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Hepatotoxicity and nephrotoxicity evaluation in Wistar albino rats exposed to Morinda lucida leaf extract

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Abstract

Background: Aqueous extract of Morinda lucida benth leaf is consumed in Southern Nigeria in the treatment of malaria without any regard for its safety. **Aim:** The aim of the study was to investigate the effects of ingestion of the ethanolic leaf extract of the plant on liver and kidney functions in Wistar albino rats. **Materials and Methods:** Acute oral toxicity test was performed to determine the LD_{50}; sub-chronic toxicity study was then carried out by oral administration of different doses of the extract on daily basis to different groups of rats for 42 days. The animals were subsequently sacrificed, and liver and kidney functions assessed biochemically using standard techniques. **Results:** The acute oral toxicity result, $LD_{50,}$ revealed Morinda lucida leaf extract to be non-lethal at 6400mg/kg body weight. The results obtained for liver and kidney function parameters indicated that ingestion of Morinda lucida leaf extract has no toxic effect on liver and kidney functions. **Conclusion:** The results can form the basis for clinical trials in humans.

Keywords: Morinda lucida, leaf extract, liver, kidney, toxicity, rats.

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Introduction

Plant derived products have been used for medicinal purposes for centuries. At present, it is estimated that about 80% of the world population relies on botanical preparations as medicines to meet their health needs [1]. Herbs and spices are generally considered safe and proved to be effective against certain ailments, while literature has documented severe toxic reactions from the use of herbs on many occasions, still the potential toxicity of herbs has not been recognized by the general public or by professional groups of traditional medicine[2,3] Patients are often unaware of important similarities and differences between medicinal herbs and approved medications, some mistakenly think of herbs as natural alternative to chemicals, failing to recognize that herbs are composed of bioactive chemicals some of which may be toxic [4].

Many xenobiotics are capable of causing some degree of liver injury [5]. The liver is prone to xenobiotic-induced injury because of its central role in xenobiotic metabolism, its portal location within the circulation, and its anatomic and physiologic structure [6].

The Kidney is highly susceptible to toxicants for two reasons. A high volume of blood flows through it and it filters large amounts of toxins which can concentrate in the kidney tubules. Nephrotoxicity is toxicity to the kidneys. It can result in systemic toxicity causing: decreased ability to excrete body wastes, inability to maintain body fluid and electrolyte balance and decreased

synthesis of essential hormones [7].

Different parts of Morinda lucida benth have been reported to possess medicinal properties. The leaf extract of the plant was reported to possess trypanocidal [8], antimalarial activities [9, 10] and aortic vasorelaxant effect [11]. Oliver-Bever [12] documented the use of a weak decoction of the stem bark to treat severe jaundice. Morinda lucida leaf extract has also been reported to have a strong oral hypoglycemic property [13, 14]; Adeneye and Agbaje [14] attributed this property to increased peripheral utilization of glucose. The leaf extract of Morinda lucida has also been documented to possess reversible antispermatogenic activities in rats [15].

Many people in Southern Nigeria treat malaria by drinking aqueous leaf extract of Morinda lucida. It is well documented that Morinda lucida leaf extract has various therapeutic benefits with no known adverse effect among the users; the responses of various organs; especially liver and kidney in humans, to ingestion of this extract remain largely unknown. Hence this study was undertaken to examine to what fashion liver and kidney function parameters would be affected in rats exposed to Morinda lucida leaf extract.

Materials and Methods

Plant materials and preparation of plant extract

The leaves of Morinda lucida were collected in the month of June, 2009, at Tonkere, a village in Ile-Ife, Nigeria. The plant was authenticated at the herbarium of Botany Department, Obafemi Awolowo University, Ile- Ife, by Mr. Ademoriyo. The herbarium number is14650.

The plants were collected, washed with tap water and cut into tiny bits of about 2 cm. They were subsequently dried in a hot air oven and comminuted using a grinding machine. Four hundred and fifty grams of the ground sample was immersed in absolute ethanol for 72 hr on a mixer to ensure maximum extraction .The extract was filtered with a No 1 Whatman filter paper and the decoction was concentrated to dryness in vacuo on a rotary evaporator to obtain the crude ethanolic extract.

Experimental animals

Male and female Wister albino rats (200-260g) obtained from the animal house of the Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife were used for the study. They were housed in rat cages in well ventilated house, temperature of 27-30^{0}C, 12 h natural light and 12 h darkness, with free access to tap water and dry rat pellet (purchased at Ogo Oluwa Enterprises, Ile-Ife). They were allowed to acclimatize for three days before the experiment.

Acute oral toxicity study/LD_{50} determination

Different doses of the extract were administered orally to seven groups of rats consisting of 6 rats in a group. The animals in group A served as control and received normal saline. The animals in groups B, C, D, E, F and H received 200, 400, 800, 1600, 3200 and 6400mg/kg body weight respectively through oral administration with a canula attached to a graduated syringe. They were all placed under observation for 24 h after which the numbers of dead rats were recorded, and LD_{50} calculated [16].

Sub-chronic toxicity study

None of the animals in the oral toxicity study died. Therefore, the extract was administered to the animals for further 42 days. At the end of 42 days, the rats were weighed and blood samples collected through cardiac puncture under chloroform anesthesia into lithium heparin specimen bottles for biochemical analysis. The animals were subsequently sacrificed by cervical dislocation. Liver and kidney function tests were performed on the blood samples using standard techniques [17].

Statistical analysis

The mean and standard deviation and the level of significance for the differences between means were computed by students test SPSS 6.

Results

In the acute oral toxicity study, no death was recorded even at the highest dose of 6400mg/kg body weight (Table 1) an indication that the LD_{50} of the plant is higher than 6400mg/kg.

The values of total and conjugated bilirubins, total protein and albumin, ALT, AST, and ALP obtained for the study groups (B, C, D, E, and F) showed no statistical significant differences ($p>0.05$) between the study and control animals (A). There were also no significant differences between the study groups (Table 2).

In Table 3, there were no statistical significant differences ($p>0.05$) in the values obtained for Na^+, K^+, HCO^-_3, Cl^-, urea and creatinine between the control and study groups, and between the study groups.

Table 1 LD_{50} determination by arithmetic method of Karbar adapted by Aliu and Nwude [16]

Dose (mg/kg)	Rats	Death	Dose difference	Mean death	Dose different X mean death
Saline	6	0	-	-	-
200	6	0	200	-	-
400	6	0	400	-	-
800	6	0	800	-	-
1600	6	0	1600	-	-
3200	6	0	3200	-	-
6400	6	0	-	-	-

$$LD_{50} = \text{Maximum dose} = \frac{Y}{\text{Number of rats per group}}$$

Table 2 Effect of intake of Morinda Lucida aqueous extract on liver function profiles (N = 6)

Parameters	Group A Control	Group B 200mg/kg	Group C 400mg/kg	Group D 800mg/kg	Group E 1,600mg/kg	Group F 3,200mg/kg
Total Bilirubin (μmol/L)	12.67±3.50	10.83±1.84	12.00±2.37	11.88±2.14	10.67±1.75	10.17±3.37
Conj Bilirubin (μmol/L)	4.33±1.03	3.83±1.17	3.67±1.21	3.50±1.38	3.67±0.87	4.00±1.79
Total protein (g/L)	64.00±3.16	68.17±0.75	69.33±7.12	66.50±4.04	67.17±5.25	65.33±4.68
Albumin (g/L)	37.83±2.32	39.17±1.47	38.67±4.37	40.00±3.45	38.50±2.43	39.06±3.39
Globulin (g/L)	26.17±3.25	27.00±1.79	28.67±3.39	27.95±2.14	29.67±1.17	26.83±3.76
ALT (IU/L)	26.83±1.72	26.33±1.21	26.83±1.47	25.83±3.19	26.83±1.17	26.83±1.47
AST (IU/L)	20.83±1.47	21.17±1.47	20.83±1.47	21.00±2.10	21.00±2.37	20.83±2.32
ALP (IU/L)	77.33±2.58	76.33±2.66	75.83±3.76	74.83±3.49	75.33±3.83	76.83±3.19

Table 3 Effect of intake of Morinda Lucida aqueous extract on kidney function profiles (N = 6)

Parameters	Group A Control	Group B 200mg/kg	Group C 400mg/kg	Group D 800mg/kg	Group E 1,600mg/kg	Group F 3,200mg/kg
Sodium (mmol/L)	137.17±6.24	136.33±5.68	137.17±2.79	134.00±3.74	137.33±6.38	137.50±5.32
Potassium (mmol/L)	4.43±1.57	4.42±0.45	4.38±1.13	4.40±1.47	4.30±0.96	4.43±0.88
Bicarbonate (mmol/L)	25.33±2.16	25.17±4.88	24.83±3.31	25.17±1.94	25.17±2.32	25.67±2.81
Chloride (mmol/L)	103.50±9.33	104.33±9.59	104.17±8.31	105.17±4.71	101.00±9.49	103.83±10.63
Urea (mmol/L)	6.40±1.01	6.43±1.05	6.27±0.85	6.32±0.80	6.52±1.46	6.05±0.68
Creatinine (mmol/L)	92.83±6.37	92.17±15.09	90.83±12.17	92.83±8.47	93.00±7.24	93.67±10.27

Discussion

Investigation of the acute toxicity is the first step in the toxicological investigation of an unknown substance. The index of acute toxicity is the LD_{50}. However, LD_{50} should not be regarded as a biological constant, since differing results are obtained on repetition or when the determinations are carried out in different laboratories [18], due to many variables such as animals' species and strain, age, gender, diet, bedding, ambient temperature, and time of the day. Hence, there are considerable uncertainties in extrapolating LD_{50} value obtained for specie to other species, consequently, recognizing LD_{50} test as providing, at best, only a ballpark estimate of human lethality has been advocated [19].

The result of acute oral toxicity (LD_{50}) of Morinda lucida leaf extract was found to be greater than 6400mg/kg body weight as no mortality was recorded in any group of experimental rats. In an acute oral toxicity study by Adeneye and Agbaje [14], Morinda lucida leaf extract was documented to be non-lethal in rats at 2000mg/kg body weight.

The current LD_{50} values based on acute oral toxicity recommended by the Globally Harmonized System of Classification and Labeling of Chemicals[20] are as follows: Category 1, ≤ 5mg/kg; category 2, > 5mg/kg≤50mg/kg [they are both labeled as danger: fatal if swallowed]; category 3, >50mg/kg≤300mg/kg [danger: toxic if swallowed]; category 4, >300mg/kg ≤ 2000mg/kg [warning: harmful if swallowed], category 5, >2000mg/kg≤5000mg/kg [warning: may be harmful if swallowed] and LD_{50} >5000mg/kg [not classified: no specified label]. Hence, the LD_{50} of greater than 6400mg/kg is an indication that the extract may be safe for human consumption, confirming the belief of the herbalists that Morinda lucida leaf extract is not harmful. The values obtained for liver function parameters showed that the conjugating ability of the liver was not compromised from the total and conjugating bilirubin levels; the synthetic ability of the liver was also maintained judging from total protein and albumin values. There was also no hepatocellular damage as revealed by the ALT and AST values.

The electrolytes, urea and creatinine are markers of kidney function, the plasma levels of these parameters in all groups of experimental animals and control were within reference range throughout the period of the study.

In conclusion, from the present study, it was established that ingestion of Morinda lucida leaf extract has no adverse effect on liver and kidney function in rats.

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