±0.54
	T. test		0.25 (NS)	-0.43 (NS)
7th week	Mean±SE	19.00±.44	19.83±.16	19.66±0.33
	T. test		*-2.07	0.41 (NS)
8th week	Mean±SE	19.50±0.34	19.66±0.33	19.66±0.33
	T. test		-0.30 (NS)	0.00 (NS)

Significant differences with controls calculated by paired sample t-test; NS: Nonsignificant
, *P<0.05.
G1: Control-ve: Normal rats fed on basal diet.
G2: Control +ve: Rats supplemented with 2% cholesterol and fed on basal diet.
G3: Rats treated with 2% cholesterol and fed on desert rose leaves.

8. Daily Food intake (FI) body weight gain (BWG) and food efficiency ratio (FER)

Data recorded in Table (8) illustrates the effect of desert rose on food intake (FI), body weight gain (BWG) and food efficiency ratio (FER) in hypercholesterolemic rats for 8 weeks. As shown, the mean value of food intake of the positive control group was non significantly lower than that of the negative control group (18.63±0.18 and 18.76±0.16 g/day, respectively), and the mean value of desert rose leaves treated group was non significantly lower than that of the positive control (18.60±0.17 and 18.63±0.18 g/day, respectively).

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

Biological evaluation parameters	Treatments / Statistics	G1 -ve Control	G2 +ve Control	G3 Desert rose leaves
FI g/day	Mean±SE	18.76±0.16	18.63±0.18	18.60±0.17
	T. test		0.74 (NS)	0.16 (NS)
BWG g/8 week	Mean±SE	28.00±4.12	29.83±2.66	33.50±3.23
	T. test		-0.29 (NS)	-0.77 (NS)
BWG %	Mean±SE	14.34±2.06	14.45±1.44	18.20±1.99
	T. test		-0.03 (NS)	-1.35 (NS)
FER g/day	Mean±SE	0.024±0.003	0.026±0.002	0.029±0.002
	T. test		-0.42 (NS)	-0.75 (NS)
FER %	Mean±SE	2.40±0.36	2.63±0.23	2.95±0.28
	T. test		-0.42 (NS)	-0.75 (NS)

Significant differences with controls calculated by paired sample t-test; NS: Non significant.
G1: Control-ve: Normal rats fed on basal diet.
G2: Control +ve: Rats supplemented with 2% cholesterol and fed on basal diet.
G3: Rats supplemented with Cholesterol and treated with desert rose leaves.

Regarding body weight gain, the mean value of positive control was higher than that of the negative control (29.83±2.66, and 28.00±4.12 g/8 week, respectively), and the mean value of body weight gain in the desert rose leaves treated group was also non significantly higher than that of the positive control group (33.50±3.23 and 29.83±2.66 g/8 week, respectively).

On the other hand, the mean value of body weight gain % of positive control was non significantly higher than that of the negative control group (14.45±1.44, and 14.34±2.06 % respectively), and also the mean value of the desert rose leaves treated group was non significantly higher than that of the positive control group (18.20±1.99 and 14.45±1.44 %, respectively). The mean value of food efficiency ratio in the positive control group was non significantly higher than that of the negative control group (0.026±0.002 and 0.024±0.003 g/day, respectively), and the mean value of desert rose leaves treated group was non significantly higher than that of the positive control group (0.029±0.002 and 0.026±0.002 g/day, respectively). Consequently, the mean value of food efficiency ratio % the mean value of positive control was non significantly higher than negative control (2.63±0.23, and 2.40±0.36 % respectively), and the mean value of desert rose leaves treated group was non significantly higher than that of the positive control group (2.95±0.28 and 2.63±0.23 %, respectively).

9 Histopathological investigations

9.1 Histopathology of liver

Histopathology of liver of the normal rats from the negative control group fed basal diet for 8 weeks shows liver tissue with preserved normal architecture, the portal tracts was composed of normal bile duct, portal vein and hepatic artery. The liver cells arranged in single plate with eosinophilic cytoplasm, central nuclear and normal central veins as shown in Figure (1, A). 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 sinosiodal congestion as shown in Figure (1 B). Liver of a rat from the group that fed 2% cholesterol diet and treated with desert rose leaves for 8 weeks shows nearly normal liver with very mild hydrophic regeneration of hepatocytes as shown in Figure (1, C).

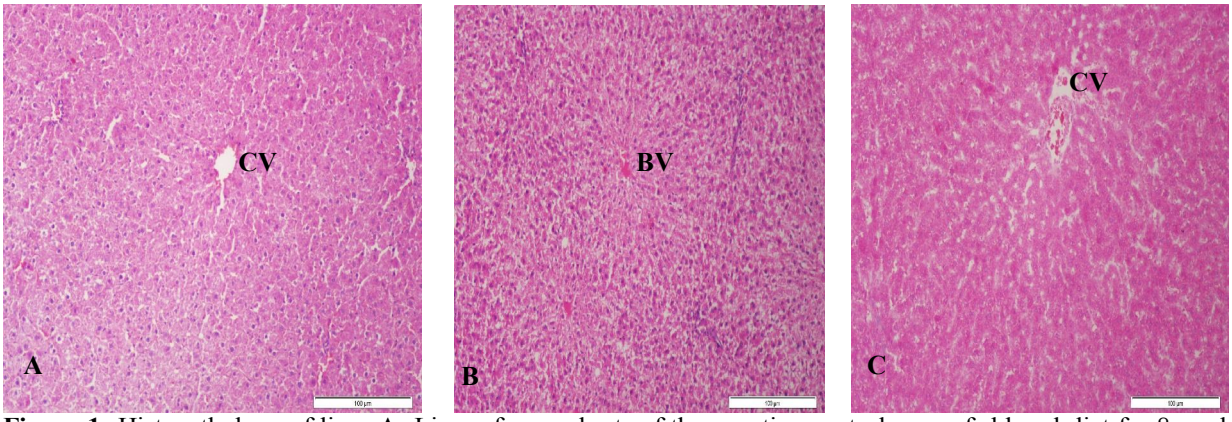

Figure 1: Histopathology of liver **A;** Liver of normal rats of the negative control group fed basal diet for 8 weeks. **B;** Liver of a rat from the positive control group fed 2% cholesterol for 8 weeks. **C;** Liver of a rat from the third group that fed 2% cholesterol diet and treated with desert rose leaves for 8 weeks. CV: central vein, BV: blood vessel. (h & e . x 100).

9.2 Histopathology of Kidney

Histopathology of Kidney of the negative control group negative control group fed basal diet for 8 weeks 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 (2, A). kidney of rat from the positive control group fed fat rich diet with 2% cholesterol for 8 weeks shows shrunkage of glomerular tuft, congestion of glomerular capillaries, mild tubular atrophy and intershilial edema with formation of scattered intratubular granules as shown in Figure (2, B). Kidney of rat from the third group that fed 2% cholesterol diet and treated with desert rose leaves for 8 weeks shows recovered kidney tissue with normal architecture and normal glomeruli with normal structure and pattern and no histopathological changes were noticed (Figure 2, C).

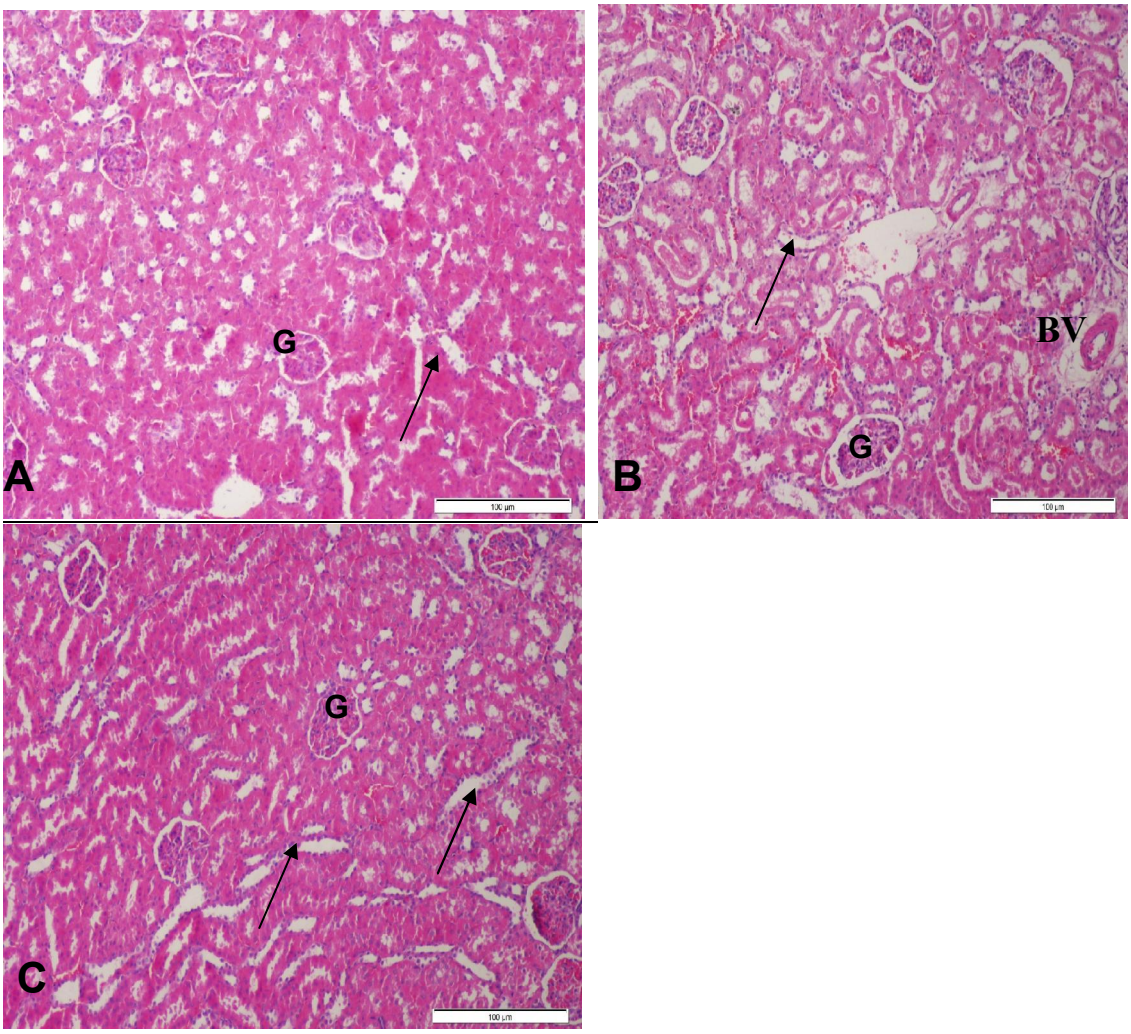

Figure 2: Histopathology of Kidney **A;** Normal kidney of a rat from negative control group fed basal diet for 8 weeks. **B;** kidney of a rat from the control positive group fed fat rich diet with 2% cholesterol for 8 weeks. **C;** kidney of a rat fed 2% cholesterol diet and treated with desert rose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

Discussion

The current study describes a new *in vivo* investigation to test the effect of desert rose leaves (*Adenium arabicum* Forssk.) supplementation on antioxidant enzymes activity and lipid peroxide in male wistar albino rats. No previous studies were recorded on this plant material in treatments of diseases except some local people who use desert rose seeds as cardiac drug with sever side effect.

Oxidative stress produced by free radicals has been linked to the development of several diseases such as cardiovascular, cancer and neurodegenerative diseases and also with ageing (Witzum, 1994). In the current study, antioxidants enzymes; glutathione reduced, serum glutathione reductase and serum superoxide dismutase were significantly decreased as a result of cholesterol supplementation (2% in the diet for 8 weeks) in the positive control group, whereas lipid peroxide (MDA) was significantly increased. This result is consistent with (Basha and Sujitha, 2011 and El Rabey et al., 2013). Treating hypercholesterolemic rats with desert rose leaves has significantly reduced the lipid profile and increased the antioxidant enzymes (Palé et al., 2004). This means that the desert rose leaves powder has a powerful antioxidant effect resisting the oxidant systems of free radicals, molecules or molecular fragments containing one or more unpaired electron (Valko *et al.,* 2007).

Biological parameters; total body weight, water consumption, organ weight (liver and right kidney and left kidney) and relative organ weight, food intake and daily body weight gain (BWG), food efficiency ratio (FER) and percentage of food

efficiency ratio (FER%) were not affected either by cholesterol supplementation or desert rose leaves treatment.

The histopathological investigations of liver and kidney showed histopathological alteration as a result of 2% cholesterol supplementation in the positive control group. This result is consistent with other studies showed a correlation between hypercholesterolemia and histological changes in these organs under study (Altunkaynak et al., 2008, Ouvrier et al., 2011 and El Rabey et al., 2013), whereas an improvement in microscopic examination of tissues of the desert rose leaves fed group.

Conclusion:

The current study presented the role of desert rose leave supplementation in treating hyper oxidative stress, as an antioxidant, improving lipid profile in hypercholesterolemic rats and improving tissues of liver and kidney as a result of induced hypercholesterolemia in male wistar albino rats.

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