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Feeding Sprague Dawley Rats With Jordanian Wild Edible Plants and a High Fat Diet Reduced the Malondialdehyde Levels

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Abstract

The objective of this study was to determine the effect of selected Jordanian wild edible plant on lipid peroxidation and lipid profile in adult male Sprague Dawley rats fed high-fat diet. Fiftysix male, adult Sprague-Dawley rats at eight weeks of age, weighing about 200g were distributed into 7 experimental groups, 7 rats each . The groups included a negative control group that was fed a normal fat diet (NFD) and a possitve control group that was fed a high fat diet (HFD) (45% calories from fat). The six treatment groups were fed a HFD for the first 4 weeks of the experiment and a HFD with 9% of one of the selected dried plants for another 4 weeks. The treatment groups are sumac, thyme, clary, gundelia, garden rocket and wild mint. Blood samples were collected from the right heart ventricle. Serum malondialdehyde, lipid profile and fasting blood glucose were measured for rats. Results showed that the addition of different dried plant powders to the HFD did not significantly affect serum levels of TG, TC, HDL, LDL and fasting blood glucose. On the other hand, malondialdehyde (MDA) levels were significantly ($p < 0.05$) higher in the HFD group (4.09±0.45 mmol/ml) than those of other groups. MDA serum levels for the other groups were as follows: NFD (2.47±0.05), sumac (2.45±0.13), thyme (2.88±0.07), clary (2.97±0.16), garden rocket (2.96±0.11), gundelia (2.92±0.16) and wild mint (2.68±0.09). These levels were not sinificantly different from each other. It is concluded that incorporating dried plant powders in rat diets had a significantly positive effect only on lipid peroxidation assay as indicated by serum MDA levels.

Keywords: antioxidants, wild edible plants, oxidative stress, malondialdehyde, high fat diet

1. Introduction

Oxidative stress could be defined as a state of imbalance between the production and neutralization of free radicals in the body (Kratchanova et al., 2014). It is a major factor for several degenerative and inflammatory diseases (Tawaha et al., 2007). Free radicals are atoms, molecules, or ions with unpaired electrons that are highly unstable and active toward chemical reactions with other molecules such as reactive oxygen species (ROS) (Lu et al., 2010). The main target molecules of ROS are nucleic acids, proteins, sugars and lipids (Craft et al., 2012).

The oxidation of polyunsaturated fatty acids (PUFAs) in biological systems by free-radicals is known as lipid peroxidation (Esterbauer & Cheeseman, 1990). The main primary products of lipid peroxidation are lipid hydroperoxides (Ayala et al., 2014). Many different aldehydes can be formed as secondary products during lipid peroxidation such as malondialdehyde (MDA), propanal, hexanal, and 4-hydroxynonenal (4-HNE) (Ayala et al., 2014). MDA has been widely used for many years as a convenient biomarker for lipid peroxidation because of its reaction with thiobarbituric acid (TBA) (Esterbauer & Cheeseman, 1990).

Antioxidants may be defined as compounds that can significantly delay or inhibit the oxidation process of lipid or other molecules and protect against free radicals by inhibiting the initiation or propagation of oxidizing chain reactions (Volf, 2014). Antioxidants are important in terms of their ability to protect against oxidative cell damage that can lead to several chronic diseases (Rajendran et al., 2014). Various types of antioxidants are present in the diet to maintain free radical concentrations at low levels (Carocho & Ferreria, 2013).

Dietary polyphenols are among the most important groups of natural antioxidants that are found in human diet. Epidemiological, clinical and nutritional studies strongly support the evidence that dietary phenolic compounds enhance human health by lowering risk and preventing the onset of degenerative diseases (Scalbert et al., 2005). Sumac, thyme, clary, garden rocket, gundelia and wild mint are among the sources of polyphenols that are consumed in Jordan.

Rhus coriaria L. belongs to Anacardiaceae family (Ali-Shtayeh et al., 2014; Al-Eiswi & Takruri, 1989). Traditionally it has been used in the treatment of diarrhea, ulcer, hemorrhoids, liver disease, dysentery, diuresis, hemorrhage, hematemesis, hemoptysis, ophthalmia, conjunctivitis, leucorrhea, and as stomach tonic (Shabbir, 2012).

Thyme (*Origanum syriacum* L.) belongs to the Lamiaceae family (Husein et al., 2014; Al-Eiswi & Takruri, 1989). It has been widely used in the traditional herbal medicine (Ayesh et al., 2014).Thyme is included in the list of The United States Food and Drug Administration (FDA) which contains more than 150 plants that present essential oils, oleoresins (solvent-free), and natural extractives (including distillates) that are safe for human consumption without limitations on intake (Costa et al., 2015).

Clary (*Salvia Judaica* Boiss.), also called Judean sage, is from Lamiaceae family (Al-Ismail et al., 2007; Al-Eiswi & Takruri, 1989). In Jordan, it grows in different areas of the country and is collected to be stuffed with rice, meat, oil and spices, then it is cooked and used in the Jordanian diet (Tukan et al., 1998).

Garden rocket (*Eruca sativa* Mill.) is a member of the Cruciferae family (Ali-Shtayeh et al., 2014; Al-Eiswi & Takruri, 1989). The leaves of this plant are traditionally used as antiscorbutic, diuretic, stimulant and stomachic. The rocket seeds oil also has glucosinolate methyl sulphinyl butyl isothiocyanate which induces enzymes activity (Gulfraz et al., 2011). The phenolic compounds in the seeds have antimicrobial properties against pathogenic bacteria while tannins are reported to have antiviral, antibacterial and antitumor activity (Khoobchandania et al., 2010).

Gundelia (*Gundelia tournefortii* L.) belongs to the Asteraceae (Compositae) family (Ali-Shtayeh et al., 2014; Al-Eiswi & Takruri, 1989). Dry seeds of *G.tournefortii* are known to be effective for the treatment of vertigo disease, in Eastern Anatolia folk medicine (Evin, 2012). Fresh seeds of *G. tournefortii* are used in pickles and also are effective diuretics (Evin, 2012). Latex obtained from roots of *G.tournefortii* is used externally (Evin, 2012). In Jordan it is fried with egg or cooked with meat to be consumed in the Jordanian diet (Tukan et al., 1989).

Wild mint (*Mentha longifolia* (L.) Hudson) is a member of the large mint family Lamiaceae (Ali-Shtayeh et al., 2014; Al-Eiswi and Takruri, 1989). Milk or water decoctions of wild mint leaves were used traditionally for coughs, colds, asthma and other bronchial illnesses. It has also been used to treat headaches, fevers, indigestion, flatulence, hysteria, painful menstruation, delayed pregnancy and urinary tract infections (Viljoen et al., 2006).

Therefore, the objective of this study was to determine the antioxidant capacity of these plants by using malondialdehyde as an indicator.

2. Materials and Method

2.1 Animals and Experiment Diets

The research was approved by the ethics committee of the Department of Nutrition and Food Technology for Animal Experimentation/ The Univarsity of Jordan. Sixty four adult male Sprague Dawley rats, weighing around 200 g were divided into 8 groups; negative control (G1), positive control (G2) and six treatment groups (G3, G4, G5, G6, G7, G8). The rats were housed individually in plastic cages with wire mesh and a plastic tray underneath under controlled conditions of: temperature (22±2 °C), relative humidity (49±5%) and hygienic conditions in ventilated room and a 12-12 hours' light- dark cycle. The animals were acclimatized for one week during which they were fed standard chow and tap water ad libitum. Weight gain and food consumption were monitored weekly.

The experimental diet mixtures were prepared according to the guidelines of American Institute of Nutrition 1993 for adult animals (AIN-93M) (Reeves, 1997). These diet mixtures used in the experiment were as follows; standard AIN-93M diet; high fat diet (HFD) which was a modified AIN-93M diet for the fat (45% of the calories from fat); HFD with 9% sumac; HFD with 9% thyme powder; HFD with 9% clary powder; HFD with 9% garden rocket powder; HFD with 9% gundelia powder and HFD with 9% wild mint powder. The plants powders were obtained by sun-drying of the fresh plants, grinding them using a domestic coffee mill. The composition of the diet mixtures is shown in Table 1.

G1 received standard AIN-93M diet for the 8 weeks period and G2 was fed HFD for the 8 weeks period while G3, G4, G5, G6, G7 and G8 were fed HFD for the first 4 weeks followed by HFD with 9% plant powder for another 4 weeks. Weight gain and food consumption were monitored weekly. At the end of the experiment, the rats were sacrificed and blood samples were collected, after 8 hours fasting by cardiac puncher, in plain separate gel tubes. Then the samples were centrifuged at 3000 rpm for 10 minutes (HERMLE Z200A centrifuge, Labor Technik, Wehingen, Germany). Finally serum samples were stored at -18 °C until analysis.

Table 1. Experimental diets composition

Ingredient	Normal diet	HFD	Sumac	Thyme	Garden rocket	Clary	Gundelia	Wild mint
Corn starch (g)	612.5	341.7	277.7	302.4	304.4	308.2	297.8	307.4
Egg white (g)	140	172.5	170.3	159.7	162.1	160.1	166.2	158.2
Sucrose (g)	100	125	125	125	125	125	125	125
Fiber(g)	40	64.9	55.5	50.9	50.2	52	53.3	48.2
Soybean oil (g)	40	50	43.5	46.5	48.3	48.2	48.5	47.8
Sheep tallow (g)	-	187.9	187.9	187.9	187.9	187.9	187.9	187.9
Choline (g)	2.5	3	3	3	3	3	3	3
Vitamin mix (g)	10	10	10	10	10	10	10	10
Mineral mix (g)	35	35	35	35	35	35	35	35
Biotin premix$_1$ (g)	10	10	10	10	10	10	10	10
TBHQ$_2$ (mg)	8	47.58	47.58	47.58	47.58	47.58	47.58	47.58
Plant (g)	-	-	90	90	90	90	90	90
Total (g)	1000	1000	1008.06	1020.35	1025.84	1029.47	1026.71	1022.40
Kcal/g $_3$	3.8	4.7	4.7	4.6	4.6	4.6	4.6	4.6

Note. Biotin pre-mix composed of 130 mg of biotin with 999.87 g of corn starch. TBHQ: tertiary butylhydroquinone; Kcal/g: kilo calorie per gram diet.

2.2 Lipid Peroxidation Assay

Serum malondialdehyde (MDA) was determined according to thiobarbituric reactive substance assay (TBARS) described by Esterbabuer and Cheeseman, (1990) which is based on the reaction of MDA with thiobarbituric acid (TBA) producing a pink pigment.

For that, 0.5 ml of the serum sample was mixed with 2.5 ml 10% trichloroacetic acid solution in a centrifuge tube, then the tube was placed in a boiling water bath for 15 min. After that it was cooled in cold water and centrifuged at 3000 rpm for 5 min using (HERMLE Z200A centrifuge, Labor Technik, Wehingen, Germany). Then 2 ml of the supernatant was added to 1 ml of 0.67% TBA solution in a test tube and placed in a boiling water bath for another 15 min and finally cooled in tap water. The absorbance was measured at 532 nm using UV/Visible spectrophotometer (Lambda 3b, PERKIN ELMER, USA) against a sample blank of distilled water.

Serum lipids and fasting blood glucose were measured in a Medical Laboratory (Mega Lab, Amman, Jordan). An automated clinical analyzer (Mindray BS 380, China) was used for the analysis.

2.3 Statistical Analysis

Statistical analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS) version 19. Analysis of variance (ANOVA) along with LSD test were used to determine any significant differences among the variable means of the experimental groups. The data was presented as mean±SEM. P value of less than 0.05 was considered significant.

3. Results

3.1 Weight Gain, Food Intake and Food Efficiency Ratio (FER)

Table 2 shows the mean initial and final body weights, weight gain, accumulative food intake and food efficiency ratio for the groups. Initial body weights among the different groups were close to each other with no significant differences. Final body weight values among experimental groups were also not significantly different from each other ($p > 0.05$). For weight gain, it also was not significantly different among the studied groups ($p > 0.05$). Accumulative food intake (g) of NFD group (1096.47±118.6) was significantly higher than that of the other experimental groups ($p < 0.05$), while there were no significant differences among the other

groups. Regarding food efficiency ratio (g/100 g), there were no differences among the studied groups ($p > 0.05$).

Table 2. Initial and final body weights, body weight gain, accumulative food intake and food efficiency ratio among experimental groups for eight weeks*

Group (Diet)	Initial weight (g)**	Final weight (g)	Weight gain (g)	Accumulative food intake (g)	Food efficiency ratio (g/100g)***
G1 (NFD)	195.03±7.11[a]	355.64±12.70[a]	160.61±6.84[a]	1096.47±44.82[a]	14.65±0.76[a]
G2 (HFD)□	202.44±8.65[a]	338.97±16.03[a]	136.53±14.16[a]	869.01±25.56[b]	15.71±1.56[a]
G3 (Sumac) †	197.63±8.34[a]	323.29±16.81[a]	125.66±11.66[a]	844.94±48.17[b]	14.96±1.34[a]
G4 (Thyme) †	197.70±4.68[a]	331.71±8.08[a]	134.01±9.10[a]	879.94±25.21[b]	15.25±1.00[a]
G5 (Clary) †	196.94±4.82[a]	328.57±7.94[a]	131.63±8.08[a]	841.51±31.90[b]	15.70±0.95[a]
G6 (Garden rocket) †	201.10±5.62[a]	356.47±7.70[a]	155.37±10.30[a]	905.93±31.93[b]	17.34 ±1.43[a]
G7 (Gundelia) †	196.40±9.56[a]	345.16±9.68[a]	148.76±13.30[a]	912.06±32.04[b]	16.34±1.50[a]
G8 (Wild mint) †	201.56±6.28[a]	348.64±14.96[a]	147.09±11.34[a]	887.83±26.82[b]	16.62±1.26[a]

Note. NFD: normal fat diet; HFD: high fat diet. * Values are presented as mean±SEM. Different superscript letters in the same column indicate significant difference at $p < 0.05$; ** Initial body weight was measured after one week acclimatization; *** Food efficiency ratio = body weight gain (g)/100 g food intake; □High fat diet: 45% of calories from fat; † Dried plants comprise 9% of the diet (90 g/kg diet).

3.2 Serum Glucose, Lipid Profile and Lipid Peroxidation Assay

Table 3 shows the lipid profile, serum glucose and lipid peroxidation assay for rats blood serum among different experimental groups at the end of the experiment.

Garden rocket group had the lowest fasting glucose value (163.83±13.92) followed by gundelia group (164.17±21.28), sumac group (166.00±4.95), clary group (169.33±9.37), wild mint group (170.00±9.47), NFD group (174.17±17.22), thyme group (174.50±4.43) and finally HFD group which had the highest fasting glucose value (176.50±17.67). However, There were no significant differences in serum fasting glucose (mg/dL) among all of the studied groups ($p > 0.05$).

Regarding TG levels (mg/dL), there were no differences among the groups ($p > 0.05$). The values were ranged from (113.60±19.24) for the clary group to (150.83±54.99) for the gundelia group. Also, there were no significant differences in TC serum levels (mg/dL) among the groups. TC values were ranged from (64.33±7.97) for the clary group to (79.25±10.69) for NFD group.

Similarly, serum levels of HDL (mg/dL) for different experimental groups were close to each other, with no significant differences among them ($p > 0.05$). HDL levels were ranged from (45.86±3.98) for NFD group to (49.71±7.05) for the thyme group. For LDL levels (mg/dL), there were no significant differences among the groups. The values of LDL were ranged from (5.93±4.12) for thyme group to (13.90±1.41) for HFD group.

However, MDA concentration (nmol/mL) of HFD group (4.09±0.45) was significantly higher than that of the other experimental groups. MDA concentrations of sumac group (2.45±0.13), NFD group (2.47±0.15), wild mint group (2.68±0.23), thyme group (2.88±0.18), clary group (2.97±0.43), garden rocket group (2.96±0.29) and gundelia group (2.92±0.43) were close to each other, with no significant differences among them ($p > 0.05$).

Table 3. Biochemical analysis for rats fed different experimental diets for eight weeks *

Group	Blood glucose (mg/dL)	Blood Lipids (mg/dL)				MDA (nmol/ml)
		TG	TC	HDL	LDL	
G1	174.17±7.03[a]	127.40±4.41[a]	79.25±4.04[a]	46.00±1.96[a]	9.65±1.70[a]	2.47±0.05 [b]
G2	176.50±7.22[a]	133.83±25.41[a]	72.00±4.21[a]	48.00±2.66[a]	13.90±0.58[a]	4.09±0.17[a]
G3	166.00±4.95[a]	116.40±6.04[a]	65.00±3.48[a]	47.17±2.51[a]	8.75±1.95[a]	2.45±0.13 [b]
G4	174.50±2.22[a]	141.33±21.88[a]	72.17±4.99[a]	49.71±2.68[a]	5.93±1.68[a]	2.88±0.07 [b]
G5	169.33±3.83[a]	113.60±8.61[a]	64.33±3.25[a]	46.71±1.74[a]	10.23±2.84[a]	2.97±0.16 [b]
G6	163.83±5.68[a]	130.14±11.76[a]	70.14±1.57[a]	45.86±1.50[a]	8.22±1.01[a]	2.96±0.11 [b]
G7	164.17±8.69[a]	150.93±22.45[a]	68.67±4.20[a]	48.14±2.33[a]	9.04±1.81[a]	2.92±0.16 [b]
G8	170.00±3.86[a]	138.67±7.27[a]	73.50±4.09[a]	47.29±2.68[a]	12.96±2.93[a]	2.68±0.09 [b]

Note. G1: normal fat diet group (negative control); G2: high fat diet group (positive control); G3: sumac group; G4: thyme group; G5: clary group; G6: Garden rocket group; G7: Gundelia group; G8: wild mint group; NFD: normal fat diet; HFD: high fat diet; MDA: malondialdehyde. * Values are represented as mean±SEM. Different superscript letters in the same column indicate significant difference at $p < 0$.

4. Discussion

Dyslipidemia refers to disruption of lipid metabolism which is characterized by increased LDL and triglyceride serum concentrations that are often accompanied by decreased HDL levels (Wazaify et al., 2013). Oxidative stress and ROS is closely associated with dyslipidemia and the formation of oxidized LDL which stimulates the release of more ROS and promotes platelet formation (Singh et al., 2015). ROS also could oxidize lipids leading to the formation of MDA (final product of lipid peroxidation) which is known to be a marker of oxidative damage (Ayala et al., 2014).

Wild edible plants are considered as a source of bioactive components and natural antioxidants which may contribute to the reduction of oxidative stress (Romojaro et al., 2013). Therefore, the objective of the present study was to evaluate the effect of selected local wild edible plants on lipid peroxidation and lipid profile in adult male Sprague Dawley rats.

Body weight changes can be used to evaluate nutrient utilization. As presented in Table 2, there were no significant differences among all experimental groups in either initial body weight, final body weight or body weight gain ($p < 0.05$). Even when using HFD there were no differences among the groups which is inconsistent with the findings of An et al. (2011) who found that weight gain of HFD group (65% of calories from fat) was significantly higher than NFD. Also, Lee and others have concluded that weight gain of HFD group (~50% of calories from fat) was significantly higher than NFD group (Lee et al., 2006).

However, in another study Moraes et al. (2012) found that after introducing HFD containing (40% of calories from fat) to the animals there were no differences in body weight gain and final body weight (Moraes et al., 2012). Such a result is expected since total caloric intakes of groups were similar (approximately 4000 kcal).

To the best of our knowledge, there were no studies to determine the effect of any of the studied plants on body weight or weight gain in rats. However, Wang et al. (2014) showed that excessive weight gain and obesity could be prevented or slightly reversed by the intake of dietary polyphenols. This finding cannot be clearly observed in the present study since the positive control group was not significantly different from negative control group.

Accumulative food intake could be an indicator of the acceptability and palatability of food. According to results of this study accumulative food intake of NFD was significantly higher than other groups. It was reported in many studies that accumulative food intake of the group that received a standard diet was significantly higher than that of the groups which received HFD (Moraes et al., 2012; An et al., 2011; Lee et al., 2006).

In the present study, the values of fasting blood glucose among experimental groups were close to each other ($p > 0.05$) (Table 3). Feeding HFD did not raise fasting blood glucose levels in comparison with feeding NFD which is consistence with the work of Moraes et al. (2012) and An et al. (2011). However, it has been reported that HFD in general can activate Toll-like receptors resulting in an immune response that promotes the activation of pro-inflammatory pathways leading to insulin resistance (Pfluger et al., 2008).

Serum lipids are used as an indicator for cardiovascular diseases. In this study, it was observed that HFD did not affect any parameter of blood lipids including TG, TC, HDL and LDL (Table 3). This result was consistent with what was observed by An et al. (2011) and Moraes et al. (2012). Lee and coworkers who used ~ 50% of calories

from fat obtained similar results regarding blood lipids except for HDL which was significantly higher in HFD group than NFD group (Lee et al., 2006). There are several possible reasons for this result. Firstly, the time period may have been insufficient to induce dyslipidemia. Furthermore, food intake of the HFD was lower than that of NFD (Table 2); thus it needs more time to show an effect. In addition, the amount and type of fat used may have a role in affecting blood lipids. On the other hand, other studies indicated that rats which received HFD have significantly higher TG, TC and LDL than those that received standard diet (Karam et al., 2016; Guo et al., 2011).

The addition of different plants to the HFD did not result in significant effect on blood lipids although it was expected that the addition of the plants may have a protective effect against dyslipidemia. This observation may be explained by the possibility that the four weeks period (first four weeks of the experiment) was not sufficient to produce hyperlipidemia at the beginning of the experiment and that the last four weeks period of the experiment may had not been enough to exhibit a significant effect on blood lipids.

Moreover, introducing the plants into the diet at the middle of the experiment and not from the beginning could elucidate such an observation. Besides that, the bioavailability of phenolic compounds could be a problem since it is absorbed in very little amounts and its concentration in the plasma and tissues was very low too (Cartea et al., 2010). It is reported that cell wall-bound phenolic compounds differ from those of free phenolic compounds in terms of bioavailability (Cartea et al., 2010). In addition to that sun drying was found to decrease polyphenols content as a result of increased enzyme activity (Kamiloglu et al., 2016).

Ingesting HFD increases the oxidative stress and ROS production in the body (Nappo et al., 2002). Lipids are considered as a target for ROS which oxidize it in a process called lipid peroxidation (Ayala et al., 2014). Malondialdehyde (MDA) is a secondary product of lipid peroxidation process and it can be measured by TBARS assay (Ayala et al., 2014; Esterbauer & Cheeseman, 1990). In this study serum MDA was measured as an indicator of lipid peroxidation which reflects the degree of antioxidant capacity in vivo. Based on what we found in the present study (Table 3), all of the tested plants were found to decrease the serum levels of MDA compared with HFD group.

Up to our knowledge, no researchers have investigated the antioxidant capacity of the tested plants by measuring MDA serum concentration in rats fed HFD. However, some studies reported the protective effect of some of the plants or its extracts against oxidative stress induced by different ways in animals and humans.

Results from Candan and Sökmen study suggest that the methanolic extracts of sumac fruits have considerable antioxidant activity against lipid peroxidation process in vitro (Candan & Sökmen, 2004). Salimi et al. (2015) studied the antioxidant effect of sumac aqueous extract in diabetic rats. Their results showed that MDA levels of the liver and kidney in diabetic animals treated with the extract were found to be lower than the non-treated diabetic group. Another study conducted by Pourahmad et al. (2010) investigated the hepatoprotective activity of aqueous extract of sumac and found that it is effective in lowering lipid peroxidation in liver cells indicated by MDA concentrations along with other oxidative stress biomarkers.

According to Viuda-Martos and others, thyme was found to prevent peroxidation of lipids in vitro (Viuda-Martos et al., 2010). Thyme powder, extract and oil were found to reduce liver MDA levels in rats with CCl_4 induced liver injury (Al-Badr, 2011). Wild mint extract (500 mg/kg/day) was proven to decrease MDA serum levels in rats with acetic acid-induced colitis (Murad et al., 2016).

Seham et al. (2015) found that garden rocket oil lowered plasma MDA levels in rats on hypercholestrolic diet. In another study, liver MDA levels were lower in rats treated with ethanol, ethyl acetate and n-butanol gundelia extracts at doses of 200 and 300 mg/ kg in comparison with the positive control rats with CCl_4 induced liver damage (Niknahad et al., 2016). Unfortunately, there were no studies to compare the effect of clary on lipid peroxidation using MDA as an indicator in vivo or in vitro. In this study, clary was found to significantly lower serum MDA levels (Table 3).

The antioxidant effect of phenolic compounds is mainly due to their redox properties and their capacity to block the production of ROS. There are various possible mechanisms of this including: free radical scavenging activity, transition-metal-chelating activity, and/or singlet-oxygen-quenching capacity (Rubio et al., 2013).

A few limitations should be noted in this study. The duration of the animal experiment and the fat content in the diet possibly were not enough to produce dyslipidemia in the animals. In addition, using the dried plant powder which contains cell wall-bound phenolic compounds decreases their bioavailability. Furthermore, other biochemical tests could be used to evaluate oxidative stress in the body rather than using serum MDA only.

5. Conclusions

Incorporating dried plant powders in rats diet did not significantly affect serum fasting glucose and lipid profile. However, its positive effect was observed only on lipid peroxidation assay as indicated by serum MDA levels.

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