Journal of Pharmaceutical, Chemical and Biological Sciences
ISSN: 2348-7658
Impact Factor (GIF): 0.615
Impact Factor (SJIF): 2.092
March-May 2016; 4(1): 76-87

Original Research Article

Effects of *Moringa oleifera* Lam (Moringaceae) Seeds in Rats Fed with High Fat Diet

Mustafa A. Hammam, Gaber A. Kalil , Salah M. El-Sayed*, Ibrahim A. Ahmed

Biochemistry Department, Faculty of Agriculture, Menofia University, Shibin El-Kom, Egypt

***Corresponding Author:** Salah M. El-Sayed, Biochemistry Department, Faculty of Agriculture, Menofia University, Shibin El-Kom, Egypt

Received: 07 March 2016 Revised: 24 March 2016 Accepted: 30 March 2016

ABSTRACT

The present work aims to study the potential effect of *Moringa Oleifera* (*MO*) seed oil and seed ethanol extract to give more protection against hyperlipidemia . Thirty male albino rats were used over 30 days period. The animals were divided into (5) groups, wherein groups number (1) represent control which were fed basal diet, while group number (2) was received high fat diet to serve as hyperlipidemic group. Other three groups allowed to feed high fat diet supplemented with seed oil , seed ethanol extract and atrovastatin, group number (3) treated simultaneously with moringa seed oil, fourth group treated with moringa seed ethanol extract while the last group treated with atrovastatin. At the end of the experimental period, blood samples were collected to determine lipid profile and determine the kidney functions include urea nitrogen and creatinine. Also liver was removed surgically for histopathological observation. From the obtained results we concluded that group of rats fed on high fat diet were considered as a major risk factor for hyperlipidemia disease. Our results could be summarized that moringa seed oil and moringa seed ethanol extract were considered the best for causing a reduction of TC, TG and LDL . Also, kidney function has been improved and there were significant reduction urea than that of positive control group. It could be concluded that moringa seed oil and moringa seed ethanol extract under study are useful for the treatment of hyperlipidemia.

Keyword: Moringa; Oil; Extracts; Triglycerides; Cholesterol

INTRODUCTION

Moringa oleifera L (Moringaceae) known commonly as Ben oil tree or drumstick tree in English language, 'Okwe oyibo' in Igbo, 'Gawara' or 'Habiwal' in Hausa and'Adagba maloye' or 'Ewe Igbale' in Yoruba grows rapidly in most regions and climatic conditions of Nigeria. M. oleifera is an important food commodity which has had enormous attention as the 'natural nutrition of the tropics' [1]. A number of

medicinal properties have been ascribed to various parts of this tree. Most parts of this plant: root, bark, gum, leaf, fruit (pods) flowers, seed and seed oil have been used in folk medicine in Africa and South Asia [2[. The plant seeds contain hypotensive activity, strong antioxidant activity and chelating property against arsenic toxicity [3]. Moringa is rich in many vitamins, including vitamin A, several forms of vitamin B, vitamin C, vitamin D and vitamin E. In fact, it has more of these vitamins than a variety of foods (such as carrots, oranges and milk) that claim to be excellent source of these vitamins [4]. Furthermore, M. oleifera has been found to be a potential new source of oil especially with the advent of the need for oleo-chemicals and oils/fats derived fuels (Biodiesel) all over the world [5]. Mature seeds yield 38–40% edible oil called ben oil from its high concentration of behenic acid. The refined oil is clear, odorless and resists rancidity. The seeds oil can also be used as a natural source of behenic acid [6]. Various extraction methods can be used in the extraction of oil and the method is normally dependent on what type of botanical material is been used. These methods include mechanical, traditional and solvent extraction. Solvent extraction method involves the counter – current flow of solvent and out bearing materials in the extraction vessel. It is usually used to recover a component from either a solid or liquid. The sample is contacted with a solvent that will dissolve the solutes of interest. Solvent extraction is of major commercial importance to the chemical and biochemical industries, as it is often the most efficient method of separation of valuable products from complex feed stocks or reaction products. The yield using this process is usually higher than that of mechanical method; and the residue usually contains less than 2% oil. Common solvents used are hexane and benzene (hydrocarbons) both of which are petroleum derivatives. Solvent extraction plants are becoming more as processing industries now aim to produce meals with minimum oil contact for commercially acceptable production levels without impairing oil or meal quality.

Serum lipid abnormalities result in increasing vascular risks; hence, aggressive treatment of hyperlipidemia is recommended. Advancing age and hypercholesterolemia have been widely considered cardiovascular risk factors in the elderly [7]. Hyperlipidemia is a powerful and extremely one of the major causes of the development of cardiovascular disorders [8]. High fat diet is the term used to denote raised serum levels of one or more of total cholesterol, low-density lipoprotein cholesterol, triglycerides, or both total cholesterol and triglyceride (combined hyperlipidemia), [9].

Moringa oleifera is one such tree which is used for various purposes like, medicinal, cooking, oils, cosmetics, etc. Extracts from different parts of this plant have shown radical scavenging & antioxidant properties. It has also been reported that the leaves possess hypo-cholesterolemic activity & hypolipidemic effects as it is found to lower serum cholesterol, triglyceride, VLDL & LDL cholesterol levels with an increase in the HDL cholesterol levels [10].

The aim of the present investigation was to evaluate the influence of Moringa *oleifera* seed oil and seed ethanol extract on serum lipids, and kidney function of hyperlipidemic rats on biochemical, biological and histopathological changes that may occur to rats fed on high fat diet.

MATERIALS AND METHODS

Materials

Moringa seeds were collected from research center department of medical and aromatic plants. Giza Egypt. The seeds were dried and milled.

Preparation of *Moringa olifera* seeds oil

The dried powder of the seeds was placed in a Soxhlet and subjected to extraction using petroleum ether (40–60 °C) to prepare the oil. The solvent was completely removed by

evaporation under reduced pressure at a temperature not exceeding 40 °C.

Preparation of crude extract from Moringa olifera seeds

Hydroalcoholic extract of Moringa olifera seeds were prepared using 70% ethanol by cold maceration technique. The percentage yield of the extract was found to be 8.8% respectively

Identification of fatty acids

Saturated, unsaturated and total fatty acids were determinate in the oil by using methyl esters boron trifluoride method [11], the oil is saponified with sodium hydroxide in methanol .the fatty acids are methylised with boron tri fluoride in methanol, extracted with heptanes and determined on a gas chromatograph with FID detector (PE auto system XL) with auto sampler and Ezchrom integration system. Carrier gas (He), ca.25 Psi –air 450 ml/min –Hudrogen 45 ml – split 10 ml/min. oven temperature 200 C injector and detector 250 C.

Identification of Moringa *oleifera* ethanol extract

GC / MS analysis : the analysis was carried out using a GC (Agilent Technologies 7890 A) interfaced with a mass – selective detector (MSD,Agilent 7000) equipped with an apolar Agilent HP-5ms (5%-phenyl methyl poly siloxane)capillary column (30 m×0.25 mm i.d. and 0.25 um film thickness) the carrier gas was helium with the linear velocity of ml/min . The identification of components was based on compartion of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by compartion of the fragmentation pattern of the mass spectral data with those reported in the literature.

Proximate composition

The methods of the Association of Official Analytical Chemists (AOAC, 1990) were used for proximate analysis. Moringa seeds samples (5 grams) was used for determination of moisture content by weighing in crucible and drying in oven at 105^{o}C, until a constant weight was obtained. Determination of ash content was done by ashing at 550^{o}C for 3h. The Kjeldah method was used to determine the protein content. The crude fibre content of the samples was determined by digestion method and the fat was done by Soxhlet extraction method. All determinations were done in triplicate. The carbohydrate content of the test sample was determined by estimation using the arithmetic difference method [12].

%CHO = 100 – (% fat. + % ash + % fiber + % protein)

Physico-chemical properties of the oil

The oils were extracted using Soxhlet extractor and the physico-chemical properties (saponification value, acid value, ester number, peroxide value, iodine value , specific gravity and refractive index).of the oil were carried out using the method described by [13].

BIOLOGICAL EVALUATION

Animals

Adult male albino rats Sprague Dawely strain weighing between (90 – 100) gm, were obtained from the animal house of Egyptian Organization for biological Products and Vaccines (VACSERA) Cairo, Egypt. The animals study were taken after the prior approval of Animal Ethics Committee. The animals were kept in wire cages with wire bottom. The diet was introduced to the rats in special feed cup that kept food spilling to a minimum, water was provided to the rats by means of glass tube projecting through wire cage, an inverted bottle supported one side of the cage.

Experimental Design

Thirty rats were divided into five groups: group (A) control fed on basal diet, groups (B, C, D and E) were allowed to feed hyperlipidemic diet to

induce hyperlipidemic through the feeding period. One of each experiment continued feeding hyperlipidemic diet without any supplementation saved as hyperlipidemic group (B) and the other three groups of each experiment were allowed to feed hyperlipidemic diet with M.oleifera oil (300 mg kg^{-1} b. wt., p.o.) as group (C), M.oleifera extract (300 mg kg^{-1} b. wt., p.o.) as group and standard atorvastatin (10 mg kg^{-1} b.wt., p.o.) as group (E). Standard diet composition was described in table 1 by Campbell et al [14] and hyperlipidemic diet was described by Nakamura et al [15] as follows:

Table 1: Standard and hyperlipidemic diets

Ingredient	Standard diet	Hyperlipidemic diet
Carbohydrates as starch	80 %	72.75 %
Protein as casein	10 %	10 %
Fats as corn oil	5 %	5 %
Salt mixture	4 %	4 %
Vitamins mixture	1 %	1 %
Cholesterol	0	2 %
Bile salts	0	0.25 %
Sheep tail fat	0	5 %

Blood sampling and analysis

Blood samples were collected after six weeks in tubes contain heparin as an anticoagulant from the eye plexuses under diethyl ether anesthesia and then centrifuged at 3000 rpm for 20 min. to obtain plasma, which was kept frozen until analysis. The total cholesterol was analyzed calorimetrically according to [16] method. HDL - cholesterol was determined according to [17] method. LDL cholesterol was calculated as:

LDL-C = Total C - (HDL-C + triglyceride/5). Risk ratio was calculated according the formula of [17] which Risk ratio = total cholesterol /HDL cholesterol. Atherogenic Index (AI) was calculated according to [17] using following equation: Atherogenic index = LDL-C / HDL-C. The triglycerides were analyzed according to [18] method. Alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) activities were measured according to the method described by [19]. Alkaline phosphatase (ALP) activity was measured by the method of [20]. Albumin was determined according to [21] method. Urea and creatinine were determined according to [22].

Histopathology

Liver from the experimental groups were immediately fixed in 10% formalin, then treated with conventional grades of alcohol and xylol, embedded in paraffin and sectioned at 4–6 lm thickness. The sections were stained with Hematoxylin and Eosin (H&E) stain for studying the histopathological changes according to [23].

Statistical analysis

The results of the animal experiments were expressed as the Mean ± SE and they were analyzed statistically using the one-way analysis of variance ANOVA followed by Duncan's test. In all cases $p<0.05$ was used as the criterion of statistical significance.

RESULTS AND DISCUSSION

Fatty acids composition

As shown in Table (2) Moringa seed oil was found to contain a high level of oleic acid (C18:1 n-9), which accounted for (66.12 %) of the total fatty acid. Thus, Moringa oil is belongs to the oleic acid oil category [24]. The presence of polyunsaturated (PUFAs, 1.03 %) and saturated

fatty acids (SFs, 21.73 %) were very low in comparison with the monounsaturated fatty acids (MUFAs. 76.51 %). Thus, Moringa oil presented relatively low contents of saturated fatty acids (SFAs) and high contents of unsaturated fatty acids (UNFAs) compared with other common vegetable/fruit seed oils, such as corn, olive and sesame and soybean [25]. High oleic acid in moringa oil makes it desirable in the term of nutrition and high stability cooking and frying oil [26]. The unsaturated fatty acids are very important for the stability of oils because of the chemical reactions occurring at the double bonds. The rate of those oxidation reactions depend on the number of double bonds in the carbon chain. Therefore, Moringa oils with high proportion of oleic acid are more stable than the others. Also, oleic acid is less susceptible to oxidation than polyunsaturated fatty acid from the n-6 series (linoleic acid). Another interesting fact is that considerable content of linoleic acid (C18:2) as an essential fatty acid in the Moringa oil may be provide high nutritional remuneration and render beneficial healthy effect on blood lipid, blood pressure and cholesterol contents [27] and it is preferred by industries when oil hydrogenation is required.

Table 2: Fatty acids composition of Moringa oil

Fatty acids	Name	Relative distribution
C 22	Behenic acid	6.36 %
C16	Palimitic acid	6.61 %
C18	Stearic acid	5.30 %
C:20:0	Arachidic acid	3.46 %
C 20:1 n−9	Gondoic acid	2.75 %
C 16:1 n−7	Palmitoleic acid	1.78 %
C 18:1 n−7	Vaccenic acid	5.86 %
C 18:1 n−9	Oleic acid	66.12 %
C 18:2 n−6	Linoleic acid	1.03 %

Chemical composition of Moringa ethanol extract

Gas Chromatography and Mass spectroscopy analysis of compounds was carried out in ethanol extract of Moringa shown in Table (3). In the GC- MS analysis, thirty bioactive phytochemical compounds were identified in the ethanol extract of Moringa . The identification of phytochemical compounds is based on the peak area, retention time , molecular weight and molecular formula. The fraction for Moringa ethanol extract was characterized by large amounts of Palmitic acid - ethyl ester which constituted 31.47% , m-cresol,4,6-di-tert-butyl- and Cis 9-Hexadecenoic acid which constituted 20% and 19% respectively.

Table 3: **Phytocomponents identified in the ethanol extract of Moringa by GC-MS**

No	Compound	%	No	Compound	%
1	β-Hyddoxyisovaleric acid	0.37	16	Tymol	0.07
2	Ascorbic acid, permethyl-	7.2	17	Carvacrol	0.04
3	Butanoic acid	0.45	18	Geranyl isovalerate	0.20
4	2-Chloro-3-Hydroxybutanoic acid	0.09	19	Cis 9-Hexadecenoic acid	19
5	linalool	6		m-cresol,4,6-di-tert-butyl-	20
6	L- lactic acid	0.18	21	Bulelated Hydroxytoluene	2.36
7	L-Aspartic acid	3.89	22	Limonen-6-ol-di-tert-butyl-	0.25
8	D-(+)-Arabitol	11.68	23	Ferulic acid	3.76
9	2- pentanol	0.24	24	Cis-9-Octadecinioc acid	0.76
10	Phenol-4(2-aminopropyl)-	0.13	25	Pentadecanoic acid	1.29
11	Dodecanoic acid	0.41	26	Palmitic acid, ethyl ester	31.47
12	ESculetin	0.22	27	Flavanone	15.01
13	p-cimene	0.20	28	Scopoletin	0.59
14	4- Hydroxycoumarin	1.10	29	n-hexadecanoic acid	4.70
15	4-tert-Butylcatechol	0.35	30	Kaempferol	3.58

Proximate composition of Moringa seeds

Proximate compositions of Moringa seeds are presented in Table 4. The moisture contents (MS) of seeds was (1.518%). The low moisture content is an indication that the seeds could be stored for longer period of time. The low ash content value of (2.252%) is correspondent to some staples as being good source of minerals. while seeds contained appreciable amount of crude protein content (29.4%) making it to be a good source of supplementary protein for man and livestock. The fat content of the Moringa seed under study (33.29%) is higher than that reported for melon seeds (17.36-25.06%) by [28]. This is also in line with the results given by [26] that gave the value of oil yield from mature seeds of any plant to be between 22 – 43%. Variation in oil yield may be due to differences in variety of plant, cultivation climate, ripening stage and the method of extraction used. The high potential of oil makes this seed a distinct potential for the oil industry. The carbohydrate content of dried moringa seeds was (28.558%). Sufficiency of Carbohydrate is however necessary for optimum functioning of the brain, heart, nervous, digestive and immune systems [29].

Table 4: Proximate composition of Moringa seeds (w/w %):

Constituents	Percentage (w/w %)
Moisture content	1.518
Crude fibre content	6.5
Total ash	2.252
Protein content	29.4
Crude Fat content	33.29
Carbohydrate content	28.558

Physico-chemical properties of the oil

Table 5 showed the physico-chemical properties of moringa seeds oil. Acid value was 0.57(mg/g). The acid value was lower than the Codex standard value for virgin vegetable oils (4.0 mg KOH/g Oil). The peroxide value was 2.27 Meq/kg. The value was lower than the codex standard value (up to 10Meq/kg) for refined vegetable oil and lower than the maximum value (20Meq/kg) allowed for unrefined olive oil [30].

This implies that the moringa seeds oil have lower degree of rancidity. Also, iodine value was very low when compared to oil from other oil seeds. Mabalaha et al [31] reported iodine values of 95.8 Wijs in Tsama melon and 124.0 Wijs in Desert melon. The lower iodine value signifies low degree of unsaturation and the lesser the liability of the oil to become rancid by oxidation. The saponification value (160.31 mg/g) was low when compared with the values recorded for moringa oil (190.4-191.2 mgKOH/g) [32], soyabean, Peanut and cotton seed oil Codex, 1993. The refractive index at 25 oC (1.471) and specific gravity (0.903). The refractive index of moringa oil was slightly higher than the value reported by [5] at 40oC. Both the refractive index and specific gravity of moringa seed oil were similar to that of palm oil and groundnut oil reported by [28].

Table 5: Physico-chemical properties of moringa oil

Parameters	Moringa oil
Acid value (mg/g)	0.58
Iodine value (g/100g)	65.27
Peroxide value (Meq/kg)	2.37
Saponification value (mg KOH/g)	160.32
Refractive Index 25oC	1.471
Specific gravity	0.903

Impact of moringa oil, moringa ethanol extract and atorvastatin supplementation on the plasma lipid profile

The data in Table 6 show the concentrations of different plasma lipids in all the groups. The results revealed that moringa oil, moringa ethanol extract and atorvastatin groups showed decreases in plasma triglycerides, total cholesterol , LDL–C , risk ratio and atherogenic index , and increases in HDL-C in comparison with the high fat group, especially atorvastatin group appeared high significant effect compared with all treated groups. Hyperlipidemia mainly increased the levels of cholesterol or LDL-C which is an important risk factor in the initiation and progression of atherosclerotic lesions [33]. The moringa groups showed lower levels of lipid profile parameters which are in line with [34] who reported that feeding linoleic and oleic acid (5%) decreased levels of triglycerides, total cholesterol and LDL-C. Also [35] found that the serum cholesterol, triacylglyceride, VLDL, LDL, and atherogenic index were reduced by M. Oleifera but HDL level was increased as compared to the corresponding high fed cholesterol diet group (control). MO was also found to increase the excretion of fecal cholesterol. Thus, it can be concluded that MO possesses a hypolipidemic effect. Confirmed by [36] they showed reduction in cholesterol levels in rats on oral supplementation of MO leaves powder.

Table 6: Effect of moringa oil, moringa ethanol extract and atorvastatin on lipid profile in rats feed on high fat diet:

Group	Triglycerides (mg/dl)	Cholesterol (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	Risk ratio	Atherogenic index
Control	160b	163ab	58.5ab	72.5b	2.8c	1.25b
High fat	222.5c	203.3c	54.17a	104.67c	3.77d	1.94c
M . oil	149.17ab	158.17ab	65cd	63.33ab	2.44ab	0.98ab
M . extract	163.83b	166b	61.5bc	71.73b	2.7bc	1.17b
Atorvastatin	137.67a	153.3a	68d	57.8a	2.26a	0.85a

Means in the same column followed by the same letters do not differ significantly, and when the means followed by different letters differ significantly at ($p \geq 0.05$)

Impact of moringa oil, moringa ethanol extract and atorvastatin supplementation on liver function

In Table 7 the hyperlipidemic control group showed decreases in the levels of albumin level compared with the control group. A low serum albumin indicates poor liver function. These results agree with [37,38] who reported that the high-fat diet reduced serum albumin. In addition, the plasma AST , ALT and ALP activities were a significant ($P<0.05$) increases in hyperlipidemic group when compared with control group [39]. recorded significant increases in the serum AST and ALT activities in rabbits fed a high fat diet (35% palm oil) compared with the control group, fed a standard diet. Increases in serum activities of these enzymes are usually indicative of possible liver damage. In rats subjected to high fat diet with moringa oil, seed ethanol extract and atorvastatin the enzyme liver marker indicate a decrease of AST, ALT and ALP as compared with hyperlipidemic group . However, Moringa and atorvastatin groups caused significant elevation ($P<0.05$) in mean value of albumin parameter. Earlier studies demonstrated that root and flower of M. oleifera had reduced elevated AST, ALT and ALP levels in rodents [40] .Also another study has showed that leaf extracts of M. oleifera had significantly restored the elevated AST, ALT and ALP enzyme levels to the normal levels [41].

Table 7: Effect of moringa oil, moringa ethanol extract and atorvastatin on liver function in rats feed on high fat diet

Group	Albumin (mg/dl)	ALT activity (U/L)	AST activity (U/L)	ALP activity (U/L)
Control	3.77b	30.33a	37a	81.67a
High fat	3.32a	78.83c	83.17c	96.83b
M . oil	3.89b	29.33a	30.5a	82.33a
M . extract	3.72b	56b	58b	88.17ab
Atorvastatin	3.88b	28.5a	30.33a	86.67ab

Means in the same column followed by the same letters do not differ significantly, and when the means followed by different letters differ significantly at ($p \geq 0.05$)

Effect of Moringa oil and ethanol extract on kidney function of hyperlipidemia rats.

Mean values of urea nitrogen and creatinine (mg/ dl) for the groups of rats are illustrated in Table 8 . From these results, it could observe that there was non-significant difference ($P< 0.05$) between positive control group and group treated with moringa or atorvastatin for

creatinine. While, there was significant difference between positive control group and group treated with moringa or atorvastatin for urea nitrogen which reduced the urea nitrogen level significantly. It appears from our results that *Moringa oleifera* improved the nutritional value and realized the best effect on kidney functions. On the contrary, in a previous study by [42] they concluded that the administration of the mice treated with doses of 600, 750 and 900 mg/kg bw of M. stenopetala aqueous leaf extract did not show significant change on urea, creatinine, total protein and uric acid as compared to the control group.

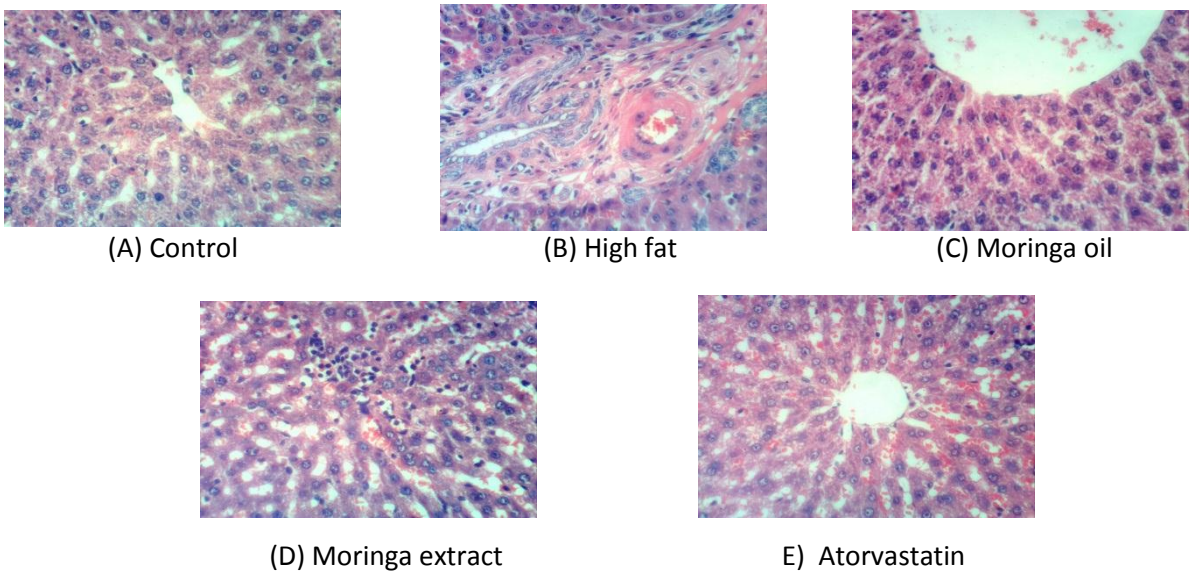
Table 8: Effect of moringa oil, moringa ethanol extract and atorvastatin on kidney function in rats feed on high fat diet

Group	Creatinine (mg/dl	Urea nitrogen (mg/dl
Control	0.9[a]	30.83[ab]
High fat	0.97[a]	33.33[b]
M . oil	0.92[a]	28.83[a]
M . extract	0.88[a]	30.67[ab]
Atorvastatin	0.9[a]	28[a]

Means in the same column followed by the same letters do not differ significantly, and when the means followed by different letters differ significantly at ($p \geq 0.05$).

Effect of Moringa oil and ethanol extract on histopathological changes of liver

Fig. 1 presents the histopathological observation of liver revealed the accumulation of triglycerides and fatty changes. Groups (A and C) showing no histopathological changes ,while group (B) high fat diet showing showing focal hepatic necrosis associated with inflammatory cells infiltration as well as congestion of hepatic sinusoids , group (D and E) showing congestion of hepatic sinusoids. The administration of moringa oil reversed the pathological changes and brought back the normal architecture of the liver.



(A) Control (B) High fat (C) Moringa oil

(D) Moringa extract E) Atorvastatin

Fig. 1: Histopathological changes detected in the liver of (A) control group, (B) high fat diet group, (C) moringa oil , (D) moringa ethanol extract and (E) Atorvastatin group

CONCLUSION

The effect of moringa seed oil and moringa seed ethanol extract were studied in experimental rats, where hyperlipidemia was induced through high fat diet. The administration of moringa seeds to the hyperlipidemic rats significantly reduced total cholesterol, TG, and LDL .The oil administration of moringa seeds revealed maximum protective effect in comparison with ethanol extract. Further in-depth studies can result in the development of an effective moringa seeds as anti-obesity drug.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

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Cite this article as:
Mustafa A. Hammam, Gaber A. Kalil, Salah M. El-Sayed, Ibrahim A. Ahmed. Effects of Moringa oleifera Lam (Moringaceae) Seeds in Rats Fed with High Fat Diet. J Pharm Chem Biol Sci 2016; 4(1): 76-87