Asian Journal of Medical Research Original Article

Biochemical Effects of Methanolic Extract of *Morinda Morindoides* And *Morinda lucida* Leaves on Lipid Profile, Bilirubin and Some Marker Enzymes

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Abstract

Objective: The present work investigated the biochemical effects of *Morinda morindoides* and *Morinda lucida* methanolic leaf extracts on lipid profile, bilirubin and some marker enzymes level in the plasma of male albino rats.

Methods: Phytochemical screenings were carried out on the leaves of *M.morindoides* and *M.lucida* samples.Thirty five (35) male albino rats were divided into seven groups and administered with distilled water as the control, 50,100,200mg/Kg *M.lucida methanolic* extracts and 50,100,200mg/Kg of *M.morindoides* methanolic extracts respectively.Lipid profile, bilirubin and some marker enzymes (Alanine transaminase-ALT, Aspartate transaminase-AST, Alkaline phosphatase-ALP) assays were determined in the plasma using standard techniques.

Results: Majority values of the parameter taken were not significantly ($p<0.05$) higher than normal control.The results from the parameters showed that both species (*Morinda morindoides* and *Morinda lucida*) have similar effects by not having pronounced adverse effect on the animals (rats), and that both species contain cardenolides and saponins.

Conclusion: It was therefore concluded that *M.morindoides* and *M.lucida* could be safe at the tested dosages (50,100 and 200mg/Kg b.wt).

Keywords: *Morinda morindoides, Morinda lucida,* methanolic, Leaf extracts, Phytochemical screening, Adverse effects, rats.

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body.

The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds.[1] Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes.[2,3]

Medicinal plants are used to treat many conditions such as asthma, premenstrual syndrome, rheumatoid arthritis, menopausal symptoms, malaria chronic fatigue and irritable bowel syndrome, among others.[4] Much of the medicinal use of plant seem to have been developed through observation of wild animal and by trial and error. Many drugs commonly used today are of herbal origin and it has been estimated that about 25% of all prescribed medicine today are substances derived from plants. Such drugs are aspirin from Filipendula ulmaria, the anti-malarial agent, quinine from Cinchina sp, the anti-hypertensive principles, reserprine from Pauwolfia serpetina,as well as the anti-neoplastic alkaloids, vincristine and vinblastine from Catharanthus roseus.[5]

Morinda morindoides a medicinal plant used to treat diseases and ailments such as malaria, worm, amoebiasis, haemorrhoids, gonorrhea and scabies.[6; 7] While Morinda lucida which is traditionally used to treat diseases and ailments such as malaria, arthritis, rheumatism, leprosy, pulmonary troubles.[8]

The use of medicinal plants is popular among the rural communities simply because of the popularity and acceptability of belief that all natural products are safe. However, this is not correct because plants often contain toxins for their self-protection which could be dangerous and of deleterious effects to humans consuming them. This implies that different herbal medicine act in different ways causing harmful effect to human physiology. Many commonly used herbal medicine in their irregular high doses or with other medications in long term are toxic. Toxic effects of herbal medicine range from allergic reactions to cardiovascular, hepatic, renal, neurological and dermatologic toxic effects. Also, some herbal medicine cause abnormal laboratory results in form of alternation in liver function tests, electrolyte disturbances and blood sugar level changes.

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Larrey [4] reported and recommended discontinuation of herbal and other products before proceeding for surgery and anesthesia to avoid undesirable and unexpected side effects, complications and delayed outcome.

Hence the need for continued research into medicinal plants, especially those that are used in traditional medicine practices across the developing countries of Africa, Asia and South-America cannot be over emphasized. Such research endeavours are geared towards discovering new therapeutic agents or newer and richer sources of known drugs of natural origin. The basic goal of such drug discovery efforts always hinges on developing new products with enhanced therapeutic benefits, that is higher efficacy and low toxicity profile. Hence, in this study, an investigation of the biochemical effects of the methanolic extracts of *M.morindoides* and *M.lucida* on lipid profile, bilirubin and some marker enzymes and the examination of the bioactive substances of the plants species qualitatively were carried out.

MATERIALS AND METHODS

Plant materials and preparation of methanolic extracts

Different herbs namely M.morindoides leaves(Yoruba-ewe ponju owiwi)andM.lucida leaves(Yoruba-ewe oruwo)used were purchased from herb sellers at Itoku and Lafenwa markets in Abeokuta, Ogun state, Nigeria. The herbs samples were identified in the Department of Botany, University of Agriculture, Abeokuta(UNAAB) by a taxonomists.

The plants leaves were collected, washed and air dried for seven daysuntil constant weightwere obtained. The air dried herbs were milled to powdery form using grinding machine.

Two hundred gram(200g) of ground leaves were suspended in absolute methanol (1.5 litre) separately. The mixtures were then left separately in shaker at 190 rev/min for 48hours at room temperature. The extracts were decanted separately, and then filtered through Whatman filter paper No 1. These were then allowed to dry (by evaporation) at room temperature, given solid methanolic extracts. Each solid extracts was reconstituted in respective quantity to obtain a stock solution of reasonable amount.

Phytochemical screening

The phytochemical tests for active substances were carried out on portions (100gram each) 0f the powdery samples using standard phytochemical procedures according to Sofowara [9],as described by Edeoga et al [10].

Experimental animals

Thirty five (35) male albino rats used were purchased from faculty of Veterinary medicine, Department of Anatomy, University of Ibadan, Ibadan, Nigeria. The animals (150-180g) were acclimatized for two weeks, after which they were grouped according to their relative weights into seven groups (that is five rats per group) and were given adequate supply of food and water.

Extracts administration

Each extract solution prepared was administered orally to the experimental animal in each group thus; group A served as control and were given distilled water equivalent to the highest volume of extract administered to rats, group B,D and F were administered *Morinda morindoides* extracts at 50,100 and 200mg/Kg bodyweight respectively, while group C,E and G received 50,100 and 200mg/Kg body weight of *Morinda lucida* respectively. The administration of these extracts were for two weeks(14days).

Biochemical analysis

The male albino rats were weighed and blood samples collected through cardiac puncture under diethyl ether anesthesia into lithium heparin specimen bottles for biochemical analysis on the 15th day after the last herbs administrations. The animals were subsequently sacrified by cervical dislocation. Lipid profile, bilirubin and some marker enzymes assays were carried out on the blood samples using standard techniques, as described by Ranjna. [11] and Sucheta et al.[12]

Statistical analysis

Results were expressed as mean $\pm$ standard error of mean(S.E.M). Statistical comparison was made by one-way ANOVA and followed by least significant difference (LSD).$P<0.05$ was considered significant.

RESULTS

In phytochemical screening carried out qualitatively, both plants species of *Morinda* leaves contained Cardenolides and Saponins.

The majority values of the lipid profile, bilirubin and some marker enzymes assays obtained showed no statistical significant differences($P<0.05$) between the study groups and control animals(A).

Table 1: Phytochemical constituents of *M.morindoides* and *M.lucida* leaves.

Test of active substances	*Morinda morindoides*	*Morinda lucida*
Alkaloids	-ve	-ve
Cardenolides	+ve	+ve
Anthraquinones	-ve	-ve
Saponins	++ve	++ve
Tannins	-ve	-ve

Table 2: The effect of methanolic extracts on bilirubin and some marker enzymes

Parameters	Group A Control	Group B 50mg/Kg of *M.m*	Group C 50mg/Kg of *M.l*	Group D 100mg/Kg of *M.m*	Group E 100mg/Kg of *M.l*	Group F 200mg/ Kg of *M.m*	Gr0up G 200mg/Kg of *M.l*
Bilirubin(mg/dl)	1.62 ± 0.13^{c}	0.33 ± 0.08^{a}	0.74 ± 0.04^{b}	0.79 ± 0.11^{b}	0.92 ± 0.00^{b}	0.69 ± 0.09^{b}	0.81 ± 0.13^{b}
ALP(IU/L)	13.81 ± 0.47^{b}	4.33 ± 0.54^{a}	36.40 ± 2.27^{d}	23.31 ± 1.67^{c}	23.79 ± 2.78^{c}	7.26 ± 0.94^{a}	3.32 ± 0.67^{a}
ALT(IU/L)	15.01 ± 0.20^{cd}	14.15 ± 1.29^{c}	10.74 ± 0.95^{b}	16.80 ± 0.86^{d}	6.07 ± 0.45^{a}	5.32 ± 0.44^{a}	10.70 ± 1.12^{b}
AST(IU/L)	42.81 ± 1.33^{d}	42.44 ± 1.21^{d}	16.80 ± 1.87^{b}	41.34 ± 0.53^{d}	30.10 ± 1.40^{c}	8.75 ± 0.86^{a}	11.81 ± 1.37^{a}

However, groupB of triacylglycerols in HDL, groupC of triacylglycerols in VLDL+LDL, groups(B,C,Dand E) of cholesterol in HDL, groups(C,D,E and F) of cholesterol in VLDL+LDL, groups(F and G)of phospholipids in red blood cells, groups(B,C and D)of phospholipids in HDL and groups(C,D and E) of alkaline phosphatase activities, showed slight increase in the concentrations and enzymes activities from the control animals(A)

DISCUSSION

The phytochemical screening of the plants studied showed that the leaves were rich in cardenolides and saponins. This suggests that they can be used for medicinal purpose since some extracts containing these active substances are being used as medicine [9].

Themedicinaluses of the plants (M.morindoides and M.lucida)couldbe due to their high level of saponins. This is in agreement with the report of Duke [13]; and Shibata et al [14],that from the medicinal view point, the most widely used saponins are the ginenosides of ginseng, which are reputed to prolong human life and aid survival to stress.

It was also found that crude extract from *M.morindoides* exhibited invitro and invivo antimalarial activity [15-18].

Very low level of bilirubin concentration in the plasma which falls within normal values, indicates that the leaves extracts did not cause any haemolysis and liver damage which is in agreementwith Suchetal et al [12] that reported that the production of bilirubin was increased due to haemolysis and the liver lost its ability to metabolise it.

The results obtained from the assays of the aminotransferases (ALT and AST) were within normal values and were not higher than that of the control which suggest that the leaves extracts did not cause any tissue damage as reported by Sucheta et al [12].

Sucheta et al [12] reported that decrease in SGOT (AST) was observed in drug therapy, such as intake of opiates, erythrocin and penicillin. Also decrease in SGPT (ALT) was observed in combination with increased cholesterol levels in cases of congested liver. Therefore,

Table 3 The effects of methanolic extracts on lipid profile (N=6)

Parameters	Group A Control	Group B 50mg/Kg of *M.m*	Group C 50mg/Kg of *M.l*	Group D 100mg/Kg of *M.m*	Group E 100mg/Kg of *M.l*	Group F 200mg/Kg of *M.m*	Gr0up G 200mg/Kg of *M.l*
Triacylglycerols in plasma(mg/dl)	63.00±6.38b	35.51±4.99a	35.47±4.88a	62.50±6.83b	65.88±7.85b	22.86±6.70a	74.91±8.20b
Triacylglycerols in VLDL+LDL (mg/dl)	296.22±15.97d	230.38±2.81c	356.64±13.55e	114.50±10.47a	141.62±7.48ab	295.34±13.76d	258.56±5.10c
Triacylglycerols in HDL(mg/dl)	57.03±5.21c	70.95±5.77d	54.50±2.35c	54.83±4.96c	39.56±2.23b	36.56±2.73ab	26.45±1.10a
Triacylglcerols in RBC (mg/dl)	55.35±1.91d	47.83±3.06d	28.91±0.45c	8.33±0.52a	51.14±1.56d	16.64±1.98b	18.46±0.00b
Cholesterol in plasma(mg/dl)	89.65±5.50d	93.43±2.53d	34.51±7.26a	68.12±1.57c	31.63±0.87a	52.71±4.35b	27.19±1.74a
Cholesterol in VLDL+LDL (mg/dl)	30.13±2.74a	44.35±5.56ab	66.95±7.79c	55.53±7.32bc	51.21±6.25bc	50.74±2.47bc	39.83±5.38ab
Cholesterol in HDL(mg/dl)	16.67±2.40a	32.19±0.91cd	37.14±2.58d	40.27±3.96d	25.88±2.71bc	17.84±2.68ab	17.84±0.62ab
Cholestero l in RBC(mg/dl)	23.94±o.57bc	10.93±2.52a	23.75±3.57bc	23.57±3.57bc	18.15±1.36ab	31.20±1.39c	28.24±2.62c
Phospholipids in plasma(mg/dl)	175.77±2.92d	134.73±0.71d	36.45±4.21a	123.93±5.61d	87.48±1.40c	72.9±4.86b	26.73±1.40a
Phospholipids in HDL(mg/dl)	72.90±2.81b	123.93±1.40c	115.42±6.31c	121.50±1.40c	77.76±4.32b	69.25±4.91b	50.22±4.29a
Phospholipids in VLDL+LDL (mg/dl)	303.75±14.03f	258.39±3.53e	170.10±2.81c	204.12±1.40d	170.10±5.06c	125.14±2.10b	105.70±4.91a
Phospholipids in RBC(mg/dl)	247.22+3.63d	190.35±4.51c	151.88±4.91b	156.74±4.91b	107.73±1.62a	278.24±6.31e	400.95±5.61f

the reduction in AST and ALT activities caused by M.morindoides and M.lucida could be due to their low level toxicity. This is also in agreement with the report of Adeneye and Agbaje [19];Oduola et al [20], that reported that ingestion of M.lucida leaf extract had no adverse effect on liver and kidney functions in rats.

Alkaline phosphatase (ALP) had been known to be associated with the lipid transport in intestine and with calcification process in bone. The slight increase and decrease in the ALP activities being not significant, suggest that the integrity of various membrane systems were not compromised.

The plasma level of lipid profile parameters in all groups of study animals and control were within reference range according to Ranjna.[11] These results of the parameters obtained were inversely correlated with the report of Conderce , that high level of LDL-cholesterol and low level of HDL-cholesterol have been strongly associated with the risk of coronary heart disease(CHD).[12] The leaves extracts could be useful in treatment of coronary heart disease(CHD) and for assessing cardiovascular risk, since the administration of the leaves extracts resulted in high concentration of HDL cholesterol(good cholesterol).This agrees with Suchetal et al [12] that HDL cholesterol concentration is considered as a tool for assessing cardiovascular risk. Also Ranjna[11] reported that HDL-cholesterol and coronary heart disease (CHD) are inversely related.

Finally, the mild toxicity effects of the plants may be due to the presence of saponins. This agrees with Casarett et al [22], that there is usually a latent period of two to three hours prior to the onset of symptoms of toxicity during which the saponins are hydrolysed to their active triterpene components. Also, Molt [23]reported that saponins are toxic to insects and molluscs and some of the most useful natural agents for controlling schistosomiasis in snails are saponins in nature.

In conclusion, the data generated from this study Show that administration of methanolic extracts of these plants (M.morindoides and M.lucida) to rats are not injurious to the tissue at the tested dosage .

ACKNOWLEDGEMENT

The authors are grateful to Dr(Mrs) Ugbaja R.N, Mr Awoyemi O.A, Mr Olurinde O.J, Mrs Adebawa J.O and Miss Adeleye T.O for their technical assistance. We appreciate the financial support of Fatunbi J.O, Fabusoro M.T, Adegbasa L.C, Aina O.B and Adesanya A.F.

We also wish to appreciate the authority of the University of Agriculture, Department of Biochemistry, Abeokuta, Nigeria, for making their laboratory and some facilities available for use.

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