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RESEARCH ARTICLE

The Antioxidant Activity of Low Doses of Moringa Seeds (*Moringa oleifera* Lam.) in Hypercholesterolemic Male Rats

Haddad A. El Rabey[1,2], Jalaluddin A. Khan[3], M I. Sakran[1,4], and Maryam A. Al-Ghamdi[3]

[1]Department of Biochemistry, Faculty of Science, University of Tabuk, Tabuk, Saudi Arabia; [2]Genetic Engineering and Biotechnology Institute, Sadat City University, Sadat City, Minufiya, Egypt; [3]Biochemistry Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia; [4]Biochemistry Section, Chemistry Department, Faculty of Science, Tanta University, Tanta, Gharbia Governorate, Egypt

Correspondence: helrabey@ut.edu.sa (H.A.ER.)

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ABSTRACT | The present study evaluated the antioxidant activity of two low doses of moringa seed powder (50 and 100 mg/kg body weight) in hypercholesterolemic male rats. Twenty-four albino rats were divided into four groups. The first group (G1) was the negative control group and was fed fat-rich diet. The second group (G2) was the hypercholesterolemic positive control and was fed 2% cholesterol in the fat-rich diet. The third group (G3) and the fourth group (G4) were also fed 2% cholesterol in the fat-rich diet and co-supplemented with 50 and 100 mg/kg body weight (b.w.) moringa seed powder, respectively, for 8 weeks. The hypercholesterolemic positive control group (G2) showed increase in lipid peroxidation and decrease in antioxidants in the serum and kidney tissue homogenate compared with the negative control group. In addition, the serum urea, uric acid, and creatinine were increased in G2. The histology of the kidney showed some pathological alterations compared with the negative control group. Treating the hypercholesterolemic rats with 50 or 100 mg moringa seeds powder/kg b.w. in G3 and G4, respectively, ameliorated the above altered parameters restoring them nearly to the normal levels, and also restored the normal histology of the kidney. The overall study showed that moringa seed powder has antioxidant activity in hypercholesterolemic male rats.

KEYWORDS | Antioxidants; Kidney disease; Lipid peroxidation; Moringa seed

ABBREVIATIONS | b.w., body weight; CAT, catalase; G1, group 1; G2, group 2; G3, group 3; G4, group 4; GR, glutathione reductase; GSH, reduced form of glutathione; HbA_{1c}, glycated hemoglobin; MDA, malondialdehyde; SOD, superoxide dismutase

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1. INTRODUCTION

The tree of *M. oleifera* (*Moringa oleifera* Lam.) is described as the miracle tree, tree of life, and God's gift to man [1]. Moringa has many health benefits when supplemented as leaves, roots, seeds, or their extract [2, 3]. Moringa root wood could reduce the elevated urinary oxalate and lower the deposition of stone-forming constituents in the kidney of calculogenic rats resulting from ethylene glycol-induced toxicity [4]. Moringa seed extract could ameliorate liver fibrosis in rats and reduce liver damage and symptoms of liver fibrosis and decrease the CCl_4-induced elevation of hepatic hydroxyproline content and myeloperoxidase activity [5]. The leaf extract of *M. oleifera* was also effective in protecting against liver injury induced by CCl_4 [6, 7]. It was also used in protecting against liver toxicity induced by various toxicants such as cadmium [8] and antimalarial combination of artesunate/amodiaquine [9]. The aqueous extract of moringa leaf was effective in treating streptozotocin-induced diabetic albino rats, showing antioxidant and antihyperglycemic effects [2]. The crude extract of moringa is a good scavenger for nitric oxide radicals and a potential source of natural antioxidants [10]. Moringa has nutraceutical uses and is used in the treatment of hypercholesterolemia and hyperglycemia. As a nutritional supplementation, it can be prescribed as food appendage for coronary artery disease patients along with their regular medicines [11]. Ghiridhari et al. [12] reported that medication with *M. oleifera* rendered diabetic patients better glucose tolerance by increasing treatment time. Moringa also increased wound healing of normal and dexamethasone-suppressed wound in rats [13]. In addition, moringa was effective in wound healing and as a food appendage for coronary artery diseases [11, 13]. Vongsak et al. [14] suggested that leaf maceration of *M. oleifera* and the 70% ethanol extract have high quality antioxidant raw material for pharmaceutical and nutraceutical development. While the high doses of moringa exert taxological effects in mice and rats [10, 15], the low doses of moringa seed powder (50 and 100 mg/kg body weight) were active against hyperglycemia [3] and hyperlipidemia [16]. Accordingly, this study aimed to test the antioxidant activity of two low doses of moringa seeds powder (50 and 100 mg/kg body weight) in hypercholesterolemic male rats.

2. MATERIALS AND METHODS

2.1. Animals

Twenty-four adult male albino rats of East China origin, weighing 180–200 g, were obtained from Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia. Animal experiments were conducted under protocols approved by the Institutional Animal House of the University of King Abdulaziz at Jeddah, Saudi Arabia. The rats were kept for two weeks for acclimatization before the start of the experiments. During all experiments, rats were housed 6 per cage and received fat-rich diet and tap water ad libitum in a constant environment (room temperature: 28 ± 2°C; room humidity: 60 ± 5%) with a 12 h light and 12 h dark cycle.

2.2. Experiment Design

The animals were divided into four groups. The first group (G1) serving as negative control was fed fat-rich diet. The second group (G2) of hypercholesterolemic positive control was fed 2% cholesterol in the fat-rich diet to induce hypercholesterolemia [17]. The third group (G3) and the fourth group (G4) were also fed 2% cholesterol and co-supplemented with 50 and 100 mg/kg body weight (b.w.) moringa seed powder, respectively, for 8 weeks. At the end of the experiment, animals were sacrificed under ether anesthesia. One kidney was kept on ice for tissue homogenate preparation and the other one was kept in 10% formalin for histopathological examination.

2.3. Kidney Homogenate Preparation

The homogenate was prepared under cooling condition (4°C). The kidney was cut into small pieces, washed by phosphate-buffered saline, and then grinded in a homogenization buffer consisting of 0.05 M Tris-HCl, pH 7.9, 25% glycerol, 0.1mM ethylenediaminetetraacetic acid, 0.32 M $(NH_4)_2SO_4$, and a protease inhibitor tablet (Roche, Basel, Switzerland). The lysates were homogenized on ice using a Polytron homogenizer. The suspension was sonicated in an ice bath to prevent overheating for 15 s followed by 5 min centrifugation at 12,000 rpm and 4°C. The supernatant was aliquoted and stored at −80°C until use.

2.4. Blood Sampling and Biochemical Analysis

Blood samples of rats were centrifuged at 2,000 *g* for 10 min at 4°C, and aliquoted for the respective analytical determinations.

2.4.1. Glycated Hemoglobin

Glycated hemoglobin (HbA_{1c}) was determined according to the method described by Antunes et al. [18] using a glycohemoglobin reagent kit from Pointe Scientific (Canton, MI, USA) according to the manufacturer's instructions.

2.4.2. Fasting Blood Glucose

Fasting blood glucose was determined using a glucose kit from Human Diagnostics (Wiesbaden, Germany) based on the method of Barham and Trinder [19], following the manufacturer's instructions.

2.4.3. Lipid Peroxidation and Antioxidants Activity

Lipid peroxidation was determined by measuring malondialdehyde (MDA) according to the method of Ohkawa et al. [20]. The activity of catalase (CAT)

TABLE 1. Effect of moringa seed powder supplementation for 8 weeks on serum lipid peroxidation and antioxidants in hypercholesterolemic male rats

Parameters	Statistics	G1	G2	G3	G4
MDA (nmol/ml)	LSD 0.05 = 0.467	2.06 ± 0.08^a	10.68 ± 0.21^d	6.28 ± 0.13^b	3.35 ± 0.18^c
	t-test		*–3.22****	*1.87****	*1.52****
CAT (U/ml)	LSD 0.05 = 0.247	4.64 ± 0.11^a	0.583 ± 0.03^d	1.73 ± 0.04^c	3.36 ± 0.08^b
	t-test		*0.03****	*–1.44****	*–1.77****
SOD (U/ml)	LSD 0.05 = 9.578	851.38 ± 2.42^a	385.08 ± 2.05^d	518.92 ± 2.79^c	757.28 ± 4.22^b
	t-test		*125.99****	*–38.04****	*–66.73****
GSH (nmol/ml)	LSD 0.05 = 1.545	1.37 ± 0.043^a	0.064 ± 0.001^c	0.414 ± 0.009^d	0.728 ± 0.031^b
	t-test		*1.78****	*–3.42****	*–2.75****
GR (U/ml)	LSD 0.05 = 40.994	617.11 ± 20.86^a	216.12 ± 4.24^d	416.83 ± 16.41^c	512.68 ± 4.26^b
	t-test		*79.77****	*–35.51****	*–56.00****

Note: Data are represented as mean ± SE. t-test values: ***, significant at $p < 0.001$. ANOVA analysis: within each row, means with different superscript (a, b, c, or d) are significantly different at $p < 0.05$, whereas means having superscripts with the same letters mean that there is no significant difference at $p > 0.05$. LSD, least significant difference.

and superoxide dismutase (SOD) were determined according to the method of Aebi [21] and Nishikimi et al. [22], respectively, and the amount of the reduced form of glutathione (GSH) and the activity of glutathione reductase (GR) were estimated in the serum and the kidney homogenate according to the method of Saydam et al. [23]. The above antioxidant and lipid peroxidation tests were performed using the specified assay kits purchased from Biodiagnostic (Giza, Egypt) according to the instructions of the manufacturer.

2.4.4. Renal Functions

Serum urea, uric acid, and creatinine were determined according to the methods of Fawcett and Scott [24], Fossati et al. [25], and Tietz [26], respectively, using a kit from Human Diagnostics according to the instructions of the supplier.

2.5. Histopathological Examination

The fixed kidney tissue was processed routinely and embedded in paraffin. Sections of 5 µm thick were prepared and stained with hematoxylin and eosin (H&E) dye for microscopic investigation [27]. The stained sections were examined and photographed using an Olympus microscope with a fixed digital camera.

2.6. Statistical Analysis

Data were analyzed using the SPSS software (Chicago, IL, USA) to calculate values for t-test and the mean ± SE, and then analyzed using one way analysis of variance (ANOVA) using Duncan's multiple range test [27].

3. RESULTS

3.1. Serum Lipid Peroxidation and Antioxidants

Table 1 shows the effect of moringa seed powder supplementation for 8 weeks on serum lipid peroxidation and antioxidants in hypercholesterolemic male rats. The oral administration of 2% cholesterol in G2 for 8 weeks significantly ($p < 0.001$) increased serum lipid peroxidation. The co-supplementation with 50 mg/kg b.w. of moringa seed powder in G3 and 100 mg/kg b.w. of moringa seed powder in G4 significantly ($p < 0.001$) decreased serum lipid peroxidation. The higher dose of moringa in G4 was more effective in decreasing lipid peroxidation than the lower one in G3. **Table 1** also shows that the oral administration of 2% cholesterol in G2 for 8 weeks significantly ($p < 0.001$) decreased serum catalase, SOD, GSH, and GR. The co-supplementation with 50 mg/kg b.w. of moringa in G3 and 100 mg/kg b.w.

TABLE 2. Effect of moringa seed powder supplementation for 8 weeks on lipid peroxidation and antioxidants of kidney tissue homogenate in hypercholesterolemic male rats

Parameters	Statistics	G1	G2	G3	G4
MDA (nmol/g)	LSD 0.05 = 0.709	3.10 ± 0.09^{a}	13.01 ± 0.20^{b}	6.80 ± 0.21^{c}	4.26 ± 0.28^{d}
	t-test		*–44.76****	*17.87****	*27.15****
CAT (U/g)	LSD 0.05 = 2.929	25.93 ± 0.90^{a}	4.01 ± 0.21^{d}	12.85 ± 0.26^{c}	19.51 ± 1.63^{b}
	t-test		24.85***	–25.07***	–9.67***
SOD (U/g)	LSD 0.05 = 47.839	111.68 ± 3.04	644.67 ± 4.01^{d}	814.73 ± 3.54^{c}	958.68 ± 29.81^{b}
	t-test		*138.76****	*–55.90****	*–9.70****
GSH (nmol/g)	LSD 0.05 = 5.606	2.782 ± 0.045^{a}	1.117 ± 0.045^{c}	1.784 ± 0.024^{d}	2.337 ± 0.112^{b}
	t-test		*15.70****	*–19.19****	*–7.92****
GR (U/g)	LSD 0.05 = 52.813	780.47 ± 13.64^{a}	342.90 ± 8.48^{d}	424.02 ± 29.96^{c}	682.75 ± 198.37^{b}
	t-test		*205.72****	*–29.83****	*–17.84****

Note: Data are represented as mean ± SE. t-test values: ***: significant at $p < 0.001$. ANOVA analysis: within each row, means with different superscript (a, b, c, or d) are significantly different at $p < 0.05$, whereas means having superscripts with the same letters mean that there is no significant difference at $p < 0.05$. LSD, least significant difference.

in G4 significantly ($p < 0.001$) increased the serum catalase, SOD, GSH, and GR.

3.2. Lipid Peroxidation and Antioxidants in the Kidney Tissue Homogenate

Table 2 shows the effect of moringa seed powder supplementation for 8 weeks on lipid peroxidation and antioxidants in kidney tissue homogenate of hypercholesterolemic male rats. The oral administration of 2% cholesterol to the rats of G2 for 8 weeks significantly ($p < 0.001$) increased lipid peroxidation and decreased catalase, SOD, GSH, and GR in the kidney tissue homogenate. The co-supplementation with 50 mg/kg b.w. of moringa seed powder in G3 and 100 mg/kg b.w. of moringa seed powder in G4 significantly ($p < 0.001$) decreased lipid peroxidation and increased catalase, SOD, GSH, and GR in the kidney tissue homogenate. The higher dose of moringa in G4 was more effective in increasing antioxidants and decreasing lipid peroxidation than the lower one in G3.

3.3. Kidney Functions

Table 3 shows that serum creatinine, uric acid, and urea were significantly increased as a result of hypercholesterolemia in G2. The concurrent treatment with the low doses of moringa seed powder in G3 and G4 significantly decreased the above elevated kidney function parameters and nearly restored them to the normal values as in G1. **Table 3** also shows that fasting glucose level was not significantly affected by hypercholesterolemia in G2 or by treatment with moringa in G3 and G4. Similarly, the level of glycated hemoglobin was not affected either by induced hypercholesterolemia in G2 or by treating with the two doses of moringa in G3 and G4 (**Table 3**).

3.4. Kidney Histology

The kidney of the negative control rats shows normal renal histological structure of parenchyma and glomeruli (**Figure 1A**). **Figure 1B** shows the kidney from the positive control group exhibiting structural changes including thickened glomerular basement membrane, vacuolated endothelial lining glomeruli, and vacuolated epithelial lining renal tubules. The co-supplementation with 50 mg/kg b.w of moringa seeds powder in G3 nearly restored the renal tissue to its normal histology with no histopathological changes (**Figure 1C**). In **Figure 1D**, the kidney tissue of hypercholesterolemic rats in G (4) treated with a higher dose of moringa (100 mg/kg b.w.) exhibited normal histological structure with no histopathological changes.

TABLE 3. Effect of moringa seed powder supplementation on kidney functions in hypercholesterolemic male rats for 8 weeks

Parameters	Statistics	G1	G2	G3	G4
Uric acid (mg/dl)	LSD 0.05 = 0.930	4.01 ± 0.19^{a}	4.55 ± 0.26^{b}	4.28 ± 0.24^{d}	4.16 ± 0.38^{c}
	t-test		*–0.57****	*0.92****	*0.74****
Creatinine (mg/dl)	LSD 0.05 = 4.477	0.74 ± 0.03^{a}	0.95 ± 0.03^{d}	0.88 ± 0.03^{b}	0.80 ± 0.04^{c}
	t-test		*–0.22****	*–0.46****	*–0.30****
Urea (mg/dl)	LSD 0.05 = 5.194	23.94 ± 1.64^{a}	26.63 ± 1.72^{d}	23.26 ± 1.66^{c}	22.95 ± 1.72^{b}
	t-test		*–0.14****	*0.13****	*0.42****
FBG (mg/dl)	LSD 0.05 = 0.257	113.17 ± 4.16^{a}	115.50 ± 3.41^{a}	112.50 ± 4.52^{a}	113.50 ± 3.78^{a}
	t-test		*–0.34*^{NS}	*0.82*^{NS}	*0.46*^{NS}
HbA_{1C} (%)	LSD 0.05 = 12.603	5.01 ± 0.10^{a}	5.08 ± 0.09^{a}	5.03 ± 0.08^{a}	5.08 ± 0.06^{a}
	t-test		*–0.39*^{NS}	*0.48*^{NS}	*0.00*^{NS}

Note: Data are represented as mean ± SE. t-test values: ***, significant at $p < 0.001$. ANOVA analysis: within each row, means with different superscript (a, b, c, or d) are significantly different at $p < 0.05$, whereas means having superscripts with the same letters mean that there is no significant difference at $p < 0.05$. LSD, least significant difference; FBG, fasting blood glucose; NS, nonsignificant.

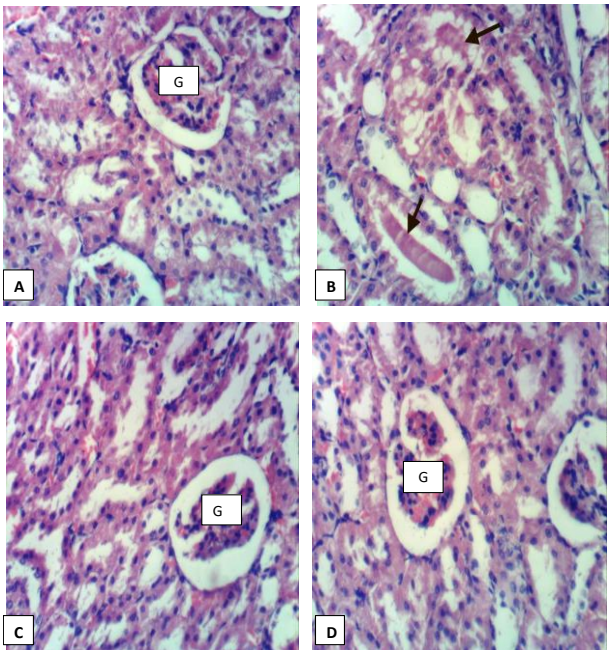

FIGURE 1. Photomicrographs of sections of the kidneys from rats in different groups. A: The rat kidney from the negative control group (G1) showing normal histological structure; B: The rat kidney from the hypercholesterolemic group (G2) showing protein cast in the lumen of renal tubules (arrows), focal tubular necrosis, and thickening of parietal layer of Bowman's capsule; C: The rat kidney from group 3 (G3) showing no histopathological changes; D: The rat kidney from group 4 (G4) showing no histopathological changes. G indicates glomeruli (H & E, ×400).

4. DISCUSSION

In the current study, induction of hypercholesterolemia by feeding rats of the positive control group on 2% cholesterol in the fat-rich diet for 8 weeks [17, 28, 29] caused detrimental effects as indicated by increased lipid peroxidation, decreased antioxidants (catalase, GSH, GR, and SOD), and compromised renal functions [28, 29], whereas glycosylated hemoglobin (HbA_{1c}) and fasting blood glucose were not significantly affected. Treating the hypercholesterolemic male rats under the present study with 50 or 100 mg moringa seeds powder/kg b.w. in G3 and G4, respectively, ameliorated the induction of lipid peroxidation and reduction of antioxidants, with the parameters approaching the negative control values. This beneficial effect may be ascribed to the scavenging activity of moringa seed towards free radicals, including nitric oxide radicals [10, 14]. Indeed, Kumbhare et al. [10] reported that moringa is a good scavenger for nitric oxide radicals and a potential source of natural antioxidants. The leaves, roots, seeds, bark, fruit, flowers, and immature pods of *Moringa oleifera* have important medicinal properties and a high nutritional value [30]. Moringa seed powder contains glucosinolates such as glucomoringin, flavonoids (e.g., quercetin and kaempferol), and phenolic acids (e.g., chlorogenic acid) [1, 2], and is characterized by the high antioxidant, hypoglycemic, hypotensive, anti-dyslipidemic, anticancer, and anti-inflammatory properties [31–33]. The kidney function parameters (uric acid, creatinine, and urea) were significantly compromised with hypercholesterolemia. Indeed, elevated total cholesterol and the low-density lipoproteins (LDL), as well as the reduced high-density lipoprotein (HDL) all increase the risk of developing renal dysfunction [28, 29, 34, 35]. The co-supplementation with moringa seed powder for 8 weeks significantly decreased serum uric acid and serum creatinine. These results agree with findings from previous investigations [3, 36, 37]. The histology of the kidney also showed pathological alteration compared with the negative control group and this was in line with the increase of lipid peroxidation and the decrease of antioxidants activity [28, 35]. Restoring the kidney tissue to its normal histological morphology upon the co-supplementation with moringa seed powder in G3 and G4 is likely due to the antioxidant activity of moringa [3, 10, 14].

5. CONCLUSION

The present study demonstrated that the low doses of moringa seed powder increased antioxidants and decreased lipid peroxidation in the serum and kidney tissue homogenate in hypercholesterolemic male rats. The low doses of moringa also restored the kidney function and morphology of the hypercholesterolemic male rats nearly to the normal state as in the negative control group.

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