

## **Assessment of the antioxidant activity of parsley and carob in hypercholesterolemic male rats.**

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### **Abstract**

**Herbal antioxidants have been successfully used to reduce oxidative stress and treat many diseases such as diabetes, obesity and coronary heart diseases. The antioxidant activity of the methanolic extract of carob legume and parsley seed was evaluated in hypercholesterolemic male rats in a study conducted for 8 w. 24 male rats were randomly divided into four 6-rat groups. The 1<sup>st</sup> group (G1) was fed fat rich diet, the 2<sup>nd</sup> group (G2) was the hypercholesterolemic positive group fed 2% cholesterol in the fat rich diet, and the 3<sup>rd</sup> (G3) and the 4<sup>th</sup> (G4) groups were supplemented with 2% cholesterol in the fat rich diet as in G2 and co-treated with 20 mg/Kg bw parsley seeds methanol extract and carob legume methanol extract, respectively. The G2 hypercholesterolemic rats showed significant increase in serum lipid peroxidation and kidney function, and decrease in antioxidants. While the methanolic extract of parsley and carob extract in G3 and G4, respectively significantly decreased lipid peroxidation and kidney functions, and increased antioxidants. In conclusion, the methanolic extracts of both parsley and carob have an antioxidant activity and a protective effect against oxidative stress. The methanolic extract of parsley seed appeared more efficient than that of carob legumes.**

**Keywords:** Hypercholesterolemia, Antioxidant, ROS, Rat, Carob, Parsley.

### **Abbreviations**

ANOVA: Analysis of Variance; BW: Body Weight; CAT: Catalase; G1: Negative control group; G2: Positive control group supplemented with 2% cholesterol in the fat rich diet; G3: Hypercholesterolemic rats supplemented with 2% cholesterol and treated with 20 mg/Kg bw of parsley seeds methanolic extract; G4: Hypercholesterolemic rats

supplemented with 2% cholesterol and treated with 20 mg/Kg bw of carob legume methanolic extract; GR: Glutathione Reductase activity; GSH: Glutathione Reduced; HDLC: High Density Lipoproteins Cholesterol; LDLc: Low Density Lipoprotein Cholesterol; MDA: Malondialdehyde; SOD: Superoxide Dismutase; TG: Triglyceride; VLDLC: Very Low Density Lipoproteins Cholesterol.

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### **Introduction**

The high level of serum low-density lipoprotein (LDL) and the low level of high density lipoprotein (HDL) are markers for coronary heart diseases [1-3]. In addition, the high fat intake of saturated fatty acids leads to a higher incidence of atherosclerosis and coronary heart disease (CHD) [4].

Alteration in oxidative stress induced by reactive oxygen species (ROS) and impairments of the antioxidant system contributes to the incidence of hypercholesterolemia and subsequent cardiovascular diseases [5]. Free radicals (Reactive

Oxygen Species (ROS)) are products of normal cellular metabolism, and are extremely reactive and potentially damaging transient chemical species [2]. Vaughan et al. [4] and Custodio et al. [6] stated that the process of atherosclerosis begins with the oxidation of low-density lipoprotein cholesterol by free radicals; this is then taken into the lining of the arteries by scavenger cells which form lipid-loaded cells called 'foam cells' that accumulate cholesterol and form fatty streaks, narrowing the arteries. The second reason is primary lipotoxicity caused by oxidative stress from increased lipid

peroxidation, highly reactive oxygen species production within hepatocytes, mitochondrial dysfunction, and inflammation [7].

Antioxidants are *in vivo* defense systems, they reduce the oxidative stress by stopping the formation of free radicals, inhibit the formation of reactive oxygen species and reduce hydro peroxides, hydrogen peroxide, singlet oxygen and quenching superoxide formation [8]. The intake of food containing antioxidant compounds protects the low and the very low-density lipoproteins from oxidation and reduces lipid levels in plasma, and thus may reduce the risk of cardiovascular disease [9]. Lipid profile can be ameliorated by adjustment of diet through minimizing consumption of cholesterol and saturated fatty acids, and increasing physical activity [10].

The seeds and taproots of parsley (*Petroselinum crispum*) possess many therapeutically important constituents such as anti-oxidative, menorrhagic, antimicrobial, anticoagulant, antianemic, antihyperlipidemic, diuretic effects, antihypertensive antihepatotoxic, hypoglycaemic, hypouricemic and estrogenic activities [11]. Furthermore, the major constituents are coumarins, ascorbic acid, flavonoids, phenyl propanoids, phthalides, various terpenoid compounds, carotenoids and tocopherol [12]. Furanocoumarins (psoralen, bergapten, isoimperatorin, oxypeucedanin, xanthoxin, trioxalen and angelicin) are also important constituents of parsley. On the other hand, the essential oil (apiol and myristicin) of parsley is responsible for its toxicity [12].

Carob is the fruit of an evergreen (*Ceratonia siliqua* L.) is a rich source of minerals, such as potassium, calcium, magnesium, sodium, copper, iron, manganese and zinc [13]. Seven amino acids were extracted from carob pods (alanine, glycine, leucine, proline, and valine, tyrosine and phenylalanine). Also, carob contains insoluble dietary fibers such as cellulose, hemicelluloses and lignin, as well as water-insoluble polyphenols [14]. The aqueous extract of carob pods decreases lipid peroxidation in the cerebrum and myocardia as well as the level of hydrogen peroxide in liver, brain and kidney, and cannot decrease it in heart [15].

In this study, the antioxidant activity of parsley seed and carob legume methanol extracts was evaluated in hypercholesterolemic male rats.

## Materials and Methods

### Tested plant materials

The seeds of parsley seeds and the legumes of carob were purchased from a herbal medicine shop in Jeddah (Saudi Arabia).

### Composition of the fat rich diet

The diet consisted of 64.6% corn starch, 16% casein, 10% corn oil, 4% N.N cellulose, 4% salt mixture, 1% vitamin mixture, 0.2% choline chloride and 0.2% DL methionine.

## Animals

Twenty four (150-200 g) male rats (*Rattus norvegicus*) of East China origin weighing were purchased from Faculty of Pharmacy, King Abdulaziz University, and Jeddah, Saudi Arabia. The experiments were performed under approved protocol No. 319-34 (The Institutional Animal House of the University of King Abdulaziz at Jeddah, Saudi Arabia).

### Housing condition

The rats were housed six per polycarbonate cage. Cages, bedding and glass water bottles were replaced twice per week. The rats were kept under observation for two weeks before the beginning of the experiment to exclude any undercurrent infection.

### Preparation of the methanolic extract

The methanolic extract of dry parsley seed powder and carob legumes powder was prepared by soaking 200 g of each in 1 l of 90% methyl alcohol under shaking for 5 d and then kept in a refrigerator. A rotatory evaporator apparatus attached to a vacuum pump was used to evaporate methanol. Two ml of suspending agent tween 80 was added to 20 g of the methyl extract, and then suspended in 100 ml distilled water to prepare a 20% methanolic extract [16].

### Design of the experiment

The 24 rats were divided randomly into four groups (n=6 rats) as follows: the 1<sup>st</sup> group (G1) was the negative control group fed fat rich diet, the 2<sup>nd</sup> group (G2) was the hypercholesterolemic positive control fed 2% cholesterol in the fat rich diet for 8 w [17]. The 3<sup>rd</sup> and the 4<sup>th</sup> groups (G3 and G4) fed 2% cholesterol as in G2 and co-treated with 20 mg/Kg bw parsley seeds and carob legumes methanolic extracts using stomach tube, respectively.

### Blood collection

For biochemical analysis, at the end of the experiment, blood was collected from fasted animals (14-16 h after their last feeding) from the heart of dimethyl-ether pre-anaesthetized rats in plain tubes. Serum was obtained by centrifugation at 1000 rpm for 10 min at room temperature, and then stored at -20°C until analysis. To estimate the antioxidant and lipid peroxidation in liver and heart tissue homogenate, the abdomen was opened and the heart and the liver were saved in ice-cold for tissue homogenate preparation.

### Tissue homogenate preparation

Heart and liver tissue homogenates were prepared according to the method described in Abulnaja et al. [2] from ice cold liver and heart tissues.

**Estimation of serum proteins**

A spectrophotometric method was used in quantification of proteins as described by Peterson [18] using Sigma kit (USA). Serum albumin was also estimated spectrophotometrically according to the method described by Rebecca [19] using Sigma-Aldrich (USA) kit. Serum globulin was calculated as follows: serum globulin (g/dl)=total protein (g/dl)-albumin (g/dl).

**Total bilirubin estimation**

The total bilirubin was estimated spectrophotometrically using spectrum kit (Germany) as described by Balistreri et al. [20].

**Kidney function**

Urea and uric acid were estimated in serum spectrophotometrically according to the method described by Fawcett et al. [21], Fossati et al. [22], respectively using Human kit (Germany), whereas creatinine was estimated according to the method described by Tietz [23] using Moody International creatinine kit, UKAS (Germany).

**Serum electrolytes (Na<sup>+</sup> and K<sup>+</sup>) estimation**

Sodium and potassium ions (Na<sup>+</sup> and K<sup>+</sup>) were estimated in serum colorimetrically using Electrolytes Test Kit (India) according to Schoenfeld et al. [24] and Terri et al. [25], respectively.

**Estimation of antioxidants and lipid peroxidation**

The activities of serum, and heart and liver tissue homogenate of non-enzymatic glutathione reduced (GSH), and the antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) were assayed colorimetrically according to Beutler et al. [26], Aebi [27] and Nishikimi et al. [28], respectively using Biodiagnostic kit (Egypt). In addition, glutathione reductase activity (GR) was also assayed spectrophotometrically in

serum, and liver and heart tissue homogenate according to the method described by Goldberg et al. [29] using Biodiagnostic kit (Egypt). Lipid peroxidation was also estimated in serum, and liver and heart tissue homogenate by measuring the value of malondialdehyde (MDA) according to the colorimetric method of Satoh [30] using Biodiagnostic kit (Egypt), too.

**Statistical analysis**

The SPSS program was used to calculate mean values, standard error and test of significance, whereas, one way analysis of variance of SAS package was used to calculate the least significant difference and Analysis of Variance (ANOVA).

**Results**

**Total bilirubin**

Table 1 shows the effect of administration of 20 mg/Kg bw of parsley and carob methanolic extracts for 8 w on serum total bilirubin in hypercholesterolemic male rats. Bilirubin was non-significantly increased in G2 as a result of hypercholesterolemia. After treatment with 20 mg/Kg bw of parsley and carob methanolic extracts for 8 w in G3 and G4, respectively the level of total bilirubin was nonsignificantly decreased in both groups approaching the negative control.

**Serum protein**

Table 1 also shows the effect of administration of 20 mg/Kg bw of parsley and carob methanolic extracts for 8 w on serum proteins in hypercholesterolemic rats. The total protein (albumin, globulin, and their A/G ratio) were nonsignificantly decreased by hypercholesterolemia in G2 and nonsignificantly increased by the concurrent supplementation with 20 mg/Kg bw of parsley seed methanolic extract in G3 and 20 mg/Kg bw of carob legume methanolic extract in G4 approaching the negative control.

**Table 1.** Effect of administration of 20 mg/Kg bw of parsley and carob methanolic extracts for 8 w on serum total protein and bilirubin in hypercholesterolemic male rats.

Parameters	Statistics	Negative group (G1)	control	Positive group (G2)	control	20 mg/Kg bw of parsley methanol extract (G3)	20 mg/Kg bw of carob methanol extract (G4)
Total bilirubin (mg/dl)	Mean ± SE	0.49 ± 0.07 <sup>a</sup>		0.55 ± 0.06 <sup>a</sup>		0.48 ± 0.06 <sup>a</sup>	0.52 ± 0.05 <sup>a</sup>
	LSD 0.05=0.198						
	t-test	-		-0.51 NS		0.63 NS	0.42 NS
Total protein g/dl	Mean ± SE	6.48 ± 0.09 <sup>a</sup>		6.26 ± 0.21 <sup>a</sup>		6.43 ± 0.11 <sup>a</sup>	6.47 ± 0.09 <sup>a</sup>
	LSD 0.05=0.465						
	t-test	-		0.98 NS		-0.60 NS	-0.72 NS
Albumin (g/dl)	Mean ± SE	4.28 ± 0.14 <sup>a</sup>		4.06 ± 0.16 <sup>a</sup>		4.11 ± 0.13 <sup>a</sup>	4.23 ± 0.13 <sup>a</sup>
	LSD 0.05=0.393						
	t-test	-		1.73 NS		-0.20 NS	-0.62 NS

Globulin (g/dl)	Mean ± SE	2.31 ± 0.15 <sup>a</sup>	2.19 ± 0.26 <sup>a</sup>	2.36 ± 0.11 <sup>a</sup>	2.23 ± 0.06 <sup>a</sup>
	LSD 0.05=0.750				
	t-test	-	0.60 NS	-0.60 NS	-0.15 NS
A/G ratio	Mean ± SE	1.85 ± 0.15 <sup>a</sup>	1.85 ± 0.58 <sup>a</sup>	1.74 ± 0.14 <sup>a</sup>	1.89 ± 0.10 <sup>a</sup>
	LSD 0.05=0.399				
	t-test	-	-1.01 NS	1.02 NS	1.00 NS

Data are represented as mean ± SE. t-test values; \*\*\*significant at P<0.001. ANOVA analysis: within each row, means with different superscript (a, b, c or d) are significantly different at P<0.05, whereas means superscripts with the same letters mean that there is no significant difference at P>0.05. LSD: Least Significant Difference.

**Kidney function**

Table 2 shows the effect of administration of 20 mg/Kg bw of parsley and carob methanolic extracts for 8 w on kidney function in hypercholesterolemic male rats. Serum uric acid, creatinine and urea in G2 were significantly increased (at <0.001 for uric acid and creatinine, and at P<0.05 in urea). The concurrent supplementation of 20 mg/Kg bw of parsley and

carob methanolic extracts for 8 w in G3 and G4, respectively significantly decreased serum uric acid and creatinine compared to G2 approaching the negative control G1. Whereas serum urea was non-significantly decreased in both G3 and G4. Parsley seed methanolic extract was more efficient than carob legumes methanolic extract in restoring the altered kidney function indices.

**Table 2.** Effect of administration of 20 mg/Kg bw of parsley and carob methanolic extracts for 8 w on kidney functions and serum electrolytes (Na<sup>+</sup> and K<sup>+</sup>) in hypercholesterolemic male rats.

Parameters	Statistics	Negative control group (G1)	Positive control group (G2)	20 mg/Kg bw of parsley methanol extract (G3)	20 mg/Kg bw of carob methanol extract (G4)
Uric acid (mg/dl)	Mean ± SE	4.11 ± 0.04 <sup>b</sup>	4.35 ± 0.04 <sup>a</sup>	4.15 ± 0.06 <sup>b</sup>	4.18 ± 0.07 <sup>a</sup>
	LSD 0.05=0.126				
	t-test	-	-4.62***	**3.26	***5.65
Creatinine (mg/dl)	Mean ± SE	0.55 ± 0.02 <sup>c</sup>	0.77 ± 0.03 <sup>a</sup>	0.60 ± 0.02 <sup>bc</sup>	0.63 ± 0.02 <sup>b</sup>
	LSD 0.05=0.071				
	t-test	-	-5.30***	10.51***	*2.95
Urea (mg/dl)	Mean ± SE	20.78 ± 0.88 <sup>b</sup>	24.73 ± 1.30 <sup>a</sup>	23.01 ± 0.35 <sup>ab</sup>	24.23 ± 0.11 <sup>b</sup>
	LSD 0.05=2.339				
	t-test	-	-2.84*	1.26 NS	0.39 NS
Na <sup>+</sup> (mmol/l)	Mean ± SE	140.00 ± 0.42 <sup>a</sup>	140.67 ± 0.61 <sup>a</sup>	140.17 ± 0.45 <sup>a</sup>	140.50 ± 0.87 <sup>a</sup>
	LSD 0.05=1.837				
	t-test	-	-0.71 NS	0.82 NS	0.21 NS
K <sup>+</sup> (mmol/l)	Mean ± SE	4.75 ± 0.04 <sup>a</sup>	4.70 ± 0.05 <sup>a</sup>	4.71 ± 0.04 <sup>a</sup>	4.73 ± 0.06 <sup>a</sup>
	LSD 0.05=0.171				
	t-test	-	0.59 NS	-0.19 NS	-0.37 NS

Data are represented as mean ± SE. t-test values \*significant at P<0.05, \*\*significant at P<0.01, \*\*\*significant at P<0.001. ANOVA analysis: within each row, means with different superscript (a-d) are significantly different at P<0.05, whereas means superscripts with the same letters mean that there is no significant difference at P>0.05. LSD: Least Significant Difference, N.S: Non-Significant.

**Serum electrolytes**

Table 2 also shows the effect of administration of 20 mg/Kg bw of parsley and carob methanolic extracts for 8 w on serum Na<sup>+</sup> and serum K<sup>+</sup> in hypercholesterolemic male rats. The levels of Na<sup>+</sup> and K<sup>+</sup> in the serum were non-significantly

affected either by induced hypercholesterolemia in G2 or treating with 20 mg/kg bw of parsley and carob methanolic extracts for 8 w in G3 and G4, respectively.

**Serum antioxidants**

Table 3 shows the effect of administration of 20 mg/Kg bw of parsley and carob methanolic extracts for 8 w on serum antioxidants enzymes in hypercholesterolemic male rats. The oral administration of 2% cholesterol in G2 for 8 w significantly (at <0.001) decreased serum catalase, SOD, GSH, and GR. The concurrent supplementation with 20 mg/kg bw of

parsley seed methanolic extract in G3 and carob legume methanol extract in G4 significantly (at <0.001) increased serum catalase, SOD, GSH, and GR in the hypercholesterolemic male rats. Parsley seed methanolic extract was more efficient than carob legume methanol extract in restoring the antioxidants activity.

**Table 3.** Effect of administration of administration of 20 mg/Kg bw of parsley and carob methanol extracts for 8 w on serum antioxidants in hypercholesterolemic male rats.

Parameters	Statistics	Negative control group (G1)	Positive control group (G2)	20 mg/kg bw of parsley methanol extract (G3)	20 mg/kg bw of carob methanol extract (G4)
Catalase (U/ml)	Mean ± SE	2.00 ± 0.05 <sup>a</sup>	0.20 ± 0.01 <sup>d</sup>	1.45 ± 0.08 <sup>b</sup>	0.47 ± 0.02 <sup>c</sup>
	LSD 0.05=0.175				
	t-test	-	***26.48	***-12.87	***-6.09
Superoxide dismutase (U/ml)	Mean ± SE	312.60 ± 4.35 <sup>a</sup>	127.63 ± 1.72 <sup>d</sup>	242.47 ± 3.14 <sup>b</sup>	188.60 ± 2.28 <sup>c</sup>
	LSD 0.05=8.504				
	t-test	-	***39.93	***-38.22	***-44.81
Glutathione reduced (GSH) (mmol/ml)	Mean ± SE	162.57 ± 40.74 <sup>a</sup>	36.84 ± 2.62 <sup>d</sup>	136.64 ± 14.80 <sup>b</sup>	100.81 ± 22.45 <sup>c</sup>
	LSD 0.05=79.110				
	t-test	-	92.22***	***-187.30	***-87.68
Glutathione reductase (GR) (U/ml)	Mean ± SE	5.90 ± 0.22 <sup>a</sup>	1.36 ± 0.08 <sup>d</sup>	4.63 ± 0.11 <sup>b</sup>	3.11 ± 0.11 <sup>c</sup>
	LSD 0.05=0.359				
	t-test	-	***21.18	***-22.48	***-15.65

**Antioxidant enzymes in liver and heart tissues homogenate**

Tables 4 and 5 show the effect of 20 mg/kg bw of parsley and carob methanolic extracts for 8 w on antioxidant enzymes in liver and heart tissues homogenate of hypercholesterolemic male rats. Catalase, SOD, GSH, and GR in the liver and heart tissues homogenate were significantly (at <0.001) decreased in the positive control group (G2) as a result of cholesterol

administration for 8 w. The concurrent supplementation with 20 mg/Kg bw of parsley and carob methanolic extracts for 8 w in G3 and G4, respectively significantly (at <0.001) increased catalase, SOD, GSH, and GR in the liver and heart tissue homogenate in the hypercholesterolemic rats. Parsley seeds methanolic extract was more efficient than carob legume methanol extract.

**Table 4.** Effect of administration of 20 mg/Kg bw of parsley and carob methanol extracts for 8 w on liver tissue homogenate antioxidants in hypercholesterolemic rats.

Parameters	Statistics	Negative control group (G1)	Positive control group (G2)	20 mg/kg bw of parsley methanol extract (G3)	20 mg/kg bw of carob methanol extract (G4)
Catalase (U/g)	Mean ± SE	5.36 ± 0.11 <sup>a</sup>	0.88 ± 0.02 <sup>d</sup>	3.95 ± 0.13 <sup>b</sup>	2.86 ± 0.10 <sup>c</sup>
	LSD 0.05=0.314				
	t-test	-	***44.65	***-19.85	***-16.46
Superoxide dismutase (U/g)	Mean ± SE	1014.50 ± 10.57 <sup>a</sup>	562.68 ± 3.61 <sup>d</sup>	822.45 ± 5.33 <sup>b</sup>	629.58 ± 6.26 <sup>c</sup>
	LSD 0.05=20.822				
	t-test	-	***33.15	***-54.08	***-13.26
Glutathione reduced (mmol/g)	Mean ± SE	262.4 ± 24.57 <sup>a</sup>	143.92 ± 34.67 <sup>c</sup>	214.86 ± 28.61 <sup>b</sup>	190.68 ± 17.12 <sup>d</sup>
	LSD 0.05=24.57				
	t-test	-	***33.15	***-54.08	***-13.26

		LSD 0.05=66.134			
t-test		-	103.20***	***-47.50	***-71.34
Glutathione reductase (U/g)	Mean ± SE	9.41 ± 0.08 <sup>a</sup>	3.13 ± 0.12 <sup>d</sup>	6.37 ± 0.10 <sup>b</sup>	5.60 ± 0.13 <sup>c</sup>
	LSD 0.05=0.305				
t-test		-	***40.89	***-33.02	***-17.54

Data are represented as mean ± SE. t-test values: \*\*\*: significant at P<0.001. ANOVA analysis: within each row, means with different superscript (a-d) are significantly different at P<0.05, whereas means superscripts with the same letters mean that there is no significant difference at P>0.05. LSD: Least Significant Difference.

**Table 5.** Effect of 20 mg/kg bw of parsley and carob methanol extracts for 8 w on antioxidants of heart tissue in hypercholesterolemic male rats.

Parameters	Statistics	Negative control group (G1)	Positive control group (G2)	20 mg/Kg bw of parsley methanol extract (G3)	20 mg/Kg bw of carob methanol extract (G4)
Catalase (CAT) (U/g)	Mean ± SE	4.51 ± 0.07 <sup>a</sup>	0.69 ± 0.02 <sup>d</sup>	3.60 ± 0.15 <sup>b</sup>	2.15 ± 0.10 <sup>c</sup>
	LSD 0.05=0.309				
	t-test	-	***58.36	***-19.96	***-12.35
Superoxide dismutase (SOD) (U/g)	Mean ± SE	9415.30 ± 21.32 <sup>a</sup>	4296.20 ± 35.81 <sup>d</sup>	6567.70 ± 21.58 <sup>c</sup>	5417.50 ± 15.65 <sup>c</sup>
	LSD 0.05=84.917				
	t-test	-	***102.95	***-42.44	***-24.28
Glutathione reduced (GSH) (µmol/g)	Mean ± SE	247.14 ± 14.88 <sup>a</sup>	139.21 ± 10.72 <sup>d</sup>	201.78 ± 8.78 <sup>b</sup>	171.42 ± 13.70 <sup>c</sup>
	LSD 0.05=36.469				
	t-test	-	140.74***	***-114.75	***-47.86
Glutathione reductase (GR) (U/g)	Mean ± SE	8.58 ± 0.10 <sup>a</sup>	2.20 ± 0.12 <sup>d</sup>	6.16 ± 0.08 <sup>c</sup>	7.50 ± 0.05 <sup>b</sup>
	LSD 0.05=0.296				
	t-test	-	***41.54	***-26.09	***-32.05

Data are represented as mean ± SE. t-test values: \*\*\* significant at P<0.001. ANOVA analysis: within each row, means with different superscript (a-d) are significantly different at P<0.05, whereas means superscripts with the same letters mean that there is no significant difference at P>0.05. LSD: Least Significant Difference.

**Lipid peroxidation**

Table 6 shows the effect of 20 mg/kg bw of parsley and carob methanolic extracts for 8 w on lipid peroxide in serum, and liver and heart tissue homogenate in hypercholesterolemic male rats. The lipid peroxidation in the serum, liver and heart tissue homogenate were significantly (at <0.001) increased in the positive control group (G2), as a result of cholesterol supplementation for 8 w. The concurrent supplementation with 20 mg/kg bw of parsley seeds methanolic extract in G3 and carob legume methanolic extract in G4 significantly (at <0.001) decreased lipid peroxidation in the hypercholesterolemic rats. Parsley seed methanolic extract was also more efficient in reducing oxidative stress as revealed by MDA levels.

**Table 6.** Effect of administration of 20 mg/kg bw of parsley and carob methanol extracts for 8 w on lipid peroxidation in hypercholesterolemic male rats.

Parameters	Statistics	Negative control	Positive control	20 mg/kg bw of parsley	20 mg/kg bw of carob
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		group (G1)	group (G2)	methanol extract (G3)	methanol extract (G4)
Plasma (nmol/ml)	Mean ± SE	1.400 ± 0.131 <sup>d</sup>	8.281 ± 0.169 <sup>a</sup>	5.200 ± 0.196 <sup>b</sup>	2.833 ± 0.144 <sup>c</sup>
	LSD 0.05=0.4605				
	t-test	-	-31.162***	***10.781	***33.342
Liver tissue (nmol/g)	Mean ± SE	8.766 ± 0.367 <sup>d</sup>	26.983 ± 0.439 <sup>a</sup>	20.966 ± 0.454 <sup>b</sup>	15.900 ± 0.367 <sup>c</sup>
	LSD 0.05=1.227				
	t-test	-	-32.668***	***9.230	***22.517
Heart tissue (nmol/g)	Mean ± SE	6.366 ± 0.348 <sup>d</sup>	22.716 ± 0.578 <sup>a</sup>	17.016 ± 0.258 <sup>b</sup>	10.466 ± 0.326 <sup>c</sup>
	LSD 0.05=1.1969				
	t-test	-	-23.393***	8.909***	***16.343

Data are represented as mean  $\pm$  SE. t-test values: \*\*\* significant at  $P < 0.001$ . ANOVA analysis: within each row, means with different superscript (a-d) are significantly different at  $P < 0.05$ , whereas means superscripts with the same letters mean that there is no significant difference at  $P > 0.05$ . LSD: Least Significant Difference.

## Discussion

Feeding rats with 2% cholesterol in the fat rich diet for two months induce hypercholesterolemia causing elevated total cholesterol, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and decreased high-density lipoprotein (HDL) [2,3,17,31]. In this study, the total protein, albumins, globulins, albumins/globulins ratio total, bilirubin and serum electrolytes, were not significantly affected by either hypercholesterolemia or the concurrent supplementation with parsley seed methanolic extract and carob legume methanolic extract.

The significant decrease of the enzymatic and nonenzymatic antioxidants and decrease of lipid peroxidation as a result of induced hypercholesterolemia in serum, and heart and liver tissue homogenate of the positive control group is in agreement with previous studies [2,32-34]. The alteration in oxidative stress induced by reactive oxygen species (ROS) and impairments of the antioxidant system contributes to the incidence of hypercholesterolemia and subsequent cardiovascular diseases [5]. Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) and non-enzymatic antioxidants play an important role in alleviating tissue damage due to the formation of free radicals [2].

Increasing the antioxidant enzymes activity and decreasing lipid peroxidation in serum, and liver and heart tissue homogenate with the concurrent supplementation with 20 mg/kg bw of parsley seeds methanolic extract in G3 and carob legume methanolic extract in G4 for 8 w is consistent with previous studies due to their content of phenolic and other antioxidant compounds [11,15,35]. Parsley seeds showed more effective to improve serum antioxidant enzymes and lipid peroxide than carob legume.

The increase in serum creatinine, uric acid, and urea as a result of induced hypercholesterolemia is consistent with Schaeffner et al. [36], El Rabey et al. [31] and Abulnaja et al. [2]. The elevated total cholesterol and the low density lipoproteins in particular were significantly associated with an increased risk of developing renal dysfunction. The alleviation of the adverse effects on kidney function with the concurrent supplementation with 20 mg/kg bw of parsley seed methanol extract in G3 and carob legume methanolic extract in G4 for 8 w agrees with Haidari et al. [37].

In conclusion, parsley seed and carob legume methanolic extract succeeded in ameliorating the adverse effects resulted from hypercholesterolemia by increasing the antioxidant activity of the enzymatic antioxidants and the level of the nonenzymatic antioxidants, and decreasing the oxidative stress by lowering lipid peroxidation in hypercholesterolemic rats. Parsley appeared more efficient than carob.

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