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Effect of Methanolic Extract of Colocasia Gigantea on the Histology of Liver of Male Wistar Rats

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Abstract: Background and Objective

Colocasia gigantea is an erect herbaceous perennial tropical root crop of the family Aracea, produced majorly for its underground corms. It is locally called 'ede nwokuko' or kochu because of its believed healing power to cure several illnesses. The aim of the research work is to determine the effect of methanolic extract of Colocasia gigantea on the histology of Liver of Male Wistar Rats.

Methods

Twenty 20 male wistar rats were randomly grouped into four groups of five animals each (n=5) which weighed 160 - 199 gram. Group A, served as control received water and feed throughout the experiment only. Group B, Group C, and Group D received oral administration of 100mg/kg, 250mg/kg and 500mg/kg of methanolic extract, 500 mg/kg of Colocasia gigantea respectively. The administration lasted for 90days.

Results

Findings from the histological results showed normal hepatocytes and normal portal tracts in both control group and treatment groups B, C, and D.

Conclusion

The adminstration of methanolic extract of Colocasia gigantea on male wistar rats, showed no toxic effect on the liver.

Keywords: Colocasia gigantea, liver histology, wistar rats.

BACKGROUND

Herbs and plants are known to have played vital roles in preserving human health and improving quality of human life since ancient times as herbs have served humans well as important constituents of medicines, seasoning, beverages, cosmetics, and dyes (Rashmi *et al*., 2018; Toiu *et al*., 2018). The popularity of herbal medicine in recent times is based on the premise that plants contain natural bioactive substances capable of promoting health and managing diseases (World Health Organization, 2018).

Colocasia gigantea is an erect herbaceous perennial tropical root crop of the family Aracea, produced majorly for its underground corms which are estimated to contain 70–80 % starch (Kaushal *et al*., 2013). It is described as the 'elephant ear' leave due to the shape and size of its green leaves, and can reach up to 1-3 m high during growth (Lewu *et al*., 2009; Patil and Ageely, 2011). Colocasia gigantean, also called giant elephant ear, or indian taro, is a 1.5 – 3m tall herb with a large, fibrous, inedible corm, producing at its apex a whorl of large leaves (Ivanic et al 2008). Colocasia gigantea, is called 'ede-nwokuko' or 'isi-apupa' in some parts of Igboland.

The Liver is the largest glandular organ in the body and the second largest organ. It is located in the upper right portion of the abdominal cavity under the dome of the diaphragm (Nega *et al.*, 2003). The Liver is one of the most important organs in the body and plays a vital role in the regulation of some metabolic processes, and also in the secretion and storage of some substances and storage. The Liver is however predisposed to a number of diseases such as Cirrhosis, Hepatitis and Liver Cancer (Guyton, 1996). More so, Liver diseases pose a significant health problem worldwide and account for approximately 2 million deaths each year, either due to cirrhosis complications, viral hepatitis or hepatocellular carcinoma; and are statistically responsible for 4% of all deaths i.e. 1 out of 25 deaths (Devarbhavi *et al*., 2023).

Colocasia gigantea leaves when used as leafy vegetables have been noted as being rich in nutrients including minerals and vitamins such as calcium, phosphorous, iron, vitamin C,thiamine, riboflavin and niacin (Lewu *et al*., 2009; Patil and Ageely, 2011).

In Asia and Africa, the specie is used in traditional medicine to treat arterial hypertension, liver problems, ulcers, snakebites and rheumatism (Safo-Kantaka, 2004)

The aim of this study is to determine the effect of methanolic extract of *Colocasia gigantea* on the histology of the liver of adult male wistar rats.

MATERIALS AND METHODS

Experimental Animals

Twenty male wistar rats with weight of 160 to 199gram was obtained from the Animal House Department of Zoology, University of Nigeria Nsukka. and were housed in the Animal House Faculty of Basic medicine Sciences Nnewi Campus. The animals were kept in standard cages at room temperature of 27±2°C and was acclimatised for two weeks in the Animal House Faculty of the College of health Sciences, which was maintained with normal laboratory feed (Grower feed) and water *ad libitium*. After acclimatization, the animals were administered the methanolic leaf extract of Colocasia gigantea, and were kept on 12-hours light and dark cycles to maintain their normal physiology. Handling and Treatments in the Study conformed to current national and international guidelines for the care and use of Laboratory animals in Biomedical Research which are the guidelines of the National Institute of Health; NIH publication 85-23, (1985) for laboratory animal care and use. The adminstration lasted for 90 days and was done during the hours of 8-10am every morning. At the expiration of the 90 days the animals where sacrificed, blood collected for biochemical test, organs where harvested and fixed with 10% formal saline for histological studies.

Procurement of Materials and Preparation of Leaf Extract

Fresh leaves of *Colocasia gigantean* were harvested from a local farm in Otolo Nnewi, Nnewi North LGA of Anambra state. Twenty male Wistar rats, cocoyam leaves (*Colocascia gigantea*), standard plastic cages, water can, distilled water, methanol (JHD Chemicals, Guangdong, China), electronic weighing balance (M-Metlar M311L, China), oral cannula, slide, microscope (Olympus XSZ-107BN), and Neubauer counting chamber (England) were used. Plain blood tubes and EDTA tubes (Fantastik, China), 2 ml hypodermic syringe, Pyrex beakers (Techmel, USA), measuring cylinder (MINGE, Germany), Sysmex autoanalyzer 300 (Japan), vital feed (Jos, Nigeria), and dissection kits (Redcross, England). An automatic water distiller,

spectrophotometer (Yoke Instrument, China), rotary evaporator (Digital, TT-52), Randox reagent kits, and thermostat oven (DHG-9023APEC-MEDICAL, USA) were used..

Chemicals and Reagents used were; Thiobarbituric acid (TBA) (99% purity) was purchased from BDH (BDH, England); malondialdehyde tetrabutylammonium salt (MDA salt) (96% pure) and methanol (99.8% pure) were purchased from Sigma-Aldrich (Steinheim, Germany). Glacial acetic acid (99–101% pure) was purchased from Sigma-Aldrich (USA). Sodium bicarbonate and phosphate buffer were freshly prepared by the Human Physiology Laboratory.

Plant procurement and identification

Samples of cocoyam leaves were obtained from a local farm in the Otolo community, Nnewi, Nnewi north local government, Anambra State. The leaves were identified in the Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State, and the herbarium number obtained was deposited in the herbarium catalogue.

Methanolic extraction of cocoyam leaf

Leaves of cocoyam were obtained from a farm in Otolo community, washed in running water tap water to remove debris and air dried under ambient temperature. The dried leaves of cocoyam were milled into a powder form using a grinder. About 250-gram of the milled form of cocoyam leaves were macerated in 1000mls of 95% absolute methanol for 48hours. It was filtered using a clean white cloth and further filtration using Whatman No 1 filter paper. The filtrate was concentrated using a rotatory evaporator and dried further using a laboratory oven at 45°C into a gel-like form. The extract was preserved in airtight container and kept in a refrigerator f3or further usage. The extraction method was done with modifications as described according to the method employed by Al-Attar and Abu Zeid (2013).

Experimental Design

The experimental animals were divided into four groups of five animals each as follows:

Group A received feed and distilled water *ad libitum*

Group B received 100 mg/kg of methanolic extract of *Colocascia gigantea*

Group C received 250 mg/kg of methanolic extract of *Colocascia gigantea*

Group D received 500 mg/kg of methanolic extract of *Colocascia gigantea*

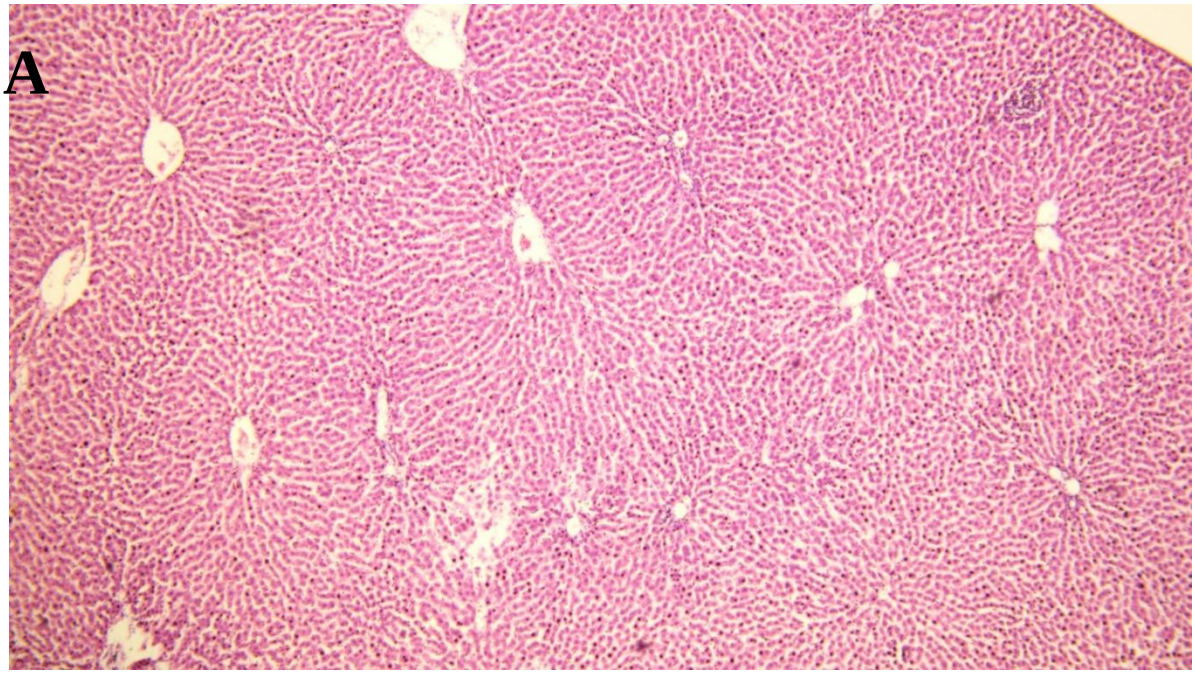
All experimental protocols were observed under strict supervision, the experiment lasted for sixteen weeks, and administration was done through oral gavage four times weekly for a period of 90-days. Administration of extract was with reference to the derived LD50 which was greater than 5000

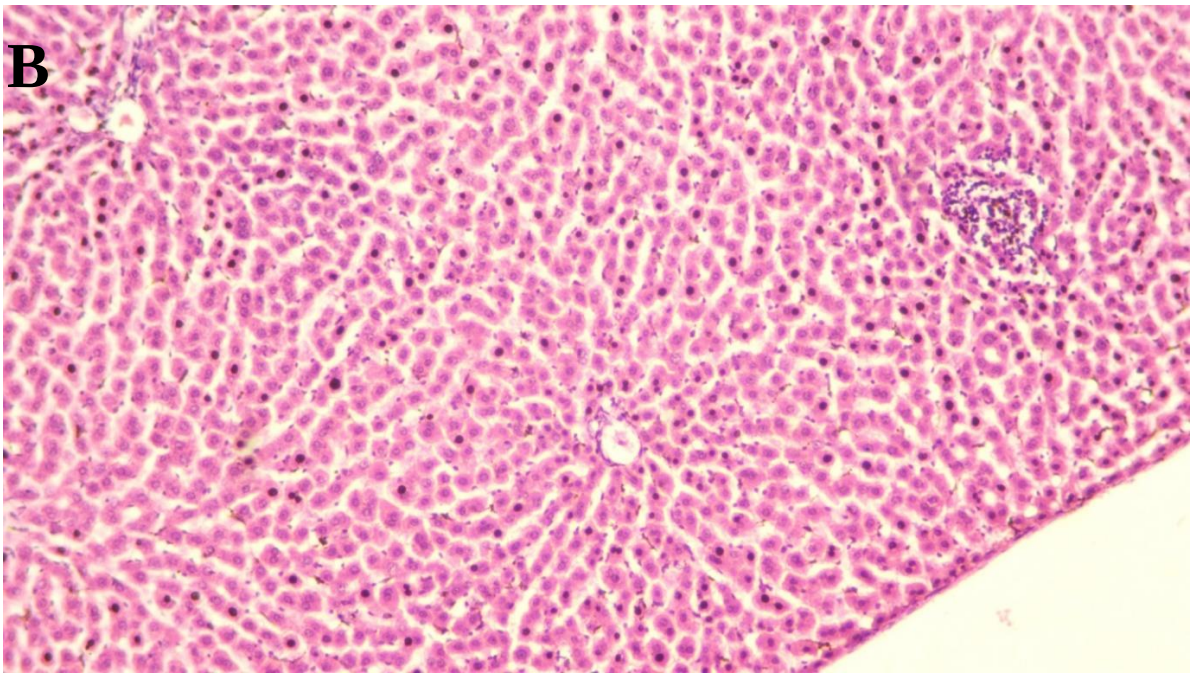
Ethical considerations of the study were carried out according to the guidelines of the Animal Ethics Committee of NAUREC, which conformed to the guidelines of rat handling and treatment of the National Institute of Guidelines for Laboratory Animal Care and Use as described by Carbone and Austin (2016).

Tissue sections were produced by normal histochemical methods of dehydration, clearing, impregnation, embedding, sectioning and H&E staining after fixation. The micrographs of the relevant stained sections were subsequently taken with the aid of a light microscope and microscope camera.

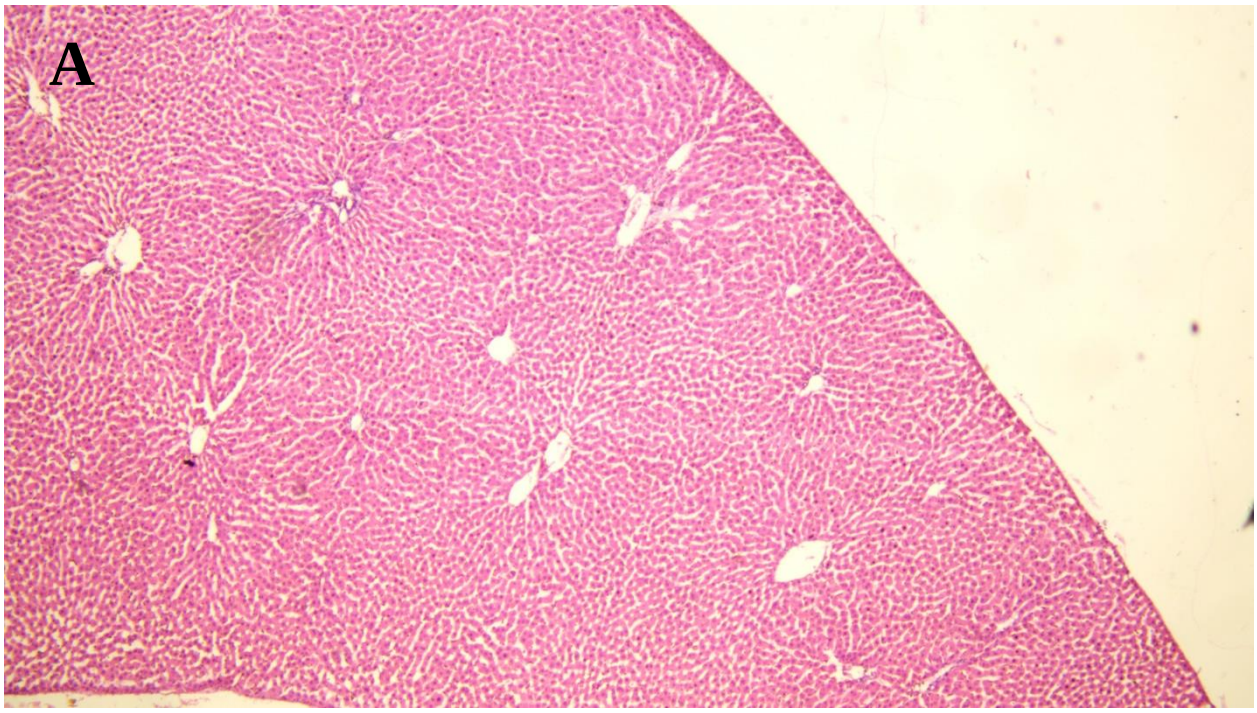
RESULTS

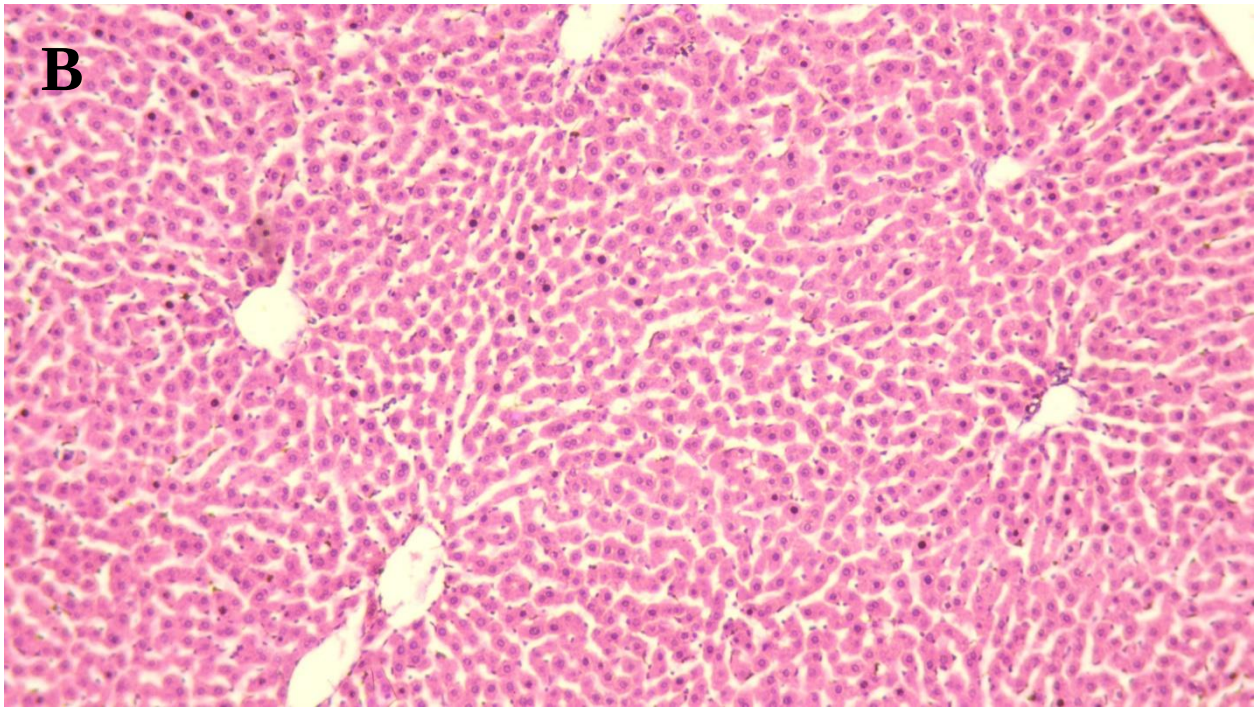
Shown below are the photomicrographs of the liver of the male wistar rats based on the experimental groups



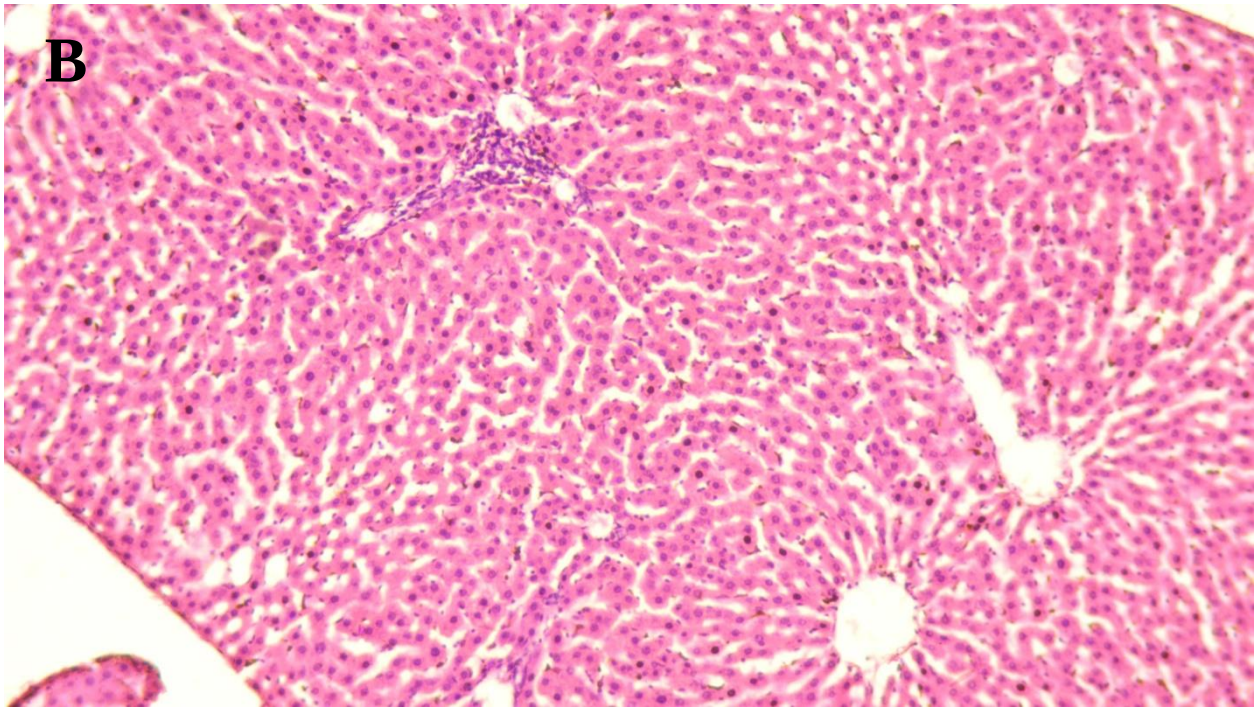


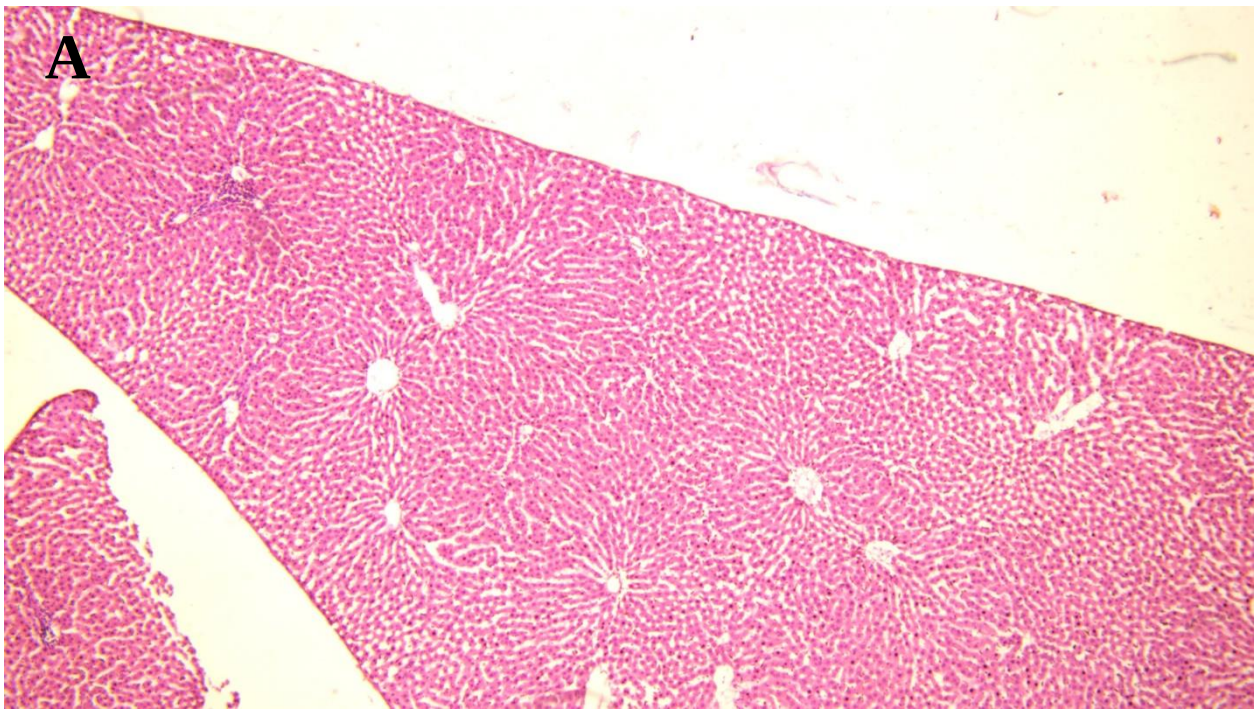
A. LIVER: Section shows normal liver with normal portal tracts (PT), central vein (CV) and liver plate of hepatocytes one-to-two-cell thick. H&E: A=X40; B=X100



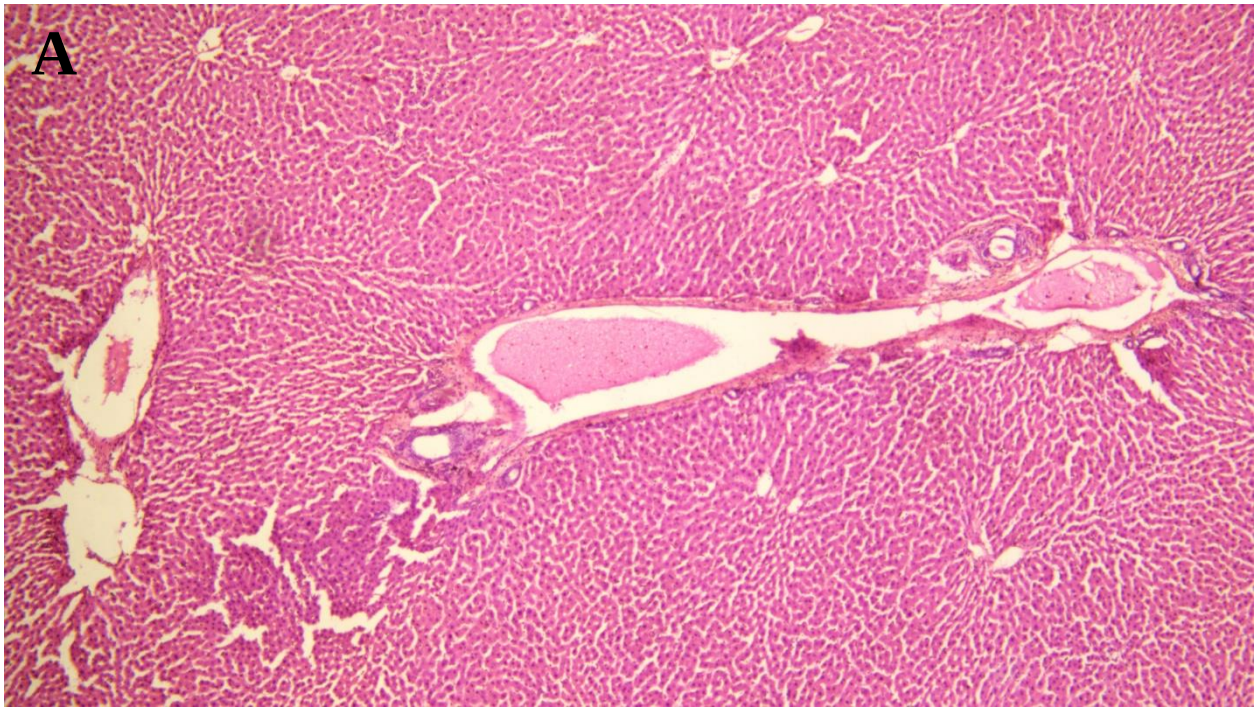


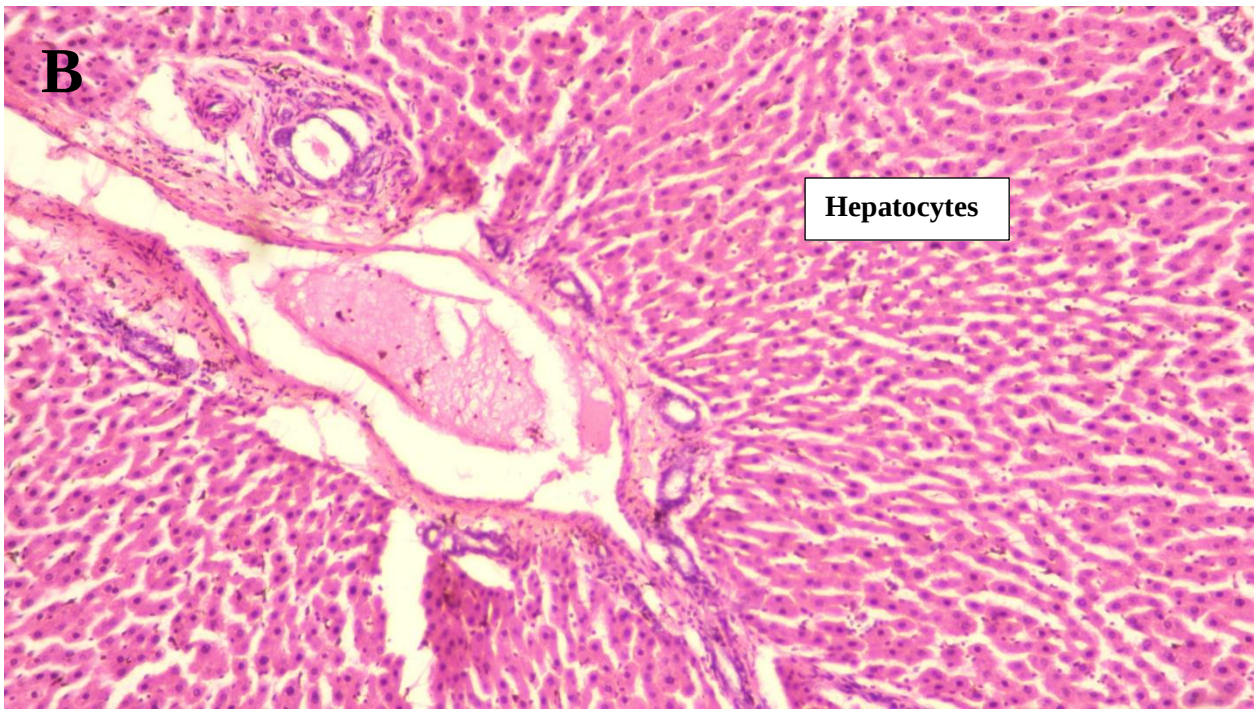
B.LIVER: Section shows normal liver with normal portal tracts, central vein and liver plate of hepatocytes one-to-two-cell thick. H&E: A=X40; B=X100





C.LIVER: Section shows normal liver with normal portal tracts, central vein and liver plate of hepatocytes one-to-two-cell thick. H&E: A=X40; B=X100





D. LIVER: Section shows normal liver with normal portal tracts, central vein and liver plate of hepatocytes one-to-two-cell thick. H&E: A=X40; B=X100

DISCUSSION

The Structural unit of the liver is the liver Lobule, a roughly hexagonal column of liver cells called hepatocytes (Nega *et al*., 2003). Branches of the hepatic artery and portal vein are located between adjacent lobules (Nega *et al*., 2003). The capillaries of a lobule are sinusoids, large and very permeable vessels between the rows of liver cells that receive blood from both the hepatic

artery and portal vein (Nega *et al.,* 2003). Each liver lobule has a central vein and the aggregation of the central veins forms the hepatic veins, which take blood out of the liver to the inferior vena cava (Nega *et al*., 2003). Histologically, the Liver is composed of the following structures;

i. Parenchyma: The Parenchyma is composed of hepatocytes (Nega *et al*., 2003).

ii. Stroma: The Stroma is the continuation of the surrounding capsule of Glisson. It consists of connective tissue and contains the vessels (Deepak, 2020). The capsule is also covered by a layer of mesothelium, which arises from the peritoneum covering the Liver (Nega *et al*., 2003). The connective tissue of the stroma is type III collagen (reticulin), which forms a meshwork that provides structural backing for the hepatocytes and sinusoids (Deepak, 2020)

iii. Sinusoids: These are capillaries that pass between hepatocytes (Nega *et al*., 2003).

iv. Spaces of Disse (perisinusoidal spaces): These are found between the hepatocytes and the Sinusoids (Nega *et al*., 2003).

A major part of the hepatic cell population is made up of hepatocytes which form 70% of Liver Mass (Zorn, 2008). The hepatocytes face the persinusoidal space on one side, while the other faces the bile canaliculi and is lined with microvilli (Deepak, 2020). Subcellular Contents of the hepatocytes include;

a) Smooth endoplasmic reticulum which are vital in toxin degradation and conjugation, as well as cholesterol synthesis (Deepak, 2020).

b) Mitochondria which can be up to 1000 per cell (Deepak, 2020)

c) Golgi network, composed of approximately 50 small Golgi units and contain granules with very low density lipoprotein and bile precursors (Deepak, 2020).

d) Peroxisome consisting of oxidases and catalases, enzymes responsible for detoxification reactions in the liver (Deepak, 2020)

e) Glycogen deposits are normally lost in H&E Procedures and leads to irregular staining in some areas(Deepak, 2020)

f) Lipid droplets

Kupffer cells are phagocytic cells vital for the phagocytosis of infectious microbes, foreign particles, and Cytokine end products (Nega *et al*., 2003). They are attached to the luminal surface of the sinusoidal endothelium (Zorn, 2008).

Hepatic stellate cells: These cells are located within persinusoidal space of Disse, in the recesses between hepatocytes (Zorn, 2008). These cells are associated with several functions such as secretion of Cytokines, storage of vitamin A and synthesis of hepatic extracellular matrix (Deepak, 2020). They gets activated during liver injury and play a key role in progression of fibrosis (Depaak, 2020).

Biliary epithelial cells: Biliary epithelial cells or cholangiocytes line the bile duct in the portal triads and modify bile composition (Deepak, 2020). It has also now become evident that during liver transplantation. They might be targeted during liver transplanting by leukocytes (Deepak, 2020).

Endothelial cells are the biggest group of non-parenchymal cells of liver and line the intrahepatic circulatory vessels of liver and provide a large surface area for nutrient absorption (Deepak, 2020). They form a pathogenic and selective barrier during separation of hepatocytes from sinusoidal blood by exchange of molecules (Depaak, 2020). Lymphocytes from the innate immune system which are rich in natural killer cells

Colocasia gigantea is known for nutritive and health promoting contents as the corm, for instance is relatively low in protein (1.5%) and fat (0.2%) as noted in other tuber crops, but it is a great

starch source (70–80 g/100 g dry taro), fiber (0.8%), ash (1.2%), thiamine, riboflavin, iron, phosphorus, zinc, Vitamin B6, vitamin C, niacin, potassium, copper, and manganes (Quach *et al*., 2003; Rashmi *et al*., 2018). The leaves when used as leafy vegetables have been noted as being rich in nutrients including minerals and vitamins such as calcium, phosphorous, iron, vitamin C,thiamine, riboflavin and niacin (Lewu *et al*., 2009; Patil and Ageely, 2011). Many plants are touted for their pharmacological properties including *Colocasia gigantea* which is a tuber crop that is third in importance after Yam and Cassava in West Africa (Onyeka, 2014). In Asia and Africa, this species is used in traditional medicine to treat arterial hypertension, liver problems, ulcers, snakebites and rheumatism (Safo-Kantaka, 2004). Colocasia gigantean is locally named Kochu because of its healing power, and has been autenticated to have antibacterial, antidiarrhoeal, cytotoxic and so many useful properties (Safaet et al 2021, Obonti et al 2021, MEng and Sereemaspun 2015).

The findings from the present research work as shown in the photomicrograph B, C, and D showed no obvious abnormality with the cells of the liver nor portal tract sequel to the administration of methanolic extract of Colocasia gigantea in graded doses of low(100mg/kg), moderate(250mg/kg) and high(500mg/kg) quantity of extracts. The micrograph was normal in groups B, C, D compared to the control A. which received only feed and water. The portal tracts were also not altered either in the 40x or 100x slide magnifications.

These findings agree with the findings of Safaet et al (2021) who researched on the antidiarrheal, antimicrobial and antioxidant potentials of methanol extract of Colocasia gigantea. Safaet et al, confirmed the pharmacological effect of Colocasia gigantea in treating diarrhoea, microbes and bacterial infections as well as the safety in the use of plant, without any recorded toxicity (Safaet et al 2021). Also Obonti et al in the research work on antimalarialaction, advocated the safety of Colocasia gigantea leaves (Obonti et al., 2021). MEng and Sereemaspun (2015), studied on the cytotoxic properties of Colocasia gigantean and identified the bioactive ingredients that demonstrated cytotoxicity on tested cells. Also, Bhagyashree and Hussein (2011), studied the effect of methanolic extract of C. gigantea on rodent liver cells with CCl4 and acetaminophen induced cytotoxicity. The extract stopped increases and expectations of exhaustion of complete tissue; glutathione were seen within the sight of *Colocasia* entire leaf juice (Bhagyashree and Hussein, 2011).

CONCLUSION

Colocasia gigantea locally named Kochu because of its healing power, has been autenticated to have antibacterial, antidiarrhoeal, anticancer, many useful properties and nutritional benefits. The plant has also been established to be frequently used both in Africa and Asia, as edible for food and treatment of ailments and various diseases. This research showed that methanolic extract of Colocasia gigantea administered for 90days had no toxic effect on the histology of the liver of male wistar rats studied.

FUNDING

This research received no funding and the study was conducted by self-funding of the author

ETHICAL CONSIDERATION

Ethical consideration and guidelines for the study was got from Nnamdi Azikiwe University Animal Research Ethics Committee (NAUREC) which conformed to the guidelines of rat handling and treatment of the National Institute of Guidelines for Laboratory Animal Care and Use as described by Carbone and Austin (2016).

CONFLICT OF INTEREST

Authors of the manuscript declare no conflict of interest.

ACKNOWLEDGEMENT

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