Int.J.Curr.Microbiol.App.Sci (2024) *13*(04): 15-25

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 13 Number 4 (2024)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2024.1304.003

Proximate Analysis and Pathological Studies of Extracts of *Cola acuminata* and *Cola nitida* in Some Visceral Organs (Kidney and Liver) of Albino Rats in Southern Nigeria

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Keywords

Areca nut, UHPLC, Redox titration, Vitamin B_6, Vitamin C

Article Info

Received:
22 February 2024
Accepted:
28 March 2024
Available Online:
10 April 2024

A B S T R A C T

The proximate values and pathological effects of the consumption of kolanuts were determined. A total of thirty albino rats aged 8-16 weeks were procured from the Animal House of Department of Biochemistry University of Nigeria Nsukka. LD50 was carried out using eighteen kmn albino rats within 14 days. Rats were divided into 3 groups A, B, and C. Group A were given 0.02g of *Cola acuminata* and *Cola nitida* paste daily while group B consumed 0.04g daily and group C (Control) were fed on pellets. Drinking water was given to both the control rats and experimental rats and libitum. Statistical analysis of the weight using student 't' test for comparison of weight before experimentation and after showed significant decrease in rats weight $P<0.05$. The results obtained revealed normal liver tissues in control with normal parenchyma. However, the liver of rats exposed to kola nuts showed evidence of vacuolation, infiltration of inflammatory cells and mild to moderate necrosis as shown in the photomicrographs. The kidney tissues revealed the presence of casts in the tubular lumen, mild erosion of tubules and constriction of capillary turf. However, the proximate values of kolanuts revealed Moisture, Ash, Protein, Fat, Fibre, Carbohydrate and Calories.

Introduction

According to (Iyere, 2011), Kola nuts have been used by the African natives from immemorial as a necessity and a luxury, before the emergence of Western civilization and religion, kola nuts played a very significant role in the daily life of the people of West Africa, including Nigeria. Nothing was considered complete without kola nut. Kola was offered to a very important guest as a mark of respect and reconciliation, agreements are never considered sealed without kola nuts shared and the gods cannot be appeased without kola nuts. Similarly, marital rites are not complete without kola nuts being presented (Iyere, 2011). Shrines are adorned with kola nuts as a means of sacrifice (Ratsch, 2005). It figured in compacts of friendship and mark of hospitality as it was readily served to visitors, especially among the Igala people of Kogi state and Igbo tribe in south eastern Nigeria as a sign of peace and acceptance of visitors (Mboto, 2014). Some studies have revealed that the social significance of the use of the kola nut may be because it contains substances that stimulate the central nervous system

(CNS). In addition, the nut has been revealed to contain caffeine which may help in relieving migraine, also, contains theobromine which acts as a cerebra vasodilator and thought to relive pain and neuralgia (Mboto, 2014). Moreover, the production of free radicals in living organisms is a vital phenomenon for the cell regulation through various biochemical or enzymatic detoxification process, (Efe *et al.,* 2016).

The free radicals are reported to be involved in many serious illnesses such as cancer and cardiac disease and constitute even aggressive form factors to DNA, proteins and other macro molecules (Efe *et al.,* 2016). It is necessary therefore that some of the plants used as remedies for treating infectious disease be investigated in order to validate their efficacy (Efe *et al.,* 2016). Kola nut has been used in folk medicine as an aphrodisiac, an appetite suppressant, nausea, migraine, indigestion treatment, and in some cases, it is used to control vomiting in pregnant women (Esimone *et al.,* 2017). The plant was also used in treating skin wounds and infections. However, kola nut extract and caffeine showed toxic effects that may lead to death in rats within 48 hours, and the study also stated that the oral LD50 dosage of kola nut and caffeine was about 150 – 200mg/kg (Salahdeen *et al.,* 2015). A study was conducted to determine the effect of kola nut extract on the total body weight of rats and pointed that it decreased with an increase in the size of the liver, kidneys, brain, and testes (Ikegwuonu *et al.,* 1981). Clinico-pathological manifestations of aqueous extract of kola nut (*Cola nitida*) on four month puppies in Edo Nigeria showed histopathological leisions; neuronal necrosis and pyknosis in the brain, gastric erosion, with haemorrhage in intestine, necrosis of the renal tubular epithelia cells of the kidney, necrotic myocyte in the heart, thickening of the alveolar septa in the lungs and in the liver necrosis of the hepatocytes. Some of the biochemical parameters showed great statistical significance: AST, Urea, Total protein and Creatinine are high compared with the control group (Barde *et al.,* 2022). Other studies recorded a decline in the mobility of mice after chronic consumption of kola nut, in addition to a reduced intake of food and water that led to weight loss, and attributed to kola nut may have an appetite suppressant effect.

Conversely, the consumption of kola fruits had a toxic effect on cancer cells in the human liver (Endrini *et al.,* 2009). A recent study concluded that kola nut has a toxic effect on kidney and liver functions in rats (Adeosun *et al.,* 2018). Despite the extensive uses of the kola nut in traditional medicine, few studies have evaluated its pathological potentials.

This study is therefore aimed at pathological studies of different varieties of kola nuts extracts. LD50 was carried out using eighteen (18) albino rats within 14 days. The rats were shared into 6 (A to F) and were given the kola nut extract in the concentration 1000-10,000 mg/kg body weight as shown below.

Materials and Methods

Collection and identification of *Cola* species

Fresh kola nuts of *Cola acuminata* and *Cola nitida* species were procured from farmers in Udi Enugu State, Ikom Cross River State and Ado-Ekiti Ekiti State Nigeria between the months of April and June 2023. Samples of each kola were identified by a taxonomist at the department of plant science and biotechnology, university of Nigeria Nsukka. A voucher specimen (UNH NO. 1836[5]) was deposited at their herbarium for future references.

Sample population

Thirty albino rats were used for the animal study.

Sample size

One kilogram of *Cola acuminata* and *Cola nitida*

Proximate analysis

Standard methods of the Association of Official Analytical Chemist (AOAC, 1984) were used to determine the moisture, crude protein, crude fat, total ash, and crude fibre contents of each sample.

Experimental Design

A total of thirty albino rats (male) aged 8-16 weeks were procured from the Animal House of Department of Biochemistry University of Nigeria Nsukka. They were housed at the animal house of College of Medicine (at old site) to acclimatize for 2 weeks (14 days). On the last day of acclimatization the rats were weighed and caged individually in stainless steel experimental cages. Room temperature was regulated between 25 + 1^0C. The room had electric bulb illuminating with light at a light : dark

cycle of 13:11 hour. The rats were divided into 3 groups of 4 controls, group A and group B, on each day, Group A (2 males and 2 females) were given 0.02g of kola nut slurry (paste) daily while group B (2 males and 2 females) consumed 0.04g daily and group C (Control) were fed on pellets and tap water.

The weighed quantities of kola nut thoroughly mixed with one quarter of the feed and 0.5ml of tap water were given to the animals. The remaining three quarters feed was later given without kola nut. The above experimental procedure was carried out for both *Cola acuminata* and *Cola nitida* respectively for 42 days (six weeks). Drinking water was given to both the control rats and experimental rats ad libitum.

Determination of Body Weight

The live body weights of animals from each experimental group were measured every three days throughout the experimental period. The body weight measurement were done using an analytical electronic balance (Mettler PM34, DoltaRange).

Kola nut paste (methanol and acetone extract)

Five hundred grams (500g) of *Cola acuminata* and *Cola nitida* respectively were weighted out in two portions using mettler balance. Five hundred (500g) of each kola nut specie (fresh) were ground respectively using a thermo mixer grounding machine for 4 minutes to obtain a homogenous mixture of the paste (slurry) of each kola nut respectively. The pastes were reconstituted using 1000ml of distilled water respectively.

Determination of (LD50)

LD50 was carried out using eighteen (18) albino rats within 14 days. The rats were shared into 6 (A to F) and were given the kola nut extract in the concentration 1000-10,000 mg/kg body weight as shown below.

Euthanization of Albino Rats and Sample Collection

On Day 42 of post-treatment, the rats were weighed and euthanized using chloroform and dissected out. The Liver and Kidney were carefully exercised. Connective tissues adherent on the organs were stripped off and transverses section of the organ cut and fixed in formol saline.

Data Analysis

Data was analyzed using simple percentages, mean, standard deviation and student t-test at $P<0.05$ using Statistical Package for social sciences(Version 13.0) soft ware (SPSS, Chicago, IL.).

Results and Discussion

This study revealed proximate values/contents of *Cola acuminata* and *Cola nitida* which showed that *Cola acuminata* contained Moisture 92.3%, Ash content 4.0%, Protein 1.4%, Fat 0.3%, Fibre 1.2%, Carbohydrate 0.8%, Calories 24% while *Cola nitida* contained Moisture 94%, Ash content 2.4%, Protein 1.2%, Fat 0.3%, Fibre 1.0%, Carbohydrate 1.1%, Calories 25.3%. This agreed with Odebunmi *et al.,* (2009) who carried out study in Ilorin, Nigeria and evaluated the proximate composition and showed that *C. nitida* has the highest moisture, crude fat and crude fibre contents of 66.4, 5.71 and 7.13%, respectively.

Highest percentage of Moisture in the proximate analysis carried out in this study agrees with (Almustapha *et al.,* 2022) who carried out studies on Proximate and mineral analysis of kola nuts (*Cola acuminata* and *Cola nitida*) in Offa, Kwara State, Nigeria where they reported proximate composition of *Cola nitida*: moisture 53.8, dry matter 46.2, ash 1.94, crude protein 9.2, crude fat 1.25, carbohydrate 33.81%. *Cola acuminata*: moisture 54.4, dry matter 45.6, ash 1.46, crude protein 8.8, crude fat 1.5, carbohydrate 33.84%. The results from this study is in disagreement with Ajai *et al.,* (2012); Akinpelu, *et al*., (2018) and Benjamin *et al.,* (2022) who reported highest percentage of Carbohydrate in proximate values of *Cola acuminata* and *Cola nitida* extracts.

Result of weights

The 8 Albino rats used in this study showed apparent decrease in their weights following administration of kola nut paste when compared with the control (4 Albino rats) which showed apparent increase in body weight. Statistical analysis of the weight using student 't' test for comparison of weight before experimentation and after showed significant decrease in rats weight $P<0.05$.

The increase in weight of the rats in control group is as a result of increase feeding. However, the decrease in weight of rats could be physiological, especially

reduction in feeding. In addition, the rats might have identified the kola nut substance as a strange and foreign substances in their system.

Tissue Results

The effects of Kola nut (*Cola acuminata* and *Cola nitida*) were evaluated in the liver and kidney. The samples obtained from these organs were processed histologically according to Paraffin wax embedding technique and stained using Haematoxylin and Eosin procedure.

The stained tissue slides were examined under the microscope for changes and alterations in the tissues studied. The results obtained revealed normal liver tissues in control with normal parenchyme. However, the liver of rats exposed to *C. acuminata* and *C. nitida* showed evidence of vacuolation, infiltration of inflammatory cells and mild to moderate necrosis as shown in the photomicrographs below:

The kidney tissues of the rats treated with the kola substance also revealed the presence of casts in the tubular lumen, mild erosion of tubules and constriction of capillary turf

From our study, *Cola nitida* and *Cola acuminata* induced hepato-renal anomalies in albino rats. The Albino rats used in this study showed apparent decrease in their weights following administration of kolanut paste when compared with the control (which showed apparent increase in weight). Statistical analysis of the weight using student 't' test for comparison of weight before experimentation and after showed significant decrease in rats weight $P<0.05$. The increase in weight of the rats in control group is as a result of increase feeding. However, the decrease in weight of rats could be physiological, especially reduction in feeding. In addition, the rats might have identified the kola nut substance as a strange and foreign substances in their system.

The results obtained revealed normal liver tissues in control with normal parenchyme. However, the liver of rats exposed to *C. acuminata* and *C. nitida* showed evidence of vacuolation, infiltration of inflammatory cells and mild to moderate necrosis as shown in the photomicrographs. This is in agreement with Hummdi *et al.,* (2020) in Jeddah Saudi Arabia where they reported severe congestion of the liver tissue, dilation of central veins, congestion with edema, invasion of inflammatory cells, and a significant decline in the Kupffer cells.

These findings were in agreement with previous studies, which determined the occurrence of cellular proliferation and the necrosis of Kupffer cells in some different cases of hepatotoxicity (Luty *et al.,* 2000).

Rats treated with feed pellets + 0.4g of *Cola acuminata* extract + water daily shows fatty deposit of the hepatocyte, this agreed with Hummdi *et al.,* (2020); Oyedeji *et al.,* (2012); Ikechukwu *et al.,* (2010) who reported histological changes in rats treated with kola nuts had an increased severity represented as vacuolar cytoplasmic degenerations, fatty infiltration, nuclear karyolysis, fibrosis of the portal tract, and bile duct proliferation. Others stated that treating rats with extracts of Kola nuts caused cellular necrosis and cytoplasmic degenerations in seminiferous tubules.

Table.1 Animal test details

Group	Conc.mg/kg)	No animals before test	No of rats alive after test
A	1000	3	3
B	2000	3	3
C	4000	3	3
D	6000	3	3
E	8000	3	3
F control	-	3	3

LD50 = $\sqrt{Test\ Conc.with\ highest\ mortality\ x\ Highest\ Conc.wihout\ motality}$

= $\sqrt{8000x\ 5000}$

= $\sqrt{40,000,000}$

= 6320mg/kg Body Weight.

Table.2 Proximate value/content of *Cola acuminate* and *Cola nitida*

Substance (parameter)	Concentration % Distilled Water	
	Cola acuminata (CA)	*Cola nitida* (CN)
Moisture	92.3	94
Ash content	4.0	2.4
Protein	1.4	1.2
Fat	0.3	0.3
Fibre	1.2	1.0
Carbohydrate	0.8	1.1
Calories	24	25.3%

Value expressed as % net weight

Table.3 Animal experimentation analysis report

Group	No of rats	Mean weight before experimentation(g)	Mean weight after experimentation	p- value
A	4	181.0±1.3	174.2± 1.0	p>.0.05
B	4	193.5+0.9	188.6 ±6.5	P>0.05
C	4	184.3± 11	186.7±5.2	P<0.05

Plate.1 Control Liver: Photomicrograph of liver tissue showing control (A) normal hepatocyte (B) (arrow). Stain H&E. X400

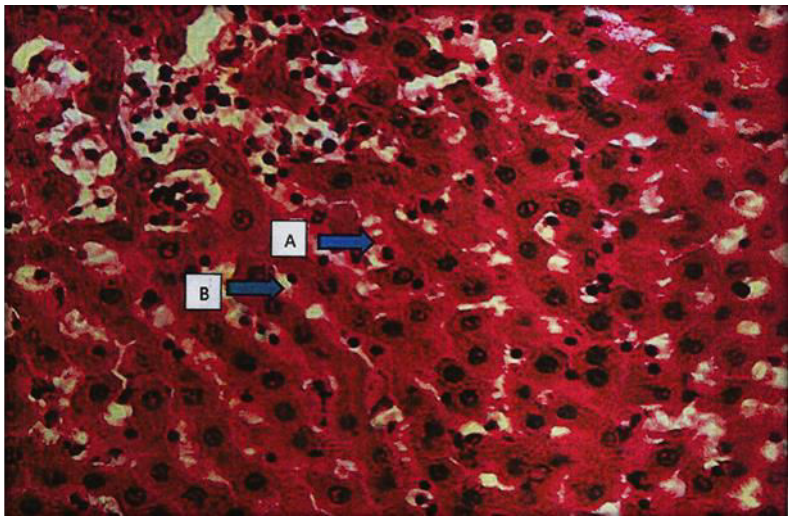


Plate.2 Control Liver: Photomicrograph of liver tissue showing control (A) normal hepatocyte (B) (arrows). Stain H&E. Stain H&E. X400

Plate.3 Group A Liver: Photomicrograph of liver tissue (A), rats treated with 0.2g of *Cola nitida* extracts/ rat feed pellets + water daily, shows fatty deposit of the hepatocyte (B) (arrows) Stain H&E. X400.

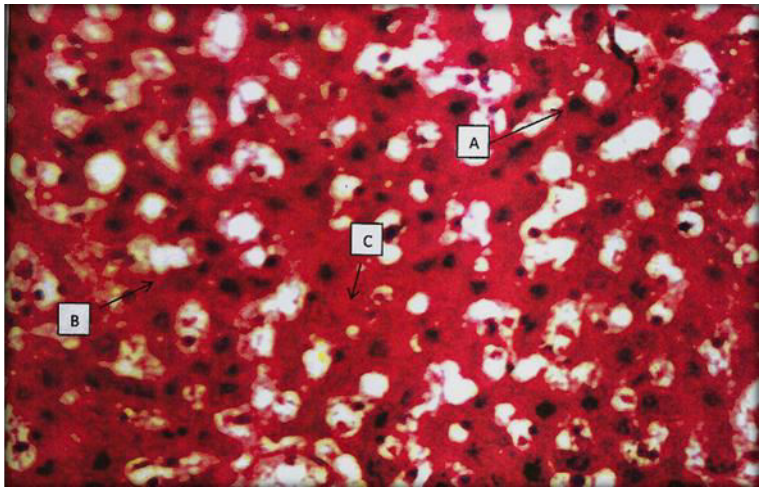


Plate.4 Group A Liver: rats treated with 0.2g /*Cola acuminata* extracts + rat feed pellets + water daily, reveals fatty deposit(c) (arrows). Stain H & E. X400

Plate.5 Group B Liver: Rats treated with feed pellets + 0.4g of *Cola acuminata* extract + water daily shows fatty deposit of the hepatocyte (B). Stain H&E. X400.

Plate.6 Kidney tissue of group B : Photomicrograph of rats treated with 0.4 g of *cola acuminata* + pelleted rat feed + water daily, showing cellular reduction in the tubules (A) bowman capsule (B) (arrows)

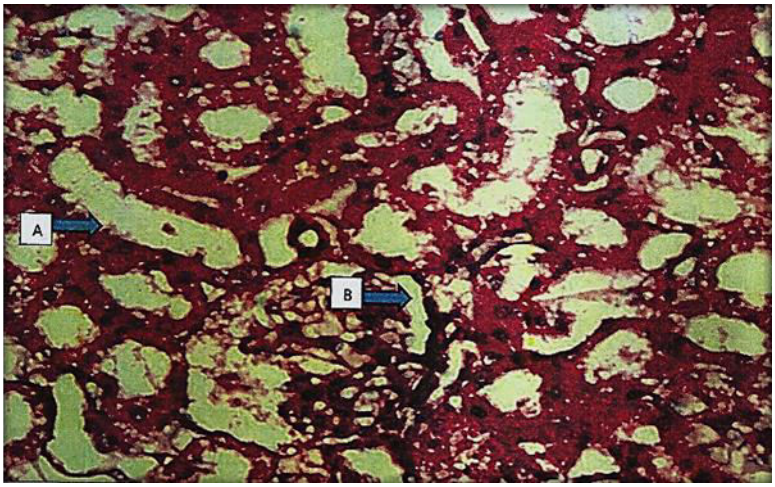


Plate.7 Kidney group C control slide: Photomicrograph of kidney tissues showing the control with normal cellular architectures (A and B). Stain H & E. X400.

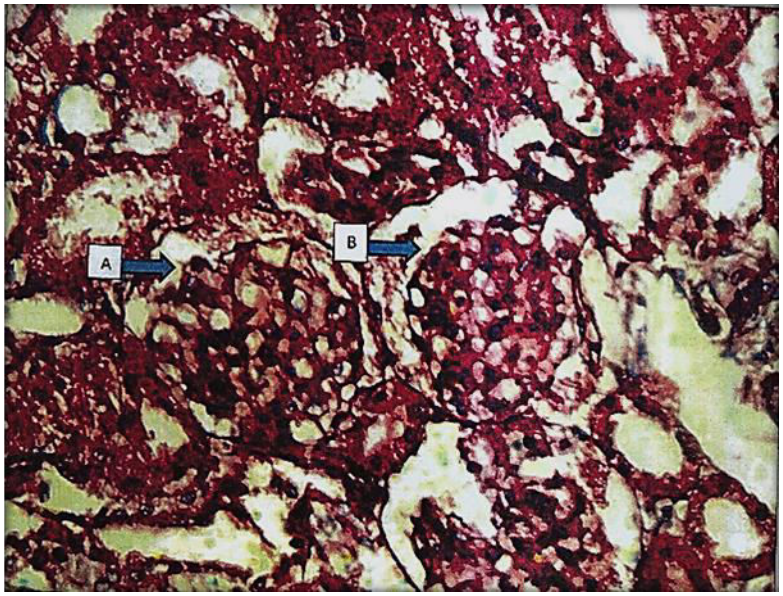


Plate.8 Kidney tissue of group A: Photomicrograph of rats treated with 0.2 g of *Cola acuminata* + rat pellet feed + water daily (B) showing cellular reduction in bowman capsule and convoluted tubules (arrows). Stain H & E. X400.

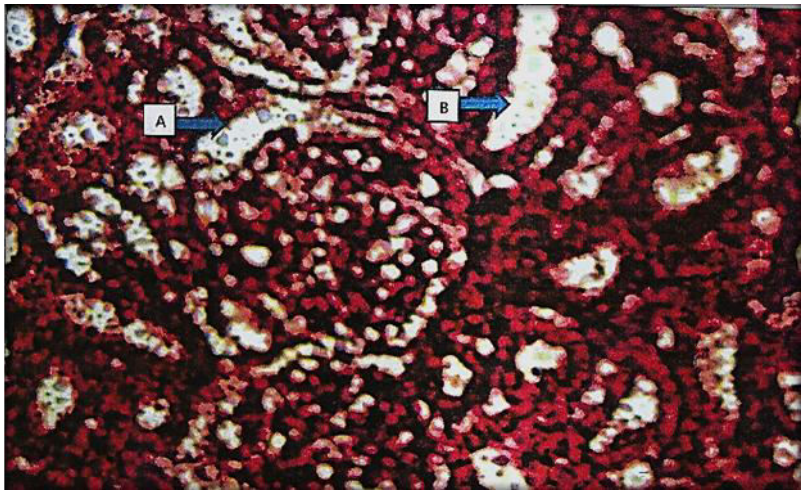
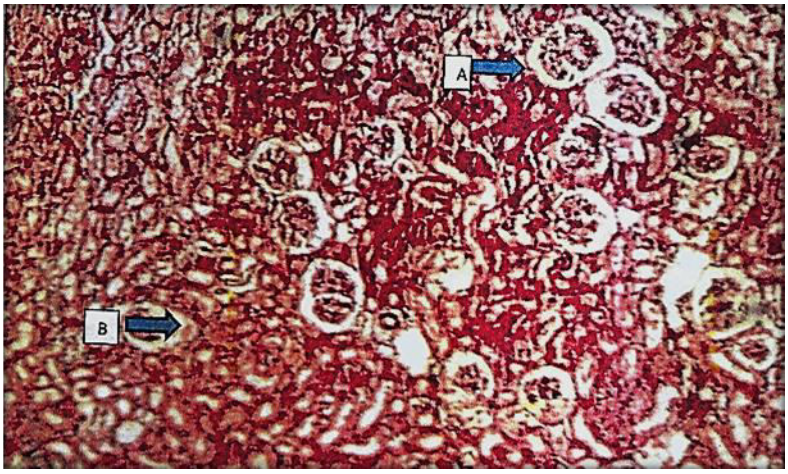


Plate.9 Kidney tissue of group B: Photomicrograph of rats treated with 0.4 g of *Cola nitida* + rat pellet feed + water daily, showing cellular reduction in bowman capsule and glomeruli (A and B). Stain H & E. X400.

Plate.10 Kidney tissue of group A: Photomicrograph of rats treated with 0.2 g of *Cola nitida* + rat pellet feed + water daily, showing cellular reduction in bowman capsule and glomeruli (A and B). Stain H & E. X400.



The kidney tissues of the rats treated with the kola substance also revealed the presence of casts in the tubular lumen, mild erosion of tubules and constriction of capillary turf agreed with Egoro *et al.,* (2023); Ikegwuonu *et al.,* (2016) in Bayelsa State Nigeria where they reported significant elevation ($p<0.05$) of alanine amino transferase, aspartate aminotransferase, alkaline phosphatase, creatinine and urea.

Author Contribution

Chinedu Habakkuk Ozoffor: Investigation, formal analysis, writing—original draft. Felicia Ngozi Onyemelukwe: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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How to cite this article:

Chinedu Habakkuk Ozoffor, Ngozi Felicia Onyemelukwe and Elochukwu Ogbuagu. 2024. Proximate Analysis and Pathological Studies of Extracts of *Cola acuminata* and *Cola nitida* in Some Visceral Organs (Kidney and Liver) of Albino Rats in Southern Nigeria. *Int.J.Curr.Microbiol.App.Sci.* 13(4): 15-25.
doi: https://doi.org/10.20546/ijcmas.2024.1304.003