

## SHORT COMMUNICATION

# Hypocholesterolemic Effect of *Moringa oleifera* Polyphenols in Rats Fed High Fat-Cholesterol Diet

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### ABSTRACT

**Introduction:** One of the greatest risk factors for cardiovascular diseases is hypercholesterolemia. *Moringa oleifera* is a good source of phytochemicals and is well explored for its antioxidant properties. **Methods:** The main aim of the present study was to assess the potential cholesterol lowering effect of *Moringa oleifera* leaf polyphenols (MOP) in an animal model. Five groups of male Wister rats were fed for 45 days as follows: a standard diet (GI); high fat-cholesterol diet (GII); high fat-cholesterol with MOP (100 and 200mg/kg body wt GIII & GIV respectively); and high fat-cholesterol with statins (Atorvastatin) (G-V). **Results:** Administration of MOP rich extract (GII and GIV) significantly ( $p \leq 0.05$ ) lowered the serum cholesterol, triglycerides and low-density lipoprotein cholesterol. A significant ( $p \leq 0.05$ ) decrease in the activity of the HMG CoA reductase enzyme was observed in GIII, GIV and GV but not in GI & GII. **Conclusion:** The results demonstrate that the polyphenol extract of *Moringa oleifera* leaves has a significant cholesterol lowering effect through inhibiting HMG CoA reductase activity and faecal bile acid binding.

**Key words:** Bile acids hypercholesterolemia, HMG CoA reductase, lipid profile

### INTRODUCTION

Disorders of cholesterol metabolism are treated by various drugs such as bile sequestering agents, nicotinic acid, statins, inhibiting 3-Hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase), the rate-limiting enzyme, which catalyzes the conversion of HMG-CoA to mevalonic acid (Rang, Dale & Ritter, 2007). Phenolic compounds are common constituents of our diet and are abundant in grains, vegetables, fruits, seeds, nuts and processed foods (Balasundram, Sundram & Samman, 2006).

The leaves of *Moringa oleifera* Lam (MO, Moringaceae) are popularly used in India

as herbal medicine for various ailments/disorders viz., antidiabetic, antioxidant and hypocholesterolemic. Our earlier studies have demonstrated the antioxidant efficacy (*in vitro*) inhibition of the rate limiting enzyme, HMG CoA reductase) in cholesterol synthesis as well as cholesterol reduction in rats on oral supplementation of *Moringa* leaves powder (Arabshahi-Delouee, Devi & Urooj, 2007) Reddy, Ahmed & Urooj, 2012; Oinam *et al.*, 2012). This study plans to elucidate the mechanism of anti-hypercholesterolemic action of *Moringa oleifera* polyphenols (MOP) in rats fed a high-fat cholesterol diet.

## METHODS

### Preparation of *Moringa oleifera* leaf extract

The leaves were collected locally and identified by Dr Shivamurthy, Department of Studies in Botany, University of Mysore and a voucher specimen was retained in the laboratory for future reference. The leaves were washed and dried at 50 °C. A 15 g dehydrated sample was extracted with 100 ml 80 % methanol (Methanol 80 ml and water 20 ml).

### Chemicals and reagents

All chemicals used were of analytical grade. HMG-CoA was obtained from sigma chemicals, Bangalore. The diagnostic kits of total cholesterol, HDL, triglycerides, protein and albumin were obtained from Agappe Diagnostics Ltd. Kerala.

### Animals and Induction of hypercholesterolemia

Forty male Wister rats weighing between 100-130 g were collected after getting the Animal Ethics Committee clearance from the Central Animal House of the University of Mysore (MGZ/1041/08-09, 25/08/08). The rats were grouped randomly into 5 groups of eight each. Group I was fed with a standard diet with serum cholesterol levels of 62 mg/dl, Groups II to V were fed a high fat-cholesterol diet (HFCD) and hypercholesterolemia condition was confirmed at 95 ± 19 mg/dl serum cholesterol levels.

### Treatment with MO polyphenols (MOP) and Atorvastatin

GI was fed with a standard diet (healthy control); GII was fed with a high fat - cholesterol diet (HFCD); GIII and GIV were given HFCD with 100 mg and 200 mg/kg body wt. of polyphenols, administered orally. GV was also fed with HFCD with the rats being given Atorvastatin orally at 10 mg/60 kg body weight. The rats were given food and water *ad libitum* during the

experimental period (45 days). At the end of the experimental period, the rats were starved and sacrificed at night after 9pm.

### Biochemical parameters

The serum protein, albumin, total cholesterol, triglycerides and HDL-cholesterol were analysed using diagnostic kits. The VLDL-cholesterol was calculated as TG/5.LDL-cholesterol. LDL-C levels were calculated using Friedwald's formula (Hermida *et al.*, 2008). HMG-CoA reductase was determined in liver microsomes by a spectrophotometric assay (Hulcher & Oleson, 1973). Dried faecal bile acid levels were estimated by colorimetric method and expressed as cholic acid equivalent per g of faecal matter (Snell & Snell, 1954).

### Statistical analysis

Each experiment was conducted in triplicates and data expressed as Mean ± SD. Data was subjected to two way ANOVA and Tukey's multiple comparison tests using SPSS software (11<sup>th</sup> version)

## RESULTS

Prior to the hypocholesterolemic treatment, the baseline mean serum total cholesterol levels of the standard diet fed GI group was 62 mg/dl, while it was 95 ± 19 mg/dl for Group II to Group V fed with high fat -cholesterol diet.

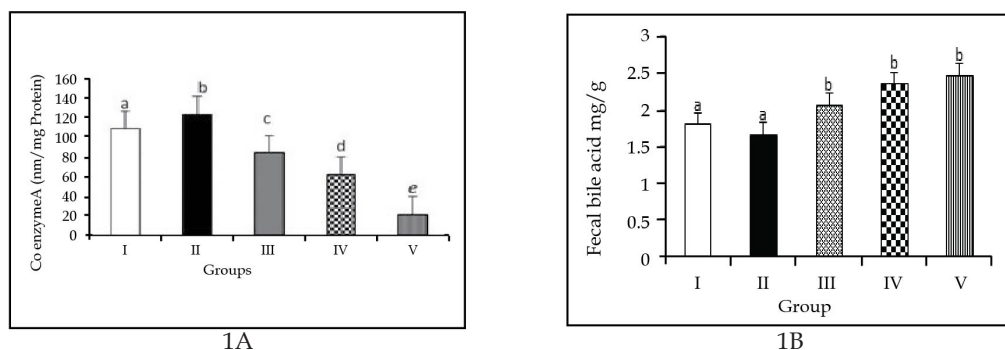
### Biochemical parameters of rat serum treated with MOP

After the 8-week experimental period, on an average, total cholesterol (TC) decreased from 95.19 to 82.17 in GIII, GIV and GV. The TC, LDL-C and other lipid parameters were significantly ( $p \leq 0.05$ ) low in all groups except in GII (positive control). In particular, the administration of 200 mg of *Moringa oleifera* polyphenols (MOP) (Group IV) reduced the TC and LDL-C levels to a greater extent. The HDL-C levels were high in control group (GI) and in experimental groups (GII, GIII, GIV

**Table 1.** Influence of *Moringa oleifera* polyphenols on serum protein and lipid profile of rats

| Group | TC <sup>ε</sup>           | HDL <sup>ε</sup>        | LDL <sup>ε</sup>         | VLDL <sup>ε</sup>        | TGL <sup>ε</sup>         | Protein <sup>ε</sup>    | albumin <sup>ε</sup>   |
|-------|---------------------------|-------------------------|--------------------------|--------------------------|--------------------------|-------------------------|------------------------|
| G-I   | 59.18±9.70 <sup>a</sup>   | 39.47±6.29 <sup>a</sup> | 8.76±2.45 <sup>a</sup>   | 10.96±6.52 <sup>a</sup>  | 39.65±9.45 <sup>a</sup>  | 6.641±0.64 <sup>a</sup> | 3.58±0.22 <sup>a</sup> |
| G-II  | 114.95±11.02 <sup>b</sup> | 27.72±6.80 <sup>b</sup> | 72.65±15.73 <sup>b</sup> | 14.55±6.625 <sup>a</sup> | 45.90±6.42 <sup>ab</sup> | 6.66±0.70 <sup>a</sup>  | 3.43±0.27 <sup>a</sup> |
| G-III | 85.31±9.48 <sup>c</sup>   | 25.75±3.88 <sup>b</sup> | 45.85±10.37 <sup>c</sup> | 13.71±5.79 <sup>a</sup>  | 50.94±6.93 <sup>b</sup>  | 6.65±0.30 <sup>a</sup>  | 3.34±0.32 <sup>a</sup> |
| G-IV  | 76.79±3.87 <sup>c</sup>   | 24.85±4.16 <sup>b</sup> | 19.58±7.58 <sup>d</sup>  | 32.36±9.57 <sup>b</sup>  | 50.85±7.10 <sup>b</sup>  | 6.62±0.26 <sup>a</sup>  | 3.52±0.27 <sup>a</sup> |
| G-V   | 84.41±2.43 <sup>c</sup>   | 24.87±3.94 <sup>b</sup> | 51.32±12.58 <sup>c</sup> | 8.7±4.22 <sup>a</sup>    | 36.39±6.05 <sup>a</sup>  | 6.51±0.32 <sup>a</sup>  | 3.52±0.19 <sup>a</sup> |

GI: Standard diet; GII: High fat and cholesterol diet (HFCD); GIII: HFCD+ Polyphenols (100mg/kg BW); GIV: HFCD+ Polyphenols (200mg/kg BW); GV: HFCD+ Atorvastatin (10mg/60kg bw); E- Expressed as mg/dl; ε - expressed as g/dl; (p≤0.05)



GI: Standard diet; GII: High fat and cholesterol diet (HFCD); GIII HFCD+ Polyphenol extract (100mg/kg BW); GIV: HFCD+ Polyphenol extract (200mg/kg BW); GV: HFCD+ Atorvastatin (10mg/60kg bw ( $p \leq 0.05$ )). Bile acid expressed as cholic acid equivalent.

**Figure 1.** Influence of *Moringa oleifera* polyphenols on cholesterol metabolism in rats

and GV) and did not differ significantly ( $p \leq 0.05$ ), however, VLDL and TGL levels were significantly ( $p \leq 0.05$ ) low in GI and GV (Table 1). The serum TGL levels were also high in GII, GIII & GIV. There was no significant ( $p \leq 0.05$ ) difference in serum protein and albumin levels among the groups.

#### HMG-CoA reductase activity and faecal bile acid

The rate of release of Coenzyme A was significantly low in GIII, GIV and GV compared to GI and GII. At the end of the assay, that is, at the 4<sup>th</sup> min, Coenzyme A released from GV was least, followed by GIV < GIII < GI < GII. The faecal bile acid increased in all experimental groups (GII, GIII, GIV and GV) compared to control (GI) (Figure 1A & 1B).

#### DISCUSSION

Feeding of MOP and high fat-cholesterol diet did not have any adverse effects on the apparent growth of rats. In the present study, treatment with MOP resulted in lowering of total cholesterol (TC) in HFCD fed groups after 45 days and also was equally potent compared to statins in reducing serum total cholesterol.

Cholesterol acts as starting material for bile acids synthesis in the liver, by promoting bile acid synthesis from cholesterol in the liver; the hypercholesterolemic condition can be reversed to some extent. In the present study, MOP reduced TC and LDL by inhibiting cholesterol synthesis and promoting bile acid synthesis by cholesterol. Other researchers have reported cholesterol lowering potency in rats and rabbits fed with different parts of *Moringa oleifera*, viz., crude leaf extract and cooked fruit (Mehta *et al.*, 2003; Ghasi, Nwobodo & Ofili, 2000)

Cholesterol homeostasis is maintained by controlling two processes, viz. cholesterol biosynthesis in which HMG CoA reductase catalyses the rate limiting process controlled by nutritional and hormonal state of animals and cholesterol absorption of both dietary cholesterol and cholesterol cleared from the liver through biliary secretion (Trapani, Segatto & Pallottini, 2012). In the present study, the activity of the enzyme is more significantly inhibited in GII, GIV & GV (Atorvastatin and MOP treated groups) than in GI & GII. Also, the hypocholesterolemic activity of the MOP and statins has been attributed to an increase in biliary cholesterol and

bile acids concentrations and a subsequent increase in the faecal excretion of these compounds.

Normal hepatobiliary secretion and enterohepatic circulation are required for the elimination of endogenous compounds such as cholesterol and bilirubin and their metabolites from the body, as well as the maintenance of lipid and bile acid homeostasis (Kosters & Karpen, 2008). As an important component of the enterohepatic circulation is the apical sodium co-dependent bile acid transporter (ASBT) which mediates the active re-absorption of conjugated bile acids in the terminal ileum (Izzat, Deshazer & Loose-Mitchell, 2000). A specific inhibitor of the ASBT protein would block the re-absorption of bile acids in the ileum and promote their excretion in the faeces, thereby reducing the amount of bile acids returning to the liver. The reduction in the bile acid pool due to increased faecal loss following administration of an ASBT inhibitor is expected to result in increased hepatic oxidation of cholesterol to bile acids, eventually depleting the liver pool of esterified cholesterol (Fki, Sahnoun & Sayadi, 2007).

In the present study, *Moringa* feeding was found to increase the excretion of faecal bile acids in GIII & GIV compared to the GI & GII. These results indicate that the MOP may have inhibited ASBT protein resulting in blockage of the re-absorption of bile acids in the ileum and promoting their excretion in the faeces, thereby reducing the amount of bile acids returning to the liver.

## CONCLUSION

The polyphenol extract of *Moringa oleifera* exhibited definite cholesterol lowering activity in rats with high serum cholesterol levels by influencing lipid metabolism as evidenced by inhibiting the key enzyme and faecal excretion of cholesterol metabolites. Thus, *Moringa oleifera* can be

explored as a potent functional ingredient in food formulations. Further studies are recommended on identification of the active phenolic acids and other phytochemicals to establish the plant as a potential nutraceutical with ethno-medicinal values.

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