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The Effect of Aqueous Extract of Lonchocarpus Cyanencens Leaf on the Histology of the Liver of Wistar Rats

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Abstract
This study was carried out to evaluate the effect of aqueous extract of Lonchocarpus cyanescens on the histological architecture of the liver. The histological effect of consumption of Lonchocarpus cyanescens leaf extract on the liver of Wistar rats was carried out on thirty-five (35) Wistar rats. The Wistar rats were divided into seven groups with five rats per group. Group A serves as control, Group B received 200mg/kg body weight of L. cyanescens, Group C received 500mg/kg body weight of the extract, Group D were given 1000mg/kg body weight of the extract while group E, F and G received 2000mg/kg, 3500mg/kg, and 5000mg/kg body weight of Lonchocarpus cyanescens aqueous leave extract respectively. The administration was implemented once daily by oral compulsion. After 42 days of treatment, the Wistar rats were given chloroform anaesthesia, dissected and their liver were harvested in the histology laboratory and put in 10% formal saline. The liver was processed in the histopathology laboratory for microscopic examination of the tissue followed by photomicrographs of selected slides, which were recorded. The results from the histopathological examination showed normal liver histology in tissue of group A. The group B, C and D revealed mild vascular dilatation, mild portal vascular congestion and mild Kupffer cell activation normal with intact sinusoidal spaces showing that the extract is not deleterious even at low doses. Group D, E and F (2000mg/kg, 3500mg/kg and 5000mg/kg body weight respectively) shows mild portal congestion and moderate activation of Kupffer. The different immunological systems of the rats and the concentration of Lonchocarpus cyanescens may account for the variations in Kupffer cell activation and the immunologic response to the concentration supplied, confirming the safety of our extract up to 5000 mg/kg body weight. Based on the histological findings, Lonchocarpus cyanescens aqueous leave extract is not toxic to the liver tissue and is safe for consumption.

Keywords: Aqueous extract, Lonchocarpus Cyanencens, histology, liver, Wistar rat

Introduction

One of the first methods of treating a wide range of illnesses, herbal medicine has a comparatively high patient base due to its affordability, accessibility, and ability to fit into the sociocultural fabric of the populace (Butani and Verma, 1981). According to the WHO, the greatest place to find a wide range of medications is from herbal or medicinal plants (Doughari *et al.,* 2008). The exploration of substitute routes for the ever-questionable adverse effects of synthetic drugs has attracted increasing attention. Despite the advancements in technology, intelligent people in many parts of the world, particularly developing nations, still turn to herbs to treat specific illnesses. This practice persists due to both poverty and the effectiveness of herbal remedies (Etetim *et al*., 2008).

Lonchocarpus cyanescens is known as Elu in Yoruba, Anunu in Ibo, Talaki in Hausa, Suru in Tiv and Ebelu in Edo languages of Nigeria. Lonchocarpus cyanescens also known as "ALU" is a deciduous scandent shrub. The plant features oblong pods with points on both ends, alternating leaves, and flat fruits with one to five seeds. The aerial sections produce

indigo, which has long been used in West Africa as a helpful colorant for dying fabrics. The plant is used in traditional medicine; Lonchocarpus cyanescens's bioactivity effects have been shown to be anti-inflammatory, anti-arthritic, anti-diabetic, and ulcer-relieving. It has some additional pharmacological properties including antiviral, antifungal, anti-protozoal, and antibacterial activities (Manoj and Aquad, 2003). It has been shown that the plant leaves contain reducing sugars, flavonoids, tannins, saponins, and cardiac glycosides (Iyoha and Onoagbe, 2016). It is crucial to remember that despite Lonchocarpus cyanescens' well-known excellent healing qualities, there is insufficient evidence available on its safety, effectiveness, and various other interactions and contraindications. This is the justification for the study, which centers on the liver as a major organ prone to insult by toxicity. Similarly, an acute toxicity assessment and biochemical data by (Iyoha *et al.,* 2023) showed that the plant is practically non-toxic. The nutritional and antidiabetic properties have well been documented (Amu *et al.,* 2019). However, there are little or no scientific data to prove its effect on the histomorphology of liver status in rats as a pivotal organ for metabolism, hence this study was undertaken to determine the effects of aqueous extract of L. cyanenscens leaves on histomorphology of liver status in normal Wistar albino rats.

Materials and Methods

Collection of Plant Materials

Fresh leaves of Lonchocarpus cyanescens were obtained from Abavo in Ika South Local Government Area of Delta State, Nigeria. The plant was identified in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. A herbarium specimen with voucher number UBHf0291 was deposited at the herbarium of the University of Benin.

Preparation of plant extracts

The plant leaves were washed and air-dried at room temperature for a period of three weeks, before it was pulverized into powder. The 100g of the powdered leaves were weighed with electrical weighing balance into sterilized conical flask and 500ml of distilled water was poured into the flask, the content of the flask was shaken, and the top were covered with aluminum foil and kept at ambient temperature for 48 hours after which the extract was obtained by filtering using clean cloth with fine pores. The extracts were then concentrated in crucible using water bath set at temperature of 45^{o}C. The weight of concentrated extract was taken and then stored in air-tight sample bottle in refrigerator till it is time to be analyzed according to the method of Amu *et al.* (2019).

Experimental animals

Male albino rats weighing between 150 and 220 g were housed in wooden cages in the Animal House of Anatomy Department, University of Benin, Benin City. The rats were allowed to acclimatize to the environment for a period of two weeks before the start of the experiment. They were placed on commercial feed (growers pellet) and drank water ad libitum.

Groups and Dosage

Group A Received 1 ml distilled water only
Group B Received 200 mg/kg of extract L. cyanescens
Group C Received 500 mg/kg of extract L. cyanescens
Group D Received 1000 mg/kg of extract L. cyanescens
Group E Received 2000 mg/kg of extract L. cyanescens
Group F Received 3500 mg/kg of extract L. cyanescens
Group G Received 5000mg/kg of extract L. cyanescens

Tissue collection, processing and staining: After being exposed to the extract for 42 days, the rats were killed by cervical dislocation while sedated with ether. The rat's heart was quickly removed. The heart tissues underwent a 72-hour fixation in 10% buffered formalin prior to histological processing and staining with haematoxylin and eosin. Tissues were stained using approved techniques (Drury *et al*., 1976).

Photomicrography: The heart's H&E-stained slides were viewed by a histopathologist using a Leica DM750 research microscope with a Leica CC50 digital camera connected. Digital photos of the tissues were taken at x400 magnifications.

Results

Plate 1: Liver of Control rat composed of hepatocytes A, sinusoids B and portal vein C (H&E x 400)

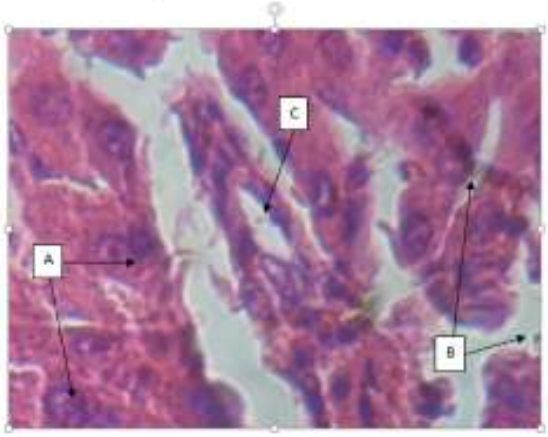


Plate 2: Liver of rat given 200mg/kg aqueous extract of L. cyanescens showing mild portal vascular dilatation and congestion A and mild kupffer cell activation B (H&E x 400)

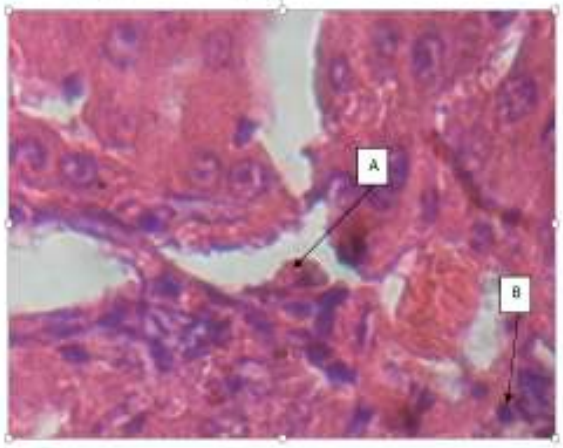


Plate 3: Liver of rat given 500mg/kg aqueous extract of L. cyanescens showing moderate vascular congestion A and mild kupffer cell activation B (H&E x 400)

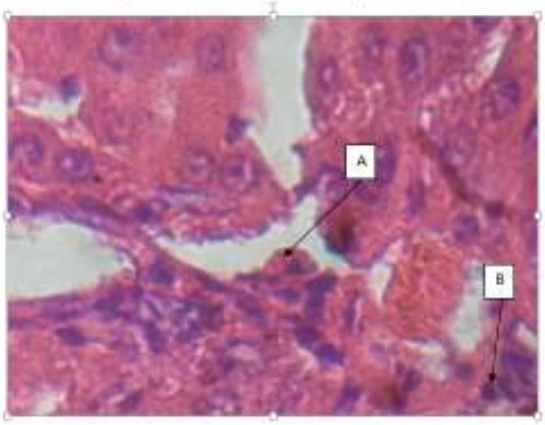


Plate 4: Liver of rat given 1000mg/kg aqueous extract of L. cyanescens showing mild kupffer cell activation A (H&E x 400)

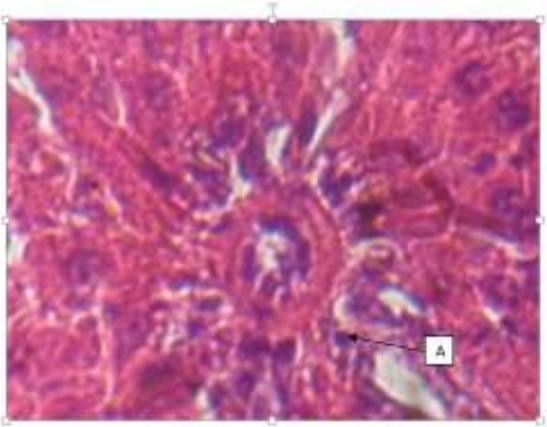


Plate 5: Liver of rat given 2000mg/kg aqueous extract of L cyanescens showing mild portal congestion A, kupffer cell activation B and periportal lymphocytosis C (H&E x 400)

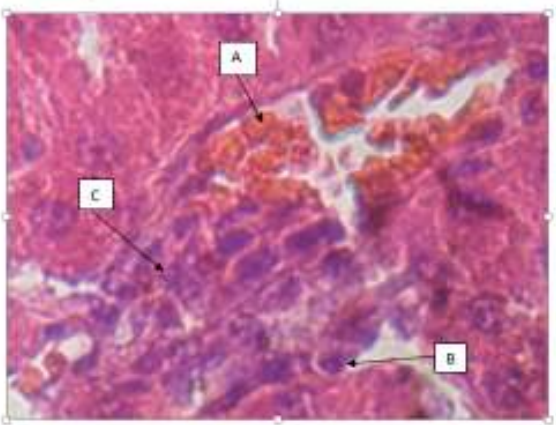


Plate 6: Liver of rat given 3,500mg/kg aqueous extract of L. cyanescens showing mild periportal lymphocytosis A, portal congestion B and kupffer cell activation C (H&E x 400)

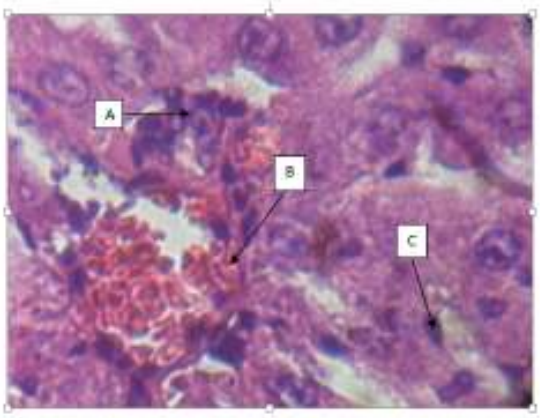


Plate 7: Liver of rat given 5,000mg/kg aqueous extract of L. cyanescens showing mild portal congestion A and kupffer cell activation B (H&E x 400)

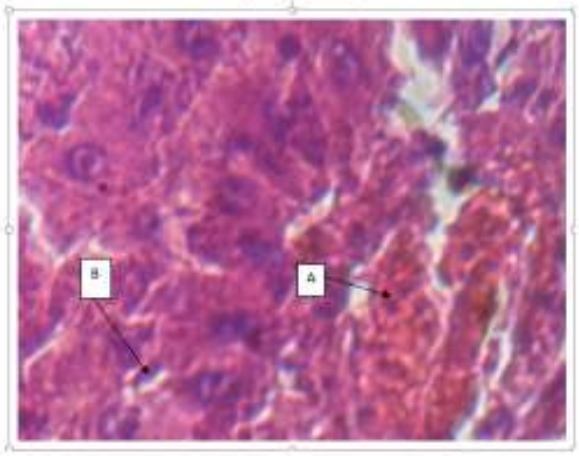
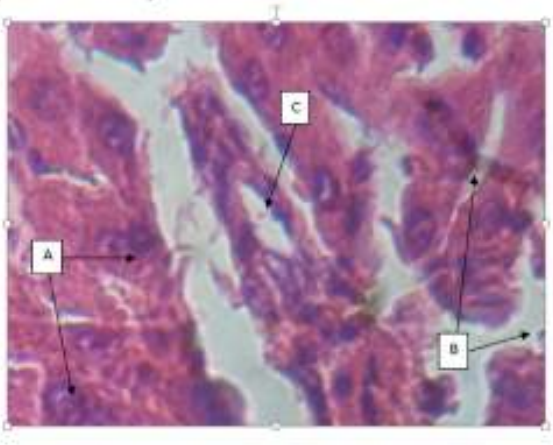


Plate 1: Liver of Control rat composed of hepatocytes A, sinusoids B and portal vein C (H&E x 400)



Discussion

The majority of people in poor nations frequently employ medicinal herbs as an alternate form of treatment. The potential negative effects of using these plants have not been thoroughly studied, yet, as plant extracts may be hazardous by nature because they contain chemicals that are either cytotoxic or carcinogenic (Humphrey and Mckenna, 1997). Additionally, the majority of herbal remedies lack drug regulatory certifications attesting to their efficacy and safety (Seth and Sharma, 2004). While facing the threat of maximum exposure to xenobiotics and their metabolic by-products, the liver, the key organ involved in numerous metabolic functions (such as the production and secretion of bile, the production of fibrinogen, prothrombin, heparin, and sulfuric acid ester, and the conversion of sugar into glycogen), plays a central role in the detoxification process (Meyer and Kulkarni, 2001). Plate 1 is an x 400 photomicrograph of the liver stained with H&E that displays the typical architecture of the liver tissue, with the sinusoids, portal vein, and hepatocytes visible. It is evident that the Kupffer cell activation was deemed mild in the body weight concentrations of 200 mg/kg, 500 mg/kg, and 1000 mg/kg, and moderate in the body weight concentrations of 2000 mg/kg, 3500 mg/kg, and 5000 mg/kg.

The difference in the immunologic response to the concentration administered could be due to the varying immune system of the rats and concentration of Lonchocarpus cyanescens, affirming that our extract is safe even to 500mg/kg body weight. These results agree with those reported by Omonkhua and Onoagbe (2012) where the roots of Urena lobeta, the bark of Irvingia gabonensis and the leaf of Carica papaya did not have any deleterious effect on oxidative status and lipid peroxidation in normal rats.

According to earlier studies on the function of Kupffer cells in the etiology of liver disease, these cells are the first to be exposed to substances taken up from the gastrointestinal tract. A key physiological role of the Kupffer cells is their capacity to eradicate and detoxify pathogens, endotoxins, degenerated cells, immunological complexes, and toxic substances. Further histopathological results shown that all the groups treated with Lonchocarpus cyanescens aqueous leaves extract at various doses revealed mild and moderate activation of Kupffer cells with mild portal congestions and lymphocytosis. The current study is consistent with earlier research on the effects of Gongronema latifolium leaf extract on the histopathology of various wistar rats' organs (George *et al*., 2021) and the effects of Lonchocarpus Cyanencens Leaf on Oxidative Status in Normal Albino Wistar Rats (Iyoha *et al.,* 2023).

Our study further proves that Lonchocarpus cyanescens aqueous leaf extract itself is not a toxic agent. Put another way, administering different concentrations of the extract boosted the rat's immune system, allowing the liver to eliminate the hazardous chemical through blood circulation. This implies that, although

administration at lower dosages will be preferred, particularly on a long-term use, the aqueous leaf extract of Lonchocarpus cyanencens may be safer at all tested doses. This is consistent with other research that found no overt adverse responses in either the acute or sub-acute phases. Therefore, it was observed that the leaf extract of Lonchocarpus cyanencens aqueous would be fairly safe to eat.

Conclusion: This study demonstrated that the aqueous extract of Lonchocarpus cyanescens leaf may not cause significant deleterious effects on the liver of Wistar rates based on the histological findings and therefore recommended for consumption and for the management of acute liver conditions. Further research should be directed towards the mechanism of action of the extract

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