

Hypocholesterolemic effects of crude extract of leaf of *Moringa oleifera* Lam in high-fat diet fed wistar rats

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Abstract

The leaves of *Moringa oleifera* Lam (Moringaceae) are used by the Indians in their herbal medicine as a hypocholesterolemic agent in obese patients. The scientific basis for their use in hypercholesterolemia was therefore examined. It was found that administration of the crude leaf extract of *Moringa oleifera* along with high-fat diet decreased the high-fat diet-induced increases in serum, liver, and kidney cholesterol levels by 14.35% (115–103.2 mg/100 ml of serum), 6.40% (9.4–8.8 mg/g wet weight) and 11.09% (1.09–0.97 mg/g wet weight) respectively. The effect on the serum cholesterol was statistically significant. No significant effect on serum total protein was observed. However, the crude extract increased serum albumin by 15.22% (46–53 g/l). This value was also found to be statistically significant. It was concluded that the leaves of *Moringa oleifera* have definite hypocholesterolemic activity and that there is valid pharmacological basis for employing them for this purpose in India. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Numerous population studies have linked elevated concentration of total cholesterol or LDL-cholesterol in plasma with increased incidence of atherosclerotic events (Goldstein et al., 1973; Keys, 1975). It has further been shown that the clinical complications of atherosclerosis could be diminished and life prolonged when plasma lipids are lowered by hypocholesterolemic agents (Lipid

Research Clinics Program, 1984a; Lipid Research Clinics Program 1984b; Helsinki Heart Study, 1987).

Many drugs with proven hypocholesterolemic activity are available clinically to ameliorate cases of individuals with premature arterosclerosis and those with other risk factors, such as hypertension or diabetes mellitus (Brown and Goldstein, 1992).

In many cultures of the world, herbal remedies are increasingly being employed in an attempt to achieve the same purpose. In India, for instance, the leaves of *Moringa oleifera* Lam is claimed to

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possess cholesterol-reducing effect and is used to treat patients with heart disease and obesity. For this reason it was decided to resolve this claim by investigating the effects of the crude extract of leaves of *Moringa oleifera* Lam (English: Horseradish plant or drumstick tree) on the serum, liver and kidney cholesterol of the wistar rat. Its effects on serum total protein, and albumin, were also examined in the same animal model.

Moringa oleifera Lam is native to south Asia, but grows in tropical Africa and Latin America (Ramachandran et al., 1980; Sofowora, 1982). Its potential medicinal value can be exploited in these areas to prevent, even manage incidences of hypercholesterolemia. In Nigeria, *Moringa oleifera* leaves are eaten as vegetables without any side effects being reported. These leaves are also eaten commonly as a food by infants and children in south India, because the high content of β -carotenes helps to prevent the development of vitamin A deficiency blindness.

2. Methods and materials

2.1. Preparation of crude leaf extract of *Moringa oleifera*

The identity of *Moringa oleifera* Lam (horseradish plant) was confirmed by Dr J.C. Okafor of the Forestry herbarium, Ministry of Agriculture and Natural Resources, Enugu, Nigeria. A voucher specimen is deposited in the forestry herbarium of the ministry (No.18/7830). The horseradish leaves were collected and the stalk removed. A total of 10 g of the leaves was ground and homogenised with 50 ml of distilled water in an electric blender. The decoction thereafter was filtered through a sterilised cheese cloth. The clear filtrate was stored in a refrigerator at 4°C and served as the stock crude extract. The weight of the extract was expressed as weight of leaves of *Moringa oleifera* Lam that produced that quantity of crude extract. The final concentration of the extract was 200 mg/ml.

2.2. Preparation of standard and high-fat diets

A standard diet and a high-fat diet (Adamu et al., 1982) were prepared by mixing calculated amounts of corn meal, milk powder, palm oil, millet husk, salt mixture and multivitamin tablets such that the final percentage of each component in the diets was as follows: carbohydrate, 73%; protein 16%; fat, 3%; crude fibre 5%; mineral salt, 2%; vitamins 1%, for the standard diet and carbohydrate, 56%; protein, 16%; fat, 20%; crude fibre, 5%; mineral salt, 2%; and vitamins, 1%, for the high-fat diet.

2.3. Condition and preparation of the animals

Fifteen male wistar rats weighing between 150 and 250 g were divided into three groups of five rats each. The first group of rats were fed ad libitum with standard diet. This was the first control group. The second and third groups were fed ad libitum with the high-fat diet. The third group of rats was given, in addition to high-fat diet, a daily dose of the extract, 1 mg/g body weight administered orally. The second group served as another control group. Normal and high-fat diet control groups were not administered with the extract but received instead equivalent volumes of normal saline everyday.

All animals were kept in well ventilated cages. They were fed with their respective diets and either extract or normal saline as the case may be for 30 days. At the end of this period, the animals were kept for overnight fasting. They were thereafter anaesthetised with ether and blood samples taken by cardiac puncture. The animals were then sacrificed and the livers and the kidneys were harvested for various estimations.

2.4. Serum, Liver and Kidney cholesterol estimation

Serum, liver and kidney cholesterol were determined by the method of Zlatkis et al. (1953).

2.4.1. Total serum cholesterol estimation

The following were added using volumetric pipettes: test sample (T): 0.05 ml Serum; 6.0 ml

glacial acetic acid; and 4.0 ml colour reagent; the standard sample (S): 1.0 ml 0.2 mg/ml cholesterol standard; 0.05 ml distilled water; 5.0 ml glacial acetic acid; and 4.0 ml colour reagent; while for the blank sample (B) the following reagents were added: 0.05 ml distilled water; 6.0 ml glacial acetic acid; and 4.0 ml colour reagent.

The two layers which formed between the colour reagent and glacial acetic acid were thoroughly mixed as soon as they were formed to ensure even distribution of heat. The test tubes were cooled and absorbance read with a 540 nm filter. Final values were obtained by using the relation: Total serum cholesterol = $T - B/S - B \times 0.2 \times 100/0.05 = T - B/S - B \times 400$ mg/100 ml.

2.4.2. Total tissue cholesterol estimation

The method of Zlatkis et al. (1953) was used for this estimation. 0.4 g of the tissue was weighed and refluxed with 20 ml of ethanol-ether (3:2 v/v) for 15 min. The infusion was filtered and the volume adjusted to 25 ml with ethanol ether. 0.1 ml of crude leaf extract of *Moringa oleifera* was evaporated to dryness in a boiling tube and to it was added 6.0 ml glacial acetic acid and 4.0 ml colour reagent in a test tube to serve as the test sample (T). The standard sample (S) contained 1.0 ml cholesterol (0.2 mg/ml); 0.1 ml distilled water; 5.0 ml glacial acetic acid and 4.0 ml colour reagent. The blank sample (B) test tube contained 0.1 ml distilled water, 6.0 ml glacial acetic acid and 4.0 ml colour reagent. The absorbance was measured at 540 nm. Final values were obtained by using the relation: Total tissue cholesterol = $T - B/S - B \times 0.2 \times 100/0.1 = T - B/S - B \times 200$ mg/g tissue.

2.5. Serum albumin and total protein determination

Serum albumin and total protein were determined by the method of Reinhold, 1953.

2.5.1. Total protein

The following were added into five different test tubes with the aid of volumetric pipettes (1 ml and 5 ml volumes): Biuret blank, 5.0 ml Biuret reagent

and 0.1 ml distilled water; Serum blank, 5.0 ml tartrate-iodide solution and 0.1 ml serum; Standard blank, 5.0 ml Biuret reagent and 0.1 ml distilled water; Protein standard, 5.0 ml Biuret reagent and 0.1 ml protein standard; Test sample, 5.0 ml Biuret reagent and 0.1 ml serum. The test tubes were placed in a water bath at 30°C for 10 min and absorbance measured with a 540 nm filter, using biuret blank for the zero setting.

2.5.2. Total protein and albumin

Total protein and albumin were determined by the method of Reinhold (1953). A sulphate-sulphite solution of 7.5 ml was measured into a test tube and 0.5 ml of serum was slowly added. The test tube was stoppered and the content mixed by inverting the tube. A volume of 2 ml of the mixture was added to 5.0 ml of biuret reagent in another test tube for total protein determination.

For albumin estimation, 3.0 ml of ether was poured into the test tube containing the remaining serum-sulphate-sulphite mixture. The test tube was stoppered and shaken thoroughly. This was centrifuged at 2500 rpm for 7 min and the supernatant used for albumin estimation. The following were added into five different test tubes using a 5 ml volumetric pipette. Biuret blank, 5.0 ml Biuret reagent and 2.0 ml sulphate-sulphite solution; Serum blank, 5.0 ml tartrate-iodide solution and 2.0 ml supernatant; Standard blank, 5.0 ml Biuret reagent and 2.0 ml distilled water; Protein standard, 5.0 ml Biuret reagent and 2.0 ml supernatant.

The test tubes were placed in a water bath at 30°C for 10 min and absorbance measured at 540 nm using biuret blank for zero setting. The serum blanks were read first. In all cases of protein and albumin estimation, the following relations were used: Protein, g/100 ml = $(U - Bu) \times C/S - Bs$, where U stands for absorbance of the test sample, S is the absorbance of the standard serum, Bs is the absorbance of the standard blank, Bu is the absorbance of the serum blank and C is the concentration of protein in the standard serum in g/100 ml. Albumin equals protein remaining in sulphate-sulphite solution.

All results were expressed as mean \pm SEM and significance of statistical difference between con-

trol and test means were determined using student's *t*-test.

3. Results

3.1. Effects on cholesterol levels

In this analysis, the values obtained from the high-fat diet exclusively fed rats are compared with those of the normal group, while the values for the group that were fed high-fat diet plus crude extract of *Moringa oleifera* are compared with those of the high-fat exclusive group.

Keeping the rats on high-fat diet significantly increased the total cholesterol levels in serum ($P < 0.0005$), liver ($P < 0.0005$), and kidney ($P < 0.01$) compared to rats on a normal diet. The increase was 28% in serum, 38% in liver, and 24% in kidney. When high-fat diet was co-administered with the crude extract of leaf of *Moringa oleifera* Lam, the cholesterol-increasing effect of high-fat diet was decreased. The cholesterol-lowering action of the crude extract was found to be significant ($P < 0.001$) in serum, but not in liver and kidney ($P < 0.1$). The percentage decrease in serum, liver and kidney was 14.35% (115–103.2 mg/100 ml of serum), 6.40% (9.4–8.8 mg/g wet weight) and 11.09% (1.09–0.97 mg/g wet weight) respectively (Tables 1 and 2).

3.2. Effects on protein and albumin levels

Neither high-fat diet nor high-fat diet plus

crude extract had any effect on serum total proteins. But on the other hand, high-fat diet reduced serum albumin by 20% ($P < 0.001$). The high-fat diet mixed with the crude extract resulted in a 15.22% increase in serum albumin compared to the high-fat diet alone ($P < 0.05$).

4. Discussion and conclusion

Crude extract of leaf of *Moringa oleifera* Lam has been shown to possess hypocholesterolemic activity. A dose of 1 mg/g extract when co-administered with high-fat diet, daily for a period of 30 days, had a cholesterol-reducing effect in serum (14.35%), liver (6.40%), and kidney (11.09%), compared to the high-fat fed group. The decrease was very significant in serum ($P < 0.001$) but not statistically significant in liver and kidney ($P < 0.1$).

The results of the Lipid Research Clinics Primary Prevention Trial indicate that there is a positive correlation between plasma concentrations of LDL-cholesterol and risk of coronary artery disease (Lipid Research Clinics Program, 1984a,b). This work, which was a multicentre, randomised, double-blind study showed that a 20% drug-induced reduction in LDL-cholesterol concentrations resulted in the reduction of newly positive exercise tests (indicative of myocardial ischemia), angina pectoris, and coronary bypass surgery in the treated group by 25, 20, and 21%

Table 1

Effects of crude extract of leaf of *Moringa oleifera* Lam on serum total cholesterol, albumin, and total protein of rats kept on high-fat diet^a

Parameter studied	Normal rats	High-fat diet fed rats	High-fat diet plus extract
Total cholesterol (mg/100 ml)	90.0 ± 3.1	115.0 ± 2.7 ^b	103.2 ± 2.2 ^c
Albumin (g/l)	57.2 ± 2.0	46.0 ± 2.6 ^c	53.0 ± 2.4 ^d
Total protein (g/l)	73.0 ± 2.9	74.7 ± 3.3ns	74.0 ± 4.1ns

^a Values are mean ± standard error of mean of five wistar rats. Significant changes are shown by: ns, not significant;

^b $P < 0.0005$;

^c = $P < 0.001$;

^d = $P < 0.05$.

Table 2

Effects of crude extract of leaf of *Moringa oleifera* Lam on the liver and kidney cholesterol (mg/g) of rats fed high-fat diet^a

Tissue	Normal rats	High-fat diet fed rats	High-fat diet plus extract
Liver	6.82 ± 0.22	9.4 ± 0.31 ^b	8.8 ± 0.16ns
Kidney	0.88 ± 0.04	1.09 ± 0.07 ^c	0.97 ± 0.06ns

^a Values are mean ± standard error of mean of five rats. Significant changes calculated by student's *t*-test. ns, not significant;^b *P* < 0.0005;^c *P* < 0.01.

respectively. This work showed that a 25% reduction of the total cholesterol in plasma would reduce the incidence of coronary events by nearly 50% (Lipid Research Clinics Program 1984b).

Considering the low dose of the extract used (1 mg/g), and the level of serum cholesterol-lowering effect achieved (14.35%), it can be concluded that leaf of *Moringa oleifera* is a potent hypocholesterolemic agent. Although the effect of increasing doses of the extract on the cholesterol concentration was not pursued, still, greater reductions in cholesterol level may be achieved with higher doses of the extract.

High-fat diet plus crude extract of *Moringa oleifera* Lam had no significant effect on serum total proteins. The high-fat diet reduced serum albumin by 20% (*P* < 0.001). However, the high-fat diet mixed with the crude extract produced a 15.22% increase in serum albumin compared to the high-fat diet alone (*P* < 0.05).

Saluja et al. (1978) reported isolating β-sitosterol from the stem of a hybrid variety of *Moringa oleifera* Lam. β-sitosterol is a plant sterol with a structure similar to that of cholesterol, except for the substitution of an ethyl group at C24 of its side chain. It is believed to lower cholesterol by lowering plasma concentrations of LDL (Kane and Malloy, 1982). Therefore β-sitosterol may be a bioactive phytoconstituent in the leaves of *Moringa oleifera* Lam.

Conclusively, the observed cholesterol-reducing action of the crude leaf extract of *Moringa oleifera* Lam indicates that this leafy vegetable possesses some potential medicinal value and could validate and explain its ethnomedical use on the obese and heart patients in India. We plan to conduct further studies to better understand the mechanisms of action of this medicinal plant.

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