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Acute Toxicity of Methanol Extract of *Cola nitida* Treatment on Antioxidant Capacity, Hepatic and Renal Functions in Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author EUE designed experimental protocol. Authors SOE, OCA and ONF performed actively in plant material extraction and administration of extract to animals. Author OCE carried out biochemical analysis. Author OYN read overall manuscript and corrected discussion section. Author IC carried out biochemical analysis. Author UIR performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The study investigated the effect of methanol crude extract of *Cola nitida* on some liver function enzymes and antioxidant in wistar rats.

Place and Duration of Study: The study was carried out at the Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Nigeria, in the year 2015.

Methodology: LD₅₀ was carried out by administering 1000, 2000, 4000, 5000, 8000 and 10000 mg/kg of the *Cola nitida* extract to twenty-four animals of four animals per group. For biochemical analysis, fifteen animals were divided into three groups of five animals each, group A served as control group, group B and C received 300 and 500 mg/kg B.W of *Cola nitida* respectively.

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Results: Result indicated an LD₅₀ of 6320 mg/kg B.W. Catalase, revealed a significant ($P = .05$) decrease in test group (36.7 ± 21.1) for 300 mg/kg and (29.46 ± 18.8) for 500 mg/kg body weight when compared to the control group (59.7 ± 4.3). Superoxide dismutase concentration was also significantly ($P = .05$) lower in the 500 mg/kg group (10.32 ± 4.90) when compared to the control group (24.94 ± 2.39). GSH concentration was significantly ($P = .05$) lower in the 500 mg/kg group (21.1 ± 1.91) when compared to the control group (23.3 ± 1.28). Result for MDA significantly ($P = .05$) decreased in the control group (0.08 ± 0.03) when compared to the 300 mg/kg group (0.11 ± 0.00) and 500 mg/kg group (0.12 ± 0.03). ALT and AST concentrations were significantly ($P < 0.05$) higher in the 500 mg/kg group (43.60 ± 20.47 and 30.60 ± 20.99) when compared to the control group (23.40 ± 3.36 and 11.20 ± 5.84).

Conclusion: Result obtained from this study showed that administration of *Cola nitida* induced lipid peroxidation, and also some signs of hepatotoxicity.

Keywords: *Cola nitida*; catalase; lipid peroxidation; ALT; AST; SOD.

1. INTRODUCTION

Kolanut, the caffeine nut of *Cola acuminata* and *Cola nitida* is native to tropical Africa and cultivated extensively in the American tropics where it is widely used as an ingredient of soft drinks and used extensively in traditional medicine [1].

The nutritive and medicinal potential of Kolanut has been well documented in literature. They are commonly chewed by local labourers as a stimulant to diminish sensations of hunger, small pieces of kolanut chewed before meal act as an aid to digestion [2]. In Brazil and the West Indies, the astringent tasting nuts are used as a botanical drug to combat intoxication, hangover and diarrhea. Kolanut also contains the stimulant caffeine, theobromine and theophylline. Theophylline relaxes smooth muscle and dilates bronchioles in the lungs to benefit sufferers of asthma and bronchitis [3]. Theobromine makes the heart beat faster, dilates blood vessels and reduces blood pressure as well. Kolanut is a central nervous system stimulant that suppresses appetite, and weight loss, provides energy to those suffering from chronic fatigue and has been used as a treatment for migraine headaches.

Cola nitida has also been used in traditional medicine as an aphrodisiac, an appetite suppressant, to treat migraine headache, morning sickness and indigestion [4]. It has also been applied directly to the skin to treat wounds and inflammation on the teeth and gums. Report by Abiodun et al. [5] showed that kolanut is rich in alkaloids, tannins, phytates, flavonoids and polyphenols and thus is responsible for its pharmacological and nutritive potentials.

In Nigeria, kolanut is considered a very important traditional item which is used in hospitality, social and religious activities. However, in the Eastern region of Nigeria, kolanut is consumed indiscriminately especially by aged people and traditionalist. In previous literature, toxicity studies on the aqueous and ethanol extract of kolanut has been documented. The study is aimed to acute toxicity of methanol extract of *Cola nitida* treatment on antioxidant capacity, hepatic and renal functions in experimental wistar animals.

2. MATERIALS AND METHODS

2.1 Plant Material

The kolanut were collected in September, 2015 from Ngoro market, in Ikwano L.G.A of Abia State Nigeria. The plant material was identified by Dr. Garuba Omosun, a Taxonomist at the Department of Plant Science and Biotechnology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike as *Cola nitida*. The plant material was given voucher specimen number MOUAU/COLNAS/15/079

2.2 Preparation of Plant Extract

The plant material was washed with distilled water, and chopped into small pieces with a kitchen knife. Drying was achieved under room temperature for 7 days in an open space. Dried sample were pulverized and extraction was carried out by cold extraction method for 72 hours using 80% methanol (Sigma-Aldrich, Germany) in a glass bottle. The *Cola nitida* was filtered with Whatman No. 1 filter paper. The filtrate was concentrated to dryness in hot air oven at 40°C to give residue. The residue was stored in a refrigerator.

2.3 Experimental Animals

Thirty nine male albino wistar rats (120-125 kg) was obtained from the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The animals were housed in stainless steel cages and were allowed acclimatization period of two weeks before commencing the experiment. The animals were fed *ad libitum* with standard commercial pellet growers feed (Vital Feed, Nigeria) and with free access to clean drinking water.

2.4 LD₅₀

For LD₅₀, twenty four animals were randomly divided into six groups consisting of four animals per group. The animals were administered widely spread doses of 1000, 2000, 4000, 5000, 8000 and 10000mg/kg body weight of extract and were observed for signs of toxicity up to 48 hours.

2.5 Experimental Design

Fifteen male rats were randomly distributed into three groups of five animals per group. The weight of the animals were checked during distribution to ensure a weight difference of +/- 5 (grams) inter and intra cages.

Group A was administered distilled water only.

Group B received 300 mg/kg of *Cola nitida* extract

Group C received 500 mg/kg of *Cola nitida* extract.

2.6 Blood Collection and Sample Preparation

Experimental period lasted for twenty-one days, after which blood samples were collected through ocular puncture. Blood was spun at 895x g for 10 mins to obtain serum. The animals were later euthanized and the liver was dissected out immediately for preparation of liver homogenate used in antioxidant assay. The protocol was approved by the Animal Ethical Committee of the University and was allocated reference number MOUAU/COLNAS/BCM/2015/316

2.7 In vivo Antioxidant

- Catalase activity was determined using the modified method described by Atawodi [6]
- Superoxide dismutase (SOD) activity was

determined by the method described by Sun et al. [7]

- The level of thiobarbituric acid reactive substance (TBARs) and malondialdehyde (MDA) production was determined by the method described by Draper and Hadley [8].
- GSH activity was determined using method described by Owen and Belcher [9].

2.8 Liver Enzymes and Creatinine Assay

- Alanine Amino Transferase, Aspartate Amino Transferase and Creatinine in serum was determined using Randox commercial kits.

2.9 Statistical Analysis

Data obtained were statistically analyzed using one way analysis of variance (SPSS software, Ver. 21). The variant mean were separated by least significance difference. Significance was accepted at 95% confidence level. The result was reported as Mean \pm SD.

3. RESULTS AND DISCUSSION

The study examined the acute toxicity of methanol extract of *Cola nitida* on liver enzymes and antioxidants in wistar rats. For LD₅₀, administration of doses up to 5000 mg/kg BW *Cola nitida*, caused no death or any observable signs of toxicity even beyond 48 hours. Signs of toxicity were observed at 8000 mg/kg and 10000 mg/kg BW extract administration drowsiness, heavy breathing, paralysis, convulsions followed by death with stomach opened and tissues and intestine out. From the result shown in Table 1, the LD₅₀ was calculated as 6320 mg/kg B.W. Studies by Ayebe et al. [10] reported an LD₅₀ of 5012 mg/kg BW of water extract of *Cola nitida*. Salahdeen et al. [11] reported an LD₅₀ of \geq 200 mg/kg BW for ethanol extract of kolanut in rats. This gives an indication that methanol extract of *Cola nitida* is relatively safe.

Liver enzymes result (Table. 3) showed a significant increase in AST concentration in the 500 mg/kg group when compared to the control group. AST, is an enzyme associated with liver parenchymal cells and it catalyzes the transfer of an amino group between aspartate and glutamate. Burtis and Ashwood [12] reported an increase in AST concentration in blood serum during liver damage and certain disease

condition such as hepatitis, and myocardial infarction. This indicates that prolonged consumption of *Cola nitida*, could lead to certain disease conditions or hepatotoxicity. AST concentration is raised in acute liver damage, but is also present in red blood cells, and cardiac and skeletal muscle source [13]. The result from this study showed that the ALT level in group administered 500 mg/kg *Cola nitida* extract was significantly higher when compared to the control group. ALT is found in plasma and in various body tissues but is most common in the liver. Studies by Saluhdeen et al. [11] indicated an increase in AST and ALT concentration in rats treated with ethanol extract of *Cola nitida*. It could be suggested that acute administration of methanol extract of *Cola nitida* extract promoted cholestasis, as seen in the elevated transaminases.

Result for creatinine (Table 3) showed no significant ($P < 0.05$) differences in the studied groups. Creatinine is synthesized primarily in the liver and a breakdown product of creatine phosphate in muscle and can serve as biomarker for kidney injury. An increase in creatinine indicates kidney and nephron damage. Creatinine is removed mainly through the kidney by the process of glomerular but also via proximal tubular secretion. Creatinine concentration in living system can be altered by various muscle sizes or decreased muscular activity [14]. Saluhdeen et al. [11] reported high creatinine concentration in rats administered ethanol extract of *Cola nitida*.

Anti-oxidant play defense mechanism and promotes activities for metabolism of xenobiotic in living system. They are free radical scavengers that interact and degrade free radicals, thus preventing them from causing cellular damage [15]. The *In vivo* antioxidant activity showed *Cola nitida* reduced the concentration of superoxide dismutase (SOD), catalase and promoted lipid peroxidation.

Result for catalase (Table 2), showed a significant ($P < 0.05$) decrease in the 500 mg/kg and 300 mg/kg extract administered group when compared to the control group. Catalase an antioxidant enzyme, catalyzes the breakdown of hydrogen peroxide, a reactive oxygen species generated from normal aerobic metabolism and also known to be a toxic compound [16,17]. Increase in free radicals and imbalance in the normal redox state of tissues can lead to reduction of catalase activity [18]. Result of this study indicated that prolonged consumption of

methanol extract of *Cola nitida* led to an increase in free radical generation which can thus lead to the development of oxidative stress.

Result for SOD (Table 2), showed a dose dependent effect. SOD concentration was significantly ($P < 0.05$) higher in the control group when compared to 500 mg/kg group. Also, SOD concentration was significantly ($P < 0.05$) high in the 300 mg/kg group when compared to the 500 mg/kg group. SOD catalyzes the dismutation of super-oxide to hydrogen peroxide and oxygen [17]. Reduction in SOD concentration in serum suggests that *Cola nitida* induces production of reactive oxygen species which lead to production of hydrogen peroxide. However, SOD and catalase work collectively to achieve their desired aim, considering the point that the product of SOD activity generates hydrogen peroxide which is a potent toxic free radical that is mopped up by catalase.

Result for GSH was significantly ($P < 0.05$) higher in the control group when compared to the group administered 500 mg/kg B.W of the plant extract. GSH is an antioxidant which is important for cellular defense against ROS and lipid peroxidation. GSH is important in donating it electron, thereby putting GSH in its oxidized form. GSH also facilitates metabolism of xenobiotic by promoting phase 2 biotransformation process [19]. Result obtained for GSH indicates that the plant extract exhibit some level of toxicity in the 500 mg/kg B.W group.

Malondialdehyde (MDA) has been generally considered a biomarker for lipid peroxidation. Increase in MDA concentration has been linked with increased lipid peroxidation which in turn signals development of oxidative stress, cellular and DNA damage. MDA concentration (Table 2) was significantly ($P < 0.05$) high in the extract administered group when compared to the control group. ROS results from most biological compounds; the most vulnerable ones are polyunsaturated fatty acids (PUFAs). Reactions with these essential membrane constituents can generate lipid peroxidation (LPO). Result from this study indicated that the extract promoted peroxidation of lipid in the test groups. Increase in LPO impairs membrane activity of membrane bound proteins and receptor [20]. Also, the reduction in GSH concentration, can be added to increase in LPO reactions [21], and this was evident in this study. Studies by Muhammad and Fatima [22] indicated that methanol extract and water extract of *Cola nitida* showed little or no presence of flavonoids.

Table 1. Result for LD₅₀ showing number of mortality

Group	Conc. (mg/kg)	No. of animal	No. of mortality
A	1000	4	0
B	2000	4	0
C	4000	4	0
D	5000	4	0
E	8000	4	4
F	10000	4	4

$$LD_{50} = \sqrt{\text{Test Conc. with highest mortality} \times \text{Highest Conc. without mortality}}$$

$$LD_{50} = \sqrt{8000 \times 5000}$$

$$LD_{50} = \sqrt{40,000,000}$$

$$LD_{50} = 6320\text{mg/kg BW}$$

Table 2. Effect of methanol extract of *Cola nitida* on the level of antioxidants and lipid peroxidation in test and control animals

Group	MDA ($\mu\text{Mole/mg protein}$)	Catalase (Unit/g protein)	SOD ($\mu\text{Mole/g protein}$)	GSH ($\mu\text{Mole/g protein}$)
Control	0.08 \pm 0.03	59.74 \pm 4.34	24.94 \pm 2.39	23.30 \pm 1.28
300 mg/kg	0.11 \pm 0.00 ^c	36.70 \pm 21.10 ^a	18.66 \pm 7.24 ^b	22.14 \pm 1.05
500 mg/kg	0.12 \pm 0.03 ^c	29.46 \pm 18.80 ^a	10.32 \pm 4.90 ^a	21.10 \pm 1.91 ^a

Each value represent Mean \pm SD for each experimental group (n=5). (^a) indicates significantly (P=.05) lower when compared to control group. (^b) Significantly (P=.05) higher when compared to 500mg/kg group. (^c)

Significantly (P=.05) higher when compared to control group. SOD: Superoxide Dismutase, MDA: Malondialdehyde, GSH: Gluthathione.

Table 3. Effect of methanol extract of *Cola nitida* on the level of some liver enzymes and creatinine concentration in test and control animals

Group	AST (U/L)	ALT (U/L)	Creatinine (mg/dL)
Control	23.40 \pm 2.36	11.20 \pm 5.84	0.64 \pm 0.45
300 mg/kg	29.80 \pm 5.58	13.60 \pm 10.04	0.64 \pm 0.29
500 mg/kg	43.60 \pm 20.47*	30.60 \pm 20.99*	0.86 \pm 0.21

Each value represent Mean \pm SD for each experimental group (n=5). (*) indicates mean value is significantly different (P=.05) when compared to control group. AST: Aspartate Amino Transferase, ALT: Alanine Amino Transferase.

4. CONCLUSION

From the above data, it could be concluded that continuous or prolonged consumption of *Cola nitida* may lead to oxidative stress, causing significant challenges and decrease in anti-oxidant status.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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