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Research Article

Safety Evaluation of Methanolic Leaf Extract of *Lonchocarpus cynanecens* on the Liver of Wistar Rat

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ABSTRACT

This study was designed to safety evaluate the methanolic leaf extract of *Lonchocarpus cyanescens* on the liver of the Wistar rat. A total of 28 Wistar albino rats were used for the study. They were divided into seven groups, consisting of four rats each and were administered graded doses of the methanolic leaf extracts of 200 mg/kg, 500 mg/kg, 1000 mg/kg 2000 mg/kg 3500 mg/kg and 5000 mg/kg body weight, orally for 28 days while group one which serve has the control was administered distilled water. Rats had free access to feed and water *ad libitum*. The administration of 200 mg/kg, 1000 mg/kg, and 2000 mg/kg methanolic leaf extract showed no observable significant difference ($P>0.05$) in ALT, ALP, GGT activities, total bilirubin, direct bilirubin, and indirect bilirubin concentrations. Administration of 3500 mg/kg of the extract significantly ($P<0.05$) increases the activity of ALT and GGT. AST activity was significantly ($P<0.05$) decreased when compared to control at dose 3500 mg/kg; however, administration of 2000 mg/kg of the extract significantly ($P<0.05$) increased the activity of AST. Administration of 500 mg/kg of the extract significantly ($P<0.05$) increases the concentration of total bilirubin and indirect bilirubin. No significant ($P>0.05$) difference was observed in ALP and albumin across the graded doses administered to the rats. The results from this study, therefore, suggest that some doses of the extract might be safe while others might induce liver damage under sub-chronic toxicity.

Keywords: Albumin; Bilirubin; Enzymes; Hepatotoxicity; *Lonchocarpus cyanescens*

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INTRODUCTION

Medicinal plants, since their discovery in prehistoric times, have been very vital and used in traditional medicine and as folk medicine in non-industrialized societies due to their availability and the ability to afford them compared to modern medicine. To manage varieties of diseases, dating back since the existence of humankind, people relied on the use of plants for nourishment and medicine for treatment control **(**Onyegeme-Okerenta *et al.,* 2018**).** Hence, it is necessary to study the safety and mechanism of actions by which some of these therapeutic plants or products exert effects on tissues or organs. One of such plants is *Lonchocarpus cyanescens. Lonchocarpus cyanescens* is a shrub belonging to the tribe Dalbergiae of the order Leguminosae (Sonibare *et al.,* 2014 and Akunne *et al.,* 2014), family of Fabaceae, and it is widely grown in Nigeria, Ghana, Cameroon, Ivory Coast, Togo, Sierra Leone, Benin, and Guinea. It's a native of West Africa. It is known to improve the health care system in Nigeria as one of the medicinal plants used in traditional medicine (Ajibesin *et al*., 2008). The plant has alternate leaves, flat fruits which are 1-5cm long, long pods, and pointed at both ends (Schrire, 2002, and Burkil, 1985). The plant grows in the fringe, deciduous and savannah forest. It is commonly called West Africa wild indigo and a shrub of 5 m high and 50 cm in growth. The popularity of *L. cyanescens* is attributed to its usage in dye production. The indican-containing leaves and young sprouts are used after fermentation to

obtain the blue indigo dye, which is used for colouring textile and other materials (Ajibesin *et al.*, 2008).*Lonchocarpus cyanescens* is commonly referred to as Indigo vine or West African Indigo, because the local people extract dyestuff from the leaves of the plant, with a brilliant indigo-like true colour, which could be as a result of the presence of indigotin compound as claimed by some scientists, used for various domestic needs. (Moronkola, 2013). The bark and leaves of *L. cyanescens* are traditionally used for the treatment of diseases like ulcers, arthritis, intestinal disorder, diabetes, dysentery, psychotic disorder, leprosy, and others *(*Sonibare, 2012*)*, treatment of diseases such as bone pain, yaws (Umoh and Nwafor, 2013) and mental disorder (Sonibare *et al.,* 2014). The decoction from the root and leaves is given to women during and after child birth, used in the treatment of venereal diseases and stomach ache (Moronkola and Oladosu, 2013). Aqueous extract of the root possess anti-ulcer and anti-analgesic effects (Adegbolagun *et al.,* 2018). Phytochemical screening of the plant leave revealed the presence of secondary metabolites tannins, flavonoids, saponins, cardiac glycosides, steroids and reducing sugars (Iyoha and Onoagbe, 2016). Acute toxicological analysis of plant extracts shows that it is not toxic, as both aqueous and methanolic leaf extracts have LD50> 5000mg/kg orally (Iyoha and Onoagbe 2016). Aqueous extract of the plant has no deleterious effect on the liver of wistar rat (Iyoha and Onoagbe 2024) and aqueous and methanolic leaf extract has no effect on the oxidative stress status in normal rat (Iyoha et al 2023).

However, there is no scientific data to support the safety of methanolic leaf extract in the liver, hence this study was design to monitor the medium term effect of methanolic leaf extracts of *Lonchocarpus cyanescens*, orally administered daily for a period of 28days.

MATERIALS AND METHODS

Chemical

All reagents used were of analytical grades and were obtained commercially.

Medicinal plant

The fresh leaves of *Lonchocarpus cyanescens*, were obtained in Kwara State from a herbal dealer. The plant was identified in the Department of Plant Biology and Biotechnology, University of Benin, Benin City by Dr. O. Timothy. Herbarium specimen was deposited at the herbarium of the University of Benin with voucher number UBH$_f$0291.

Preparation of plant extracts

Under the room temperature the plant leaves were air dried for a period of two to three weeks and were further pulverized, using commercial pulverizer. In absolute methanol (lg/ 5mls), the powdered leaves were then macerated and stirred periodically for 72 hours. To remove debris and to obtain the methanolic leaf extracts, the sample was filtered using a cheese cloth and then through a Whatman No.1 paper filter respectively. The filtrates obtained were freeze dried using a freeze drier to produce a dark green powder.

Animals

Twenty-eight (28) male albino rats weighing between 100 g to 150 g were used for the experiment. The rats were weighed and housed in wooden cages in the Animal House of Biochemistry Department, University of Benin, Benin City. They were allowed to acclimatize for a period of two weeks. The rats were placed on commercial feed (Ewu grower pellet from the Bendel feed and flour mill, Ewu, Nigeria). They were allowed food and water *ad libitum*

Experimental design

A total of 28 rats were used. They were divided into seven groups of four rats each and were administered graded doses, 200mg/kg, 500mg/kg, 1000mg/kg, 2000mg/kg, 3500mg/kg and 5000mg/kg of extracts orally for a period of 28 days. Control group which serve as group one was administered distilled water for same number of days.

Blood Collection

At the end of 28 days of the study, rats were euthanized with chloroform blood was collected through a cardiac puncture using a 5ml syringe into a lithium heparin tube. The blood samples were then centrifuged at 3000g for 15min after which, the supernatants were collected and designated plasma stored in the refrigerator (-40C) for further analyses

Biochemical Analyses

The following analyses were carried out. Aspartate aminotransferase and Alanine aminotransferase (Reitman and Frankel, 1957), gamma-glutamyltransferase by Szas, (1969), Albumin by Douman *et al.,* (1971). Bilirubin according to the method of (Jendrassik and Grof, 1938).

Data Analysis

Data were expressed as mean ± S.E.M. (Standard error of the mean) of four rats and were analyzed for statistical differences using one way Analysis of Variance (ANOVA). Post hoc test multiple comparison using LSD was utilized to determine the level of significance between each treatment group and control group. At $p<0.05$, differences between groups were considered statistically significant.

RESULTS

Findings from this study reveals that administration of 200 mg/kg, 500 mg/kg,1000 mg/kg and 2000

mg/kg did not significantly($p>0.05$) alter the activity of ALT when compared to control except at dose 3500 mg/kg were significant increase ($p< 0.05$) occurred.

The administration of 200 mg/kg, 1000 mg/kg 2000 mg/kg and 3500 mg/kg of the extract significantly ($p< 0.05$) increase and decrease the activity of AST when compared to control except at dose 500 mg/kg were no significant ($p>0.05$) alteration was observed when compared to control.

The activities of GGT was not significantly($p>0.05$) alter in rats administered 200 mg/kg, 500 mg/kg,1000 mg/kg and 2000 mg/kg when compared to control except at dose 3500 mg/kg were significant ($p< 0.05$) increased was observed. The plant extract did not significantly ($p>0.05$) alter the activity of ALP across the various doses administered when compared to control as shown in Table 1.

The administration of varying doses of the extract did not significantly ($p>0.05$) alter the concentration of albumin, total bilirubin, direct bilirubin and indirect bilirubin concentration except 500 mg/kg which significantly ($p< 0.05$) increase the concentration of total bilirubin as shown in Table 2.

Table 1: **Assessment of liver functions of Rats Administered Methanolic Leaf Extracts of *Lonchocarpus cyanescens***

Parameter	Ext.	Control	200mg/kg	500mg/kg	1000mg/kg	2000mg/kg	3500mg/kg
ALT(U/I)	Mth	28.52±2.45^{a}	26.36±1.26^{a}	28.84±0.62^{a}	29.40±0.62^{a}	28.44±2.55^{a}	38.20±2.58^{b}
AST(U/I)	Mth	69.46±1.96^{a}	60.03±1.07^{b}	62.82±0.74^{a}	60.20±2.14^{b}	77.7±0.71^{b}	64.98±1.17^{b}
ALP(IU/I)	Mth	45.05±2.48^{a}	38.60±3.37^{a}	60.68±6.32^{a}	44.45±1.49^{a}	45.25±2.12^{a}	45.62±1.60^{a}
GGT(U/I)	Mth	1.45±0.29^{a}	1.74±.33^{a}	1.74±0.33^{a}	2.02±0.55^{a}	1.45±0.29^{a}	4.05±1.11^{b}

Values are expressed as Mean ± S.E.M.

Values with different superscript from control are significantly different, $p<0.05$

Values with same superscript as control are not significantly different, $p>0.05$,

n=4, Mth= Methanolic, Ext= Extract. Extract was administered for a period of 28 days

Table 2: Albumin (mg/dl) and Bilirubin (mg/dl) Levels in Rats Administered Methanolic Leaf Extracts of *Lonchocarpus cyanescens*.

Parameter	Ext.	Control	200mg/kg	500mg/kg	1000mg/kg	2000mg/kg	3500mg/kg
Alb (mg/dl)	Mth	31.65±2.80^{a}	31.97±2.53^{a}	29.44±2.68^{a}	34.95±2.36^{a}	36.49±1.46^{a}	34.43±6.0^{a}
TB (mg/dl)	Mth	0.81±0.08^{a}	0.98±0.15^{a}	1.38±0.12^{b}	0.99±0.06^{a}	0.90±0.06^{a}	0.69±0.04a
DB (mg/gl)	Mth	0.76±0.02^{a}	0.83±0.09^{a}	0.77±0.04^{a}	0.73±0.02^{a}	0.75±0.02^{a}	0.73±0.01^{a}
INB (mg/dl)	Mth	0.17±0.05^{a}	0.15±0.02^{a}	0.61±0.10^{b}	0.27±0.06^{a}	0.15±0.04^{a}	0.08±0.009^{a}

Values are expressed as Mean ± S.E.M.

Values with different superscript from control are significantly different, $p<0.05$,

Values with same superscript as control are not significantly different, $p>0.05$, n=4, Mth=Methanolic. Extract was administered for a period of 28 days TB = Total Bilirubin, DB = Direct Bilirubin INB = Indirect Bilirubin.

DISCUSSION

Hepatic tissue damage causes enzymes AST, ALT and ALP to find their way into the plasma, resulting to the elevation of plasma activities of these enzymes which are considered reliable biochemical makers of liver damage, since these enzymes are not extracellular (Harris 2005). Primarily, ALT is localized to the liver, while AST is found in a wide variety of tissues such as the heart, skeletal muscles, kidney, brain and liver (Rosen and Keefe, 2000; Friedman and scoot 2003). The increased level of plasma concentrations of ALT and AST are indicators of liver and heart damage (Wasan *et al.*, 2001 and Mythilypriya *et al.*, 2007). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are widely used in the assessment of hepatic damage by drugs or other hepatotoxins (Ramaiah, 2011).

The activity of ALT was not significantly ($p>0.05$) different when compared to the control except at dose 5000mg/kg were the rats died may be as a result of the high dosage. Significant ($p<0.05$) decrease in the activity of AST was observed in rats administered methanolic leaf extracts at certain doses when compared to control. Dose 3500mg/kg of methanolic extract significantly ($p<0.05$) decreased the activity of AST when compared to control while the administration of 2000mg/kg significantly ($p<0.05$) increased AST activity when compared to control. In this study, the administration of some doses of methanolic leaf extract did not show hepatotoxic effect while some doses administered might cause liver damage. The doses may have a protective effect by stabilization of the plasma membrane which preserves the cell's structural integrity as well as the repair of hepatic tissue damage (Pari and Murugan, 2004).

Gamma glutamyltransferase is a microsomal enzyme mainly found in the hepatocytes, biliary epithelial cells, renal tubules, pancreas and intestine. Its activity is mainly attributed to hepatobiliary system. High levels of GGT in the blood can be a sign of liver disease or damage to the bile ducts, hence, specific and a sensitive marker for cholestatic damage than ALP. The methanolic extract did not significantly ($P>0.05$) alter the GGT activity except at dose 3500 mg/kg. From the study, methanolic extract of *Lonchocarpus cyanescens* might not have any negative effect on the bile duct at some doses except at doses 3500 mg/kg of methanolic extract.

Alkaline phosphatase is an enzyme that's most concentrated in the liver, bone and bile ducts. It is a useful diagnostic tool for screening of cholestatic hepatobiliary lesion and osteoblastic bone disease (Brichacek and Brown, 2018). Elevated plasma alkaline phosphatase activity is caused as a result of Cholestasis which is the main liver disease .Liver pathology other than obstruction will occur when alkaline phosphatase activity is normal while other liver function parameter are abnormal. (Tilkian *et al*, 1979). The methanolic leaf extract of the plant did not significantly ($p>0.05$) did not alter the activity of ALP across the various doses administered when compared to control.

Albumin is a protein synthesized by the liver and can be measured in the blood or urine. It is helpful in substance transportation, fluid balance and indication of nutritional status as well as liver and kidney functions. Albumin, is known to be one of the parameters used to evaluate the normal functioning of the liver (Rasekh *et al*., 2008). The level of plasma albumin declines due to massive liver necrosis but shows no effect on mild liver injury (Johnston, 1999; Rothschild *et al.*, 1988).

From this study, the oral administration of methanolic extract in rats showed no significant difference in albumin concentration at various doses which is indicative that it has no effect on the synthetic function of the liver.

Bilirubin is a vital index of excretory function of the liver and diagnostic values. Elevation of plasma bilirubin levels is a frequent finding both in primary and hospital care (Mendez *et al*., 2019). All liver lesions induce a decrease in the hepatocyte cell count, which may cause hyperbilirubinemia (Dufour, 2003). Hyperbilirubinemia can originate from an alteration in any stage of bilirubin metabolism: excess production, impaired liver uptake, conjugation defects, or biliary excretion defects (Fevery, 2008). The methanolic extract did not have any effect on the excretory function of the liver.

CONCLUSION

From this study, certain doses of the plant leaf extract of might be safe under sub-chronic toxicity studies however some doses of methanolic leaf extract might induce damages in the liver.

REFERENCES

Ajibesin, K. K., Ekpo, B. A., Bala, D. N., Essien, E. E., and Adesanya, S. A.
(2008). Ethnobotanical survey of Akwa Ibom State of Nigeria. *Journal of Ethnopharmacology*, **115**: 387-408.

Akunne, C. E., Ezu, V. A., Mogbo, T. C., Ononye, B. U., and Ngenegbo, U. (2014).
Comparative evaluation of the root powder of *Lonchocarpus cyanescens* for the control of *Sitophilus zeamais* (Motschulsky) in maize and wheat. *American Journal of Life Sciences,* **2**(2): 53-56.

Adegbolagun, O. M., Olanrewaju, R. B., and Ogunremi, Y. (2018). Physicochemical, antioxidant

and anti-inflammatory properties of a multipurpose herbal formulation (Hb-01). *International Journal of Pharmaceutical Sciences,* **9**(1): 105-13.

Brichacek, A. L. and Brown, C.M. (2018). Alkaline phosphatase; a potential biomarker for stroke and implication for treatment. *Metabolic Brain Disease* 34(1): 3-9.

Doumas, B. T., Watson, W. A., and Biggs, H. G. (1971). Determination of Albumin. *Clinica Chimica Acta*. **31**: 87.

Fevery, J., (2008). Bilirubin in clinical practice: a review. *Liver International*, **28**:592–605.

Friedman, L., and Scoot, L. (2003). Live fibrosis, from bench to bedside. *Journal of Hepatology.* **28**(1) 38-53.

Harris, E. H. (2005). Elevated liver function tests in Type 2 diabetes. *Clinical Diabetes*, **23**(3):115-119.

Iyoha, A. I., and Onoagbe, I.O. (2024). Hepatotoxicity effects of aqueous leaf extract of *Lonchocarpus cyanescens* in normal rats. *Wellspring University Journal of Science and Computing*, **1**(1): 19-27.

Iyoha, A. I., Onoagbe, I.O. and Abu, O.D. (2023). Effect of aqueous and methanolic leaf extracts of *Lonchocarpus cyanescens* on oxidative stress status in normal albino wistar rats. *Nigerian Journal Life Sciences.* **13**(1): 7-10.

Iyoha, A. I. and Onoagbe, I. O. (2016). Acute toxicity of aqueous and methanolic leaf extracts of *Lonchocarpus cyanescens* in wistar albino rats. *Nigerian Journal of Life Sciences.* **6**: 39-44.

Jendrassik, L., and Grof, P. (1938). Colorimetric determination of bilirubin. *Biochemistry.* **297**: 81.

Johnston, D. E., (1999). Special consideration in interpreting liver function test. *American Family Physician.* **59** (8): 2223-2230.

Mendez-Sanchez, N., Qi, X., Vitek, L., and Arrese, M. (2019). Evaluating an outpatient with an elevated bilirubin. *American Journal of Gastroenterology.* **114**:1185–8.

Moronkola, D. O., and Oladosu, I. A. (2013). Chemical compositions of *Lonchocarpus cyanescens* benth.,(Fabaceae) – case study of its volatile oils, and two triterpenoids. *American Journal of Plant Sciences*, **4**: 1653-1659.

Mythilypriya, R., Shanthi, P., and Sachanandam, P. (2007). Oral acute and subacute toxicity studies with Kalpaamruthaa, a modified indigenous preparation on health. *Journal of Health Science.* 53:351-358.

Onyegeme-Okerenta, B. M., O., Ogboye, P., and Monago-Ighorodje, C. (2018). Ameliorative Effect of Aqueous Extracts of Seeds of *Delonix regia* (Hook) raff on the Liver, Kidney and Spleen of High-fat Diet Streptozotocin-induced Diabetes in Female Wistar Rats. *European Journal of Medicinal Plants*, **25**(2), 1–14.

Pari, L., and Murugan, P. (2004). Protective role of tetrahydrocucumin against erythromycin estolate- induced hepatotoxicity. *Pharmacological Research.* **49**:481-486.

Ramaiah, S. K. (2011). Preclinical safety assessment: current gaps, challenges and approaches in identifying translatable biomarkers of drugs-induced liver. *Clinics in Laboratory Medicine.* **31**:161-172.

Rasekh, H. R., Nazari, P., Kamil-Nejad, N., and Hosseinzaden L. (2008). Acute and subchronic toxicity of Galega officinalis in rats. *Journal of Ethnopharmacology*. **116**:21–26.

Reitman, S., and Frankel, S. (1957). A colorimetric method for the determination of serum glutamate – oxaloacetate and pyruvate transaminase. *American Journal of Clinical Pathology.* **28**: 56 – 63.

Rosen, H. R., and Keefe. E. B. (2000). Evaluation of abnormal liver enzymes, use of liver test and serology of viral hepatitis: liver disease, diagnosis and management. 1ST Edition. New York, Churchill living stone publisher 24-35pp.

Rothchild, M. A., Oratz, M., and Schreiber, S. S. (1988). Serum albumin. *Hepatology*. **8**(2):385-401.

Schrire, B.D. (2000). A synopsis of the genus *Phileno*ptera (Leguminosae-Millettieae) from Africa and Madagascar. *Kew Bull*, **55**: 81-93.

Sonibare, M. A., Umukoro, S. and Shonibare, E. T. (2012). Antipsychotic Property of Aqueous and Ethanolic Extracts of Lonchocarpus cyanescens (Schumach and Thonn.) Benth. (Fabaceae) in Rodents. *Journal of Natural Medicines,* **66**: 127-132.

Sonibare, M. A., Oke, T. A., and Soladoye, M. O. (2014). A pharmacobotanical study of two medicinal species of Fabaceae. *Asian Pacific Journal* of *Tropical Biomedicine*, **4** (2):131-136.

Szasz, G. (1969). Colorimetric determination of gamma-glutamyltransferase. *Clinical Chemistry,* **22**: 124 – 136.

Tilkian, S. M., Conover, M. B., and Tilkian, A. G. (1979). Clinical implications of laboratory tests; C.V. mosby company; St louis. Toronto. London; 3-44; 117-132; 154-159.

Umoh, U. F., and Nwafor, P. A. (2013). Anti-inflammatory and analgesic effects of *Lonchocarpus cyanescens* root in mice. *African Journal of Pharmacology and Therapeutics*, **2**(3): 88-93

Wasan, K. M., Najafi, S., Wong, J., and Kwong, M. (2001). Assessing plasma lipid levels, body weight, hepatic and renal toxicity following chronic oral administration of a water soluble phytostanol compound FM-VP4 to gerbils. *Journal of Pharmaceutical Sciences*. **4**: 228-234.