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Hypolipidemic and hepatoprotective effects of ethanol leaf extract of *Moringa olifera* (LAM)

Patrick .O. Igbinaduwa *, Ebhotemhem FJ

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

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***Corresponding author:**

Email : pigbinaduwa@yahoo.com
patrick.igbinaduwa@uniben.edu
Tel.: +234(0)7063806665

ABSTRACT

Moringa oliefera have had a long history of use in traditional medicine. This work seeks to examine the phytochemical composition of the leaf of *Moringa oliefera* and the effects of its ethanol leaf extract on biochemical parameters (urea, alanine aminotransferase, aspartate transferase, alkaline phosphate, total globulin, total cholesterol, sodium, potassium) in Wistar Albino rats administered different concentrations of the extract. The rats were assigned into five groups of six (6) rats each. Group A were given 0.9% normal saline and they served as control. Group B, C, D and E were administered 100 mg/kg, 250 mg/kg, 500 mg/kg, and 1 g/kg of extract respectively for 28 days. The blood of the animals was taken for biochemical studies. Results of phytochemical screening reveal that the plant contains alkaloids, cardiac glycosides, phenols, tannins, carbohydrates, reducing sugar, flavonoids. Biochemical studies show a significant difference ($p < 0.05$) in the aspartate transferase (AST) between the control and treated groups. Significant difference ($p<0.05$) was also noticed for alanine aminotransferase in the group treated with 1000 mg/kg of extract. It can therefore be concluded that oral administration of ethanol leaf extract of *Moringa oleifera* may be safe for the animals at the administered doses, since no adverse effects were detected from biochemical evaluation. It is also hepatoprotective on the animals as it enhances the functions of the liver enzymes.

INTRODUCTION

The use of herbal medicine to treat diseases is almost universal. According to World Health Organization (WHO) estimates, 80% of the populations of some Asian and African countries presently use herbal medicine for some aspect of primary health care [1].Herbal medicine has been commonly used over the years for the treatment and prevention of diseases and health promotion as well as for enhancement of the quality of life. When an herbal product is ingested, the body interacts with it in several ways. These processes are commonly manifested by changes in enzyme levels such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), amylase, and alkaline phosphatase (ALP). Also, components like urea and uric acid are vital diagnostic tools for toxicity. The vital organs that are commonly affected are heart, liver, pancreas, and kidney.

The holistic approach to health care makes herbal medicine very attractive to many people, but it also makes scientific evaluation very challenging because so many factors must be taken into account. Although many believe herbal medicines are safe, they are often used in combination and are drawn from plant sources with their own variability in species, growing conditions, and biologically active constituents. Herbal extracts may be contaminated, adulterated, and may contain toxic compounds. The quality control of herbal medicines has a direct impact on their safety and efficacy. But, there is little data on the composition and quality of most herbal medicines not only due to lack of adequate policies but also due to a lack of adequate or accepted research methodology for evaluating traditional medicines [5-7].There is therefore a need to continually evaluate the safety of various herbal preparations from different localities to evaluate their safety in man and animals.The present study is designed to carry out phytochemical and biochemical evaluation of the ethanol leaf extract of *M. oleifera.*

MATERIALS AND METHODS

REAGENTS

Reagents used for this study include dragengorff's reagent, wagner's reagent, hager's reagent, ferric chloride, dilute sodium hydroxide solution, concentrated and diluted hydrochloric acid,

concentrated and diluted sulphuric acid, sodium hydroxide, 2,4 dinitrophenylhydrazine, L-alanine, phosphate buffer, α-oxoglutarate, L-aspartate, 4-aminoanipyrine, phenol and cholesterol esterase. All reagents were of analytical grades and were purchased from local distributors and were used without further purification.

Plant collection

The leaves of the plant, *Moringa olifera* were collected at Eyaen village, Benin-Auchi road, Benin city and was identified by the curator, department of Pharmacognosy, Faculty of Pharmacy, University of Benin. A voucher specimen was deposited in the herbarium.

Preparation of plant Extract

The leaves were collected and air-dried for two weeks, homogenized and subsequently prepared for extraction. Four hundred grams (400 g) of the powdered leaves of *Moringa olifera* were extracted in 99% ethanol using a soxhlet apparatus.The extraction process was carried out using soxhlet extraction method. It was then concentrated at 50°C in a rotary evaporator. The combined extract was 85 g (21.3%).

PHYTOCHEMICAL SCREENING

Chemical tests were carried out to detect the presence of alkaloids, tannins, saponins, carbohydrates, reducing properties, anhraquinones and other phenolic compounds in accordance with standard methods [9].

Experimental animals

Thirty (30) Wistar rats of either sex were purchased from the University of Benin animal house and kept under standard environmental conditions (25 ± 21°C; 12/12 h light/dark cycle). The animals were divided into five groups of six rats each and kept in their respective cages and fed with standard diet and clean water. The animals were allowed to acclimatize for three weeks prior to the experiment. All groups were closely observed for behavioural changes and signs of abnormalities throughout the study. The experimental animals were handled and used in accordance with the international guide for the care and use of laboratory animals [10]. *Moringa Olifera* leaf extract was administered for 28 days at doses of 100, 250, 500 and 1000 mg/kg body weight and control group received 0.9% Normal saline.

Collection of blood samples

At the end of 28 day, the animals were fasted for 12 hours, anesthesized with chloroform and subsequently sacrificed. Blood was collected from each animal by cardiac puncture using sterile needle and 5ml syringe. The samples were collected in an ethylene-diamine-tetra-acetic acid (EDTA) bottles and sent for biochemical analysis.

Determination of biochemical parameters

Serum alkaline phosphatase (ALP):

Alkaline phosphatase substrate was pipetted into a test tube and equilibrated to 37°C for 3 minutes. Deionized water was added and mixed. This was incubated for 10 minutes at 37°C. Alkaline phosphatase developer was added, mixed and the resulting solution was read against blank at 590 nm.

Alanine aminotransferase (ALT):

The sample (0.05 mL), buffer (phosphate buffer (100 mmol/L, pH 7.4), L-alanine (200 mmol/L), α- oxoglutarate (2 mmol/L)) were pipetted into a test tube and incubated for 30 minutes at 37°C. 0.25 ml of 2,4-dinitrophenylhydrazine (2 mmol/L) and 2.5 mL of sodium hydroxide were added to the test-tube. The absorbance of the sample against the blank was taken at 546 nm.

Aspartate aminotransferase (AST):

The sample (0.05 mL), phosphate buffer (100 mmol/L, pH 7.4), L-aspartate (100 mmol/L), α- oxoglutarate (2 mmol/L) were pipetted into a test tube and incubated for 30 minutes at 37°C. 0.25 ml of 2,4-dinitrophenylhydrazine (2 mmol/L) and 2.5 mL of sodium hydroxide were added to the test-tube. The absorbance of the sample against the blank was taken at 546 nm.

Total cholesterol (TC):

The total cholesterol level was determined by the enzymatic endpoint method.

Ten (10) µl of sample and 1000 µl of reagent were mixed and incubated for 10 minutes at 37°C. The absorbance of the solution was taken against reagent blank at 500 nm. The absorbance of the standard reagent was also determined.

Concentration of cholesterol in sample =

$$\frac{\text{Absorbance of sample} \times \text{concentration of standard}}{\text{Absorbance of standard}}$$

Determination of total protein:

Blank reagent (100 mmol/L), sodium hydroxide and 16 mmol/L Na-K tartrate), standard reagent (biuret reagent) and the test sample (serum or heparinized or EDTA plasma) were pipetted into 3 different test-tubes. 20 µl distilled water was added to the test-tube containing blank reagent, 20 µl of standard reagent to standard regent test-tube, 20 µl sample to test sample test-tube and 1ml of the working reagent to each of the 3 different test-tubes. The solutions were mixed and incubated at room temperature for 5-10 minutes and read against blank at 546 nm.

Protein concentration =

$$\frac{\text{Absorbance of sample} \times \text{concentration of standard (60g/L)}}{\text{Absorbance of Standard}}$$

Determination of Albumin:

Blank reagent, standard reagent and the test sample were pipetted into 3 different test-tubes. 5 µl distilled water was added to the blank test-tube, 5 µl of standard reagent to the standard test-tube, 5 µl test sample to sample test-tube and 1.5 ml working reagent to each of the test-tubes. The solutions were mixed and incubated at room temperature for 5 minutes and read against blank at 630 nnm.

Albumin concentration (g/L) =

$$\frac{\text{Absorbance of sample} \times \text{concentration of standard}}{\text{Absorbance of standard.}}$$

Total Globulin (TG):

The Total globulin fraction was determined by subtracting the albumin from the total protein.

Total Globulin = Total protein Albumin

Determination of UREA:

Sample (0.5 µl) , 0.5 µl standard reagent and 0.5 µl distilled

water were pipette into separate test tubes, 50 µl working reagent was added to each of the test-tubes, mixed and incubated at 37°C for 10 minutes. 1.25 ml phenol (120 mmol/L) and 1.25 ml of sodium hypochlorite (27 mmol/L) were added to each of the test-tubes. The solutions were mixed and incubated at 37°C for 15 minutes and read against reagent blank at 546 nm.

Urea concentration =

Absorbance of sample x Standard conc. (80 mg/dl or 13.4 mmol/L)

Absorbance of standard

The sodium and potassium content were determined using the SEAC Fp20 flame photometer using standard method.

Statistical analysis

All data were expressed as means ± standard error of mean (SEM). Comparison was by the student t test using the graph pad instat3 version for windows software. Level of significance was reported at $P < 0.05$.

RESULTS

Phytochemical Screening

Table 1. : Phytochemical screening of *Moinga olifera* leaf

TEST	RESULT
Carbohydrate	Present
Reducing sugar	Present
Saponins	Present
Flavonoid	Present
Phenols	Present
Alkaloids	Present

BIOCHEMICAL EVALUATION

Table 2. : Effects of the ethanol leaf extract of *Moringa oleifera* Biochemical parameters of the albino Wistar rats

PARAMETERS	GROUP A (Control)	GROUP B (100 mg/kg)	GROUP C (250 mg/kg)	GROUP D (500 mg/kg)	GROUP E (1 g/kg)
UREA (mmol/L)	11.2 ± 0.12	10.8 ± 0.87	08.2 ± 0.89	08.12 ± 1.18	07.6 ± 0.50
Na^+ (mmol/l)	147.3 ± 0.88	144.3 ± 2.19	144.3 ± 1.45	144.0 ± 1.20	142.0 ± 0.88
K^+ (mmol/l)	5.57 ± 0.12	5.63 ± 0.09	5.50 ± 0.21	5.50 ± 0.15	5.90 ± 0.15
ALT (iu/l)	15.7 ± 0.93	18.0 ± 1.16	18.0 ± 1.16	19.0 ± 0.58	19.3 ± 088
AST (iu/l)	27.3 ± 0.88	43.3 ± 1.86*	44.7 ± 1.86*	49.7 ± 1.86*	56.0 ± 1.45*
ALP (iu/l)	11.3 ± 0.88	17.3 ± 0.67	17.3 ± 0.88	22.9 ± 0.99*	25.3 ± 0.88*
TG (mmol/L)	0.93 ± 0.09	0.83 ± 0.07	0.63 ± 0.09	0.57 ± 0.67	0.47 ± 0.09
TC (mmol/L)	1.39 ± 0.12	1.55 ± 0.06	1.18 ± 0.09	1.11 ± 0.06	1.087 ± 0.03

Values are expressed as mean ± standard error of mean (n = 3 for each group)
Comparison were made between Group A and Group B, C, D and E
The symbol * also represents the statistical significance at $P < 0.05$

DISCUSSION

Herbal medicines have received great attention as an alternative to clinical therapy in recent years. The preliminary phytochemical test of the powdered leaf sample of *Moringa oleifera* reveals the presence of alkaloids, cardiac glycosides, tannins, phenolics flavonoids, carbohydrates, reducing sugar. These bioactive constituents may be responsible for the observed therapeutic effects of the plant. Flavonoids for example, being an

Table 3. : Effect of ethanolic extract of M. olifera on Body weight changes in control and treated rats.

	Day/ organ	Group A (control)	Group B (100 mg/kg)	Group C (250 mg/kg)	Group D (500 mg/kg)	Group E (1 g/kg)
Body	Day 0	159.67=7.29	165.67±11.03	168.50±12.69	151.00±17	169.00±11.67
Weight (g)	Day 28	160.33=7.55	167.00±12.22	165.83±10.53	154.17±8.01	167.50±11.24
Organ	Liver	5.27=0.18	4.61±0.47	6.04±0.87	5.47±o.68	5.85±0.77
Weight (g)	Kidney	0.52=0.03	0.50±0.02	0.57±0.07	0.56±0.07	0.63±0.11

anti-oxidant in the plant may contribute to the hepatoprotective effects of the plant [12].Measurement of serum biochemical parameters is useful to identify target organ of toxic effects as well as general health status of animals [13]. Alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase were investigated in these studies. These were done in order to determine the effects of the plant leaf extract on the liver. These enzymes are normally affected following liver damage [14]. Aspartate aminotransferase (AST), and alanine aminotransferase are produced in the liver cells when these cells are challenged, but usually not necessarily an indication of liver damage [15].

The studies showed increase in the level of serum alkaline phosphatase with no statistical significance (at $p<0.05$) in the rats treated with 100, 250 and 500 mg/kg of the plant extract when compared to the control group. However, there was significant difference in the group treated with 1000 mg/kg of the plant leaf extract when compared to the control group. This could suggest a dose-dependent relationship. There was also a significant increase in the level of the aspartate aminotransferase (AST), but statistics reveals no significant difference (at $p<0.05$) in the alanine aminotransferase (ALT), although the level of both enzymes has been elevated. This elevation in liver enzymes is consistent with the findings of Bharali; *et al*, 2003 that oral administration of hydroalcoholic leaf extract of *M.oleifera* enhances level of hepatic enzymes [16].The Increase may infer that induction of liver enzymes may result in enhancement of liver functions especially in the elderly where their liver function has significantly reduced by age. This helps in the detoxification of xenobiotics substances, such as carcinogens, and plant venomous compounds. These results reveal slight induction in the liver enzymes but not necessarily damage to them. The studies also reveal slight increase in the total globulin, but this was statistically significant when compared with the control group. Globulins are principally responsible for both the natural and acquired immunity that an individual has against invading organisms [17]. The slight increase in the level of globulin in the treated group compared to the control group by the plant leaf extract further support the antimicrobial actions of the plant. This finding is consistent with the studies of Lawrence and Amadeo (1989) [18].

Urea is one of a number of non-protein nitrogenous substance that accumulates in the plasma when renal excretion is reduced [19]. The current studies show that there was no adverse effect on the urea concentration in serum of the experimental rats. This is in agreement with the report of Chukkalingam *et al*; (2012) on the effect of *M. olifera* on biochemical parameters of rats [20].

There was also a decrease in the level of total cholesterol (TC) and triglycerides. This effect also tend to be dose dependent and it's consistent with a previous studies by Kumari, *et al.,* (2010) where he found out that 8 g of leaf powder *M. oleifera* daily over the course of 40 days in type II diabetes is able to reduce total cholesterol and triglyceride by 14% [21]. This was also in line with the study by Naznin, *et al* (2012) where he examined the effect of *M. oleifera* leaf exract on serum triglycerides, serum cholesterol and blood glucose. The studies found out that the leaf extract induced significant decrease of serum level of triglycerides, cholesterol, low density lipoprotein cholesterol, which correlates with significant increase in high-density lipoprotein cholesterol [22]. Thus the results from present and past studies confirm *M. oleifera* leaves as potential hypolipidemic and hypocholesterolemia agent. These effects could be linked with the presence of bioactive phytoconstituents, such as - sitosterol and can be comparable with a review of the article by Farooq, *et al* (2007), "*Moringa oleifera:* A food plant with multiple medicinal uses". In the studies, the extract was found to lower the serum level of cholesterol, phospholipid, lipoproteins, atherogenic index lipid and reduce the lipid profile of liver, heart, aorta in hypercholesteremic rabbits and increased the excretion of fecal cholesterol [23]. Clinically, the hypolipidemic effect of *M. oleifera* leaf extract may help to prevent cardiovascular disorders which are one of the leading causes of death worldwide.

The changes in the level of potassium and sodium in the rats treated with different doses of the plant leaf extract were negligeable when compared with control group. This is consistent with the publication by United Sates Department of Agriculture nutrient database. In their studies, it was found out that the plant has negligible contribution to the sodium and potassium concentration [24].

The safety of this plant at low doses is in agreement with the studies done by Adedapo *et al*., (2009) that the plant is safe for consumption up to a dose of 2 g/kg body weight [25].

CONCLUSION

Oral administration of ethanol extract of *M. olifera* leaf at low doses is safe on the treated animals as there were no significant changes on the biochemicals parameters evaluated. It is also hepatoprotective on the animals as it enhances the functions of the liver enzymes.

Author's Statements:

Competing Interest: The authors declare no conflict of interest.

Animal Rights: The institutional and international ethical guidelines for the care and use of laboratory animals were followed.

REFERENCES

1. World Health Organization. National Policy on Traditional Medicine and Regulation of Herbal Medicines, Geneva. Report of World Health Organizaion (WHO) global survey 2005.
2. Ernst E, Schmidt K, Wider B. Complementary and Alternative Medicine research in Britain: The last 10 years. Complement Therapeutic Clinical Practice; 2005: 11:1720.
3. Harrison RA, Holt D, Pattison DJ, Elton PJ. Who and how many people are taking herbal supplements? A survey of 21,923 adults. International Journal Vitamin Nutrient Research. 2004: 74:1836.
4. Qato DM, Alexander GC, Conti RM, Johnson M, Schumm P, Lindau ST. Use of prescription and over-the-counter medications and dietary supplements among older adults in the United States. 2008: 300:286778.
5. Ernst E, Schmidt K, Wider B. Complementary and Alternative Medicine research in Britain: The last 10 years. Complement Therapeutic Clinical Practice. 2005:11:1720.
6. Ribnicky DM, Poulev A, Schmidt B, Cefalu WT, Raskin I. The science of botanical supplements for human health: A view from the National institute for Health (NIH) botanical research centers: Evaluation of botanicals for improving human health. American Journal of Clinical Nutrition. 2008: 87:472S5S.
7. Ernst E. Herbal Medicine in the Treatment of Rheumatic Diseases. Rheumatic Diseases Clinics of North America. 2011: 37(1) 235-248.
8. Lovera J, Bagert B, Smoot K. Ginkgo biloba for the improvement of cognitive performance in multiple sclerosis: a randomized, placebo-controlled trial. MultScler. 2007:13(3):376-85.
9. Dos Santos-Neto LL, de Vilhena MA, Medeiros-Souza P, de Souza GA. The use of herbal medicine in Alzheimer's disease-a systematic review. Evidence Based Complement Alternative Medicine. 2006: 3(4):441-5.
10. Damery S, Gratus C, Grieve R. The use of herbal medicines by people with cancer: a cross-sectional survey. Br J Cancer. 2011: 104(6):927-33.
11. Hasan SS, Ahmed SI, Bukhari NI, Loon WC. . Use of complementary and alternative medicine among patients with chronic diseases at outpatient clinics. Complement Therapeutic and Clinical Practice. 2009: 15(3):152-7.
12. Shimazaki M and Martin JL. Do herbal agents have a place in the treatment of sleep problems in long-term care? Journal of American Medical Directors Association; 8(4):248-52.
13. Bright JJ (2007). Curcumin and autoimmune disease. Advanced Experimental Medical Biology. 2007: 595:425-51.
14. Okwu DE. Phytochemicals and vitamin content of indigenous spices of South Eastern Nigeria. Sustaining Agricultural Environment. 2004: 6: 30-34.
15. Okwu DE. Phytochemicals, Vitamins and Mineral contents of two Nigeria Medicinal plants. International Journal of Molecular Medicine and Advance Science. 2005:1(4): 375-381.
16. Bharali R, Jawahira T, Mohammed RHA. Chemomodulatory effects of Moringa oliefera, Lam on Hepatic carcinogen metabolizing enzymes, Antioxidant parameters and skin papillomagnesis in mice. Asian pacific Journal of cancer prevention. 2003: 4,131-139
17. Madziga HA, Sanni S and Sandabe UK. Phytochemical and Elemental Analysis of Acalyphawilkesiana Leaf. Journal of American Science. 2010: 6(11): 510-514
18. Lawrence AK and Amadeo JP. Clinical chemistry. Theory, analysis and correlation; The Mosby company, Philadephia 1989 pp 42-44.
19. Kar A. Pharmaocognosy and Pharmacobiotechnology (Revised-Expanded Second Edition). New Age International Limited Publishers, New Delhi. 2007 pp. 332-600
20. Chukkalingam K, Mathan R, Salyanarayanan SK, Srinivasan AL. Toxicity of Moringa Oliefera seed extract on some haematological and biochemical profiles in a fresh water fish, Cyprinus Carpio. Experimental and Toxicologic pathology. 2012: 64(7)681-687
21. Kumari DJ. Hypoglycaemic effect of Moringa oliefere and Azadirachta indica in type 2 diabetes mellitus. The Bioscan. 2010: 5(2):211-214
22. Naznin A, Mamunur R, Shah A. Comparison of Moringa oliefera leaves extract with alcohol on serum triglyceride, serum cholesterol, blood glucose, heart weight in Adrenaline induced rats. Saudi journal of Biological Sciences. 2008: 15(2):253-258
23. Farooq A, Sajid L, Muhammad A and Anwarul HG. Moringa oliefera: a food plant with multiple medicinal uses. Phytotherapy Research. 2007: 21(1)17-25.
24. Olson ME. Flora of North America Editorial Committee, ed. Moringaceae: Drumstick Family. Flora of North America North of Mexico 7. New York and Oxford. 2010 pp. 167169.
25. Rashid U, Anwar F, Moser BR, Knothe G. Moringa oleifera oil: A possible source of biodiesel. Bioresource Technology. 2008: 99 (17): 8175-9.