



EFFECTS OF CRUDE AQUEOUS EXTRACT OF MORINDA LUCIDA LEAF EXTRACT ON THE HISTOLOGY OF LIVER OF ADULT WISTAR RATS (*RATTUS NORVEGICUS*).

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Authors' contributions

This work was carried out in collaboration among all authors. Author OOA, AAA, AAO and JIA designed the study and wrote the protocol. Authors OOA, AAA, AAO managed the animals, collected all data, performed the statistical analysis and wrote the first draft of the manuscript. Authors OOA, AAA, AAO and JIA did the literature search and also wrote part of the manuscript. All authors read and approved the final manuscript.

ABSTRACT

Background: *Morinda lucida* has been reported to have anti-inflammatory effect that characterizes liver disease but from this study it shows that this report was not achieved until when the extract was withdrawn and the administration of vitamin C along with the aqueous extract acts as anti-oxidant to protect the effect of any likely damage done to the liver. This research work examined the effect of *Morinda lucida* during administration, upon withdrawal and the protective effect of concomitant administration of vitamin C and the extract.

Methods: A total of 20 wistar rats (120g-160g) were used for this research. The rats were randomly selected into four groups of five animals each. Group A were used as control given only water ad libitum. Group B were given 6400 mg/kg aqueous extract of *Morinda lucida* for 4 weeks, group C were given 6400 mg/kg/bw aqueous extract of *Morinda lucida* for 4 weeks and withdrawn for two weeks while group D were given 6400 mg/kg/bw of the extract simultaneously with 5mg of vitamin C for 6 weeks.

. The rats were then sacrificed and the organ excised, weighed and fixed for histological processing and stained with heamatoxylin and eosin. The photomicrographs of the control and the treated groups were observed and compared for changes and differences.

Results: The findings showed sign of inflammatory changes secondary to administration, upon withdrawal, there appear to be reversal of the effect. However, concormittant administration of vitamin C shows evidence of protective effect.

Conclusion: *Morinda lucida* has an inflammatory effect on the liver but there is a reversible effect upon withdrawal and the concurrent administration of vitamin C as anti-oxidant has a protective effect on the damage done to the liver.

Keywords: *Morinda lucida*; anti- inflammation; liver, heamatoxylin; eosin.

1. INTRODUCTION

The use of medicinal plants has always been part of human culture and is common in Africa (1). In some countries, like Ghana, government encourages the use of indigenous forms of medicine rather than expensive imported drugs (1). Also in Nigeria, a large percentage of the populace depends on herbal medicines because the commercially available orthodox medicines are becoming increasingly expensive and out of reach (2).

Amongst the medicinal plants commonly use in Nigeria for management/treatment of various types of ailments is *Morinda lucida* Benth. *Morinda lucida* is a tropical West Africa rainforest commonly known as Brimstone tree (3).

Herbal medicine also called botanical medicine or phytomedicine refers to the use of a plant's seed, barriers, roots, leaves, bark or flowers for medicinal purposes. It has been long practiced

outside of conventional medicine (4). Those plants used as herbs are specifically used because they contain some chemical compounds which produce some specific physiologic effect in the body such plants include *Mytragyna colata*, *Camellia assamica*, *sinensis*, *Morinda lucida* etc. The herbal plants are traditionally used in the treatment of many diseases in different part of the world (5). *Morinda lucida* is one of the most widely antimalarial drugs. The leaf is bitter but medicinal, the whole plant parts: stem, root, bark and leaves are all known for medicinal properties and so are widely used in traditional medicine as a multipurpose medicinal plant and it is believed to be rich in phytochemical components which have diverse antibacterial and anti-plasmodium activities on human and other organisms. *Morinda lucida* is a medicinal plant growing in many African countries and widely used as a medicine in West Africa. It is generally used as ingredients of fever teas, which are usually taken, for the traditional treatment of malaria (6).

In West Africa *Morinda lucida* is an important plant in traditional medicine. In Nigeria *Morinda lucida* is one of the four most used plants in the preparation of traditional medicines against fever (6). Decoctions and infusions or plasters of root, bark and leaves are recognized remedies against different types of fever, including yellow fever, malaria, trypanosomiasis and feverish condition during childbirth. In some cases, the plant is employed in the treatment of diabetes, hypertension, cerebral congestion, dysentery, stomach-ache, ulcers, leprosy and gonorrhea (7).

In Côte d'Ivoire bark or leaf decoction is applied against jaundice and in DR Congo, the decoction of the stem bark or leaf is combined with a dressing of powdered root bark against itch and ringworm (1). It was reported in their work that the bark, root and leaf are bitter (8). They stated that the infusion or decoction of these parts is used for the treatment of yellow fever and other forms of fever. They also reported that the decoction of the leaf is applied to the breast of women at weaning of their infants to prevent infections. However, *Morinda lucida* is used generally for febrifuge, analgesic and laxative while the decoction of the stem bark is used for the treatment of severe jaundice (8).

It was reported that *Morinda lucida* is used locally in the treatment of irregular menstruation, insomnia and jaundice though did not state the parts that are useful in this purpose (9). *Morinda lucida* is used locally in the treatment of wound infections, abscesses and chancre (The primary syphilitic ulcer associated with swelling of local lymph glands and is painless, indurate, solitary

and highly infectious) (10). Also amongst the Igede People in Benue State, Nigeria, it was reported that the decoction of the *Morinda lucida* is used twice or thrice daily as anti – diarrhea, while the leaves are used for treatment of infertility in women (1).

Vitamin C (ascorbic acid) is a water-soluble antioxidant occurring in the organism as an ascorbic anion. It also acts as a scavenger of free radicals and plays an important role in regeneration of α -tocopherol (11). Ascorbic acid is a powerful antioxidant because it can donate a hydrogen atom and form relatively stable ascorbic free radicals. As scavenger of reactive oxygen and nitrogen oxide species, ascorbic acid has been shown to be effective against the superoxide radical ion, hydrogen peroxide, the hydroxyl radical and singlet oxygen (12).

2. MATERIAL AND METHODS

2.1. EXTRACT PREPARATION

2.1.1 PREPARATION OF SOXHLET EXTRACTION OF *MORINDA LUCIDA*

Morinda lucida leaves were plucked, identified at the Biology department of Ladoke Akintola University of Technology Ogbomosho and air-dried, then pounded into fine powder and taken to the chemistry department of LAUTECH for soxhlet extraction using an aqueous solution (William B 2008).

2.1.2 PREPARATION OF VITAMIN C

The vitamin c also known as ascorbic acid was prepared by dissolving 5mg of vitamin c in 10ml of 0.9% w/v phosphate buffer.

3. EXPERIMENTAL ANIMALS

A total of twenty (20) Wistar rats weighing between 120-160g were used for this research work. The rats were randomly selected into four groups as follow A, B, C and D of five rats each. They were kept in the animal house of Lautech University, Nigeria and given feed and water *ad libitum*. The treatment for the various groups was administered accordingly.

3.1 CHEMICAL AND EXTRACT ADMINISTRATION

Administration of the extract commenced two weeks after of acclimatization; the extract was administered every day for a period of six weeks using oral cannula for the extract administration and with vitamin C as anti-oxidant in accordance to the grouped rats. The treatment was done every morning after the animals were fed.

3.2 ANIMAL TREATMENT

Control group A were given feed and water *ad libitum*, while B group were administered with 6400mg/kg of the extract for four weeks and sacrifice on the 28th day of the experiment. Group C were administered with 6400mg/kg of the extract for 4 weeks and withdrawn for 2 weeks before they were sacrificed on the 42nd day of experiment, group D were administered with 6400mg/kg of the extract simultaneously with 5mg of vitamin C for 6 weeks and sacrificed on 42nd day of the experiment.

Table 1. Number of animals in each group and dosage of treatment given

GROUPS	NUMBER OF ANIMALS	DOSAGES
A	5	Control Group
B	5	6400 mg/kg/bw aqueous extract of <i>Morinda lucida</i>
C	5	6400mg/kg/bw aqueous extract of <i>Morinda lucida</i> and withdrawn for two weeks.
D	5	6400mg/kg/bw of aqueous extract of <i>Morinda lucida</i> + 5mg of vitamin C for six weeks.

3.3 ANIMAL SACRIFICE.

The animals were sacrificed through cervical dislocation. The thoracic and abdominal cavities were exposed adequately by using a surgical blade to make a midline incision through the skin of the abdominal wall from the xiphisternal to the pubic symphysis. After the abdominal cavity has been adequately exposed the liver were removed and fixed in 10% formosaline for histological analysis. The treatments for the various groups were administered accordingly. All experimental investigations were done in compliance with humane animal as stated in the “Guide to the care and use of Laboratory Animals Resources”. National Research Council, DHHS, Pub.No NIH 86-23 [8] and in accordance with the guideline and approval of Nigeria Medical Ethical Association for Accreditation of Laboratory Animal Care.

3.4 HISTOLOGICAL PREPARATION OF TISSUES

The liver was fixed in 10% formosaline to prevent autolysis. The liver was passed of through ascending grades of alcohol (70%, 80%, 90% and absolute alcohol 100%) to gradually remove its water contents. The sample was placed in xylene to remove the alcohol. This improves the refractive index of the tissue. The tissue was immersing in molten paraffin was so that the holes left by the alcohol would be filled up. This gives the tissue support. The tissue is placed into an embedding mold which is filled with more paraffin wax and allowed to solidify. This is done in order to make the tissue compact for sectioning. The block is trimmed to remove the excess wax. The block of tissue was placed in a microtome and trimmed to expose the surface. The microtome was set to 3-5 microns and the tissue was sectioned. The sections were picked with forceps and placed in a water bath to float out and spread well. It was picked with slide and the slide was placed on a hot plate in order for the tissue to stick to the slide. The slides were arranged on a staining rack and dewaxed in two changes of xylene for 10 minutes. It was then hydrated in descending grades of alcohol and rinsed. The stain was applied and the slides left to dry. Photomicrographs were taken with a JVC mounted on an Olympus light microscope (Olympus UK Ltd, Essex, UK).

4. PHOTOMICROGRAPHS

GROUP A: photomicrograph of the liver of experimental control group received normal diet only.

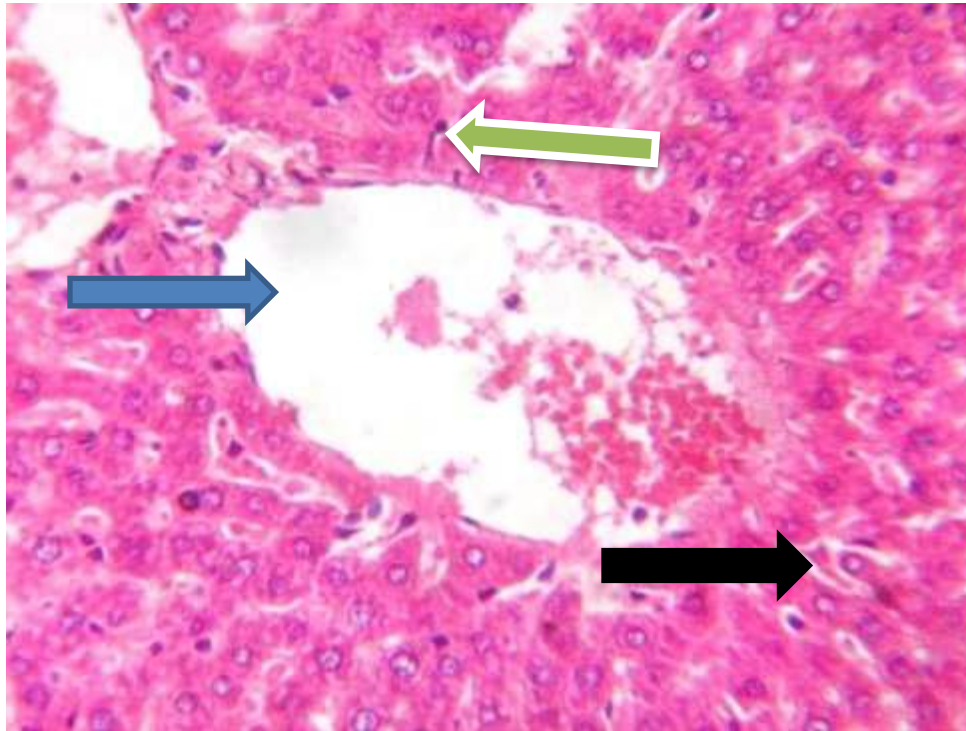


Fig. 1 Histological demonstration of the liver using H & E staining techniques [X400] showing normal central vein (CV, blue arrow) with normal sinusoid (S, black arrow around the hepatocyte(H, green arrow)

GROUP B: Photomicrograph of the liver of experimental animal administered 6400mg/kg of *morinda lucida* extract for 4 weeks.

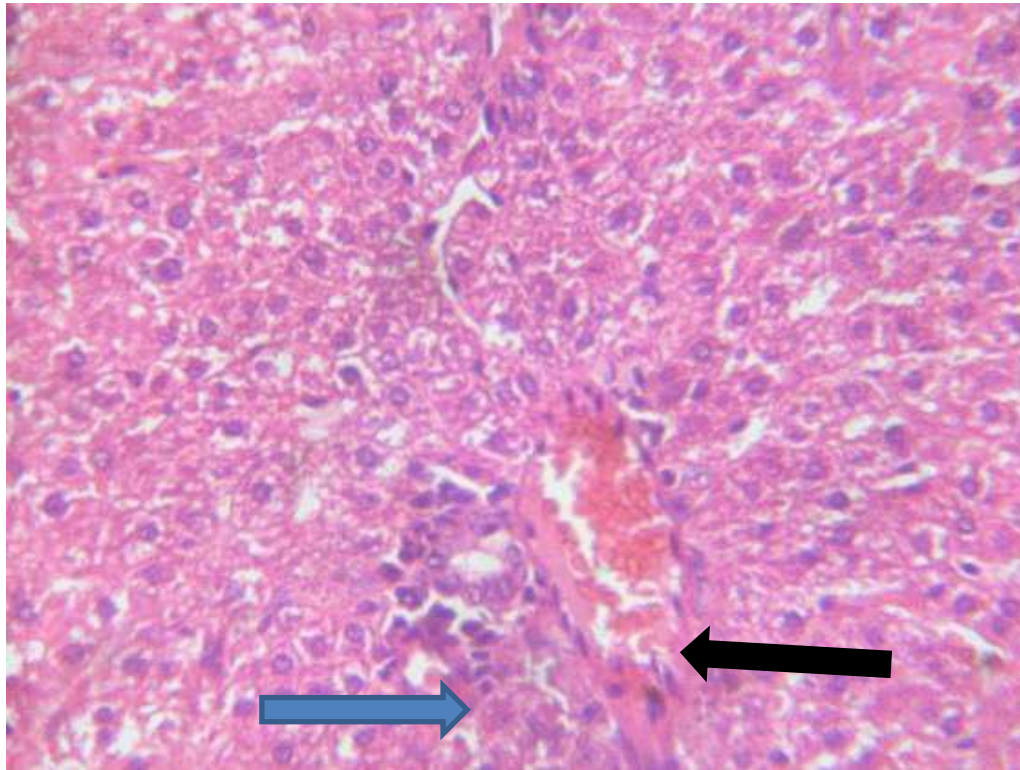


Fig. 2 Histological demonstration of the liver using H & E staining techniques [X400] showing compressed sinusoids, moderate interstitial inflammation (blue arrow) and mild periportal inflammation (black arrow)

GROUP C: Photomicrograph of the liver of experimental animal administered 6400mg/kg for 4 weeks and withdrawn for 2 weeks.

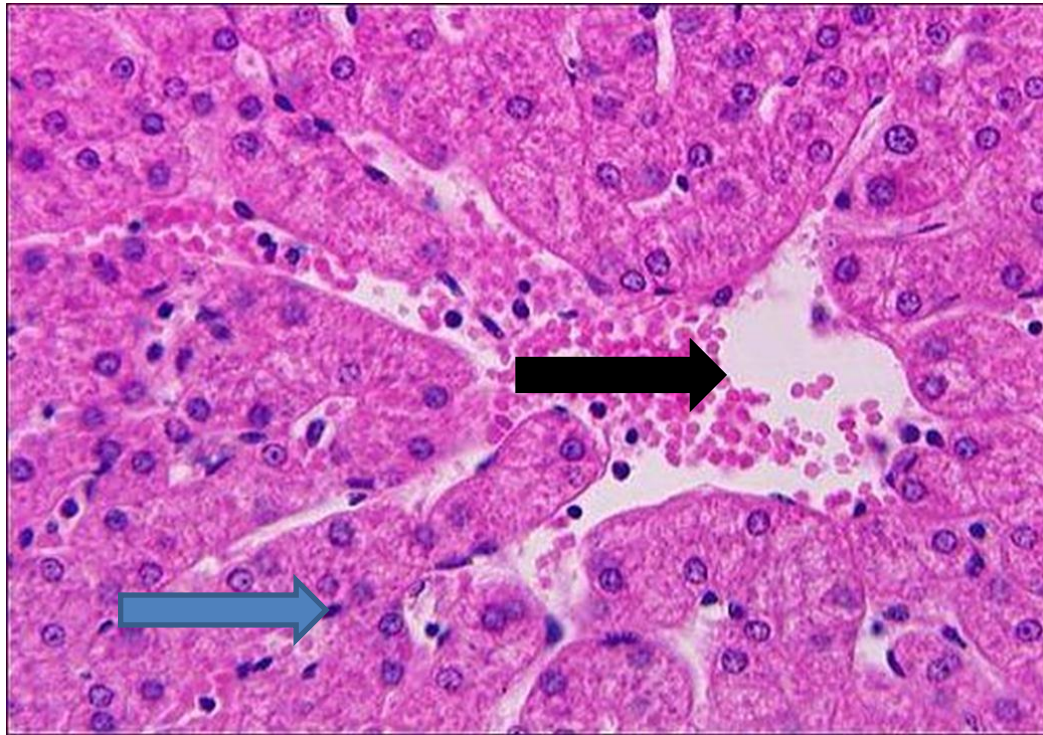


Fig. 3 Histological demonstration Section of liver using H&E staining techniques[x400] showing normal central vein (black arrow) and hepatocyte (blue arrow).

GROUP D: Photomicrograph of the liver of experimental animal administered 6400mg/kg and vitamin C simultaneously for six weeks.

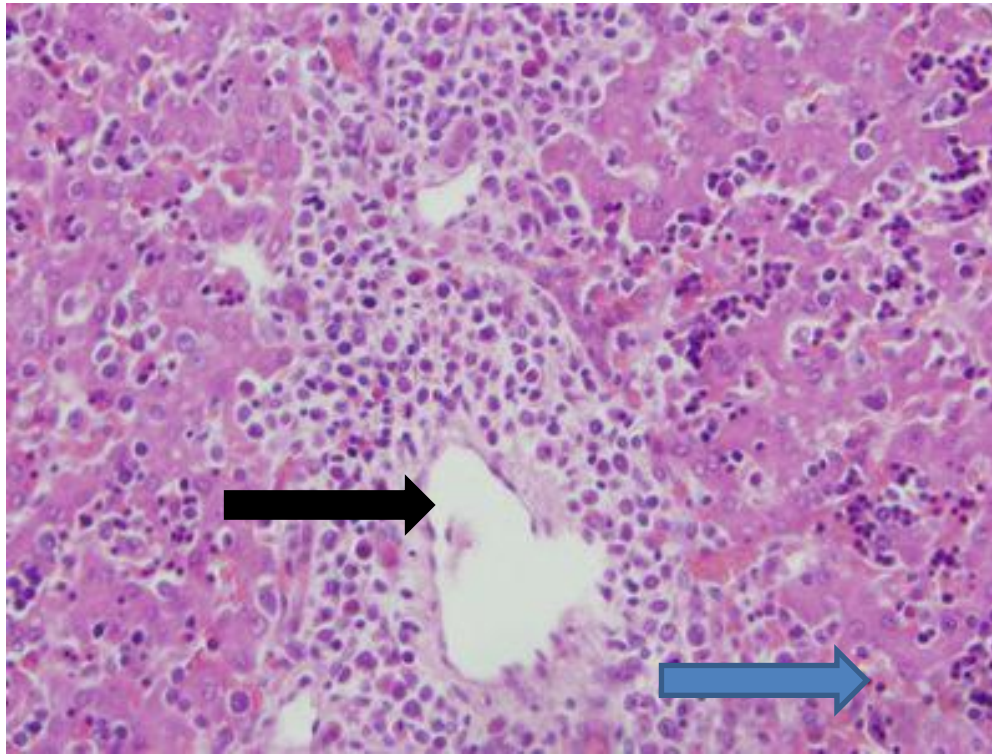


Fig. 4 Histological demonstration Section of liver using H&E staining techniques [x400] showing normal central vein (black arrow), hepatocyte (blue arrow).

5. DISCUSSION.

The results of this study points out the fact that if *morinda lucida* is administered continuously without withdrawal, it will cause mild periportal inflammation but upon withdrawal the effect is reversible and with antioxidant been used alongside the extract there is a protective effect on the liver. The control group given only feed and water shows normal central vein, normal sinusoids with spaces between plates of hepatocytes and normal hepatocytes which are radially arranged and of uniform sizes as seen in [Fig.1] The histological observation indicate that administration of 6400mg/kg of the aqueous extracts of *Morinda lucida* for 4 weeks shows proliferation of mononuclear liver cells, compressed sinusoid and mild periportal inflammation as seen in [Fig. 2] This result shows that there is inflammation within the liver which may hasten its progression to cirrhosis (Biliary cirrhosis). The withdrawal group [C] given 6400 mg/kg for 4 weeks and withdrawn for 2 weeks shows that the liver appears normal compared to observations in the control [Fig. 3]. Group D administered with 5mg of vitamin C simultaneously with 6400 mg/kg of the *Morinda lucida* extract for 6 weeks showing features in keeping with normal hepatic histology, that is, the hepatocytes to be radially arranged, of uniform sizes with nuclei varying between one per hepatocyte and distinct sinusoidal spaces between plates of the hepatocytes [Fig. 4] when compared with the control which shows that vitamin C acts an anti-oxidant.

6. CONCLUSION

The result obtained in this study shows that the aqueous extracts of *Morinda lucida* has mild inflammatory effect on the liver of adult wistar rats that is, when taken without reversal. It also shows that *Morinda lucida* has the reverse effect on the damage done to the liver upon withdrawal. Also the administration of vitamin C as anti-oxidant has protective effect on the damage done to the liver.

7. REFERENCES

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