HYPOLIPIDEMIC EFFECT OF Moringa oleifera seed oil on High Fat-Diet Induced Hyperlipidemia in Liver and Heart of Albino Rats

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Research Article

ABSTRACT

Objective: This study investigated the effect of Moringa oleifera seed oil on high fat-diet induced hyperlipidemia in liver and heart of albino rats for a period of 10 days. Methods: Twenty-four albino male Wistar rats were divided into four experimental groups of six animals each. Group 1 were fed with normal diet, Group 2 were fed with high fat-diet while Group 3 were fed with high fat-diet + Moringa oleifera seed oil extract (2ml/kg bodyweight) and Group 4 were fed with high fat-diet for 7 days + Moringa oleifera seed oil extract (2ml/kg bodyweight) later days. Results: The result shows significant (p < 0.05) increase in Triglycerides level in liver and heart (139.2±25.80 and 209.7±7.65 mmol/l) of rats fed with High fat-diet relative to control (73.93±6.63 and 58.85±4.86 mmol/l). Treatment with Moringa seed oil shows a significant (p < 0.05) decrease in the heart Triglyceride levels and Total cholesterol levels (92.14±15.97 and 183.4±4.27 mmol/l) relative to high fat-diet rat (209.7±17.65 and 197.8±1.78 mmol/l). Rats fed with high fat-diet for 7 days before treatment with Moringa seed oil shows a significant (p < 0.05) decrease in liver and heart Triglycerides (75.55±5.94 and 104.6±9.65 mmol/l) when compared to High fat-diet rats (139.2±25.80 and 209.7±17.65 mmol/l). Histopathological changes induced by high fat-diet in liver of rats were also significantly ameliorated by the Moringa oleifera seed oil. Conclusion: This finding suggests that Moringa oleifera seed oil possess a hypolipidemic effect on high fat-diet induced hyperlipidemia in the liver and heart of albino rat.

Key words: Moringa oleifera, hyperlipidemia, diet, cholesterol, triglyceride, liver and heart.

INTRODUCTION

Hyperlipidemia is a collective term used to describe conditions when the plasma level of one or more classes of lipids, namely cholesterol, triacylglycerides, phospholipids and fatty acids increases above normal levels. Hyperlipidemia is one of the major causes of the development of cardiovascular disorders [1]. Moringa oleifera, belong to the family Moringaceae, commonly known as Horse radish in English. It is known as ‘Zogalegandi’ in Hausa, ‘Ewegbele’ in Yoruba and ‘Okweyibo’ in Ibo in Nigeria. It is a small, fast, growing, evergreen, or deciduous tree that usually grows up to 10 or 12 m in height. Various parts of the Moringa oleifera Lam tree have been studied for several pharmacological actions. The aqueous extract of leaves of M. oleifera was reported to have wound healing [3] and antinursolithiasis activity [4].

In many cultures of the world, herbal remedies are increasingly being employed in an attempt to achieve the same curative or preventive purpose of treatment. In India, for instance, the leaf of Moringa oleifera Lam is claimed to possess cholesterol-reducing effect and is used to treat patients with heart disease and obesity [5].

Medicinally, the plant was reported to exhibit anti-inflammatory, antihypertensive and anti-ulcer activities [6]. It was also known to possess anti-bacterial activity against Bacillus subtilis, Mycobacterium phlei, Staphylococcus aureus, Salmonella and Shigella species [7]. The plant is also well known in traditional therapies as arbofacient and infertility control [8]. Phytochemical investigation on the plant revealed the presence of moringine and mornirine alkaloids in the root, pterygospermine alkaloid in the flower, fatty acids and fixed oils in the seed. Enzymes and bacteria substances were also found to be present in the exudates of this plant. The leaf of M. Oleifera is consumed in large quantities because of its medicinal and nutritional values in Northern Nigeria. Also, in developing countries, moringa has potential to improve nutrition, boost food security, foster rural development, and support sustainable land care. It may be used as forage for livestock, a micronutrient liquid, a natural anthelmintic and possible adjuvant [9]. Hence the purpose of this study was to investigate the hypolipidemic effect of Moringa oleifera seed oil on high fat-fed albino male Wistar rats.

MATERIALS AND METHODS

MATERIALS

Animal

Twenty four albino male Wistar rats of weight 165 ± 15 g were purchased from the Department of Animal Science, University of Ibadan, Ibadan, Oyo State. The experimental animals were kept in wooden cages at the animal house of the Department of Biochemistry, University of Benin, Benin City, Edo State. They were allowed to acclimatize to the new environment for two weeks before the study commenced.

Plant Material

Moringa oleifera Oil Extract

The Moringa oleifera seed oil was obtained from Millennium Quality Oil Factory, Gombe, Gombe State, Nigeria.

Chemicals/Reagents

All chemicals and reagents were of analytical grade. Total cholesterol, Triglyceride, and High density lipoprotein (HDL) – cholesterol assay kits were products of Randox, United Kingdom.

METHODS

Preparation of High Fat Diet- Induced Hyperlipidemia

The high fat-diet used to induce hyperlipidemia was formulated using the normal diet. The composition are made of normal diet (79%), supplemented with cholesterol (1%), egg yolk (10%) and coconut oil (10%).

Treatment of Animal

The animals were divided into four experimental groups of six animals each after two weeks acclimatization period. The animals were maintained under standard animal house condition in a well- ventilated wooden cages with 12 h light : 12 h dark schedules at 27 ± 1°C and were fed regularly with animal feed obtained from Bendel Feed Flour Mill, Ewu, Edo State and were allowed free access to water.
Group 1: Animals that feed on normal diet (control).

Group 2: Animals that feed on high fat-diet only (diet control)

Group 3: Animals that feed on high fat-diet + Moringa oleifera seed oil extract (2ml/kg body weight)

Group 4: Animals that feed on high fat-diet for 7 days + Moringa oleifera seed oil extract (2ml/kg body weight) later days.

At the end of 10 days, animals were fasted overnight and anaesthetized in a chloroform chamber.

Collection of Blood

The blood was collected by retro orbital sinus puncture, under mild chloroform anaesthesia. The blood samples were collected in lithium heparin bottles, and centrifuged for 10 minutes at 3000 rpm. The supernatants were stored frozen at –20°C until required for biochemical assays.

Tissue Collection/Preparation of Tissue Homogenates

Liver and heart collected during sacrifice were washed in ice-cold physiological saline repetitively and weighed accurately. A portion of each of the tissue was chopped into very small pieces and homogenized in ice-cold physiological saline (1g tissue: 5ml saline – 20% homogenates) using a pre-cooled mortar and pestle. The homogenates were centrifuged at 3000 rpm for 10 minutes and the supernatant stored frozen at –20°C until required for biochemical assays.

BIOCHEMICAL ANALYSIS

Determination of Total cholesterol, HDL-cholesterol and LDL-cholesterol Concentrations

Total cholesterol in the homogenate supernatants was determined using the direction described in total cholesterol assay kit on the enzymatic hydrolysis and oxidation point method of Allain et al. [10] and Roeschlaw et al. [11]. HDL-cholesterol concentration was determined following the method described by National Cholesterol Education Programme (NCEP) [12]. Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the HDL-cholesterol fraction in the supernatant were determined [13]; [14].

Determination of Triglycerides Concentrations

Triglycerides concentrations in the tissue homogenates supernatant were determined following the method described by National Cholesterol Education Programme (NCEP) [12]. The method described in the assay kit leaflets involves routine enzymatic methods exemplified by that described by Stein and Myers [15].

Histological examination of the tissue

The rat’s tissue of each rat was dissected out, trimmed into sizes and fixed in formalin. The tissue was attached to slide with the aid of moist brush and allowed to dry at about 40 – 50°C. This was latter incubated at 37°C for 12 hours and was finally stained with haematoxylin and eosin methods before viewing under microscope.

Statistical Analysis

The experimental results were expressed as mean ± standard error of mean (SEM) and were subjected to One Way Analysis of Variance (ANOVA). Significant level was set at p ≤ 0.05 using Tukey’s Multiple Comparisons Test from Graphpad Prism 6.0.

RESULTS

The effects of Moringa oleifera seed oil on rat lipid profiles for liver, heart and body/organ weight were presented in table 1.0, 2.0 and 3.0 respectively.

Relative to the control, Triglyceride level in liver was significantly (p ≤ 0.05) increase in high fat-diet group. While there was a significant (p ≤ 0.05) decrease in the Triglyceride level in animals treated with Moringa oleifera seed oil after 7 days high fat-diet compared to high fat-diet group. However, there was significant (p ≤ 0.05) increase in Total cholesterol of animals treated with Moringa oleifera seed oil after 7 days high fat-diet when compared with control but there was no significant different (p ≥ 0.05) in LDL- and HDL-cholesterols of the Moringa oleifera seed oil treated animals when compared to control and diet control group. (See table 1.0)

Relative to the control, there was significant (p ≤ 0.05) increase in triglyceride level in the heart of high fat-diet group and a significant (p ≤ 0.05) decrease in animals treated with Moringa oleifera seed oil when compared with the high fat-diet group. However, there was significant (p ≤ 0.05) increase in Triglyceride, Total cholesterol and HDL-cholesterol concentrations in the heart of high fat-diet group relative to control and a significant (p ≤ 0.05) decrease in Triglyceride and Total cholesterol levels in the animals treated with Moringa oleifera seed oil when compared with high fat-diet group while there was no significant different (p ≥ 0.05) in LDL-cholesterol of the Moringa oleifera seed oil treated groups when compared to control and diet control group. (See table 2.0)

Relative to the control, there were significant (p ≤ 0.05) increase in the body weight gain of high fat-diet rats and heart weight of Moringa oleifera seed oil treated rats after 7 days high fat-diet consumption. (See table 3.0)

Histology of the liver and heart

Prominent liver histology was seen in Group 1 (Fig. 1a) while the hepatocytes appeared distinct with well stained nucleus (blue). Distinct coarse gromeration and cell deposit was seen in Group 2 (Fig. 1b) while the hepatocytes were seen with viril fat deposit. Coarse gromeration and slight distortion in liver histology with evidence of collagen deposit and low fats deposit was observed in Group 3 (Fig. 1c). Distinct liver histology was seen in Group 4 (Fig. 1d) with evident collagens deposit around the portal tract while fat deposit was not seen. The observed deposit of collagen and low fats deposit indicate the ability of Moringa oleifera seed oil to ameliorate the induced hyperlipidemia in the liver of the rats under study.

Distinct and prominent cardiac muscle fibres were seen in Group 1 (Fig. 2a). Slight distortion in heart histology was seen with a clear companion of mild infection with waxy fibres in Group 2 (Fig. 2b). Mild area of infection mixed with normal intercalated disc spacing was seen with prominent nucleus in Group 3 (Fig. 2c). The muscles fibres appear distinct with polymorpho- nuclear leukocytic cells in Group 4 (Fig. 2d). The normality of the cardiac cell with prominent nucleus show that Moringa oleifera seed oil is a promising alternative in ameliorating high fat-diet induced hyperlipidemia in the treated rats.

DISCUSSION

The study was conducted to investigate the hypolipidemic effect of Moringa oleifera seed oil on high fat-diet fed albino Wistar rats. The results obtained from this study shows that feeding animals with high fat-diet resulted in increased triglyceride concentrations in liver and heart. High serum triglyceride levels has also been reported to be an important risk factor as it influences lipid deposition and clotting mechanism [16]. Many studies have reported high dietary fat and cholesterol induce hypercholesterolemia in animal models [17]; [18], similar results were observed with the high fat-diet fed animals having elevated lipid status than the control. Recently, a number of clinical studies suggest that the increased risk of coronary heart disease is associated with a high serum concentration of Total cholesterol, LDL-cholesterol and triglyceride. The abnormally high concentration of serum lipids is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots [19].

On the other hand, low serum concentration of HDL-cholesterol is also responsible for coronary heart disease [20]. Moringa oleifera seed Oil (MO) is a vegetable oil rich in mono-unsaturated fatty acid. It is extracted from Moringa oleifera seed, in which the crude fat content is as high as 20-45%. Oleic acid is the largest
component of its fatty acid contents. Studies show that Moringa oleifera is nutritious, have numerous antioxidants and have hypolipidemic effect [21].

Treatment with Moringa oleifera seed oil at 2ml/kg for 10 days resulted in significant decrease of triglyceride level in liver and heart, this findings is in agreement with bradycardia effect of Moringa oleifera leaves reported by Farouq et al [21], in which all parts of Moringa oleifera are reported with somewhat cardiac, circulatory stimulant activity and hypolipidemic activity [21]. Moringa oleifera has been demonstrated to prevent hyperlipidemia in male Wistar rat due to iron deficiency [22] during a study performing comparison of Moringa oleifera leaf extract with atenolol (a selective β1 receptor antagonist drug, used for cardiovascular diseases), it was reported that Moringa oleifera leaf extract possess hypolipidemic activity in lowering the body weight, heart weight, serum triglyceride level and serum cholesterol level in experimental animal [23].

The result obtained in the high fat-diet treated rats show a significantly increase in the body weight gain and heart weight when compare to control. It has been shown generally that high fat diets produce more rapid weight gain in rodents [24]. Body weight was reported to correlate with serum cholesterol levels during long term consumption of high fat diets. Long term intake of cholesterol in rats resulted in high blood cholesterol and caused accumulation of fats in tissues. The result suggests that the gain in body weight of these rats may be as a result of deposition of excess lipid that escaped the body’s threshold metabolism [25]. Increase in the body weight gain and heart weight of high fat-diet fed rats observed was able to be reversed by treatment with Moringa seed oil. Moringa seed oil is said to closely resemble olive oil in its fatty acids composition [26]. Studies have proved that the Mediterranean diet with low saturated and high monounsaturated fatty acids is effective in prevention of atherosclerosis and other diseases [27]. However, studies have shown that oils rich in monounsaturated fatty acids do not have the same effect [28]. In this present study, it was shown that Moringa oleifera seed oil has the tendency to prevent atherosclerosis but we are yet to prove which bioactive substance is responsible for its activity. Experimental studies using virgin olive oil to ascertain its effect on lipid metabolism in rats fed with high fat-diet, shows that the presence of high antioxidant phenolic compounds accounts for the cardio protective effect of Mediterranean diets [29]. Further studies might unveil the possible bioactive substance in Moringa oleifera seed oil that results in its hypolipidemic effect.

The histology of the liver reveals that high fat-diet rats show deposition of fatty cells on the tissues when compared with the control. This shows increase risk of cardiovascular disease and atherosclerosis which left untreated can lead to health risk and even dead. Treatment with Moringa oleifera seed oil shows a decrease in congested cells; fat deposit and less inflammatory cells (see Fig.1). This findings however, correlate with the hepatoprotective activity and antioxidant activity of Moringa oleifera seed oil [30]; [31]. Moringa oleifera is a rich source of antioxidant [32], recent studies comparing palm oil with Moringa oleifera seed for their antioxidant potentials found out that Moringa oleifera seeds are superior for radicals scavenging [33], for it hepatoprotective activity. Similar study also shows that hepatoprotective activity of Moringa oleifera is mediated by the levels of alanine and aspartate transferase, alkaline phosphatase and bilirubin in serum and in liver [34].

This study suggests that Moringa oleifera seed oil have a hypolipidemic effect on high fat-diet induced hyperlipidemia in rats. It therefore holds a promising effect in ameliorating hyperlipidemia in human.

CONCLUSION

In conclusion, the administrations of Moringa oleifera seed oil (MO) on high-fat-fed rats reduce heart total cholesterol and triglyceride levels. Also, the triglyceride level in the liver was reduced after 7 days MO administration. MO is beneficial in regulating lipid metabolism and in preventing hyperlipidemic complications. It is therefore recommended that consumption of MO in our daily nutrition will be a step to preventing hyperlipidemia, since Moringa seed oil possess a strong potential for use as therapeutic agent in lipid disorders.

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Results are expressed as Mean ± SEM (n=6).<sup>ab</sup>Values with different superscripts in the same column are significantly different. P set at 0.05.

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Results are expressed as Mean ± SEM (n=6).<sup>ab</sup>Values with different superscripts in the same column are significantly different. P set at 0.05.

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REFERENCES


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