

ORIGINAL ARTICLE

The effects of *Moringa Oleifera* on lipid profile status, heart histology, and liver histochemistry in adult Wistar rats

Adelaja Abdulazeez Akinlolu, Elizabeth Oluwafunmilayo Bayode, Kamal Olaide Ghazali, Mubarak Oloduowo Ameen¹

Department of Anatomy, Faculty of Basic Medical Sciences, University of Ilorin, ¹Department of Chemistry, Faculty of Physical Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria

ABSTRACT

Background: *Moringa oleifera*'s leaves are globally used for nutritional and medicinal purposes. We evaluated lipid profile status, heart and liver histology, and the activities of alanine and aspartate transaminases of the liver in *M. oleifera*-treated adult Wistar rats. **Materials and Methods:** Twenty-four adult male Wistar rats were employed in the study. Rats of control Group I received physiological saline while rats of Groups II–IV received 250, 500, and 750 mg/kg bodyweight of methanolic extract of *M. oleifera*, respectively, for 21 days. **Results:** No gross morphological or behavioral anomalies were observed in rats of Groups I–IV. Histological examinations showed normal histoarchitecture of the heart in Group I and the liver of Groups I–III. Comparative histological analyses showed dose-dependent increased nucleated cells in heart tissues of rats of Groups II–IV than those of Group I. Furthermore, histological evaluations showed hypertrophy of central vein of the liver in rats of Group IV when compared to Group I. Statistical analyses showed dose-dependent nonsignificant higher mean levels ($P > 0.05$) of total cholesterol and triglycerides in the sera of rats of Groups II–IV compared to rats of Group I. Evaluations of levels of alanine and aspartate transaminases showed a statistically significant higher mean values ($P < 0.05$) in Group II, but statistically nonsignificant higher mean values ($P > 0.05$) in Groups III–IV compared to Group I. **Conclusions:** No significant adverse effects on lipid profile status, heart histology, and liver histochemistry were observed in *M. oleifera*-treated rats.

Keywords: Heart, lipid profile, liver, *Moringa oleifera*, toxicity

INTRODUCTION

Moringa oleifera's leaves and its various parts have been traditionally used as antidiabetic, antibacterial, anti-headache, antihypertensive, antifever, and anti-inflammatory herbal drug.^[1-3] The potential toxicity of medicinal plants to body organs is, however, of great concern^[3,4] despite traditional uses and claims

of treatment potentials.^[3] In addition, exposures to cytotoxic injuries result in alterations of activities of enzymes of body organs such as the liver.^[5] These enzymes are thus used as markers to determine the type and extent of such alterations^[5] and injuries to affected body organs.

The liver equally synthesizes very low-density lipoproteins and high-density lipoproteins, triglycerides (from circulating free fatty acids and glycerol), and cholesterol (from acetyl-CoA).^[6] Hence, lipid profile tests are of importance in pathophysiological evaluations

Corresponding Author: Dr. Adelaja Abdulazeez Akinlolu, Department of Anatomy, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria. E-mail: a3akin@gmail.com

Access this article online

Quick Response Code:



Website:
www.cjhr.org

DOI:
10.4103/cjhr.cjhr_101_16

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Cite this article as: Akinlolu AA, Bayode EO, Ghazali KO, Ameen MO. The effects of *Moringa Oleifera* on lipid profile status, heart histology, and liver histochemistry in adult Wistar rats. CHRISMED J Health Res 2017;4:104-9.

of the liver. Furthermore, the risks for cardiovascular diseases are measured through lipid profile tests.^[6,7] Therefore, to examine the potential toxicity of *M. oleifera*, this study evaluated lipid profile status, heart and liver histology, and the activities of alanine transaminase and aspartate transaminase of the liver in adult Wistar rats treated with crude aqueous extracts of *M. oleifera*.

MATERIALS AND METHODS

Ethical approval

Ethical approval was sought and received from the Department of Anatomy of the University of Ilorin, Ilorin, Kwara State, Nigeria. The protocols for the use of animals in scientific research were strictly adhered to in compliance with the World Health Organization's provisions.

Animal care and feeding

Twenty-four apparently healthy adult male Wistar rats weighing 100–200 g obtained from the colony bred of the animal house of the Department of Veterinary Physiology, University of Ibadan, Oyo State, Nigeria, were employed in the study. They were housed in individual cages in a well-ventilated and fumigated room with ambient temperature and good lighting. All rats were fed with standard pellet diet (Kusa Ventures Nigeria Limited, Ilorin) and received water *ad libitum*. The rats were acclimatized for 7 days before the start of experimental procedures. The weight of each rat was taken daily. Furthermore, each rat was examined daily for possible behavioral and gross morphological or physical changes.

Chemicals, reagents, and laboratory equipment

Normal saline solution, phosphate buffer, ethylenediaminetetraacetic acid, sulfuric (VI) acid (H_2SO_4), dimethyl sulfoxide, and hydrochloric acid were products of BDH Chemical Limited, Poole, England. Assay kits for total cholesterol, triglycerides, alanine transaminase, and aspartate transaminase were products of Randox Laboratories, United Kingdom. Spectrophotometer (Jenway Model 6405, ultraviolet/visible), mortar and pestle, weighing balance, centrifuge, pH meter (Rex model pHs 25), Norm-jet needles and syringes (Norm-jet Inc., Tuttlinger, Germany), and anticoagulant tubes (Sterling products, England) were used as appropriate in the experimental procedure of the study.

Collection and preparation of plant extracts

M. oleifera leaves were purchased from local traders at Oja-Tuntun, market of Ilorin, Kwara State, Nigeria. Harvested *M. oleifera* leaves were air-dried at room

temperature 25°C–30°C for 2 weeks. Dried pieces of the plant materials were pulverized and 1.2 kg of the dried sucker extracted with 70% methanol for 72 h. The extract was filtered, concentrated with rotary evaporator, and further dried on a water bath. The final product was gel-like and had a dark green color.

Administration of drugs/extract doses

The adult male Wistar rats ($n = 24$) were divided into four groups, each comprising six rats. Rats of Group I received physiological saline. Rats of experimental Groups II–IV received oral administrations of 250, 500, and 750 mg/kg bodyweight (bw) of the methanolic extract of *M. oleifera* leaves, respectively, for 21 days (days 1–21).

Histological evaluations

The rats of Groups I–IV were sacrificed by cervical dislocation at the end of experimental procedures on day 22. The thoracic and abdominal cavities of each rat were opened to excise and remove the heart and the liver for histological analyses and to obtain blood from the ventricles of the heart for biochemical analyses. Histological examinations of the heart and the liver were carried out using hematoxylin and eosin techniques as earlier described.^[3]

Evaluations of lipid profile status

Blood samples of rats of control and experimental groups were centrifuged to separate the serum from the red blood cells, and the serum was stored away for biochemical analyses of lipid profile status. The total cholesterol and triglycerides levels were determined in sera samples of rats of Groups I–VI based on the protocols described in assay kits of Randox Laboratories, United Kingdom.

Evaluations of the activities of alanine and aspartate transaminases of the liver

Each excised liver of rats of control and experimental groups was cut into small pieces, placed in a mortar and 0.1 M phosphate buffer (extracting solution) of at least four times the volume of the organ was added. The liver was homogenized into fine solution with the use of mortar and pestle. The homogenate was poured into a test tube and centrifuged at 2000 revolutions/min for 10 min. The supernatant was carefully removed, and the residue was discarded. The supernatant served as the sample for the evaluation of activities of alanine and aspartate transaminases of the liver. The alanine and aspartate transaminases levels were determined in the supernatant based on the protocols described in assay kits of Randox Laboratories, United Kingdom.

Statistical analyses

The mean $\pm$ standard error of mean values of total cholesterol, triglycerides, alanine transaminase, and aspartate transaminase levels in rats of control Group I was compared with rats of experimental Groups II–IV for any significant difference using *t*-test for unpaired samples. $P = 0.05$ (or less) was taken as statistically significant. The Statistical Package for the Social Science software (SPSS Statistics 23 version) developed by the International Business Machines Corporation (IBM, 2015) was used for the statistical analyses of computed data.

RESULTS

Gross morphological examinations

Daily observations of fur, skin, and behavioral pattern showed no sign of toxicity to rats. The furs were well laid. No mortality was recorded during experimental procedure.

Histological examinations of the heart of rats of control and experimental groups

Histological examinations showed normal histoarchitecture of the heart of rats of control Group I [Figure 1]. Comparative analyses showed dose-dependent increased nucleated cells in heart tissues of rats of experimental Groups II–IV than those of rats of control Group I [Figures 2–4].

Histological examinations of the liver of rats of control and experimental groups

Histological examinations showed normal histoarchitecture of the liver of rats of Groups I–III [Figures 5–7]. Comparative analyses

showed hypertrophy of the central vein of the liver in rats of experimental Group IV than those of rats of control Group I [Figure 8].

Evaluations of lipid profile status

Statistical analyses showed dose-dependent nonsignificant higher mean levels ($P > 0.05$) of total cholesterol and triglycerides in the sera of rats of experimental Groups II–IV when compared to rats of control Group I [Table 1].

Evaluations of activities of alanine and aspartate transaminases

Statistical analyses showed significant higher mean values ($P > 0.05$) of alanine and aspartate transaminases in rats of experimental Groups II when compared to rats of control Group I [Table 2]. However, there were statistically nonsignificant higher mean values ($P < 0.05$) of alanine and aspartate transaminases in rats of experimental Groups III–IV when compared to rats of control Group I [Table 2].

DISCUSSION

M. oleifera leaves contain abundant glucosinolates, isothiocyanates, carotenoids, vitamins, high proteins, and carbohydrates.^[1,2] Hence, *M. oleifera* leaves have been promoted as components of foods, nutritional supplements, or medicines for patients with HIV/AIDS by several African governments^[3] and as supplement to maize traditional complementary food to improve iron status in infants in Nigeria.^[8] However, cytotoxicity was noted only with the leaf-water extract (IC₅₀ = 6 mg/ml) in a previous study which examined the effects of extracts of *M. oleifera* on CYP3A4-mediated 6 β -hydroxylation of testosterone.^[9] The widespread public

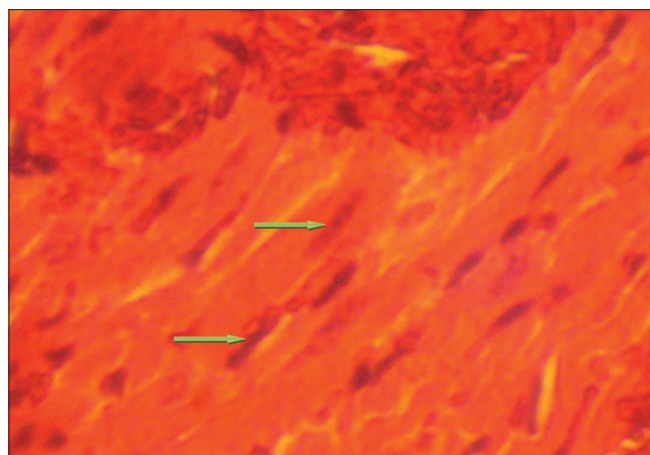


Figure 1: Photomicrograph of the heart of rats of control Group I which received physiological saline. The green arrow indicates nucleated cells. Histopathological examination showed normal histoarchitecture of the heart with adequate nucleated cells (H and E, $\times 400$)

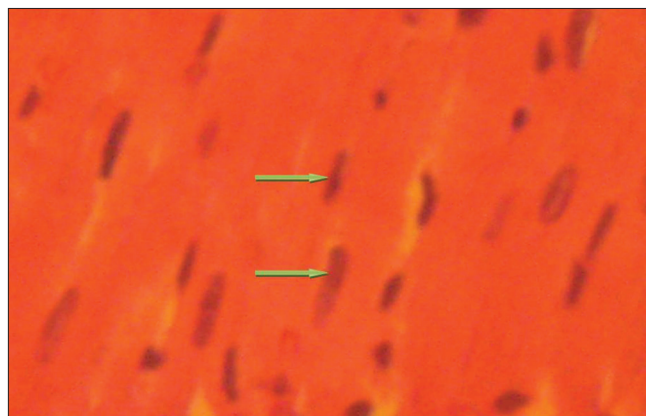


Figure 2: Photomicrograph of the heart of rats of experimental Group II which received 250 mg/kg bodyweight of *Moringa oleifera* extract. The green arrow indicates nucleated cells. Histopathological examination showed slight increase of nucleated cells (H and E, $\times 400$)

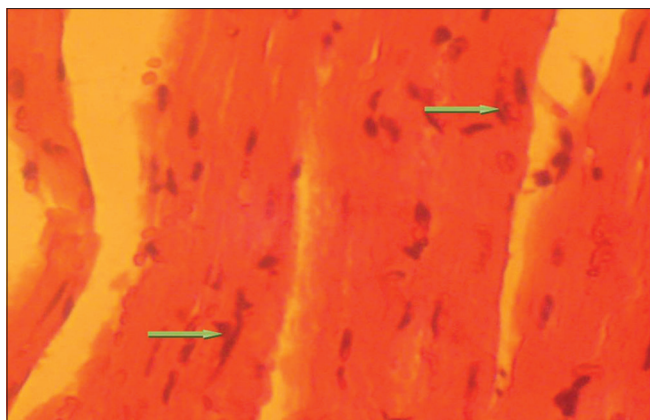


Figure 3: Photomicrograph of the heart of rats of experimental Group III which received 500 mg/kg bodyweight of *Moringa oleifera* extract. The green arrow indicates nucleated cells. Histopathological examination showed mild increase of nucleated cells (H and E, x400)

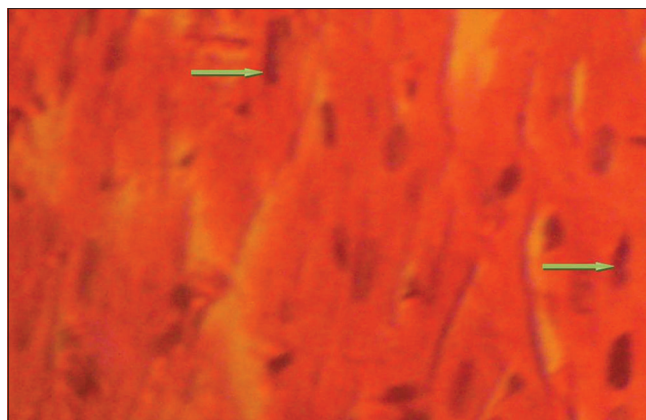


Figure 4: Photomicrograph of the heart of rats of experimental Group IV which received 750 mg/kg bodyweight of *Moringa oleifera* extract. The green arrow indicates nucleated cells. Histopathological examination showed abundant increase of nucleated cells (H and E, x400)

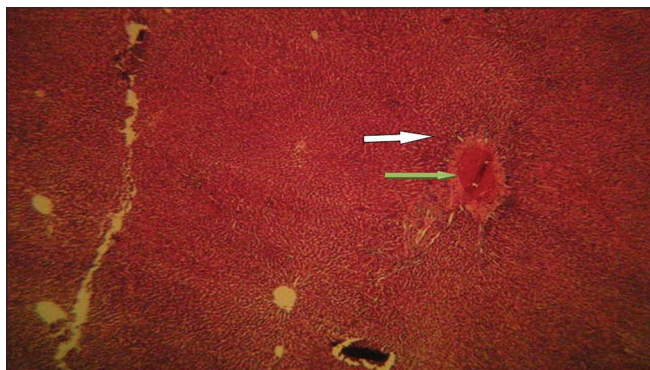


Figure 5: Photomicrograph of the liver of rats of control Group I which received physiological saline. The white arrow indicates hepatocytes while the green arrow indicates central vein of the liver. Histopathological examination showed normal histoarchitecture of the liver (H and E, x400)

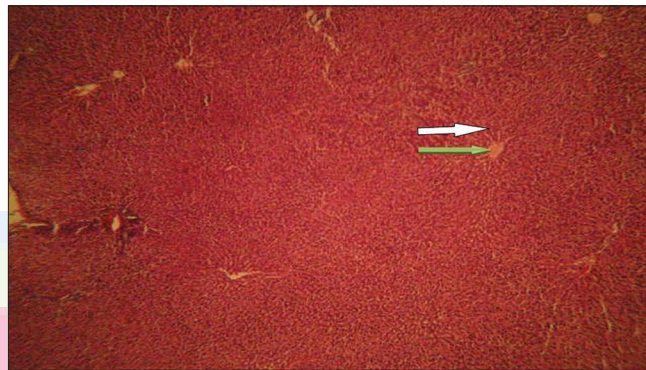


Figure 6: Photomicrograph of the liver of rats of experimental Group II which received 250 mg/kg bodyweight of *Moringa oleifera* extract. The white arrow indicates hepatocytes while the green arrow indicates central vein of the liver. Histopathological examination showed normal histoarchitecture of the liver (H and E, x400)

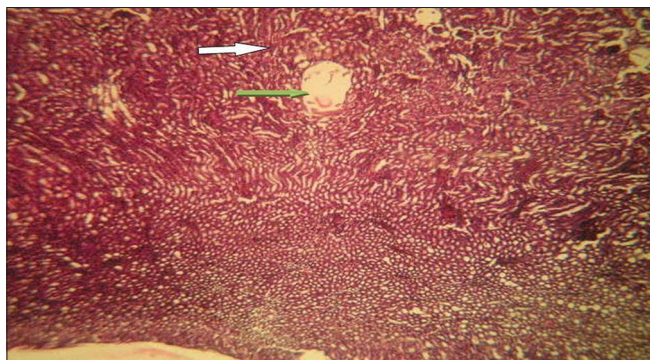


Figure 7: Photomicrograph of the liver of rats of experimental Group III which received 500 mg/kg bodyweight of *Moringa oleifera* extract. The white arrow indicates hepatocytes while the green arrow indicates central vein of the liver. Histopathological examination showed normal histoarchitecture of the liver (H and E, x400)

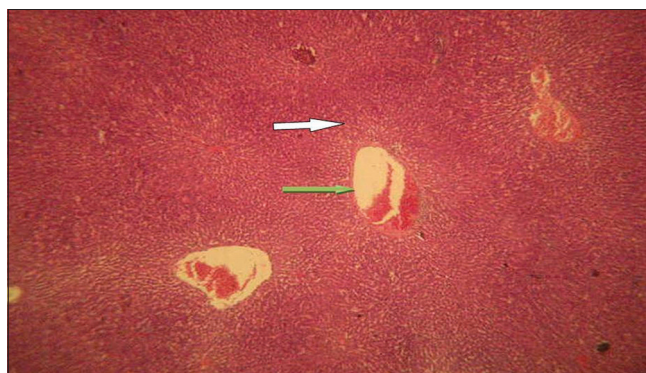


Figure 8: Photomicrograph of the liver of rats of experimental Group IV which received 750 mg/kg bodyweight of *Moringa oleifera* extract. The white arrow indicates hepatocytes while the green arrow indicates central vein of the liver. Histopathological examination showed hypertrophy of the central vein of the liver (H and E, x400)

use of *M. oleifera* leaves provides the reasons for the need to establish its safety and toxicological profile. In the present study, we observed no gross morphological or behavioral anomalies in rats of Groups I–IV. This implied that *M. oleifera* leaves possibly had no physical

adverse effects on body organs such as the skin, limbs as well as the cerebrum, cerebellum, and the internal ear which control behavioral functions, equilibrium, and balance.

Table 1: Lipid profile status in rats of Groups I-IV

Group of rats	Dose of drug/extract of <i>Moringa oleifera</i> leaves	Mean level of triglycerides (mmol/l)	P values of statistical significance (Group I vs. II-IV)	Mean level of total cholesterol (mmol/l)	P values of statistical significance (Group I vs. II-IV)
I	Physiological saline	0.189±0.09		0.11±0.06	
II	250 mg/kg bw	3.73±1.77	1.12	2.01±1.04	1.83
III	500 mg/kg bw	1.45±1.25	1.00	1.58±1.50	1.66
IV	750 mg/kg bw	1.77±1.51	1.05	1.81±1.66	1.02

Statistical significance at $P \leq 0.05$. bw: Bodyweight**Table 2: Levels of alanine and aspartate transaminases in rats of Groups I-IV**

Group of rats	Dose of drug/extract of <i>Moringa oleifera</i> leaves	Mean level of alanine transaminase (U/L)	P values of statistical significance (Group I vs. II-IV)	Mean level of aspartate transaminase (U/L)	P values of statistical significance (Group I vs. II-IV)
I	Physiological saline	113.50±6.36		251.00±9.90	
II	250 mg/kg bw	271.75±29.07	<0.01	1423.25±37.11	<0.01
III	500 mg/kg bw	157.50±86.37	0.38	482.00±32.99	0.26
IV	750 mg/kg bw	138.25±45.92	0.36	354.00±27.54	0.51

Statistical significance at $P \leq 0.05$. bw: Bodyweight

Histologically, the walls of the heart consist of three tunics: the endocardium, the myocardium, and the pericardium.^[10] The cardiac fibrous skeleton is composed of dense connective tissue and its principal components are the septum membranaceum, the trigona fibrosa, and the annuli fibrosi.^[10] Comparative histological analyses showed dose-dependent increased nucleated cells in heart tissues of rats of experimental Groups II-IV [Figures 2-4] than those of rats of control Group I [Figure 1]. Increased cellular activity is associated with increased nucleated cells, and this implied that there is an increased protein synthesis in rats treated with extract doses of *M. oleifera*. Cellular hyperplasia is physiologically normal in response to specific stimuli subject to normal body regulatory mechanisms, except in pathological conditions where hyperplasia results from excess release of hormones or growth factors.^[11]

Histological examinations showed normal histoarchitecture of the liver of rats of Groups I-III [Figures 5-7]. Comparative histological analyses showed hypertrophy of the central vein of the liver in rats of experimental Group IV [Figure 8] when compared with rats of control Group I [Figure 5]. The observed hypertrophy of the central vein of the liver could imply hepatotoxicity.

Statistical analyses showed dose-dependent nonsignificant higher mean levels ($P > 0.05$) of total cholesterol and triglycerides in the sera of rats of experimental Groups II-IV when compared to rats of control Group I [Table 1]. A marked increase in triglyceride and cholesterol levels indicates a risk factor for cardiovascular diseases.^[6,7] Similarly, persistent increased total cholesterol levels could result in hypercholesterolemia over a long period of time.^[6,7]

Statistical analyses of levels of alanine and aspartate transaminases showed significant higher mean values ($P > 0.05$) in rats of experimental Group II, but statistically nonsignificant higher mean values ($P > 0.05$) in rats of experimental Groups III-IV when compared to rats of control Group I [Table 2]. These findings are in disagreement with the results of a previous study which showed dose-dependent significant higher activities ($P < 0.05$) of alanine and aspartate transaminases in kidney tissues of rats treated with 250, 500, and 750 mg/kg bw of methanolic extract of *M. oleifera* leaves.^[3] However, in the previous study by Akinlolu *et al.* 2014^[3] and the present study, the highest values of alanine and aspartate transaminases were observed in rats treated with 250 mg/kg bw of methanolic extract of *M. oleifera* leaves. The observed trend may suggest that the limiting dose of methanolic extract of *M. oleifera* leaves in rats is 250 mg/kg bw. Alanine and aspartate transaminases are primary enzymes of the liver.^[5,12,13] Elevated levels of alanine and aspartate transaminases are, therefore, possible indicators of liver and kidney damage.^[5,12,13]

CONCLUSIONS

This study concludes that since there was a trend toward adverse effects in heart and liver physiology (especially at 250 mg/kg), long-term study or study using higher number of Wistar rats is needed. Furthermore, possible tissue hepatotoxicity was noted in rats treated with higher doses of *M. oleifera*. The observed tissue hepatotoxicity is, however, not supported by significant elevated levels of liver enzymes (alanine and aspartate transaminases) in *M. oleifera*-treated rats. Hence, moderate natural consumption of *M. oleifera* leaves is probably safe and

may not be cytotoxic to the heart and the liver in adult Wistar rats.

Recommendations for future studies

Concerted efforts should be made by scientists to determine the pharmaceutical doses of different parts of *M. oleifera* for safe nutritional and medicinal purposes and to evaluate the long-term effects of the consumption of *M. oleifera*.

Acknowledgment

The supports of the technical staff of the Departments of Anatomy and Chemistry of the University of Ilorin, Nigeria, are acknowledged.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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