Journal

J. Biol. Chem. Environ. Sci., 2009, Vol. 4(2): 695-720
www.acepsag.org

ALLEVIATION AND IMPROVEMENT OF HYPERLIPIDEMIC IN HYPERCHOLESTEROLEMIC RATS BY LENTIL SEEDS AND APPLE AS WELL AS PARSLEY AS SEMI-MODIFIED DIETS

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ABSTRACT

The study was carried out to evaluate the effects of unpeeled lentil seeds (legume), apple (fruit) and parsley (vegetable) in semi-modified diets on lipid fraction of hyperlipidemic and hypercholesterolemic male albino rats. These were based on those agents contain protein and a large amount of phenols which used as lipotropic factors and antioxidant characters. Blood lipid fractions of hyperlipidemic animals were improved by the antioxidant and protein lipotropic powers of diets used and protect animals from oxidation and precipitations on the walls of the blood vessel thus alleviated atherosclerosis. The investigation was done with semi-modified diets of unpeeled lentil seeds, dried apple and dried parsley as well as their mixtures (1:1:1) to evaluate their effects as hypolipidemic agents which amounted 10% of the diets at the expense of starch. Composition of the apple, parsley and unpeeled lentil seeds was estimated on dry matter. The results observed that three used lipotropic and antioxidant diets amounted good values of protein (especially unpeeled lentil) cured fiber and phenolic compounds (especially apple and parsley). The HPLC analysis of the three methanolic extracts of unpeeled lentil, apple and parsley for the phenolic compounds showed present of about 23 compounds varying in their levels in the extracts. It was noticed that compounds such as catechin phenol, daidzin, *p* – coumaric, genistein, ferulic, quercetin, chrysin and galangin were detected in the both of apple and parsley extracts. Rutin is detected in unpeeled lentil and apple extracts. There are other phenolic compounds were detected in the each extract which

differed between apple, parsley and unpeeled lentil extracts such as pyrogallol, chlorogenic, synergic, naringinin and luteolin were detected in unpeeled lentil extract only but P- OH benzoic, caffic and kaempherol were detected in parsley extract only. The phenolic compound such as salicylic, cinnamic, euganol and pinostrobin were detected in apple extract only.

The results of the present studies of the semi-modified diet with unpeeled lentil as well as dried apple and parsley also their mixture observed hypocholesterolemic and hypolipidemic power which alleviated the disease and improved function of liver and kidneys, blood picture, lipids profile and protein profiles, lipid peroxidation and the activities of antioxidant enzymes such as superoxide dismutase and catalase while the present results observed insignificant effects on the blood glucose levels of the experimental animals. The obtained results showed that unpeeled lentil seeds as well as dried apple and parsley semi-modified diets generally improved in the clinical blood status. Picture of blood (RBCs, WBCs and Hb), lipid fraction such as total lipid, cholesterol and triglycerides as well as HDL-C, LDL-C and VLDL-C also the treatment alleviated the risk factor of the hyperlipidemic rats. Liver function (GOT, GPT and ALP activity as well as bilirubin) and kidneys function (uric acid, urea and creatinine), total protein and its fractions (albumin, and globulin), lipids peroxidation and antioxidative enzymes activity (SOD) and catalase) were readjusted around the normal values in hyperlipidemic and hypercholesterolemic rats by the improvements of the present liportopic factors and antioxidative agents of unpeeled lentil, apple and parsley by which hyperlipidemia and hypercholesterolemia were alleviated. In connection, their semi-modified mixture diet observed the best treatment clinical effect concerning biological studies than the other treatments it means that mixture produced such synergetic effects which used semi-modified diets of unpeeled lentil, apple and parsley alone. These data of the present studies in hypercholesterolemic and hyperlipidemic male albino rats showed that the different diet treatments can be arranged in the following from increasing to decreasing order as anticholesterolemic and antilipidemic powerful:

Mixture diet > unpeeled lentil > dried parsley ≥ dried apple.

Key words: parsley; unpeeled lentil; apple; hyperlipidemia; hypercholesterolemia.

INTRODUCTION

For many years interest in dietary effects on serum lipids centered on total cholesterol levels. Unfortunately, serum lipids measurements in dietary studies generally have lagged advances in our understanding of cholesterol transport via lipoproteins. Recently, several investigations have begun to re-examine the influence of various nutrients on lipid metabolism with emphasis on serum lipoprotiens and apolipoprotiens (Katan *et al.,* 2003).

People with elevated lipid fractions levels are more likely to have atherosclerotic than people with low levels. Also, diabetes is at very high risk for atherosclerosis; those with elect triglycerides may have an increased risk as well. Atherosclerosis is a disease of large and medium sized muscular arteries and the large elastic arteries, such as aorta and iliac vessels which produced by hypercholesterolemia status (Corti *et al.,* 2003).

Oxidative stress has been implicated in diseases ranging from cancer to cardiovascular disease to age – related macular degeneration and even to aging process itself (Van – Lieshout *et al.,* 2003). Many diseases (include hyperlipidemia) related to oxidative stress have potentially serious consequences and limited treatment research has focused on arresting the oxidative damage that may be involved in disease initiation or progression. Recent research has measured both markers of oxidative stress and whole – body antioxidant capacity in response to diet, vitamin, mineral and phytochemical. There are hundreds of polyphenols with antioxidant activity that are potential contributors to the antioxidant mechanisms in animals and humans in general. These compounds are excellent candidates to explain the health benefits of diet rich in fruits and vegetables (Houston *et al.,* 2005)

Legumes are inexpensive, nutrient – dense sources of plant protein than can be substituted for dietary animal protein (Anderson *et al.,* 1999). Not only are legumes excellent sources of essential minerals, they are rich in dietary fiber and other phytochemicals that may affect health. According to the World Health Reports (2002) about the deterioration in lipid parameters in the body which leads to the incidence of atherosclerosis and cardiovascular disease, more than 75% of heart diseases, the world's leading cause of premature death, result from high blood lipid fraction and other factors such as high blood pressure, smoking and drinking alcohol.

In the present work, the main aim was to study the hypolipidemic effect of apple (fruit), parsley (vegetable) and unpeeled lentil (legume) and nutritional evaluation through an animal experiment as following studies:-

1- Chemical composition of apple, parsley and unpeeled lentil.
2- The phenolic contents in apple, parsley and unpeeled lentil constituent compounds through analysis by HPLC techniques.
3- The biological treatments of semi-modified diets of apple parsley and unpeeled lentil as antilipidemic agents on blood analysis, i.e., blood picture, glucose, lipid profile, liver function, kidneys function, protein fractions, lipid peroxidation, superoxide dismutase activity and catalase activity.

MATERIALS AND METHODS

Preparation of samples:

Samples of the present unpeeled lentil *(Lens esculenta)*, apple *(Molus pumila)* and parsley *(Petroselimun crispum)* were purchased from the local market. For apple and parsley the samples were cut and dried in an air oven at 50^{o}C till complete dryness, weighted to calculate its moisture content. The samples of dried apple, parsley and unpeeled lentil were ground to fine powder for their analysis and used in the experimental animals.

General chemical analysis:

The determination of moisture, crude protein, total lipids, ash and crude fibers were done and nitrogen free extract was calculated by difference, deducing the percentage of them 100 according to A.O.A.C (2000).

Determination of total phenols:

The determination of total phenols was done according to the procedure described in the A.O.A.C. (2000) as tannic acid

HPLC analysis of samples phenolic compounds:

Phenolic compounds of unpeeled lentil, apple and parsley samples were extracted according to the method described by Duke *et al.,* (2003) in which a known weight of dried samples was extracted by methanol. Each of phenolic compounds of the three extracts was identified and performed on JASCO HPLC using hypersil C_{18} reversed phase column (250 x 4.6 mm) with 5u particle size. Injection by means of Rhcodyne injection value with 50 PJ fixed loop was used. A constant flow rate of one ml/min. was used with two mobile phases: A 0.5% acetic acid in distilled water at pH 2.65; solvent (B) 0.5% acetic acid in pure (99.5%) acetonitrile, the elution gradient was linear starting with (A) and ending with (B) over 35 min. using and UV detector set at wavelength 254 nm. Phenolic compounds of the samples were identified by comparing their retention times with those of the standard mixture chromatogram. The concentration of an individual compound was calculated on the basis of peak area measurements and then converted.

Experimental animals:-

The Sprague – Dawley albino male rats weighing 67 – 79 were used for the present studies. The animals were obtained from Agriculture Research Center (ARC), Giza, Egypt. The animals were raised in the animal house and kept under normal laboratory conditions (temperature remain 25 ± 2^{o}C) for 48 hr before the initiation of experiment. During the period, animals were allowed free access of water and basal diet (A.O.A.C. 2000). The control diet is composed of as reported by Lane – Peter and Pearson (1971), 15% casein, 10% corn oil, 5% cellulose, 4% salt mixture (Schneeman et al., 1989), 1% vitamins mixture (Philip et al., 1993) and starch 65%. The high fat diet was similar to the control diet but differ in more fat content which was 20% sheep fat, 2% cholesterol and 0.25% bile salts and starch 42.75%.

After a period of adaptation (one week), 48 of those male albino rats were divided into two groups as following:

(Group one "first group"): Rats were fed on normal diet (8rats) as healthy normal control animals.

(Group two "second group") Rats were fed on high fat with cholesterol diet for 4 weeks (40 rats). At the end of the feeding period, blood samples were taken from the suborbital vein to test for blood cholesterol level. A high level of serum cholesterol was considered as an indication to hypercholesterolemia. The hyperlipidemic rats of the second group were subdivided into 5 subgroups (8 rats each).

Subgroup (1): Rats were fed on the high fat / high cholesterol diet without any treatment (hyperlipidemic control).

Subgroup (2): Rats were fed on high fat / high cholesterol diet with 10% dried parsley at the expense of starch (Parsley group).

Subgroup (3): Rats were fed on high fat / high cholesterol diet with 10% dried apple at the expense of starch (Apple group).

Subgroup (4): Rats were fed on high fat / high cholesterol diet with 10% unpeeled lentil at expense of starch (Lentil group).

Subgroup (5): Rats were fed on high fat / high cholesterol diet with 10% mixture of dried parsley, dried apple and unpeeled lentil by ratio of 1:1:1at the expense of starch (Mixture group).

At the end of 10 weeks interval, rats were fasted overnight and then the animals were killed by decapitation. The blood samples were collected from each rat with EDTA and subjected to centrifugation tube at 3000 rpm to obtain the plasma which was kept at 20^{o}C for the subsequent investigation.

Blood biochemical analysis:

Plasma glucose levels were determined enzymatic colorimetrically according to the method of Trinder (1969).

- For hematology analysis, blood hemoglobin (Hb %) was determined according to the method of Dacia and Lewis (1975) and the measurement of red blood cells (RBCs) count as well as white blood cells (WBCs) count were carried out according to the methods of Natt and Herrick (1952).
- For liver function, GOT (AST) and GPT (ALT) activity was determined colorimetrically according to the method of Reitman and Frankel (1957), also ALP activity was determined according to the method of Weisshaar (1975). Total bilirubin levels of plasma were determined according to the method described by Tietz (1995).
- For kidneys function, uric acid, and urea contents in plasma were determined colorimetrically according to the methods described by Caraway (1975) and the determination of plasma creatinine content

was carried out colorimetrically according to the method described by Faulkner and King (1976).

- For plasma soluble protein and its fraction, total soluble protein and albumin contents were determined according to Bradford method (1976) and Doumas *et al.,* method (1971). The globulin content was calculated by the difference between total soluble protein and albumin of the plasma.
- For plasma lipid profile, total lipids, total triglycerides and total cholesterol levels were determined colorimetrically according to the methods of Knight *et al.,* (1972), Fossati and Prencipe (1982) and Allain *et al.,* (1974) respectively. For lipoprotein profile in plasma, HDL-C and LDL-C levels were determined according to Warnick *et al.,* (1983) and Bergmeyer (1974) respectively, but VLDL-C was calculated by using the equations which described by Fiedewald *et al.,* (1972).
- For lipid peroxidation, lipid peroxide was determined according to the method of Ohkawa et al., (1979). Also, catalase and superoxide dismutase activities were determined according to the methods described by Aebi (1984) and Nishikimi et al., (1972) respectively.

All data pooled through the present studies were proceeded by General Linear Model procedures (GLM) of the statistical analysis system described in SAS user's Guide (SAS Institute, 2000). The significance of the differences among treatment groups were tested using Waller – Duncan k – ratio (Waller and Duncan, 1969) All statements of significance were based on probability of $P < 0.05$.

RESULTS AND DISCUSSION

Results

Hyperlipidemia is usually defined as a chronic disorder of lipids metabolism, characterized by hypercholesterolemia. Therefore, the present experiments were conducted to evaluate the effect of the semi-modified diets of unpeeled lentil, apple and parsley on hyperlipidemic rats as lipotropic factors and hypercholesterolemic agents. Several studies pointed out the hypolipidemic influences of medicinal plants. The chemical composition of lentil, apple and parsley are shown in Table (1).

Table (1) chemical composition of the fresh and dried unpeeled lentil seeds, parsley and apple used in the experiments.

Constituents	UnPeeled lentils		Parsley		Apple	
	Fresh	Dry	Fresh	Dry	Fresh	Dry
Water	11.50 ± 3.33	——	84.6 ± 5.19	——	84.5 ± 6.00	——
Protein	22.90 ± 6.27	25.88 ± 1.79	3.2 ± 0.20	20.77 ± 1.78	0.4 ± 0.03	2.58 ± 0.19
Fat	0.7 ± 0.03	0.79 ± 0.04	0.4 ± 0.02	2.60 ± 0.17	0.2 ± 0.01	1.29 ± 0.10
Ash	2.3 ± 0.17	2.60 ± 0.18	2.1 ± 0.17	13.63 ± 0.87	0.6 ± 0.04	3.87 ± 0.21
Fiber	2.1 ± 0.15	2.37 ± 0.17	1.3 ± 0.07	8.44 ± 0.51	0.8 ± 0.05	5.16 ± 0.32
Nitrogen free extract	60.0 ± 4.67	67.8 ± 4.41	8.00 ± 0.56	51.92 ± 3.42	13.3 ± 0.97	85.79 ± 6.16

The results showed that unpeeled lentil, dried apple and dried parsley are rich in protein, fiber, elements and carbohydrates. Dried parsley contains low amount of total phenols than dried apple but more than unpeeled lentil. (Table 2) Plant antioxidant produced a good antilipidemic and anticholesterolemic influences. For that special interest was given to the present semi-modified diets antioxidants in which the phenolic compounds of apple, parsley and unpeeled lentil methanolic extract were analyzed. HPLC analysis of the three methanolic extract for their phenolic compounds (Table 2) showed the presence 23 compounds and 14 compounds in apple and 13 compounds parsley methanolic extract respectively which were varied in amount between each other. The methanolic extract of unpeeled lentil contained 8 compounds. Total known phenolic compounds were 91.82%, 84.82% and 83.05% for apple, parsley and unpeeled lentil respectively.

It was observed that the phenolic compounds such as catechin, phenol, daidzin, *p*-coumaric, genistein, ferulic, querctin, chrysin and galangin were detected in apple and parsley methanolic extracts. More than these phenolic compounds, rutin, salicyhic, cinnamic, genstein, euganol and pinostrobin were detected in apple extract but in parsley, gallic acid, *p*-hydroxy benzoic, caffic and kaempherol were detected.

Genistein was dominating which amounted about 75% and 20% of the total phenolic compounds for parsley and apple respectively. Cinnamic was amounted about 26% of apple total phenolic compounds. This main that especially apple or parsley were considered as good sources of phenolic compounds (antioxidant). For unpeeled lentil extract pyrogallol and chlorogenic were dominating which amounted about 52 and 34% respectively but gallic acid, catechin and lutealin amounted about 5%, 6% and 3% respectively. Synergic acid, rutin and naringinin amounted less than 1% of the total phenolic compounds.

Table (2) HPLC analysis of polyphenols in Methanolic extract of the apple, parsley and unpeeled lentil

NO.	Compounds mg/100g D.W	Apple mg/100g D.W.	%	Parsley mg/100g D.W.	%	Lentil mg/100g D.W.	%
1	Gallic acid	—	—	3	0.47	15.34	4.91
2	P–OH-benzoic	—	—	1	0.16	—	—
3	Catechin	84	5.22	48	7.54	17.04	5.48
4	Pyrogallol	—	—	—	—	161.75	51.80
5	Caffic	—	—	12	1.88	—	—
6	Chlorogenic	—	—	—	—	104.68	33.52
7	Phenol	54	5.87	31	4.87	—	—
8	Daidzin	32	3.38	11	1.89	—	—
9	Synergic acid	—	—	—	—	2.48	0.79
10	Rutin	42	4.57	—	—	0.55	0.18
11	P-Coumaric	60	6.52	27	4.24	—	—
12	Genistein	182	19.77	480	75.35	—	—
13	Salicylic	130	14.13	—	—	—	—
14	Furulic	96	10.43	9	1.41	—	—
15	Cinnamic	236	25.65	—	—	—	—
16	Quercetin	22	2.39	11	1.73	—	—
17	Naringinin	—	—	—	—	0.53	0.17
18	Kaempherol	—	—	2	0.31	—	—
19	Euganol	2	0.22	—	—	—	—
20	Chrysin	4	0.43	1	0.16	—	—
21	Luteolin	—	—	—	—	9.89	3.17
22	Galangin	2	0.22	1	0.16	—	—
23	Pinostrobin	10	1.09	—	—	—	—

* Total phenols of dried Apple are amounted of 1002mg/100g. D.W.
* Total phenols of dried parsley are amounted of 751mg/100g. D.W.
* Total phenols of unpeeled lentil are amounted of 376 mg / 100g. D.W.

The treatments studied were, done on male hyperlipidemic albino rats to evaluate the effects semi-modified diets of apple, parsley and unpeeled lentil as hypolipidemic and hypercholesterolemic agents. The results of the present evaluation were statistically analyzed and summarized in Table (3). The data pointed out significant increases in total lipids, cholesterol and triglycerides when rats fed on the high fat / cholesterol diet, the values amounted 2.00, 3.50 and 3.00 fold respectively relative to that of normal control. The results summarized in Table (3) observed the effect of administration of 10% unpeeled lentil, dried apple and dried parsley as well as their mixture (semi-modified diets) on lipids profile of the hyperlipidemic and hypercholesterolemic animals. The feeding on different treatments (the three liportopic factors and their mixture for hyperlipidemic male albino rats) exhibited different influences on blood fractions, that the four treatments significantly improved and alleviated the drastic status of hyperlipidemia, where the mixture was the most effective as lipotropic factor (antilipidemia) then the three individual treatments (apple, parsley and unpeeled lentil). The blood total lipids, cholesterol, and triglycerides under the treatments of the semi-modified diets of apple, parsley unpeeled lentil and their mixture were still higher than that of control. These results are in agreement with those of blood lipoproteins profile under the same conditions. Hyperlipidemia elevated blood HDL-C, LDL-C and VLDL-C levels. The present harmful influences of hyperlipidemia were alleviated by the four treatments, but the highest elevations in lipoprotein contents were detected in hyperlipidemic animal blood. In addition, LDL-C and VLDL-C levels in hyperlipidemic rats blood were significantly improved by the present treatments of the studied lipotropic factor, but the values were still higher than those of healthy control animals. The present lipotropic factors can be arranged in the following increasing order for the semi-modified:-

Mixture > unpeeled lentil > parsley ≥ apple.

Table (4) showed that the lipidemia was accompanied by increasing in WBCs count. This elevation was also accompanied by a remarkable reduction in RBCs count and Hb content relative to the normal values. The feeding on the four treatments of the semi-modified diets induced antiblipidemic influences which improved the drastic effects on the three blood parameters (RBCs, WBCs and Hb). The four treatments produced similar improvements in the same respect.

Table (3): Lipid fraction of the experimental animal plasma.

Treatment	Total lipid		Total cholesterol		Total triglycerides		HDL-C		LDL-C		VLDL-C	
	mg/dl	%	mg/dl	%	mg/dl	%	mg/dl	%	mg/dl	%	mg/dl	%
Group (1) Normal control	251±19[a]	100	53±3.32[a]	100	57±2.94[a]	100	32±2.10[a]	100	9.6[a]	100	11.40±0.67[a]	100
Group (2) Hyperlipidaemic	503±31[b]	200	178±10.11[b]	336	167±9.12[b]	293	61±4.12[b]	191	83.6[b]	350	33.40±2.17[b]	293
Group (3) Parsley	285±21[a]	114	82±5.21[c]	155	88 ±5.55[c]	154	55±3.52[b]	172	9.4[a]	98	17.60±1.01[c]	154
Group (4) Apple	304±19[c]	121	80±4.97[c]	151	89±4.97[c]	156	48±2.39[c]	150	14.2[c]	148	17.80±1.11[c]	156
Group (5) Unpeeled lentil	321±22[c]	128	76±3.99[c]	143	78±4.17[c]	137	52±3.14[b-c]	163	8.4[a]	88	15.60±0.94[c]	137
Group (6) Mixture	235±15[a]	94	70±4.12[d]	132	67±3.94[a]	118	39±2.07[d]	122	17.6[c]	183	13.40±0.81[a]	118

* The same letters in each row represents the insignificant difference at $P < 0.05$.

Table (4): Hemoglobin, red blood cells and white blood cells of the experimental animal blood

Treatment	Hemoglobin (Hb%)		Red Blood Cells (RBCs)		White Blood Cells (WBCs)	
	g/dl	%	Count x 10^6	%	Count x 10^3	%
Group (1) Normal control	13.9±0.03[a]	100	4.1±0.29[a]	100	6.37±0.41[a]	100
Group (2) Hyperlipidaemic	12.3±0.97[a]	88	3.4±0.19[a]	83	16.3±1.01[b]	256
Group (3) Parsley	12.5±0.86[a]	90	3.67±0.20[a]	90	8.7±0.52[c]	137
Group (4) Apple	12.2±0.82[a]	88	3.48±0.18[a]	85	9.2±0.53[c]	144
Group (5) Lentil	12.4±0.74[a]	89	3.7±0.21[a]	90	7.7±0.39[c]	121
Group (6) Mixture	13.1±0.71[a]	94	3.84±0.21[a]	94	8.4±0.49[c]	132

* The same letters in each row represents the insignificant difference at $P < 0.05$.

In case of liver function, results of the studied antilipidemic semi-modified diets influences were presented in Table (5). Hyperlimidemia and hypercholesterolemia significantly stimulated GOT and GPT activities and increased plasma level of bilirubin relative to those of healthy control. The four treatments with unpeeled lentil, apple, parsley and their mixture semi-modified diets hyperlipidemic rats produced alleviation and improvement in the activity of the two transaminases (GOT and GPT) and blood bilirubin content. Alterations in GOT and GPT activity as well as bilirubin level in plasma have been thought to be the significant in the pathogenesis of lipidemia and cholesterolemia. Also, ALP activity observed the same trend like that of transaminases activity under the same conditions (Table 5). The elevations in blood bilirubin content and stimulation in GOT, GPT and ALP activities is unlikely to be due to damage in liver and RBCs (Chatterjea and Shinde, 2002). The lipotropic treatments with the present antilipidemic semi-modified diets was characterized by improvement in the three enzymes activity and bilirubin content of plasma in hyperlipidemic and hypercholesterolemic animals.

Table (5): Liver function of the experimental animal

Treatment	S.GOT		S.GPT		GOT/GPT		Bilirubin		Alkaline Phosphatase	
	U/L	%	U/L	%	Ratio	%	mg/dl	%	U/L	%
Group (1) Normal control	35±0.17[a]	100	46±3.31[a]	100	0.76[a]	100	0.47±0.03[a]	100	447±39[a]	100
Group (2) Hyperlipidemic	87±0.53[b]	249	109±6.66[b]	237	0.80[b]	105	1.06±0.07[d]	226	1557±111[b]	348
Group (3) Parsley	55±0.32[c]	157	75±4.04[c]	163	0.73[c]	96	0.65±0.04[b-c]	138	695±42[c]	155
Group (4) Apple	68±0.40[c-d]	194	80±5.12[c]	174	0.85[d]	112	0.68±0.03[c]	144.68	699±50[c]	156
Group (5) Lentil	71±0.42[d]	203	89±5.55[c]	193	0.80[c-d]	105	0.61±0.03[b]	129.79	617±39[c]	138
Group (6) Mixture	62±0.33[c]	177	78±4.17[c]	170	0.79[c]	104	0.57±0.03[a-b]	121.28	578±38[c]	129

* The same letters in each row represents the insignificant difference at $P < 0.05$.

Effects of apple, parsley, unpeeled lentil and their mixture semi-modified diets on kidneys function, and plasma protein fractions of hyperlipidemic and hypercholesterolemic male albino rats were determined and the results were statistically analyzed which summarized in Table (6). The data showed that hyperlipidemia produced a significant elevation in uric acid, urea and creatinine contents of plasma. These in creases were reduced by the treatments with the present semi-modified diets and showed significant improvements in the kidneys function. The highest treatment influence on kidneys function was observed by the mixture semi-modified diets.

On the other hands, the levels of plasma total soluble protein, albumin and globulin were significantly decreased in hyperlipidemic rats relative to those of normal healthy control. These abnormal values of plasma proteins profile was readjusted and improved by feeding on the four lipotropic semi-modified diets of the present studies. All antilipidemic diets significantly had the same improvement effects on the soluble protein profile of the experimental diseased rats. The soluble protein profile of the experimental diseased rats.

Table (6): Kidneys function and protein fraction of the experimental animals

Treatment	Urea		Creatinine		Uric acid		Total protein		Albumin (Alb)		Globulin		A/G ratio	
	mg/dl	%	mg/dl	%	mg/dl	%	g/dl	%	g/dl	%	g/dl	%	Ratio	%
Group (1) Normal control	47 ± 3.11^{a}	100	0.70 ± 0.04^{a}	100	3.5 ± 0.20^{a}	100	8.8 ± 0.51^{a}	100	3.6 ± 0.20^{a}	100	5.2 ± 0.38^{a}	100	0.69^{a}	100
Group (2) Hyperlipidaemic	89 ± 6.21^{b}	189	1.00 ± 0.06^{b}	143	6.3 ± 0.41^{b}	180	5.9 ± 0.62^{b}	67	2.8 ± 0.17^{b}	78	3.1 ± 0.21^{b}	60	0.90^{b}	130
Group (3) Parsley	58 ± 3.81^{c}	123	0.80 ± 0.03^{c}	114	4.5 ± 0.25^{c}	129	8.4 ± 0.39^{a}	95	3.9 ± 0.19^{a}	108	4.5 ± 0.28^{c}	87	0.87^{b}	126
Group (4) Apple	62 ± 3.24^{c}	132	0.78 ± 0.3^{c}	111	4.6 ± 0.24^{c}	131	8.7 ± 0.47^{a}	99	4.1 ± 0.30^{a}	114	4.6 ± 0.31^{c}	88	0.89^{b}	129
Group (5) Lentil	68 ± 4.44^{c}	145	0.84 ± 0.04^{c}	120	4.9 ± 0.31^{c}	140	8.8 ± 0.59^{a}	100	4.7 ± 0.29^{c}	131	4.1 ± 0.24^{c}	79	1.14^{c}	165
Group (6) Mixture	65 ± 4.09^{c}	138	0.74 ± 0.04^{b}	106	4.2 ± 0.19^{c}	120	8.3 ± 0.62^{a}	94	3.8 ± 0.28^{a}	106	4.5 ± 0.32^{c}	87	0.84^{b}	122

* The same letters in each row represents the insignificant difference at $P < 0.05$.

The results of the present studies of Table (7) showed that effects of hyperlipidemia on blood glucose, lipid peroxidation and antioxidative enzymes activity (catalase and SOD) in male albino rats. The data were statistically analyzed, which showed that hyperlipidemia insignificantly changed the blood glucose content. The values of blood glucose were around that of healthy normal animals. These also were observed for all experimental treatments in connection, the experimental data showed that SOD and catalase activities were significantly inhibited by hyperlipidemia and cholesterolemia. Contrary, there was a significant increase in plasma lipid peroxide level of the hyperlipidemic rats. The results also showed improvements in lipid peroxide was caused by feeding on lipotropic and antioxidative semi-modified diets of the present studies. In addition, date in Table (7) pointed out that all treatment diets alleviated the harmful influences of hyperlipidemia on the activity of catalase and SOD, but the activities were still lower – than those of normal healthy control. .

Table (7): Blood glucose, lipid peroxidation as well as the activity of superoxid dismutase and catalase

Treatment	Blood glucose		Lipid peroxide		SOD activity		Catalase activity	
	mg/dl	%	µmol/ml	%	U/ml	%	U/ml	%
Group (1) Normal control	96±6[a]	100	1.86±0.08[a]	100	481±32.01[a]	100	9.28±0.66[a]	100
Group (2) Hyper Lipidaemic	225±14[b]	234	5.01±0.31[b]	269	122±10.00[b]	25	2.00±0.12[b]	22
Group (3) Parsley	177±11[c]	184	2.49±0.09[c]	134	316±17.11[c]	66	7.51±0.52[c]	81
Group (4) Apple	159±12[c]	166	2.29±0.07[c]	123	301±18.00[c]	63	8.69±0.56[a]	94
Group (5) Lentil	167±13[c]	174	2.19±0.07[c]	118	321±20.00[c]	67	8.88±0.49[a]	96
Group (6) Mixture	170±12[c]	177	2.02±0.06[a]	109	341±21.01[c]	71	8.91±0.51[a]	96

* The same letters in each row represents the insignificant difference at $P < 0.05$.

Discussion

Apple, parsley and unpeeled lentil considered from the famous fruit, vegetable and legume in Egyptian consumption which play an important role in the Egyptian nutrition. These diets are rich in protein, fibers, minerals, vitamins and antioxidative compounds. Recently, a special interest was given to legumes protein as well as antioxidants of fruits and vegetables as a good antilipidemic anticholesterolemic and antiatherosclerosis agents. The levels of total cholesterol in blood especially lipoprotein LDL-C fraction are the most crucial health factor which are correlated with many health disorders. Liver expectancy may represents an integral response to its values too (Niu *et al.,* 2008) Dietary therapy includes several nutrients and antioxidants in the present experimental diets showed evaluated for health promotion.

Certain compounds such as pectin, phytochemicals, protein, fibers and vitamin A and C attributed the health benefits of apple. Fibers and phenolic compounds seem to be most contributing. Fibers produced a good capacity to absorb dietary fat, thus avoid its hazard on health, but the antioxidant capacity is due to the phenolic compounds of apple, which contained 1002 mg phenolic compounds/ 100 g dry weight. The analysis of them detected 23 compounds, the most abundant of which is cinnamic acid, genistein, salicylic acid, ferulic and others. These results are in agreement with those of Wolfe *et al.,* (2003), who reported the presence of several polyphenols in apple. Several articles showed a good correlation between phenolic compounds consumption and antilipidemia (Aprikian *et al.,* 2001 and Schafers *et al.,* 2004, Gosse *et al.,* 2005).

In case of parsley, this vegetable contains many natural compounds such as protein, fiber, vitamin A, β-carotene, vitamin C and folic acid as well as several phenolic compounds and others. HPLC analysis of parsley methanolic extract detected 14 phenolic compounds, the most one is genestin, then catechin and followed by small amount of phenol and *p*-coumaric. Parsley is rich in vitamin-C and β-carotene, the both had good power as antioxidant (Pattson *et al.,* 2004). Parsley reduced plasma lipid peroxides of hyperlipidemic rats and improved liver and kidneys function as well as blood picture. The enzymatic antioxidant activity and lipids profile of the diseased rats were improved. It mains that the compound present in parsley specially vitamin-C, β-carotene and phenolic compounds are of value and act to protect against diseases due to oxidative stress or disturbed lipid metabolism.

Regarding unpeeled lentil, it contains a good amount of protein β -carotene, fiber, carbohydrate as well as phenolic compounds and others. Results showed that hyperlipidemic and hypercholesterolemic animals fed on diet containing 10% unpeeled lentil (semi-modified diet) had relatively better values for parameters expressing oxidative stress (lipid proxide, enzymatic antioxidant activity, soluble protein fractions and lipid profile), liver and kidneys functions and blood picture (Hb, WBCs and RBCs). It can be reported that lentil used as therapy food against hyperlipidemia and atherosclerosis.

Analysis of phenolic compounds by HPLC showed the presence of 23 compounds. These compounds present included gallic acid catechin, chlorogenic, synergic acid, pyrogallol, rutin, naringinin, lutedin, caffic, phenol, daidzin, genistein, ferulic, cinnamic, salicylic acid, quercetin and others. The most dominate compound is pyrogallol of (52%) and chlorogenic (34%) for unpeeled lentil and genistein (20%), salicylic (14%) and cinnamic (25%) for apple but for parsley genistein (75%) catechin (8%) and phenol (5%). It is clear that these phenolic compounds detected in the unpeeled lentil with antioxidative vitamins and β-carotene are responsible for the antioxidative effect and cholesterol lowering action. These effects may be either due to retarding effect on lipids absorption or the increase in LDL – receptor – mediated cholesterol removal (Kapiotis *et al.,* 1997).

The mechanism of action especially those of the mixture (unpeeled lentil, apple and parsley) semi-modified diet may be main activity responsible for hypolipidemic activity of protein and antioxidant although other target organs (blood, liver and kidneys) cannot be discarded. The more pronounced effect of the mixture semi-modified diet than the other may be due to the synergetic effects of the three ingredients (unpeeled lentil, apple and parsley) each other which gave more effect and alleviated the hyperlipidemic drastic also improved the harmful parameter values. These may be due to that mixture was consisted of different lipotropic factors. The present suggestions are supported by the results which showed that the biological activity has been attributed to the both factors (lipotropic and antioxidative factors) either alone or synergistically. The present results are in harmony with each other. (liver and kidneys function, blood picture, lipid and protein profiles and enzymatic antioxidant activity). The stimulation of GPT activity indicated slight liver cell necrosis and the magnitude of increase correlated with the extent necrosis (Murray *et al.,* 2006).

The increases in plasma bilirubin and stimulation in transaminases activity is unlikely to be due to damage in liver and RBCs which produced under the hyperlipidemic disease (Chatterjea and Shinde, 2002). These influenced on the kidneys function by elevation blood content of uric acid, urea and creatinine. These may be due to stimulate protein metabolism which was paralleled with the decreasing in protein fractions content of blood (albumin and globulin) and inhibition in catalase and SOD activity (enzymatic antioxidant). The studied antioxidant and protein semi-modified diets alleviated the harmful influences of hyperlipidemia on these parameters. The diets protein is considered as lipotropic factors used in the protein biosynthesis including plasma enzymes which stimulated the antioxidative power. Also, antioxidant compounds that are believed to possess strong antioxidative property, including its capability to scavenge or prevent several reactive oxygen species and inhibited lipid peroxidation (Kaplan *et al.,* 2001 and Li *et al.,* 2006).

Generally, among the factors effected on LDL-C and HDL-C are proteins and antioxidants. The LDL-C value was around 4 times that of normal in hyperlipidemic rats. This may be due to a lack in total cholesterol distribution upon higher dietary level of fats. Also, the whole – body sterol (cholesterol and xenosterol) balance is delicately regulated by the gastrointestinal tract and liver, which controls sterol absorption and excretion in contribution to the cholesterol pool by whole – body cholesterol synthesis (Kidambi and Patel, 2008). The atherogenic ratio (risk factor) had been elevated in diseased rats to more than 3 times at that of control, but in the feeding on treatment semi-modified diets, the elevated value was decreased to around that of control. These may be due to the most variable food source combination of antioxidant and protein as semi-modified foods, the results showed that dietary cholesterol is a risk factor for the progression to hepatic inflammation (Weuters *et al.,* 2008).

Enzyme diagnosis is frequently used for liver function assessment. In general hyperlipidemia and hypercholesterolemia significantly stimulated especially GOT, GPT and ALP (Table 5). All semi-modified diets reduced this reverse effect with much more power effect for the mixture diet which contained protein with anti oxidative compounds included vitamins A and C as well as β-carotene and others. Yet, vitamin C fulfills both anti oxidative functions and metabolic as ones as it helps in the formation of collagen structure (Biesalski and Tinz 2008). Lin *et al.,* (2005) reported that antioxidative compounds role is most respect among all other protective factors. In another wards, feeding systems containing a particular substance is vital, a single nutrient may not work alone. This may suggests that the oxidant defense mechanisms in which these antioxidant nutrients function are sometimes independent of one another despite fighting in different areas (Burk *et al.,* 2008). The present results might add that protein and vitamin C, A and β-carotene needed a parallel phenolic compounds supplementation to perform their action as antioxidative system. It is a matter of dietary interaction that enhances the targeting of potential nutrient and medical elements (Villaverde *et al.,* 2008). The antioxidant fractions may control some specific enzymes or moreover do some up regulatory role. For example, anti oxidants significantly down – regulated the mRNA of HMG-CoA reductase (the key enzyme for cholesterol biosynthesis) and up-regulated the mRNA of cholesterol-7-alpha–hydroxylase (the key enzyme for cholesterol metabolism) in primary rat hepatocytes. These indicated that potential of antioxidant in reducing the risk of atherosclerosis through multi-mechanisms.

Accordingly, dietary therapy may act up regulatory, a result suggests that the expression of cholesterol 7 – alpha- hydroxylase gene may be directly regulated by catechins at the transcriptional level (Lee *et al.,* 2008). In case of proteins application, the packaging had to resist gastric digestion and the body must be able to absorb the particles through the intestinal mucosa without hydrolyzing the proteins in order for them to reach the systemic circulation (Biesalski and Tinz. 2008). Some proteins can promote the action of antioxidant system or even act itself as specific hormonal balancer (Ahmed *et al.,* 2003).

Liver in a special antagonistic cooperation of these hormones may mediate this control of fat homeostasis. Hepatic calls seemed to be the biological machine that control fat metabolism. When these cells are supported with special dietary antioxidant or protein of functional role, cholesterol homeostasis may obtain. Thus, the accumulation of vitamins or storage of vitamin micronutrients in single tissues is not limited to a pure storage process like the storage of vitamin A in the liver, but is often connected with important and tissue – specific metabolic functions (Ahmed et al.,, 2005). In addition, the protein moiety devoid of isoflavone compounds is likely to be responsible for this major biochemical effect of protein. Data obtained in the present studies shows a possible metabolic cross road for both antioxidant and protein in controlling cholesterolemia and liver compression of pathological disorder as well (Ahmed *et al.,* 2007).

Therefore the present studies pointed out that such food items can be used for healing of symptom due to dietary regimen that induced hyperlipidemia and hypercholesterolemia as well as atherosclerosis Also, eating semi-modified food such as apple, parsley and unpeeled lentil could protect to a certain extent against the occurrence of atherosclerosis. More studies are required to understand the interaction mechanism between protein lipotropic factors and antioxidants as hypolipidemic and anti atherosclerosis agents.

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استخدام العدس والتفاح والبقدونس في أغذية معدلة جزئياً لتخفيف وتحسين حالة مرض زيادة الليبيدات والكوليسترول في الفئران البيضاء

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استهدفت هذه الدراسة تقييم تأثير بذور العدس (بقول) والتفاح (فاكهة) والبقدونس (خضر) في الأغذية المعدلة جزئياً على مكونات الليبيدات في الفئران البيضاء المصابة بمرض زيادة الليبيدات والكوليسترول وذلك بالاعتماد علي محتوى هذه الأغذية من بروتينات ومركبات فينولية والتي تعمل كعوامل خافضة لليبيدات وكمضادات للأكسدة في تلك الحيوانات المصابة . وقد لوحظ انخفاض مكونات الليبيدات في هذه الحيوانات المصابة بالإضافة لتحسين محتوى الدم منها نتيجة فعل مضادات الأكسدة وخافضات الليبيدات في هذه الأغذية وكذلك حماية الحيوانات من هدم وأكسدة جدر الخلايا والأوعية مما يؤدي لتخفيف حالة تصلب الشرايين وقد تم إجراء هذا البحث باستخدام بذور العدس الكاملة (بدون تقشير) وثمار التفاح المجففة وكذلك البقدونس المجفف بالإضافة لمخلوط من هذه الأغذية الثلاثة بنسبة 1:1:1 كأغذية معدلة جزئياً لتقييم تأثيراتها المضادة لزيادة محتوى الجسم من الدهون والكوليسترول وذلك بنسبة 10% من غذاء الفئران على حساب محتوى الغذاء من النشا . وبالطبع تم دراسة التركيب الكيميائي للتفاح والبقدونس والعدس بدون تقشير حيث أظهرت النتائج أن هذه الأغذية تحتوي على نسبة عالية من البروتين [خصوصاً العدس] والألياف الخام والمركبات الفينولية (خصوصاً التفاح والبقدونس) كما أن الدراسة باستخدام HPLC لمستخلص كحول الميثايل لهذه الأغذية الثلاثة من المركبات الفينولية أظهرت وجود حوالي 25 مركب تختلف فيما بينهما من ناحية النوع والمحتوى مما يعني أن هذه الأغذية خصوصاً التفاح والبقدونس تعتبر من المصادر الجيدة للمركبات الفينولية [مضادات الأكسدة] وقد لوحظ أن مركب الكاتاشين موجود في المستخلصات الثلاثة(عدس وتفاح وبقدونس) ولكن الفينول والديازين والكوماريك والجستين والفيورليك والكيورستين والكريسين الجالانجين موجود في المستخلص الميثانولي للتفاح والبقدونس أما مركب الروتين موجود في مستخلص

العدس والتفاح وهناك مركبات فينولية أخرى لوحظ تواجدها في كل مستخلص على حده تختلف نسبتها فيما بينها [مستخلص التفاح والبقدونس والعدس] مثل البيروجالول والكوروجينك والسينرجيك والنارينجنين والليولين حيث لوحظ تواجده في مستخلص العدس فقط كما أن مركبات الهيدروكسي بنزوبك والكافيك والكامفيرول لوحظ تواجدها في البقدونس فقط أما المركبات الفينولية حامض الساليسليك والسيناميك والإيجانول والبنوستروبين فقد لوحظ تواجدها في المستخلص الميثانولي للتفاح فقط وأظهرت نتائج هذه الدراسة أن الأغذية المعدلة جزئياً للعدس والتفاح المجفف والبقدونس المجفف له قوة مضادة لزيادة الليبيدات والكوليسترول من شأنها تخفيف المرض وتحسين حالة جسم الحيوانات المصابة.

كما أن هذه الأغذية المعدلة جزئياً عموماً تحسن حالة الدم الطبية من محتواه من كرات الدم الحمراء والبيضاء ومحتواه من الهيموجلوبين كذلك محتوى الدم من الليبيدات الكلية والكوليسترول والجلسريدات الثلاثة كذلك مكونات الليبوبروتينات من العالية الكثافة والمنخفضة الكثافة والشديدة انخفاض الكثافة كذلك فإن هذه المعاملات العلاجية تخفف من قيمة معامل الخطورة في الفئران المصابة بزيادة ليبيدات وكوليسترول الدم. كما لوحظ أن التغذية على هذه الأغذية المعدلة جزئياً تحسن من كفاءة نشاط الكبد (إنزيمات نقل الأمين ومحتوى الدم من البيليروبين) كذلك نشاط الكيلتين (محتوى الدم من اليوريا وحامض البوليك والكرياتنين) بالإضافة لمكونات بروتينات الدم (البروتين الكلي والألبيومين والجلوبيولين) وتقلل الأكسدة الفوقية للبيدات وتحسن نشاط الإنزيمات المضادة للأكسدة حيث لوحظ أن قيمة جميع هذه التقديرات تحت الدراسة كان قريب من مثيلاتها في الفئران السليمة وارتباطاً بذلك فإن فعل العلاج بمخلوط العدس والتفاح والبقدونس أظهر أحسن النتائج وأكثرها فاعلية مقارنة بباقي المعاملات (كل مكون علي حده) وذلك لأن مكونات مخلوط للغذاء المعدل جزئياً تظهر نوع من تنشيط المكونات بعضها البعض كمضادة لزيادة الليبيدات والكوليسترول ويمكن ترتيب فعل تلك الأغذية المعدلة جزئياً حسب فعلها وقوتها التأثيرية كما يلي:-

مخلوط الأغذية > العدس الكامل > البقدونس المجفف > التفاح المجفف