

Original Article

Effect of Moringa Oleifera on Lipid Profile in Rats

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Abstract

Background: Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are primary causes of death worldwide. Moringa Oleifera tree was used as a health supplement introduced to Africa from India. Its leaves have various biological activities, including hypolipidemic effect.

Objective(s): The aim of present study was to assess the effect of Moringa Oleifera consumption on lipid profile and histopathology of the liver in hyperlipidemic rats.

Methods: A total of 50 male albino rats were divided into five equal groups. Group I was left as control and fed on standard diet only. Group II was fed on standard diet and orally injected with 400 mg/kg of rat body weight extract of Moringa Oleifera. Hyperlipidemia was induced in the remaining 30 rats by feeding an atherogenic diet for four weeks. Group III was fed on atherogenic diet only. Group IV was fed on atherogenic diet and orally injected with 400 mg/kg of rat body weight extract of Moringa Oleifera. Group V was fed on atherogenic diet with 2mg/kg of rat body weight Atorvastatin drug. Lipid profile of rat blood was measured including total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), high density lipoprotein (HDL), and very low density lipoprotein (VLDL). Liver tissue was evaluated by carrying out histopathological examination.

Results: The rats fed on Moringa Oleifera showed a significant decrease in total cholesterol and LDL by 26.8% and 40.6% respectively compared to baseline values after 4 weeks. Although VLDL and TG showed slight increase by 5.6% and 5.1% respectively, they were still within the normal range. Otherwise, there was a significant increase in LDL, VLDL, TC, and total TG levels in rats fed on atherogenic diet only by 17.13%, 195.4%, 29.2%, and 193% respectively compared to baseline values after 4 weeks. Atorvastatin drug affected the HDL level, as it increased by 16.4% % 28% from baseline after two and four weeks respectively. Atorvastatin and Moringa Oleifera decreased VLDL level by 53.9% & 36.5% respectively when compared to rats fed on atherogenic diet.

Conclusion: Moringa Oleifera was comparable to hypolipidemic medication (Atorvastatin) in improving the lipid profile of rats fed on atherogenic diet. Moringa Oleifera intake is more effective in prevention than in treatment of hyperlipidemia.

Keywords: lipid profile, atherosclerosis, Moringa Oleifera, atherogenic diet, atorvastatin.

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INTRODUCTION

Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases. Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are primary causes of death worldwide.⁽¹⁾ Currently many research studies focus on the beneficial effects of bioactive phytochemicals present in micro level in our daily diet. These phytochemicals are abundant in grains, vegetables, fruits, seeds and nuts. They are believed to contribute positively in the prevention of degenerative diseases. The mechanics behind the different beneficial effects of dietary photochemical are not fully understood. However, these compounds are

known to act as antioxidants, hypocholesterolemic, enzyme modulating agents and phytohormones.^(2,3) Moringa Oleifera is one of the 14 species of family Moringaceae, native to India, Africa, Arabia, Southeast Asia, South America, and the Pacific and Caribbean Islands.^(4,5) Its known as Drumstick tree. Various parts of this plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods are used medicinally.⁽⁵⁾ Moringa leaves contain more vitamin A than carrots, more vitamin C than oranges, more calcium than milk, more potassium than bananas and more iron than spinach. The Moringa Oleifera tree was introduced to Africa from India at the turn of the twentieth century where it was to be used as a health supplement. It has been consumed by humans throughout the century in diverse culinary ways.

Almost all parts of the plant are used culturally for its nutritional value, purported medicinal properties and for taste and flavor as a vegetable and seed. The leaves of *Moringa Oleifera* can be eaten fresh, cooked, or stored as a dried powder for many months without any major loss of its nutritional value.^(4,6)

Leaves of this plant are reported to possess various biological activities, including hypocholesterolemic, antidiabetic, hypotensive and antihyperlipidemic effect. Drugs used in treatment of hyperlipidemia currently include statins (simvastatin, pravastatin), resins (cholecystyramine) and fibrates (gemfibrozil). Less commonly used drugs include nicotinic acid, probucol, clofibrate and colestipol. Fish oils have been advocated for the treatment of increased triglyceride (TG) but were found to raise low density lipoprotein (LDL). Statins have been used for almost a decade and have not produced untoward effects.⁽⁷⁻¹⁰⁾

Therefore, the present study aimed to assess the effect of *Moringa Oleifera* consumption on lipid profile in rats.

METHODS

Study Setting & Design: An experimental animal study was conducted at the Nutrition Department Laboratory, High Institute of Public Health, Alexandria University, and the Medical Research Institute Laboratory, Alexandria University.

Preparation of *Moringa Oleifera*: Three kg of fresh leaves of *Moringa Oleifera* were collected from a private farm in El-Gharbya governorate, Ashmoun center and were taken to the Nutrition Department laboratory. These leaves were air-dried and reduced to powdered form. The powdered leaves were percolated in distilled water and ethanol 1:1 for 12 h and filtered with subsequent evaporation of the filtrate. A weighted amount of dried extract was freshly dissolved in water in order to prepare a solution to be used for treatment.^(11,12)

Experimental Animals: A total 50 healthy adult male albino rats of Wistar strain weighing around 120 to 150g were procured from the central animal house of the Medical Research Institute, "Smouha", Alexandria University. The animals were housed under laboratory conditions and kept for one week for rehabilitation. They were divided randomly into five equal groups of 10 rats each as follows: Group I (control) included 10 rats that were left as control and fed on standard feed only. Group II (control +ve) included 10 rats that were fed on standard diet and injected orally with 400 mg/kg extract of *Moringa Oleifera*. The remaining 30 rats were subjected to hyperlipidemic diet for four weeks as follow: Group III (control -ve) included 10 rats that were fed on atherogenic diet only; Group IV included 10 rats that were fed on atherogenic diet and orally injected with 400 mg/kg extract of *Moringa Oleifera*; Group V included 10 rats that were fed on atherogenic diet with 2mg/kg Atorvastatin drug as a

clinical group. The doses of *Moringa Oleifera* extract were given orally with the help of syringe directly into the esopharyngeal regions according to the animal's body weight every day for 28 days.⁽¹³⁾

Biochemical tests: The following parameters: total cholesterol (TC), TG, LDL, high density lipoprotein (HDL), and very low density lipoprotein (VLDL) were measured using spectrophotometer Microlab 300 (ELI tech semi-automated) at baseline, after two weeks, and at the end of four weeks.⁽¹⁴⁻¹⁸⁾

Histopathological study: Tissue processing was carried out using the method of Baker and Silverton (1998).^(15,19)

Statistical analysis

Data analysis was carried out using the statistical package for social sciences software version 17.0 (SPSS Inc., Chicago, Illinois, USA). Data were presented in tabular form and graphically using means and SD. For all analyses, a P value of 0.05 or less was set as level of significance. One-way analysis of variance (ANOVA) test was used to compare means of at least three independent groups.

Ethical Considerations

This study was approved by the institutional review board and the Ethics Committee of the High Institute of Public Health, Alexandria University. The study conformed to the international ethical guidelines. All laboratory biological specimens and hazardous waste were disposed of safely.

RESULTS

Table (1) shows the TC level (mg/dl) in rats fed on *Moringa Oleifera* after two and four weeks. There was no significant change with time in TC level in rats fed on normal diet. Those fed on normal diet with *Moringa Oleifera* (control +ve) showed a significant decrease in TC level by 21.6% and 26.8% after two and four weeks respectively. There was a significant increase in TC level of rats fed on atherogenic diet (control -ve) comparing to baseline level (114.4 mg/dl). It increased to 146.2 mg/dl after two weeks then to 148.1 mg/dl after four weeks. The TC level of rats fed on *Moringa Oleifera* with atherogenic diet was 107.3 mg/dl at baseline. This was slightly increased to reach 112.9 mg/dl at two weeks then decreased to reach 111.1 mg/dl at four weeks of treatment. In rats fed on atherogenic diet with atorvastatin, TC level slightly increased in a time dependent manner.

Table (2) illustrates the TG level (mg/dl) of rats fed on *Moringa Oleifera* after two and four weeks. There was no significant change in TG level among all groups except for rats fed on atherogenic diet (control -ve) where TG level increased by 73.4% to reach 76.3 mg/dl after two weeks, and by 193% to reach 129.9 mg/dl after four weeks. Baseline TG level in rats fed on *Moringa Oleifera* (control +ve) was 39.3 mg/dl. It decreased to reach 38.7mg/dl after two weeks then increased by 5.3% to reach 41.4 mg/dl after four weeks. The level of TG in rats fed on atherogenic diet + *Moringa Oleifera* increased from baseline (37.3

mg/dl) by 9.9% and 7.5% to reach 41 mg/dl and 40.1 mg/dl after two and four weeks respectively.

Table (3) reports the HDL level (mg/dl) in rats fed on Moringa Oleifera after two and four weeks. The level of HDL in rats fed on Moringa Oleifera (control +ve) group increased from baseline value (22.3 mg/dl) by 48.8% to reach 33.5 mg/dl after two weeks, and by 10.7% to reach 24.7 mg/dl after four weeks. There was a significant increase in HDL level in rats fed on atherogenic diet + atorvastatin. It increased by 16.4% from baseline after two weeks to reach 24.8 mg/dl, and by 28% after four weeks to reach 27.3 mg/dl. High density lipoprotein level changed significantly in rats fed on atherogenic diet only where its

level decreased by 9.6% after two weeks to reach 18.8 mg/dl, and increased by 10.5% to reach 23 mg/dl after four weeks. It was evident that the level of HDL increased in a time dependent manner in rats fed on atherogenic diet + Moringa Oleifera. It was 23.3 mg/dl at baseline, and increased by 18% and 20.6% to reach 27.5 mg/dl and 28.1 mg/dl after two and four weeks respectively. The baseline HDL level of rats fed on normal diet was 20.5 mg/dl. It decreased by 0.9% to reach 20.3 mg/dl after two weeks, and then increased by 5.3% to reach 21.6 mg/dl after four weeks.

Table (1): Cholesterol level of rats at baseline and after two and four weeks

Group	Cholesterol level (mg/dl)					P
	Baseline	Two weeks		Four weeks		
	Mean±SD	Mean±SD	% change	Mean±SD	% change	
Rats fed on normal diet (Control) (G I)	114.2±9.0	110.8±6.1	-3.6	116.3±4.8	1.8	0.741
Rats fed on Moringa Oleifera (Control +ve) (G II)	109.4±5.0	85.6±9.2	-21.6	80.1±7.4	-26.8	0.002*
Rats fed on atherogenic diet (Control -ve) (G III)	114.1±9.5	146.2±9.5	27.9	148.1±5.9	29.7	0.002*
Rats fed on atherogenic diet + Moringa Oleifera (G IV)	107.3±4.1	112.9±3.7	5.2	111.1±3.7	3.4	0.027*
Rats fed on atherogenic diet +atorvastatin drug (G V)	101.8±3.6	106.2±5.3	4.3	108.8±6.0	6.8	0.045*
P #	0.003*	0.001*		0.001*		

Interaction of groups and time is significant (P=0.006)*
P#: Adjusted P value for One Way ANOVA

P: Adjusted P value for repeated measures ANOVA
* P < 0.05

Table (2): Total triglyceride level of rats at baseline and after two and four weeks

Group	Total triglyceride(mg/dl)					P
	Baseline	Two weeks		Four weeks		
	Mean±SD	Mean±SD	% change	Mean±SD	% change	
Rats fed on normal diet (Control) (G I)	41.2±4.3	44.8±6.0	8	41.9±3.5	1.6	0.624
Rats fed on Moringa Oleifera (Control +ve) (G II)	39.3±10.6	38.7±10.6	-1.5	41.4±10.3	5.3	0.831
Rats fed on atherogenic diet (Control -ve) (G III)	44.0±7.7	76.3±22.0	73.4	129.9±27.2	193	0.001*
Rats fed on atherogenic diet + MoringaOleifera(G IV)	37.3±3.3	41.0±3.7	9.9	40.1±4.3	7.5	0.317
Rats fed on atherogenic diet +atorvastatin drug (G V)	36.0±3.8	36.9±3.6	2.5	39.4±5.0	9.4	0.351
P #	0.210	0.001*		0.001*		

Interaction of groups and time is significant (P=0.006)*
P#: Adjusted P value for One Way ANOVA

P: Adjusted P value for repeated measures ANOVA
* P < 0.05

Table 3: High Density Lipoprotein level of rats at baseline and after two and four weeks

Group	High Density Lipoprotein(mg/dl)					P
	Baseline	Two weeks		Four weeks		
	Mean±SD	Mean±SD	% change	Mean±SD	% change	
Rats fed on normal diet (Control) (G I)	20.5±2.8	20.3±2.0	-0.9	21.6±1.3	5.3	0.824
Rats fed on Moringa Oleifera (Control +ve) (G II)	22.3±3.0	33.5±4.1	48.8	24.7±2.3	10.7	0.001*
Rats fed on atherogenic diet (Control -ve) (G III)	20.8±2.6	18.8±1.8	-9.6	23.0±2.0	10.5	0.003*
Rats fed on atherogenic diet + Moringa Oleifera (G IV)	23.3±2.4	27.5±3.5	18	28.1±4.3	20.6	0.057
Rats fed on atherogenic diet +atorvastatin drug (G V)	21.3±1.3	24.8±3.0	16.4	27.3±2.2	28	0.001*
P #	0.300	0.001*		0.009*		

Interaction of groups and time is significant (P=0.006)*
P#: Adjusted P value for One Way ANOVA

P: Adjusted P value for repeated measures ANOVA
* P < 0.05

Table (4) shows LDL level (mg/dl) in rats fed on Moringa Oleifera after two and four weeks. The level significantly increased by 32.6% and 17.13% of baseline to reach 112.2 mg/dl and 99.1 mg/dl after two and four weeks respectively. There was a significant decrease in LDL level in rat fed on Moringa Oleifera (control +ve). It decreased by 44.5% and 40.6% from baseline to reach 44.4 mg/dl and 47.1 mg/dl after two and weeks respectively. A statistically significant irregular pattern of change in LDL level was noticed in rats fed on Moringa Oleifera + atherogenic diet. It increased by 0.78% from baseline (76.6 mg/dl) to reach 77.2 mg/dl after two weeks, and then decreased by 2% to reach 75 mg/dl after four weeks. On the other hand, there was no significant change in LDL in rats fed on normal diet where its level slightly decreased by 4.6% from baseline after two weeks to reach 81.5 mg/dl, and increased by 0.9% after four weeks to reach 86.3 mg/dl. However, its level remained within the normal range.

Table (5) shows the VLDL (mg/dl) in rats fed on Moringa Oleifera after two and four weeks. The level significantly increased in rats fed on atherogenic diet (control -ve) by 73.8% from baseline (8.2 mg/dl) after two weeks to reach 15.3 mg/dl, and by 195.4% after four weeks to reach 26 mg/dl. There was no significant increase in VLDL level in rats fed on atherogenic diet + Moringa Oleifera where it increased by 9.3% and 6.6 % of baseline level (7.5 mg/dl) to reach 8.8 mg/dl and 8 mg/dl after two and four weeks respectively. Regarding VLDL level in rats fed on Moringa Oleifera (control +ve), it decreased by 2.5% from baseline (7.9 mg/dl) after two weeks to reach 7.7 mg/dl, and increased by 5.06% from baseline after four weeks to reach 8.3 mg/dl.

Throughout the study, there was a statistical significant difference between groups as regards the change in the assessed biochemical parameters by the duration of the study (two and four weeks) ($P < 0.05$).

Table 4: Low density lipoprotein level of rats at baseline and after two and four weeks

Group	Low density lipoprotein(mg/dl)					P
	Baseline	Two weeks		Four weeks		
	Mean±SD	Mean±SD	% change	Mean±SD	% change	
Rats fed on normal diet (Control) (G I)	85.5±7.1	81.5±7.0	-4.6	86.3±5.7	0.9	0.624
Rats fed on Moringa Oleifera (Control +ve) (G II)	79.3±3.7	44.4±11.1	-44.5	47.1±7.4	-40.6	0.001*
Rats fed on atherogenic diet (Control -ve) (G III)	84.6±12.4	112.2±12.6	32.6	99.1±11.9	17.13	0.001*
Rats fed on atherogenic diet + Moringa Oleifera(G IV)	76.6±5.7	77.2±6.2	0.78	75.0±7.1	-2	0.854
Rats fed on atherogenic diet +atorvastatin drug (G V)	73.4±4.3	74.1±5.7	0.95	73.7±6.2	0.4	0.957
P #	0.085	0.001*		0.001*		

Interaction of groups and time is significant ($P=0.006$)*
P#: Adjusted P value for One Way ANOVA
P: Adjusted P value for repeated measures ANOVA
* $P < 0.05$

Table 5: Very low density lipoprotein level of rats at baseline and after two and four weeks

Group	Very low density lipoprotein(mg/dl)					P
	Baseline	Two weeks		Four weeks		
	Mean±SD	Mean±SD	% change	Mean±SD	% change	
Rats fed on normal diet (Control) (G I)	8.2±0.9	9.0±1.2	9.7	8.4±0.7	2.4	0.448
Rats fed on Moringa Oleifera (Control +ve) (G II)	7.9±2.1	7.7±2.1	-2.5	8.3±2.1	5.06	0.899
Rats fed on atherogenic diet (Control -ve)(G III)	8.8±1.5	15.3±4.4	73.8	26.0±5.4	195.4	0.001*
Rats fed on atherogenic diet + Moringa Oleifera(G IV)	7.5±0.7	8.2±0.7	9.3	8.0±0.9	6.6	0.299
Rats fed on atherogenic diet +atorvastatin drug (G V)	7.2±0.8	7.4±0.7	2.7	7.9±1.0	9.7	0.308
P #	0.274	0.010*		0.001*		

Interaction of groups and time is significant ($P=0.006$)*
P#: Adjusted P value for One Way ANOVA
P: Adjusted P value for repeated measures ANOVA
* $P < 0.05$

The histopathological analysis of liver of rats in different experimental groups revealed the presence of early steatosis within the hepatocytes and also foamy

degeneration within the liver cells as hepatocytes are widening due to deposition of fats in rats fed on atherogenic diet (control -ve group III) (Figures 1-3).

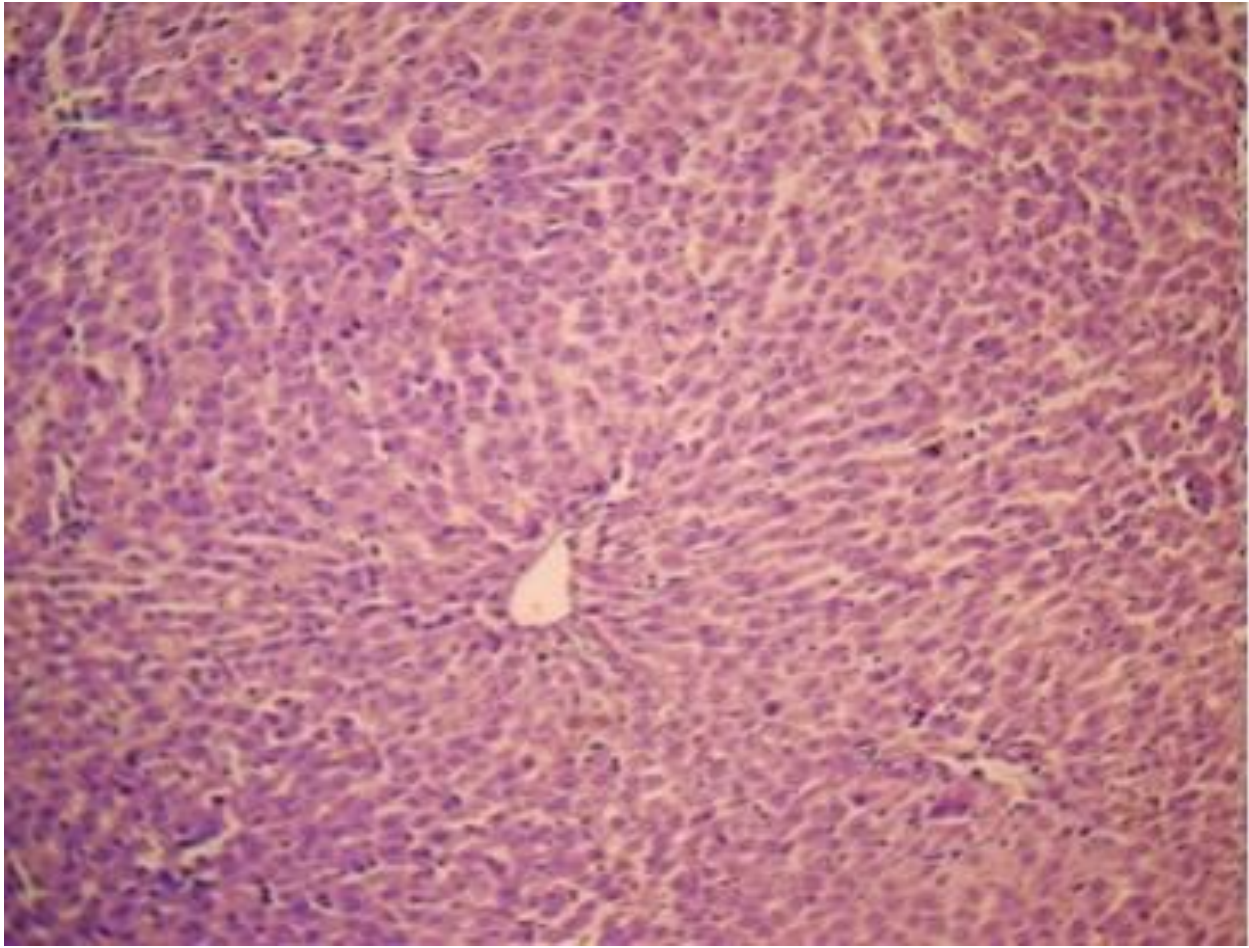


Figure 1: Transverse section in liver of rats in group I, II, IV & V (H&E X100)

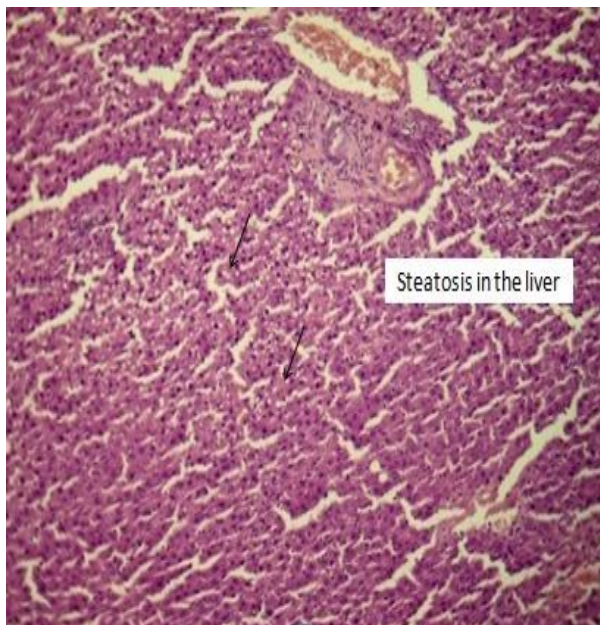


Figure 2: Transverse section in liver of rats fed on atherogenic diet (control -ve) (H&E X100)

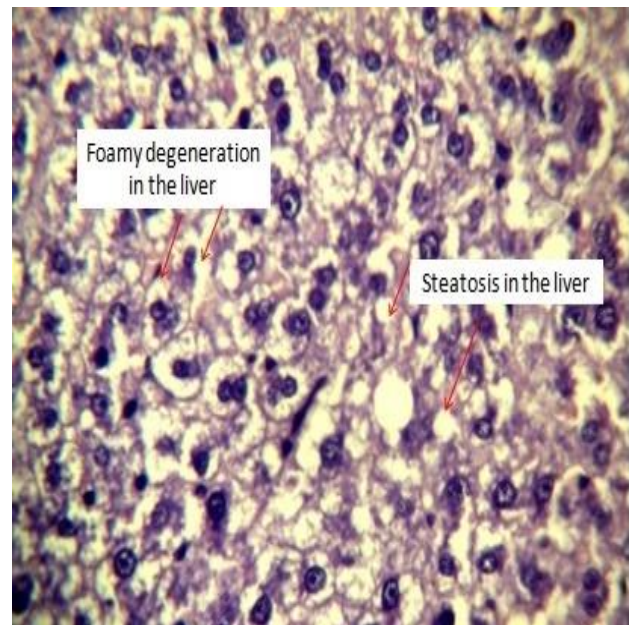


Figure 3: Transverse section in liver of rats fed on atherogenic diet (control -ve) (H&E X400)

DISCUSSION

Hypolipidaemic activity of several medicinal plants has been associated to bioactive agents like alkaloids, tannins, and cardiac glycosides, and β sitosterol. Alkaloids may mediate hypolipidaemic action either by up regulation of activities of lipolytic enzymes or by stimulating faecal bile acid excretion.⁽²⁰⁾ Also, β -sitosterol could be the major cholesterol reducing components of the ethanol leaf extract of *Moringa Oleifera* as it may work singly or in synergy with other bioactive agents.⁽²¹⁾

Plant sterols inhibit the absorption of dietary cholesterol. Beta sitosterol present in the extract of *Moringa Oleifera* is one of the plant sterol which lowers the cholesterol level by lowering plasma concentration of LDL and by inhibiting the reabsorption of cholesterol from endogenous sources in association with a simultaneous increase in its excretion into feces in the form of neutral steroids. Therefore, it can be concluded that β -sitosterol may be a bioactive phytoconstituents in the leaves of *Moringa Oleifera*.⁽²⁰⁾

The present study revealed that *Moringa Oleifera* extract had improved the serum lipid profile in hyperlipidemic rats by decreasing serum TC, TG, LDL-C and increasing serum HDL-C, thus improving the atherogenic index. This finding provides some biochemical basis for the use of *Moringa Oleifera* leaf extract as an antihyperlipidemic agent. The present findings agreed with Mehta *et al.*,⁽²²⁾ who found that cholesterol, and triglyceride levels increased by 152.9%, and 127.3% respectively compared to control group, Mehta *et al.*,⁽²²⁾ stated that cholesterol and TG lightly increased by 22% and 42% respectively in rats fed on *Moringa Oleifera* comparing to control group. Nevertheless, the results of the present study revealed a decrease in TG and TC levels among rats fed *Moringa Oleifera* by 22.7%, and 31.29% respectively comparing to control rats after two and four weeks. Moreover, the clinical group showed a decrease in TC and TG levels comparing to rats fed on atherogenic diet by 37.6%, and 36.12%, and by 106%, and 229.6% after two and four weeks respectively. The later findings agreed with Mehta *et al.*,⁽²²⁾ who found that atorvastatin decreased the levels of TC and TG comparing to rats fed on atherogenic diet by 52.2%, and 54.5% respectively.

As regards the effect on LDL, similar findings were reported by Natio *et al.*,⁽¹⁸⁾ who found that LDL increased in rats fed atherogenic diet comparing to control group by 14.8%, while it decreased by 32.5, and 19.2% in clinical group, and in rats fed on atherogenic diet + *Moringa Oleifera* respectively comparing to rats fed on atherogenic diet only.

The results concerning HDL and LDL agreed with several studies.⁽²²⁻²⁴⁾ Mehta *et al.*,⁽²²⁾ found that HDL level increased by 23.8%, and 18.4% in clinical group and rats fed on *Moringa Oleifera*, respectively comparing to rats

fed on atherogenic diet. On the other hand, VLDL increased by 14.6% in rats fed on atherogenic diet comparing to control group.

To the best of our knowledge, this is the first study to clearly demonstrate for the first time that water, and ethanolic extract of *Moringa Oleifera* leaves significantly reduced the formation of atherosclerotic plaque, along with levels of cholesterol and triglycerides. These results agree well with earlier findings where the water extract of the Leaves of this plant significantly lowers the levels of serum TC or that of TC, VLDL and LDL in hypercholesterolaemic wistar rats.^(22,25)

Atorvastatin has been known to exert its lipid lowering effect by competitive inhibition of the hepatic HMG-CoA reductase. Importantly, it was demonstrated in this study that the *Moringa Oleifera* leaf extract could reduce cholesterol and triglyceride levels in rats at degrees comparable to those of atorvastatin.

Moringa Oleifera leaf extract possessed strong radical scavenging activity and antioxidant activity. Polyphenols has been known to exert powerful antioxidant effect in vitro. They inhibit lipid peroxidation by acting as chain breaking peroxy radical scavengers, and can protect low density lipoprotein from oxidation.⁽²⁶⁾ Polyphenolic compounds also possess a variety of other biological activities, such as reduction of plasma lipids, which might be due to the up regulation of low density lipoprotein receptor expression, inhibition of hepatic lipid synthesis, lipoprotein secretion⁽²⁷⁾, and increase in cholesterol elimination via bile acids.⁽²⁸⁾ Previous studies concluded that the activity in lowering lipid levels of the *Moringa Oleifera* leaf extract may result from the phenolic compounds, poly phenolic, and flavonoids present in the extract which may agree with our study.

Dongmeza *et al.*,⁽²⁹⁾ and Fakurazi *et al.*,⁽³⁰⁾ agreed with the present study as regards liver histopathological findings. They found that the consumption of high fat diet may play a crucial role in the pathogenesis of fatty liver or hepatic steatosis. The results obtained in the present study confirmed that high fat diet causes histopathological changes in the form of hepato-cellular damage and exaggerated hepatic steatosis. However, treatment with *Moringa Oleifera* causes a momentary reduction in the enzyme levels and prevents liver damage.

CONCLUSION & RECOMMENDATIONS

Moringa Oleifera was comparable to hypolipidemic medication (Atorvastatin) in improving the lipid profile of rats fed on atherogenic diet. *Moringa Oleifera* intake is more effective in prevention than in treatment of hyperlipidemia. Therefore, awareness of people about the beneficial effect of this miracle tree (*Moringa Oleifera*) should be increased. Consumption of *Moringa Oleifera* daily as a herb or even as spices should be encouraged. Cultivation area of *Moringa Oleifera* in Egypt should be increased. Fortification of

several types of food including biscuits, breads, even cakes with *Moringa Oleifera* extract can be applied.

Conflict of Interest: None to declare.

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