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CONSTITUENTS OF APPLE, PARSLEY AND LENTIL EDIBLE PLANTS AND THEIR THERAPY TREATMENTS FOR BLOOD PICTURE AS WELL AS LIVER AND KIDNEYS FUNCTIONS AGAINST LIPIDEMIC DISEASE

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ABSTRACT

The present investigation was undertaken to evaluate the effects of unpeeled lentil seeds, apple (fruits), and parsley (vegetable) on rats suffering from hyperlipidemia and hypercholesterolemia. The studies were based on the three used edible plants amounted good values of protein (especially lentil), crude fiber and phenolic compounds (especially apple and parsley) which possess as lipotropic and antioxidant agents. 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. The treatments of the semi-modified diet with unpeeled lentil, dried apple and parsley also their mixture observed hypocholesterolemic and hypolipidemic power which alleviated the disease and improved functions of liver, kidneys and blood picture but observed insignificant effects on the blood glucose levels of the experimental animals. The obtained results showed that the semi-modified diets generally improved in the clinical blood status. Picture of blood (RBCs, WBCs and Hb), liver function (GOT, GPT and ALP activity as well as bilirubin) and kidneys function (uric acid, urea and creatinine) were improved in diseased rats treated with the semi-modified diets.

KEYWORDS

Parsley, unpeeled lentil, apple, blood picture, liver functions, kidneys functions, phenolic compounds, lipidemic disease.

INTRODUCTION

Hyperlipidemia produces atherosclerosis induced by dietary fatty diet with cholesterol. These have been reported by several investigations in animals (Lovejoy et al., 2002). Also, there are strong relationships between plasma lip levels and atherosclerosis, particularly of the coronary arteries that is of major importance. Atherosclerosis is spectrum of arterial reaction that may result from many factors acting upon the vessel wall and producing their effects through different mechanisms in different subjects which may vary significantly from one to another. Coronary atherosclerosis in primates not only resembles human lesions but can even produce myocardial infraction (McBride et al., 1998). According to the World Health Reports, more than 75% of cardiovascular diseases, the worlds leading cause of premature death result from high blood cholesterol as well as other factors such as smoking, drinking alcohols and high blood pressure (WHR, 2002). From the clinical view point, the studies need to look for natural compounds that can help the diseased to get rid and reduce the health hazard of the diseases as a care that they receiving (Turnbull, 2005). Legume seeds utilized for human consumption have been of great interest to nutritionists and agricultural scientists in providing mankind with nutritional diets particularly when protein deficits occur. Lentil seeds are not only a good choice for nutrition, but also recently, the potential role of it in hyperlipidemia has received attention. Phenols are of the major groups of nonessential dietary compounds appearing in fruit and vegetable food and they play an important role as defense compounds (El-Beltagi and Mohamed, 2010). Phenols have traditionally been considered as anti nutritive compounds (tannins) on protein digestibility. However, actually there is an increased interest in these compounds because they have been associated with the inhibition of atherosclerosis and cancer (Martine et al., 2000). Oxidative stress produces a modification of DNA, protein and lipids by reactive oxygen species (ROS) and other free radicals which play a role in aging and disease, including hyperlipidimia, atherosclerosis and cardiovascular disease. The bioactivity of phenolic may be related to their antioxidant behavior which is attributed to their ability to chelate metals, inhibit lipoxygenase and scavenge free radicals (Scalbert et al., 2005 and Mohamed et al., 2010).

The main aim of the present work, 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, liver function and kidneys function.

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).

All data pooled through the present studies were preceded 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

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	Un Peeled lentils		Parsley		Apple	
	Fresh	Dray	Fresh	Dray	Fresh	Dray
Water	11.5±3.33	———	84.6±5.19	———	85±6.00	———
Protein	22.9±6.27	25.9±1.79	3.20±0.20	20.8±1.78	0.4±0.03	2.58±0.19
Fat	0.70±0.03	0.79±0.04	0.40±0.02	2.60±0.17	0.2±0.01	1.29±0.10
Ash	2.30±0.17	2.60±0.18	2.10±0.17	13.6±0.87	0.6±0.04	3.87±0.21
Fiber	2.10±0.15	2.37±0.17	1.30±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	51.9±3.42	13.3±0.97	85.8±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).

Table 2: Total phenols content of dried apple, dried parsley and dried lentil.

Plants	Total phenols content (mg/100g. D.W.)
Dried Apple	1002
Dried parsley	751
Dried lentil	376

Plant antioxidant produced a good antilipidimic 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 3) 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.

Table 3: HPLC analysis of polyphenols in methanolic extract of the apple, parsley and lentil Cinnamic was amounted about 26% of apple total phenolic compounds. This main that

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	———	———	———	———

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 (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 antilipidemic 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 4: Hemoglobin, red blood cells and white blood cells of the experimental animal blood.

Treatments	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±0.20[a]	100	4.1±0.21[a]	100	6.37±0.25[e]	100
Group (2) Hyperlipidaemic	12.3±0.36[c]	88	3.4±0.27[c]	83	16.3±0.45[a]	256
Group (3) Parsley	12.5±0.38[c]	90	3.67±0.33[bc]	90	8.7±0.26[c]	137
Group (4) Apple	12.2±0.48[c]	88	3.48±0.24[bc]	85	9.2±0.31[b]	144
Group (5) Lentil	12.4±0.46[c]	89	3.7±0.35[bc]	90	7.7±0.48[d]	121
Group (6) Mixture	13.1±0.42[b]	94	3.84±0.35[ab]	94	8.4±0.38[c]	132
LSD 5%	0.467		0.35		0.43	

[a, b, c,.] Means within column different letter differ significantly (P> 0.5) from each other

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.

Treatments	S.GOT		S.GPT		GOT/GPT		Bilirubin		Alkaline Phosphatase	
	U/L	%	U/L	%	Ratio	%	mg/dl	%	U/L	%
Group (1) Normal control	35±4.03[e]	100	46±3.85[d]	100	0.77[b]	100	0.47±0.06[d]	100	447±18[d]	100
Group (2) Hyperlipidemic	87±6.88[a]	249	109±8.83[a]	237	0.80[ab]	105	1.06±0.10[a]	226	1557±95[a]	348
Group (3) Parsley	55±6.34[d]	157	75±4.76[c]	163	0.74[b]	96	0.65±0.04[b]	138	695±66[b]	155
Group (4) Apple	68±5.96[bc]	194	80±3.76[c]	174	0.86[a]	112	0.68±0.06[b]	145	699±64[b]	156
Group (5) Lentil	71±4.83[b]	203	89±4.30[b]	193	0.80[ab]	105	0.61±0.05[bc]	130	617±37[c]	138
Group (6) Mixture	62±4.38[c]	177	78±3.74[c]	170	0.80[ab]	104	0.57±0.04[c]	121	578±30[c]	129
LSD 5%	6.49		6.12		0.1		0.072		68.21	

[a, b, c,.] Means within column different letter differ significantly (P> 0.5) from each other

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 increases 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.

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

Treatment	Urea		Creatinine		Uric acid	
	mg/dl	%	mg/dl	%	mg/dl	%
Group (1) Normal control	47±2.45^{e}	100	0.70±0.06^{d}	100	3.5±0.40^{d}	100
Group (2) Hyperlipidaemic	89±6.55^{a}	189	1.00±0.13^{a}	143	6.3±0.44^{a}	180
Group (3) Parsley	58±3.03^{d}	123	0.80±0.04^{bc}	114	4.5±0.36^{bc}	129
Group (4) Apple	62±3.01^{cd}	132	0.78±0.03^{bcd}	111	4.6±0.40^{bc}	131
Group (5) Lentil	68±5.11^{b}	145	0.84±0.04^{b}	120	4.9±0.37^{b}	140
Group (6) Mixture	65±3.31^{bc}	138	0.74±0.04^{cd}	106	4.2±0.51^{c}	120
LSD 5%	4.91		0.077		0.49	

$^{a, b, c,.}$ Means within column different letter differ significantly (P> 0.5) from each other

Legumes, fruits and vegetables play important role in human nutrition since they are rich source of proteins, fibers, calories, certain minerals, vitamins and antioxidant agents. Certain compounds such as pectin, phytochemicals, protein, fibers and vitamin A and C attributed the health benefits of unpeeled lentil, parsley and apple. Fibers and phenolic as well as protein 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). In addition, general the 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 is 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 stimulation of GPT activity indicated slight liver cell necrosis and the magnitude of increase correlated with the extent necrosis (Murray et al., 2006). 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 are in harmony with each other. Thus the stimulation of transaminases activity and bilirubin content in blood of the diseased rats, which largely was used as indicator for liver function. Also, blood uric acid urea and creatinine (Table 6) confirmed each other and also the peripheral mechanism of the antilipidemic action of the present treatments especially the mixture maybe due to the main activity responsible for the prevent activity of against the hyoercholestrolemic disease of protein and antioxidants, although other target organs (liver and kidneys) cannot be discarded. The more pronounced effect of the mixture than the others semi-modified diet alone maybe due to the synergetic effect of the three edible diets each other. These maybe due to that, the mixture was constituted of lipotropic factors such as protein of lentil and antioxidative agents of apple and parsley.

From the present results it can be reported that the use of lentil as therapy food against lipidemia as well as choleserolemia which had a large improved effects on blood picture and functions of liver and kidneys in the present studies. The antioxidative activity of apple and parsley is suggested to be due to the capacity of phenols to transfer electron anion free radicals, chelate metal catalyst, active antioxidative enzymes as reported in our studies (Abdel-Rahim et al., 2009) and inhibit oxidases (Spencer et al., 2001). There, further of studies are required to evaluate the biochemical effects and mechanism of the studied lipotropic factors and antioxidant agents as antilipidemic and anticholestrolemic agents which maybe in edible food to recommend their use as hypolipidemic food additives.

CONCLUSIONS

The present data revealed that, the use of lentil as therapy food against lipidemia as well as choleserolemia which had large improved effects on blood picture and functions of liver and kidneys in the present studies. The antioxidative activity of apple and parsley is suggested to be due to the capacity of phenols to scavenge free radicals, chelate metal catalyst, and active antioxidative enzymes which play a role in aging and disease, including hyperlipidimia, atherosclerosis and cardiovascular disease.

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