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Effect of moringa extract against renal injury caused by high fat diet induced obesity in male rats

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Abstract: The study was performed to study the influence of *moringa* extract against renal injury caused by high fat diet in male rats. Rats randomly were divide into six groups, six animals each as following: group 1: rats not received any tratment; group 2: rats received moringa extract (ME) (300mg/kg b w) daily for 8 wks.; group 3 : rats of this group fed on HFD daily for 8 wks.; group 4: rats of this group fed on HFD concomitant with ME (300 mg/kg bw) for daily for 8 wks.; group 5: rats of this group fed on HFD concomitant with SIM (40 mg/kg bw) daily for 8 wks.; group 6: rats of this group fed on HFD concomitant with ME (300 mg/kg bw) and SIM (40 mg/kg bw) daily for 8 wks. Our results proved that the administration of high fat diet fulfilled significant rise in MDA. However, marked reduction in GSH, TAC, SOD, CAT ,GSH-Px, and GSH-Rd On the other side, the administration ME improved the deviations resulted from HFD

keywords: Moringa , high fat diet, , renotoxicity

1.Introduction

Obesity has become a global health problem, which is accompanied with various disorders such as dyslipidemia, diabetes, insulin resistance, hypertension, stroke and arteriosclerosis **[1]**. The World Health Organization (WHO) estimates that over 1 billion adults are overweight and at least 300 million are obese worldwide **[2]**

obesity are known as excessive lipids accumulation in adipose tissue throughout the body **[3]**. According to the world health organization (WHO), obesity is defined by a body mass index (BMI) of 22- 29.9 and 30 kg/m^2 respectively. BMI is an established measure for classifying weight, and is defined by the ratio between weight (kg) and the square of the height (m^2), also it is often used as a surrogate for total body fat [4] **.**

The major cause of the development of obesity is the imbalance between energy intake and energy expenditure **[5]**. This imbalance is well known to be a consequence of contributing factors, including hereditary, metabolic, behavioral and environmental factors **[6]**.

Moringa oleifera (drumstick) is medicinally important tree species of family Moringaceae and it contains several minerals **[7].** Moringa morphologically like as a stick therefore another name given as drum stick and it is referred to as a miracle tree. It is a fast growing plant and reaches to 10 m tall. The flowers long are 1.5-2 cm .The leaf long is up to 60 cm. Moringa is widely cultivated all over the plains of India and naturalized in tropical area. It grows in all types of soil and grows best in conditions provided in North India and South India **[8]** The study was performed to evaluate the influence of moringa extract againt renal disorder caused by high fat diet in male rats

2. Materials and methods

2.1. Chemicals

Zocor (Simvastatin) was obtained from Global Napi Pharmaceuticals Egypt. SIM received orally (40 mg/kg bw). The dose of SIM was according to study **[9]**.

Cholesterol was obtained from Sigma Chemical Company (St. Louis, MO, USA). It is added in high fat diet at dose 2% .The dose of cholesterol as the study [10]**.**

2.2. Moringa extract

Packed cleaned dried Leaves of moringa (*Moringa oleifera*) was obtained from local market , Egypt.

About 40 g of the powdered leaves immersing it in 250 ml of ethanol (70%). Then the mixture put for three days in refrigerator .Then by filter paper What man No 1, the mixture was filtrated. The by Rotary Evaporator, the filtrate was concentrated. The extract dissolved in distilled water . The dose moringa extract (300 mg extract /kg bw) as study **[11]**.

2.3. Animals

Rattus norvegicus, white male albino rats 8 weeks old, with average weight 160g were used for experiment. Rats were obtained from Egyptian Institute for Serological and Vaccine production, Helwan, Egypt and were housed in the animal house of the Department of Zoology, Faculty of Sciences, Mansoura University. Rats were put in cages containing wood-chip bedding, renewed every day. They were kept in a temperature-controlled environment with a 12 h light/dark cycle. All animals were placed for seven days before the experiment. The animals were given water *ad libitum, normal* diet and high fat diet in this experiment. The experimental protocol was carried out in accordance with the guide of the National Research Council for the Care and Use of Laboratory Animals and was approved by the local experimental animal ethics committee of the Department of Zoology, Faculty of Sciences, Mansoura University.

2.4. Animal grouping

After one week of acclimatization, rats were randomly subdivided into six groups, each group containing six males as follow:

1. Control group: Rats not received any treatment.
2. Moringa extract (ME) treated group: Rats received orally ME (300mg/kg bw) daily for 8 weeks.
3. High fat diet (HFD) treated group: Rats of this group fed on HFD daily for 8 weeks.
4. High fat diet and moringa extract (HFD+ ME) treated group: Rats of this group fed on HFD concomitant with ME (300 mg/kg bw) for daily for 8 weeks.
5. High fat diet and simvastatin (HFD+SIM) treated group: Rats of this group fed on HFD concomitant with SIM (40 mg/kg bw) daily for 8 weeks.
6. High fat diet and moringa extract and simvastatin (HFD+ME+SIM) treated **group:** Rats of this group fed on HFD concomitant with ME (300 mg/kg bw) and SIM (40 mg/kg bw) daily for 8 weeks.

2.5. Sample collection

After eight weeks, fasted rats were sacrificed 24 hrs after the last treatment .Rat kidney was weighed for each rat. Kidney tissue was homogenized in distilled water (10% w/v.) The homogenate of the kidney were kept at -20^{o}C till used in labeled Eppendorf's tubes for biochemical estimations. For histological studies, other samples of the kidney tissue were stored in neutral formalin (10%) .

2.6. Parameters assayed

Homogenates from the kidney were used for the determination of malondialdehyde (MDA) content according to Ohkawa, H. et al [12], glutathione (GSH) content as described by Beutler, E. [13], and total antioxidant capacity (TAC) as previously assayed by Koracevic, D., et al. [14]. The activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione reductase GSH-Rd as described previously [15], [16] [17], [18] respectively in kidney homogenates.

2.7. Statistical analysis

GraphPad Prism 5.0 software was used for all statistical analyses.The data were presented as mean and standard error (SE) (n=6). Statistical comparisons were made by one way analysis of variance (ANOVA) at $P \leq 0.05$. [19].

3. Results

Table 1 data show increase in MDA content and decrease in levels GSH, TAC, SOD, CAT , GSH-Px and GSH-Rd in HFD rats. Whereas, the treatment with ME of HFD fed rats showed

amelioration in MDA and increase of the antioxidants of kidney.

Table (1) Effect of Moringa extract on oxidative stress and antioxidants in kidney:

Parameters	Control	ME	HFD	HFD+ME	HFD+SIM	HFD+ME+SIM
MDA (nmol/g)	409.9^{a}±22.39	402.0^{a}±11.68	1478^{b}±59.46	560.2^{c}±25.31	470.0^{a}±15.28	420.2^{a}±15.44
GSH (mmol/g)	238.7^{a}±4.301	241.8^{a}±4.891	96.30^{b}±2.703	150.2^{c}±6.343	176.4^{d}±7.281	236.6^{a}±4.302
TAC (mM/g)	1.206^{a}±0.01083	1.194^{a}±0.0106	0.9755^{b}±0.0823	1.173^{a}±0.0020	1.180^{a}±0.004425	1.191^{a}±0.005625
SOD (U/g)	739.6^{a}±46.14	827.0^{a}±22.26	181.3^{b}±7.149	698.0^{a}±29.80	704.3^{a}±26.93	712.7^{a}±30.82
CAT (U/g)	180.6^{a}±6.042	180.3^{a}±6.67	43.5^{b}±4.089	126.3^{c}±8.545	133.8^{c}±4.697	188^{a}±5.422
GSH-Px (U/g)	1219^{a}±10.86	1232^{a}±10.14	762.3^{b}±17.44	1126^{c}±13.42	1176^{a}±14.97	1192^{a}±13.85
GSH-Rd (U/g)	648.6^{a}±7.400	679.4^{a}±15.01	440.5^{b}±17.64	576.2^{a}±18.47	595.1^{a}±29.56	615.6^{a}±21.48

Results were presented as means ± SE n=6.

Different letters (significant), similar letters (non-significant) change at $p \leq 0.05$

C: control ME: Moringa extract, HFD: High fat diet, SIM: Simvastatin

Plate 1 Figures A-F
Histopathological kidney change

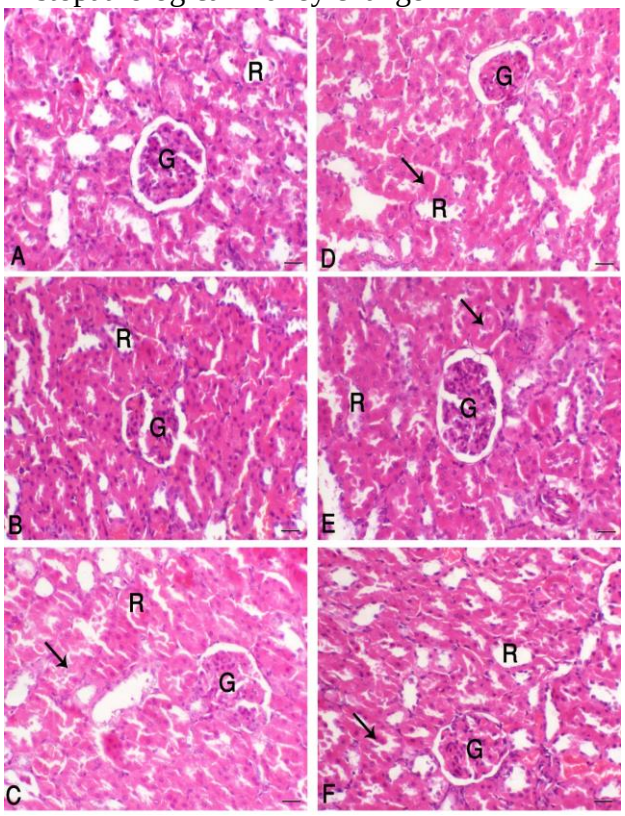

Fig. 1. Kidney sections of different treated groups. A) Kidney of control animal showed normal glomeruli (G) and renal tubules (R), B) Kidney of normal animal treated with ME extract showed normal glomeruli (G) and renal tubules (R), C) Kidney of HFD-treated animal showed marked degenerative changes within the renal tubules lining epithelium (arrow), D) Kidney of diseased animal treated with ME showed mild degree of renal tubular epithelium degeneration (arrow), E) Kidney of diseased animal treated with SIM showed mild degree of renal tubular epithelium degeneration (arrow) and F) Kidney of diseased animal treated with combination of ME and SIM showed mild degree of renal tubular epithelium degeneration (arrow), (G indicates glomeruli and R indicates renal tubules), H&E, bar= 40 µm.

4. Discussion

In this study administration of HFD showed decrease levels of kidney SOD, CAT, GSH, TAC, GSH-Rd, and GSH-Px in HFD rats and increase in oxidative stress and marked degenerative changes within the renal tubules lining epithelium in HFD rats compared to normal rats . There are several mechanisms explaining the reduction of antioxidant enzymes in obese rats, the increased lipid peroxidation lead to inactivation of the enzymes by crosses linking with MDA. This will cause an increased accumulation of superoxide, hydrogen peroxide and hydroxyl radicals which could further stimulate lipid peroxidation. Also, decreased of antioxidant enzyme may be due to rapid consumption and exhaustion of storage of this enzyme in fighting free radicals generated during development of obesity. The increase of oxidative stress may be led to damage in kidney histology represented by marked degenerative changes within the renal tubules lining epithelium [20]

On the other side, rats that received moringa extract with HFD reduced oxidative stress companied by increased in antioxidants including GSH, TAC, SOD, CAT, GSH-Rd and GSH-Px. The amelioration may be due to moringa extract contains antioxidant components, as phenol and tannins **[21]** .

Also, moringa extract with antioxidants led to recovery of damaged kidney cells. These amelioration may be due to moringa extract contains poly phenols, flavonoids β-sitosterol, kaempferol and quercetin which have hydroxyl

groups. The hydroxyl group, because of its resonance property, easily donates electrons to free radicals and effectively neutralizes them. Also, the presence of a hydroxyl group rises its antioxidant potential through intermolecular hydrogen bonding involving the -SH group of non-protein thiols and enzymes leading to the renovation of the antioxidant system against oxidative stress [7]

In addition, special phenolic compounds may induce production of antioxidant enzymes.β-Carotene from moringa leaves has shown significant effects and it is efficiently converted into vitamin A in the body [22]**.** Moreover, kidney section of the rat treated with HFD supplemented with moringa extract demonstrated restoration of normal arrangement of kidney cells, this might be due to lower fat accumulation and regeneration of the antioxidant defense system in the kidney cells through the antioxidant and renoprotective nature of ME [23]**.**

In conclusion, the data highly recommend the effectiveness of ME supplementation as useful renal protective agent against disturbance of kidney in histopathology and antioxidants caused by HFD.

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