Research Journal of Biological Sciences 8 (5): 150-154, 2013
ISSN: 1815-8846

Lipid Lowering Activity of *Moringa pergerina* Seeds in Rat: A Comparison Between the Extract and Atorvastatin

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Abstract: Hyperlipidemia is the most important cardiovascular risk factor. *Moringa peregrina* is traditionally used as antihyperglycemia and antihyperlipidemia. This study aimed to investigate, the effects of hydroalcoholic extract of *Moringa peregrina* on lipid profile and atherogenic biomarkers of hyperlipidemic rat. In this experimental study, 40 male Wistar rats were divided into four equal groups: Normal rats, high fat diet rats (hyperlipidemia induced with persintra-M) high fat diet with *Moringa peregrina*, high fat diet plus atorvastatin. The regimens were given orally (by gavage) for 40 days. Also, serum lipid profile, thiol level, Malondyaldehide (MDA), antioxidant capacity, ferritin, CRP as well as atherogenic index were determined. Treatment with *Moringa peregrina* seeds significantly decreased serum LDL, VLDL, trigeliceride and total cholesterol and increased HDL-C. The Atherogenic index decreased. Also, MDA, ferritin and CRP were reduced and plasma thiol level icreased. The data demonstrates that the leaves ethanolic extract of *Morninga peregrina* has antioxidant activity and antilipidemic effect on hyperlipidemic rats. Also, reduction of lipid and protein peroxidation as an index of oxidative stress is evident.

Key words: Cardiovascular, hypercholosterolemia, *Moringa peregrina*, hyperlipidemia, oxidative stress

INTRODUCTION

Hyperlipidemia is a heterogeneous group of disorders characterized by an excess of lipids in the blood stream (Shamir and Fisher, 2011). High lipid levels can speed up a process called a therosclerosis which lead to cardiovascular disease and diabetes (Larson *et al*., 2003). Many studies have previously discussed about the role of oxidative stress in atherosclerosis (Hasani-Ranjbar *et al*., 2009). Available lipid lowering drugs have shown common side effects including liver disorders, muscle pain, flushing and adverse fetal effects (Huseini *et al*., 2006). Therefore, it is rationale to search for new safer medicinal drugs (Marles and Farnsworth, 1995). Herbal plants have always been considered a healthy source of life and different experimental and clinical researches have shown promising effects for various conditions, such as atherosclerosis (Madihi *et al*., 2013) diabetes (Khosravi-Boroujeni *et al*., 2012), cancer (Jafarzadeh *et al*., 2012), infection (Namjoo *et al*., 2013) gastrointestinal (Huseini *et al*., 2005) and CNS disorders (Hasani-Ranjbar *et al*., 2008). However, the positive effects of a lot of these plants have not yet been scientifically studied (Liu *et al*., 2004a). Although, medicinal herbs and their derivatives have been used as a remedy for hyperlipidemia for a long time, their certain efficacies have not yet been proven by valid researches (Liu *et al*., 2004b).

Moringa or *Moringa peregrina* belongs to monogeneric family of Capparales and have long been used as herbal medicine in South Asia for the treatment of a wide range of conditions, such as inflammation, gastrointestinal, hematological, cardiovascular, hepato and renal disorders (Banejad *et al*., 2010; Alkahtani, 1995). Researches have shown that *Moringa peregrina* has high antioxidants activity and is a valuable source of vitamin C, carotenoids and flavonoids. So, it has been used for

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diabetes and hypertension, as well as liver protection (Olson, 2003; Somali *et al*., 1984; Dangi *et al*., 2002). However, no scientific report exists about the usefulness of *Moringa peregrina* in improving lipid profile. Therefore, the present study aimed mainly to examine the lipid lowering effects of hydroalcoholic extract of *Moringa peregrina* on persintra-M induced hyerlipidemic rat. Oxidative stress has been considered as one of the main risk factor for atherosclerosis. Hence, the blood thiols level and Malondialdehyde (MDA) were also evaluated as two indicators of lipid peroxidation and blood carbonyl was measured as an important indicator of protein oxidation (Khodarahmi, 2001; Heidarian *et al*., 2011; Levine *et al*., 1990). Increased CRP and ferritin were, also evaluated as important biomarkers of inflammation which is an inevitable factor of atherosclerosis plaque.

MATERIALS AND METHODS

Seeds of *Moringa peregrina* were prepared from agriculture research center of Shahrekord. The genus and species of the *Moringa peregrina* were confirmed by the botanists at the Department of Biology of Shaherkord University. A herbarium specimen of *Moringa peregrina* was prepared and deposited in Medical Plants Research Center Herbarium of Shahrekord University of Medical Sciences, Iran.

Hydroalcoholic extract preparation: First, the plant was ground into powder, allowed to pass through a special sifter and its hydro-alcoholic extract was prepared through Percolation method. At first cotton was put at the end of the percolator. Then, the powdered dried plant was weted and put on it. The hydro-alcoholic solution (20:80) was poured on it in a way that it covered 3 cm over the powder. After 72 h, extracting was done and its alcohol was evaporated by rotary machine (Goyal and Shah, 2001). Finally, the concentration of the extract reached 300 mg/1 cc and 300 mg/kg/day of the hydro-alcoholic extract of *Moringa peregrina* was considered as the chosen dose.

Experimental animals: Total 40 male Wistar rats, weighting 250-270 g, aged almost 14 weeks were used in this study. The animals were bought from Pasteur Institute and were kept in the animal lair of Shahrekord University of Medical Sciences under appropriate temperature, humidity and light conditions. All the experiments were postponed until after 2 weeks in order for the rats to become accustomed to the new environment. The conditions for protecting animals included average temperature of 22°C, 12 h of light and 12 h darkness and sawdust as bedding. Before and during experiment, the rats had free access to water from the piped water and peculiar food (pelet form). As 1 week passed and the rats got accustomed to the new environment, their weight was measured by a digital scale and recorded (Roberts *et al*., 2002). The study protocol was confirmed by Ethical Committee of Shahrekord University of Medical Sciences.

Induction of hyperlipidemia: The experimental model of hyperlipidemic rat was induced by feeding the rats by Persintra-M emulsion with 2% cholesterol which contained cholesterol of egg yolk (1 g of cholesterol in per 100 g of egg yolk) butter and palm, was prepared. Then, the suitable drug dose was chosen in terms of milliliter by calculating the dose of 25 mg/kg/day cholesterol (Bertges *et al*., 2011) and was given by gavage with specific needle.

Categorization: In this study, 40 rats were randomly divided into 4 equal groups as:

- Normal control
- Fat-rich diet by persintra-M
- Fat-rich diets by persintra-M and hydroalcoholic extract of *Moringa peregrina* at a dose of 300 mg kg^{-1} p.o.
- Fat-rich diet by persintra-M and atorvastatin at a dose of 10 mg kg^{-1} p.o.

Collection and storage of samples: On the 40th day of the study, blood samples were collected by retro orbital sinus puncture, under mild ether anesthesia. Plasma was obtained by immediate centrifugation of blood samples using REMI ULTRA cooling centrifuge at 3000 rpm for 5 min at room temperature. All samples were stored at 4°C until analysis.

Biochemical analysis: Plasma lipid levels including total cholesterol, triglyceride and HDL-c were measured (Madihi *et al*., 2013) using respective diagnostic commercial kits (Pars Azmon, Iran) and LDL-C in plasma was calculated as per friedewald estimation. VLDL was calculated as VLDL = Triglycerides/5. Atherogenic index was calculated as VLDL+LDL/HDL.

Malondialdehyde (MDA) evaluation: In order to evaluate the extent of lipids peroxidation, Malondialdehyde was analyzed as an index of lipids peroxidation. This analysis was based on Chirko method (variation coefficient of internal-assessment and external-assessment) and by the use of HPLC technic. MDA molecules react with

thiobarbituric acid in high temperature and acidic condition generating a purple (MDA-TBA2) complex, its color intensity is measurable at 532 nm. However due to existing interferences, first a Sphreisorb ODS type of Weston Chromatography of MDA-TBA complex was separated by HPLC system (Water-600E) then it was analyzed and investigated (Khodarahmi, 2001).

Measuring plasma carbonyl groups: Levine *et al.* (1990), method was used in order to measure plasma carbonyl groups. In this reagent method, 2.4 di-nitro-phenyl-hydrazine along with carbonyl groups in proteins produce schiff base and a yellow complex, the color intensity is measurable spectrophotometrically, at 380 nm (Heidarian *et al*., 2011).

Measuring plasma thiols groups (-SH): Reduction of thiols groups in plasma is an important indicator of oxidative stress. To measure thiol group, 2.2 di-theo base nitro-benzoic acid, known as elman reagent was used. Thiols groups produce a yellow complex by surviving this reagent which is measurable at 421 nm (Levine *et al*., 1990).

Statistical analysis: Results were analyzed by ANOVA and Tukey tests using SPSS software version 16.0 to determine the statistical significance of data obtained from study groups. $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

The levels of biochemical markers: In high cholesterol diet group, the glucose, total cholesterol, triglyceride, LDL-C and HDL-C levels increased significantly compared with normal diet group ($p<0.05$) (Table 1). After 40 days the mean weight in groups under treatment with *Moringa peregrine* and atorvastatin decreased significantly. On 40th day in the group, fed with *Moringa peregrina* in addition to high cholesterol diet (group 3) the levels of FBS, TG, cholesterol and LDL-C decreased compared to the rats fed with high cholesterol diet (group 4). On the other hand, the levels of HDL-C increased in group 3 ($p<0.05$) (Table 2).

As shown in Table 3, group 3 (received *Moringa peregrina* +high cholesterol diet) had the same levels of ferritin, thiol, MDA and carbonyl as group 4 (Atorvastatin+high cholesterol diet). Also, the rats fed with high cholesterol diet plus *Moringa peregrina* extract had significantly higher levels of antioxidant capacity in comparison with the rats of group 4 (received atorvastatin+high cholesterol diet).

In the present study, researchers evaluated the effect of *Moringa peregrina* on lipid profiles in hyperlipidemic rats. Remarkably, *Moringa peregrina* significantly reduced the lipid levels, i.e., decreased in the plasma total cholesterol, LDL-C and VLDL levels and increased the HDL-C levels in hyperlipidemic rats. A statistically significant decrease in lipid profile was observed with atorvastatin and *Moringa peregrina* treatments in hyperlipidemic rats ($p<0.05$). Total cholesterol and LDL-C content in plasma were reduced and reversed to normal levels in rats which indicates the lipid lowering potential of *Moringa peregrina*. Additionally, it was demonstrated that *Moringa peregrina* had comparable lipid lowering effect with atorvastatin. Few studies have done to demonstrate the effects of *Moringa peregrina* (Olson, 2003; Somali *et al*., 1984; Dangi *et al*., 2002). This is the first study done to show antihyperlipidemic effect of the

Table 1: Lipid profile levels in different groups

	Laboratory tests (mg dL^{-1})					
Groups	Glucose	TC	TG	LDL	HDL	Atherogenic index
1	130.66±48.520	55.22±14.06	89.77±27.01	22.48±4.680	27.53±9.27	17.95±5.4
2	12.19±187.55	119±7.19	178.55±12.97	5.7852.38±	42.27±1.19	35.7±12.59
3	38.15±202.20	75.4±13.27	176.6±12.970	4.6827.98±	40.46±6.02	35.32±15.61
4	214.4±44.8000	76.2±6.45	±35.74140.00	32.58±23.70	42.6±6.26	27.38±10.5

Table 2: Mean of different groups

Groups	Glucose	TG	Cholesterol	LDL	HDL	VLDL
1	130.66	89.77	55.22	22.48	27.53	17.95
2	187.55	178.55	119.00	52.38	42.27	35.71
3	214.40	140.00	76.20	32.58	42.60	27.38
4	202.20	176.60	75.40	27.98	40.46	35.32

Table 3: Inflammatory biomarkers levels and other atherosclerosis factors in the study groups

Groups	Ferritin	Thiol groups	MDA	Carbonyl plasma	CRP	Anti oxidant capacity
1	2/7±0/4	200±0/5	5/74321±	0/72±0/5	3/14±0/2	432/1±0/7
2	9/1±1/4	173/6±0/4	10/07081	0/89±0/5	3/98±0/3	282/8±0/2
3	2/7±8	207/5±0/7	5011525	0/72±0/5	3/00±0/2	399/7±0/4
4	3/1±0/2	208±6/8	5/0145±0/82	0/74±0/5	3/12±0/2	438±0/4

1 = Normal-diet; 2 = High cholesterol diet; 3 = High cholesterol diet plus *Moringa peregrina*; 4 = High cholesterol diet plus atorvastatin

the seeds of *Moringa peregrina* in Iran. Iranian population, specially in Sistan and Baluchestan Province traditionally use this herb to reduce blood lipids. Mehta *et al.* (2003), also showed blood-fat reducing effect of *Moringa oleifera* in rats. Interestingly, this plant has significantly increased HDL-C, comparing to rich-fat group ($p<0.05$). According to atherogenic index, it was recognized that this plant is probably effective in reduction of atherosclerosis.

In this study, plasma MDA and active-plasma-thiol both decreased in the group which received high cholesterol diet plus *Moringa peregrina* compared to the group which received high cholesterol diet. Dietary antioxidants are thought to be beneficial in reducing the incidence of coronary heart disease (Rafieian-Kopaei *et al*., 2013, 2011). Some investigations have shown that there is a positive correlation between the antioxidation and cardioprotective activities of phenolic compounds (Sakakibara *et al*., 2003). In addition to antioxidant effects, the extract seems to have anti-inflammatory effect, probably by flavonoid quercetin. The anti-inflammatory effect of *Moringa peregrina* may be related to its inhibitory effect on primary proinflammatory transcriptional factors. One of the major indicators of inflammation in the body is CRP. On the other hand, many now believe that there is a link between the inflammatory process and atherosclerosis. After 40 days of administration of *Moringa peregrina* extract in the present study, the rats fed with high cholesterol diet plus *Moringa peregrina* extract showed significantly lower levels of CRP in comparison with the rats fed with high cholesterol diet, the same as happened with high cholesterol diet plus atrovastatin group. Ferritin is a protein that plays an important role in the storage of iron in the body. Ferritin is now emerging as a very importantfactor in the pathogenesis of diseases, such as atherosclerosis (You and Wang, 2005). *Moringa peregrina* significantly decreased ferritin but this decrement was lower in the group 3 than group 4 that received atorvastatin+high fat diet. Further studies, are needed to identify the exact components of *Moringa peregrina* which are responsible for its hypolipidemic, anti-oxidant and anti-inflammatory actions.

CONCLUSION

The present investigation showed that *Moringa peregrina* is able to reduce the plasma lipid levels and reverse to normal levels in rats, as well as having considerable antioxidant effect. Thus, it might be beneficial in hyperlipidemic patients.

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