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Original Research Article

Acute Toxicity and Effect of Methanol Extract of *Cola nitida* on Liver Enzymes, Creatinine and Antioxidants in Animal Experimental Model

ABSTRACT

Aim: The study investigated the acute toxicity of methanol crude extract of *Cola nitida* and the effect of prolonged administration on liver 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: LD_{50} 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 500mg/kg B.W of *Cola nitida* respectively.

Result: Result indicated an LD_{50} of 6320mg/kg B.W. Catalase, revealed a significant *(P =.05)* increase in control group (59.7±4.3) when compared to the test group (36.7±21.1) for 300mg/kg and (29.46±18.8) for 500mg/kg body weight. SOD concentration was also significantly *(P =.05)* higher in control group (24.94±2.39) when compared to the 500mg/kg group (10.32 ± 4.90). Also, SOD concentration was significantly *(P<0.05)* higher in the 300mg/kg group (18.66±7.24) when compared to the 500mg/kg group (10.32 ± 4.90). GSH concentration was significantly *(P =.05)* higher in control group (23.3±1.28) when compared to the 500mg/kg (21.1±1.91). Result for MDA significantly *(P =.05)* decreased in the control group (0.08±0.03) when compared to the 300mg/kg group (0.11±0.00) and 500mg/kg group (0.12±0.03). ALT and AST concentration was

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significantly *(P<0.05)* higher in the 500mg/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 prolong administration of *Cola nitida* induced lipid peroxidation, oxidative stress and also some signs of hepatotoxicity.

Keywords: *Cola nitida*, Catalase, Lipid peroxidation, ALT, AST, SOD

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[4]. 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[5]. Report by Abiodun *et al.*[1] showed that kolanut is rich in alkaloids, tannins, phytates, flavonoids and polyphenols and thus is responsible for it 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. This study is aimed to determine the acute toxicity, and effect of methanol extract of kolanut on liver enzymes and oxidative stress.

MATERIALS AND METHODS

Plant material

The kolanut were collected in September, 2015 from Ndoro market, in Ikwuano 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*.

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 4 days in an open space. Dried sample were

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pulverized and extraction was carried out by cold maceration method for 72hours 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.

Experimental Animals

Thirty nine male albino wistar rats (120-125kg) 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 acclamitazation 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.

LD_{50}

For LD_{50}, twenty four (24) animals were randomly divided into six (6) groups consisting of four (4) 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.

Experimental Design

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

Group A was administered distilled water only.
Group B received 300mg/kg of *Cola nitida* extract
Group C received 500mg/kg of *Cola nitida* extract.

Experimental period lasted for twenty-one days, after which blood samples were collected through ocular puncture. Blood was spun at 895xg for 10mins 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

In vivo antioxidant

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

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Liver enzymes and creatinine assay

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

Statistical Analysis

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

RESULTS

Table 1: Result for LD_{50} 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

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

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

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

LD_{50} = 6320mg/kg BW

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

Group	MDA (µMole/mg Protein)	CATALASE (Unit/g Protein)	SOD (µMole/g Protein)	GSH (µMole/g Protein)
Control	0.08±0.03	59.74±4.34	24.94±2.39	23.30±1.28
300mg/kg	0.11±0.00[c]	36.70±21.10[a]	18.66±7.24[b]	22.14±1.05
500mg/kg	0.12±0.03[c]	29.46±18.80[a]	10.32±4.90 [a]	21.10±1.91[a]

Each value represent Mean±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. Result for MDA showed a significant *(P =.05)* increase in the 500 and 300 mg/kg group when compared to the control group. Catalase concentration in the control group was significantly *(P =.05)* higher when compared to the 500 and 300 mg/kg group. Concentration of GSH was significantly higher in the control group when compared to the 500mg/kg group. Result for SOD showed a significant *(P =.05)* increase in the control group when compared to 500mg/kg group. Also, SOD concentration was significantly *(P =.05)* high in the 300mg/kg group when compared to the 500mg/kg group.

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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±2.36	11.20±5.84	0.64±0.45
300mg/kg	29.80±5.58	13.60±10.04	0.64±0.29
500mg/kg	43.60±20.47*	30.60±20.99*	0.86±0.21

Each value represent Mean±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. Result for AST and ALT showed a significant *(P =.05)* increase in the 500mg/kg group when compared to the control group.

Discussion

The study examined the acute toxicity and effect of methanol extract of *Cola nitida* on liver enzymes and antioxidants in wistar rats. For LD_{50}, administration of doses up to 5000mg/kg BW *Cola* nitida, caused no death or any observable signs of toxicity even beyond 48hours. Signs of toxicity after which death was observed at 8000mg/kg and 10000mg/kg BW extract administration. Signs of toxicity observed were drowsiness, heavy breathing, paralysis, convulsions followed by death with stomach opened and tissues and intestine out. From the result, the LD_{50} was calculated as 6320mg/kg B.W. Studies by Ayebe *et al.*[10] reported an LD_{50} of 5012mg/kg BW of water extract of *Cola nitida*. Salahdeen *et al.*[11] reported an LD_{50} of $\geq$ 200mg/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 showed a significant increase in AST concentration in the 500mg/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]

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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 prolong 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 500mg/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. It catalyzes the transfer of an amino acid from L-alanine to α-ketoglutarate, the products of this reversible transamination reaction being pyruvate and L-glutamate. 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 *Cola nitida* extract promoted cholestasis, as seen in the elevated transaminases.

Result for creatinine showed no significant *(P<0.05)* difference 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 circular damage Diplock *et al.* [15]. The *In vivo* antioxidant activity showed *Cola nidita* reduced the concentration of superoxide dismutase (SOD), catalase and promoted lipid peroxidation.

Result for catalase, showed a significant *(P<0.05)* decrease in the 500mg/kg and 300mg/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 prolong consumption of methanol extract of *Cola nitida* led to an increase in free radical

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generation which can thus lead to the development of oxidative stress.

Result for SOD showed a dose dependent effect. SOD concentration was significant *(P<0.05)* high in the control group when compared to 500mg/kg group. Also, SOD concentration was significantly *(P<0.05)* high in the 300mg/kg group when compared to the 500mg/kg group. SOD catalyzes the dismutation of super-oxide to hydrogen peroxide and oxygen [17]. Reduction in SOD concentration in serum suggests that *Cola nidita* 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 500mg/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 it oxidized form. NADPH from the pentose phosphate pathway (PPP) is strongly responsible for converting the oxidized glutathione into it reduced form by serving as an electron donor. 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 500mg/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 was significantly *(P<0.05)* high in the extract administered group when compared to the control group. ROS results with all biological compounds; the most vulnerable ones are polyunsaturated fatty acids (PUFAs). Reactions with these essential membrane constituents can generated 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 adduced 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 nidita* showed no or little presence of flavonoids. Flavonoids are ubiquitous groups of polyphenolic

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compounds which are present in plants, and are highly concentrated in seeds and fruits [23]. Flavonoids possess strong antioxidant ability capable of scavenging hydroxyl radicals, superoxide anions and lipid peroxy radicals [24]. The inability of the extract to improve antioxidant defense system can be attributed to the little or no flavonoids present in the extract and also toxic compound present in the plant material. External source of dietary antioxidants is highly required to beef up antioxidant mechanism in biological systems.

CONCLUSION

Studies have shown that that *Cola nidita* has very strong and high pharmaceutical and medicinal properties. However, it toxicological profile needs to be evaluated. Result of this study revealed continuous or prolonged consumption of *Cola nitida* may lead to oxidative stress, causing significant challenges and decrease in anti-oxidants.

References

1. Newall C, Anderson LA, Phillipson JD. Herbal Medicines. In: A Guide for Health-Care Professionals, London, England, Pharmaceutical Press; 1996: 84
2. Epega AA. Obi divination, Athelia Henrietta Press. 2003: 1-2.
3. Livingstone FM, Merger MA, Northwest KK. Kolanut; its stimulants and usefulness to the society. 2003
4. Esimone CO, Adikwu MU, Nworu CS, Okoye FB, Odimegwu DC. Adaptogenic potentials *Camellia sinensis* leaves, *Garcinia kola* and *Kola nitida* seeds. Sci. Res. Assay. 2003: 2 (7): 232-237
5. Abiodun OA, Oyekanmi AM, Oluoti OJ. Biochemical and phytochemical properties of *Cola acuminata* varieties. Am. J. of Exp. Agric. 2004: 4 (11): 1280-1287.
6. Atawodi S. Evaluation of the hypoglycaemic, hypolipidemic and antioxidant effects of methanolic extract of “Ata-Ofa” polyherbal tea (A- polyherbal) in alloxan- induced diabetic rats. Drug Invention Today. 2001: 3: 270- 276.
7. Sun Y. Oberley W, Li Y. A simple method for clinical assay of superoxide dismutase. Clinical Chemistry. 1988: 34 (3): 497- 500.
8. Draper H, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods in

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Enzymology. 1990: 186: 421- 431.

9. Owen C, Belcher R. A colorimetric micro- method for the determination of glutathione. Biochemical Journal. 1965: 94 (3): 705- 711.
10. Ayebe EK, Yapi HF, Edjeme AA, Meite S, M'boh MG, Yapo AF, Monnet D, Djaman AJ, Nguessan JD. *In vivo, in vitro* antioxidant activity assessment and acute toxicity of aqueous extract of *Cola nitida* (Sterculiaceae). Asian Journal of Biochemical and Pharmaceutical Research. 2012: 4 (2): 144- 155
11. Salahdeen HM, Omoaghe AO, Isehunwa GO, Murtala BA, Alada AR. Gas chromatography mass spectrometry (GC-MS) analysis of ethanolic extracts of kolanut (*Cola nitida*) (vent) and its toxicity in rats. Journal of Medicinal Plant Research. 2015: 9 (3): 56- 70. DOI: 10.5897/JMPR2014.5711
12. Burtis C, Ashwood E. Aspartate aminotransferase (AST) (GOT), serum. In: Tietz Textbook of Clinical Chemistry. Philadelphia, WB Saunders Company, 1994
13. Nyblom H, Berggren U, Balldin J, Olsson R. "High AST/ALT ratio may indicate advanced alcoholic liver disease rather than heavy drinking". Alcohol. 2004: 39 (40): 336-339
14. Ndukaku OY, Ejiofor EU, Loveth UE, Oluchi OI. Valuation of some serum kidney functions and lipid profile of malaria patients in South Eastern Nigeria. Rom. J. Biochem. 2015: 52 (1): 39–49
15. Diplock A, Charleux J, Crozier-Willi G. Functional food science and defense against reactive oxidative. British Journal of Nutrition. 1998: 80 (1): 77- 112.
16. Kohen R, Nyska A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol. Pathol. 2002: 30 (6):620-650.
17. Maritim AC, Sanders RA, Watkins JB. Diabetes, oxidative stress, and antioxidants: a review. J. Biochem. Mol. Toxicol. 2003: 17: 24-38
18. Hala AA. Oxidative stress biomarkers in young male rats fed with stevioside. African Journal of Biochemistry Research. 2011: 5(11): 333-340
19. Emmanuel UE, Ebhohon OS, Omeh YN. Effect of fermented and unfermented cocoa bean on some liver enzymes, creatinine and antioxidant in wistar albino rats. Carpathian Journal of Food Science and Technology. 2015: 7 (4): 132-138
20. Arulselvan P, Subramanian SP. Beneficial effects of *Murraya koenigii* leaves on antioxidant

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defense system and ultra-structural changes of pancreatic β-cells in experimental diabetes in rats. Chem. Biol. Interact. 2007: 165: 155–164. DOI: 10.1016/j.cbi.2006.10.014

21. Ugochukwu NH, Babady NE, Coboure M, Gasset SR. The Effect of *Gongronema latifolium* extracts on serum lipid profile and oxidative stress in hepatocytes of diabetic rats. Journal of Bioscience. 2003: 28 (1): 1-5
22. Muhammad S, Fatima A. Studies on phytochemical evaluation and antibacterial properties of two varieties of kolanut (*Cola nitida*) in Nigeria. Journal of Biosciences and Medicines. 2014: 2: 37- 42
23. Alan L, Miller ND. Antioxidant flavonoids structure, function and clinical usage. Alternative Medicine View. 1996: 1 (2): 103- 111.
24. Amie D, Davidovic-Amie D, Beslo D, Trinajstic N. Structural-radical scavenging activity relationship of flavonoids. Croatia Chem. Acta. 2003: 76: 55-61.