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Toxicological Evaluation of Methanol Leaf Extract of *Cola hispida*

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ABSTRACT

Cola hispida is a plant that has been employed in African folkloric medicine as an analgesic plant and to prevent premature labor. The aim of the research is to assess the acute and sub-acute oral toxicity of the methanol leaf extract of *Cola hispida* in mice and rats, respectively. Pulverized leaf was extracted by maceration in 6 L of methanol (98% purity) for 72 hours, filtered and concentrated at 40ºC using a rotary evaporator. In the acute toxicity test, the extract at 10, 100, 1000, 1600, 2900, and 5000 mg/kg was administered orally to mice, while, 200, 400, 600, and 800 mg/kg doses of the extract were administered orally for 28 days in the sub-acute toxicity test. No behavioural signs of toxicity nor lethal effect were observed in the acute toxicity test indicating that the LD_{50} is greater than 5000 mg/kg. In the sub-acute toxicity evaluation, the extract showed neither mortality nor treatment-related adverse effects with regards to the cardiac, hepatic, renal, and antioxidant parameters. The histopathological examination of liver sections, however, revealed mild congestion of blood vessels and haemorrhage at 800 mg/kg indicating potential unfavorable effect on long-term administration at higher doses. The findings imply that the methanol extract of *Cola hispida* leaves is relatively safe. However, long-term use at higher doses should be avoided.

Keywords: *Cola hispida* extract, Acute toxicity, Sub-acute toxicity, Safety evaluation.

Introduction

The interest in traditional medicines is on the increase as man continues to explore and take advantage of the chemical diversity of the world around him to meet his ever-growing healthcare needs.[1] The usage of alternative medicines is no longer limited to resource-poor regions as there is a rise in demand for plant-based medicinal products even in developed regions.[2] *Cola hispida* (Sterculiaceae) Brennan and Keay, is a small forest tree, with greater distribution in the Eastern and Northern parts of Nigeria, and found in Cameroon, Gabon, and the Congo Brazzaville. The leaf decoction is used locally as an analgesic and to prevent premature labor.[3,4] The seeds have been shown to have psychoactive properties due to its high alkaloid and caffeine contents .[1,3] Apart from the reports on local usage, information is scanty on the ethnopharmacology of the plant, though recently, the cardioprotective potential of the leaf extract in doxorubicin-induced myocardial injury in rats has been reported.[1] However, concerns about potential toxicity to vital organs remain an issue with natural products despite their perceived safety in comparison to synthetic drugs.[5] Recently, there have been reports about the toxicities associated with long-term administration of medicinal plants.[5,6]It thus becomes necessary to evaluate the toxic effects that might occur with the administration of single or repeated doses of plant-derived compounds or extracts irrespective of associated pharmacological benefits. The study was carried out to evaluate the toxicological effects of methanol extract of *Cola hispida* leaves (ME-CH).

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Materials and Methods

Chemicals and reagents
The chemicals and reagents used were of analytical grade. They are products of Sigma Aldrich, Germany, Merck, USA and Janssen, Germany.

Collection and preparation of plant materials
Fresh *Cola hispida* leaves were harvested in April 2019 from OzomMgbagbuOwa, Enugu State of Nigeria and identified by Mr. A. Ozioko, a taxonomist at the Bioresources Development and Conservation Programme (BDCP) in Nsukka, Enugu State, Nigeria. The leaf samples were deposited in their herbarium with the voucher number InterCEDD/16304. The leaves were air dried and pulverized and 1080 g of the powdered leaves was extracted by maceration in 6 L of methanol (98% purity) for 72 hours. This was followed by filtration, first with a mesh and then with Whatman No 1 filter paper and filtrate concentrated using a rotary evaporator at 40 ºC to obtain the crude methanol leaf extract of *Cola hispida* (ME-CH)

Phytochemical Screening
The phytochemical screening of methanol leaf extract of the *Cola hipsida* was investigated using the methods described by Trease and Evans, Harbourne, Soni and Sosa.[7,8,9]

Experimental animals
Eighteen (18) Swiss mice of average body weight of 30 g and 20 Wistar rats (85-140 g) were used for the acute and sub-acute toxicity studies, respectively. They were acclimatized to laboratory environment for seven days before commencement of the study. Animals were maintained on standard animal feed (Pecco Foods, Enugu, Nigeria) and water ad libitum in clean, well-ventilated animal cages. The animals were handled in accordance with the relevant ethical guidelines compliant with the International Standard for the use of laboratory animals. Also, the research was approved by the Ethical Committee of the Faculty of Biological Sciences, University of Nigeria Nsukka, with an approval number: UNN/FBS/EC/1040.

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Acute toxicity study
The protocol of Lorke[10] was adopted for the acute toxicity study. The mice (n=3) were administered orally, doses of 10, 100, and 1000 mg/kg b.w. of the extract in the first phase and monitored for 24 hours for any signs of toxicity. Due to the absence of mortality nor signs of toxicity, another group of mice (n=3) were administered increased doses of 1600, 2900, and 5000 mg/kg b.w respectively in the second phase and monitored for signs of toxicity or mortality.

Sub-acute toxicity study
The method described by the Organization for Economic Cooperation and Development[11] was adopted for the sub-acute toxicity studies. Twenty (20) rats were randomly distributed into five groups of four (4) animals each. Group 1, labelled as control group received 1 mL distilled water while groups 2-5 (test groups) were administered orally 200, 400, 600 and 800 mg/kg body weight of the extract respectively for 28 days. On the 29th day, the animals were weighed and then euthanized under mild chloroform anaesthesia. Blood samples were collected from the orbital sinus into plain sample bottles for biochemical analysis. Blood samples for the biochemical analyses were allowed to clot, centrifuged at 4000 rpm for 30 min to obtain the serum which was aspirated with a syringe into clean sample bottles and stored in a refrigerator until use.

Estimation of Biochemical Parameters
The antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were assayed by methods described by Aebi,[12]Kakkar*et al*.[13] and Paglia and Valentine,[14] respectively. Kidney function parameters (urea and creatinine) were determined using commercially available test kits. Blood urea nitrogen was determined following the method of Searcy *et al*.,[15] while serum creatinine levels were determined using the method of Tietz.[16] Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were assayed using the method of Reitman and Frankel[17] as outlined in the test kits. Serum activities of creatine kinase (CK) and lactate dehydrogenase (LDH) were assayed using Randox kits as outlined by the manufacturers.

Statistical Analysis
Statistical analysis of data was carried out using the Statistical Product and Service Solution (SPSS, version 21.0). All data were expressed as mean ± standard deviation (SD) and statistical differences between means were determined by one-way analysis of variance (ANOVA). Data was considered significant at $P<0.05$.

Results and Discussion

Phytochemical composition of ME-CH
The phytochemical screening of the methanol leaf extract of *Cola hispida* (ME-CH) (Table 1) revealed the presence of phenolics (4.06 ± 0.06 mg/g), flavonoid (4.40 ± 0.23 mg/g), saponins (0.72 ± 0.06 mg/g), tannins (1.86 ± 0.04 mg/g), alkaloids (1.65 ± 0.22 mg/g) and glycosides (1.00 ± 0.14 mg/g). Several pharmacological effects of plant-derived polyphenols have been reported.[18-20] Thus, the high contents of phenolics and flavonoids in the extract relative to other detected classes of phytochemicals indicate efficacious antioxidant potential of the plant. Alkaloids have pharmacological applications as anesthetics, analgesics, anti-malaria, and CNS stimulants amongst other uses.[21,22] Hence, the presence of alkaloids in the extract supports the local use of the leaves as analgesic.

Acute toxicity study of ME-CH
There were no signs of toxicity, nor was any death recorded in the acute toxicity study even when a high dose of 5000 mg/kg body weight was administered to the mice. This indicates that the median lethal dose (LD_{50}) of the plant extract is higher than 5000 mg/kg and could be deemed safe for oral consumption, which correlates with the report of Kennedy[23] on the toxicological safety of extracts at LD_{50} higher than 5000 mg/kg b.w

Effect of ME-CH on antioxidant enzymes' activities of rats
Antioxidant enzymes: catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) are part of the body's defense system activated to counteract the toxic effects of reactive oxygen species which are produced either from normal metabolic processes or toxicant induced. Superoxide dismutase scavenges O^{-2} radicals converting them to H_2O_2 which is then detoxified by both GPx and CAT.[31] Excessive generations of toxic reactive species can, however, inactivate these enzymes, causing deleterious effect on vital cell components. In the study, treatment of experimental animals with ME-CH elicited increase in the activities of enzymatic antioxidants when compared to the normal control. Significant ($p<0.05$) increase in SOD activity was observed in the groups treated with higher doses of 600 and 800 mg/kg b.w of the extract, whereas CAT and GPx activities were significantly ($p<0.05$) enhanced in all the treated groups when compared to the control group (Table 2). The possible enhancement of antioxidant enzymes' activities evident in the treated groups indicates the potential antioxidant properties of *Cola hispida* leaf. This result is in accordance with the report on an *in vivo* study on the ethyl acetate leaf extract of *Cola hispida* in which a reversal of doxorubicin-induced reduction in the activities of these enzymes was reported.[1]

Effect of ME-CH on some biochemical parameters in male Wistar rats
The result revealed that the methanol leaf extract of *Cola hispida* (ME-CH) produced significant *($p<0.05$)* reductions in the activities of cardiac marker enzymes (creatine kinase and lactate dehydrogenase) in the treated groups (groups 2-5) when compared with the normal control (Table 3). Creatine kinase (CK) and lactate dehydrogenase (LDH) are two useful markers in the evaluation of cardiac ischemia, cardiotoxicity and cardio-protective effect of pharmacological agents using animal models. Their concentrations are usually elevated following myocardial injury[1,24]and thus are used as indicators of cardiac health. The significant reductions in the enzyme activities in this study indicate a non-toxic effect of the extract on cardiac tissues in the rats. Thus, suggesting a cardio-protective rather than cardio-toxic effect of *Cola hispida* leaves. This correlates with an earlier report on reduction in the activities of these cardiac marker enzymes in rats treated with ethyl acetate leaf fraction of *Cola hispida* following doxorubicin-induced myocardial injury.[1]

Table 1: Phytochemical screening of ME-CH

Phytochemicals	Concentration (mg/g)
Phenolics	4.06 ± 0.06
Flavonoids	4.40 ± 0.23
Tannins	1.86 ± 0.04
Alkaloids	1.65 ± 0.22
Saponins	0.72 ± 0.06
Glycosides	1.00 ± 0.14

Values are expressed as mean ± SD. (n = 3)

Table 2: Effect of ME-CH on antioxidant enzymes' activities of rats

Groups	CAT (U/L)	SOD (U/L)	GPx (U/L)
Normal control	14.97 ± 0.86^{a}	10.13 ± 0.77^{a}	37.53 ± 0.55^{a}
200 mg/kg b.w.	16.62 ± 0.66^{b}	10.25 ± 0.05^{a}	46.12 ± 0.35^{c}
400 mg/kg b.w.	18.98 ± 0.93^{c}	10.32 ± 0.05^{a}	45.13 ± 2.98^{c}
600 mg/kg b.w.	19.33 ± 1.17^{c}	10.66 ± 0.32^{b}	42.71 ± 1.03^{b}
800 mg/kg b.w.	19.43 ± 4.93^{c}	10.60 ± 0.12^{b}	42.51 ± 1.35^{b}

Values are expressed as mean ± SD. (n = 3). The mean values with different superscript letters in the columns differ significantly at $p<0.05$.

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Table 3: Effect of ME-CH on some biochemical parameters of rats

	Heart		Liver		Kidney	
Groups	**CK (U/L)**	**LDH (U/L)**	**AST (U/L)**	**ALT (U/L)**	**Urea (mmol/l)**	**Creatinine (mg/dl)**
Normal control	24.15±1.20^{c}	4.00±0.09^{c}	136.00±2.83^{d}	64.00±3.16^{c}	67.41±2.14^{c}	1.35±0.02^{b}
200 mg/kg b.w.	16.05±0.38^{b}	3.17±0.08^{b}	124.75±3.20^{c}	61.00±1.83^{c}	63.90±2.24^{bc}	1.26±0.03^{b}
400 mg/kg b.w.	13.40±1.10^{a}	3.02±0.16^{b}	96.75±2.22^{a}	57.25±1.26^{a}	56.59±1.49^{a}	1.18±0.03^{a}
600 mg/kg b.w.	13.38±1.77^{a}	2.27±0.19^{a}	93.00±5.23^{a}	58.42±1.34^{a}	56.41±1.19^{a}	1.17±0.11^{a}
800 mg/kg b.w.	15.31±0.97^{b}	3.13±0.05^{b}	106.75±2.22^{b}	57.00±1.63^{b}	53.56±2.34^{a}	1.19±0.06^{a}

Values are expressed as mean ± SD. (n = 3). The mean values with different superscript letters in the columns differ significantly at $p<0.05$.

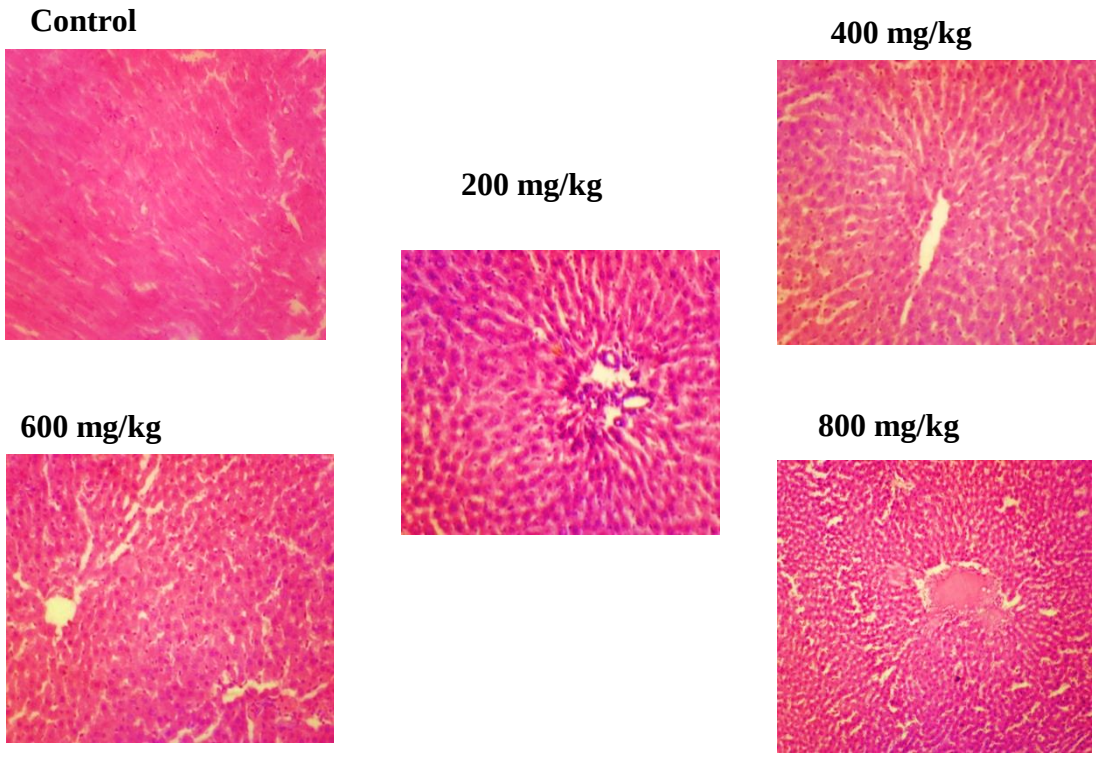

Figure 1: Photomicrograph of liver sections of control and treated groups. The liver section at 800 mg/kg b.w. showed mild congestion of blood vessels and haemorrhage whereas other sections were similar to control. H & E x 150

The liver is usually at greater risk of toxicity due to its central role in the metabolism of xenobiotic compounds and its function is primarily assessed by the presence of marker enzymes ALT and AST in serum. Elevated serum levels of these enzymes indicate liver injury and/or dysfunction. Also, serum AST level is used to assess the functional status of muscle and heart tissues.[25, 26] In the study, administration of graded doses of ME-CH significantly lowered serum AST activity relative to the control group while ALT activity was significantly ($p<0.05$) lowered in groups 3-5 administered 400 mg/kg, 600 mg/kg, and 800 mg/kg doses of the extract respectively when compared to the control group (Table 3). The reduction in the activities of these marker enzymes suggests no apparent hepatotoxicity but rather a hepato-protective effect of the plant extract in the experimental rats.

As one of the end products of protein metabolism, blood urea nitrogen is usually elevated in kidney damage, high protein intake or low fluid intake.[27] Similarly, elevated serum creatinine levels usually result from impaired renal blood flow associated with diseases such as diabetes and cardiovascular diseases.[28,29] On the renal function status of the experimental rats, there was no significant change in serum urea and creatinine levels of the animals administered 200 mg/kg b.w of the extract when compared to the control group. On the other hand, serum urea and creatinine levels were significantly ($p<0.05$) lowered in groups 3-5 which received 400, 600, and 800 mg/kg b.w of extract respectively, relative to the control group. Similar to the liver, the kidneys are strongly involved in xenobiotic metabolism and excretion and are highly susceptible to chemical toxicity. Any impairment in renal function usually results in elevated levels of blood urea nitrogen (BUN), creatinine, and serum electrolytes.[5,30] The reductions in the levels of these parameters indicate that the extract caused no alterations to the kidneys or renal function of the rats.

Histopathology of the liver revealed no significant change in the tested groups administered 200, 400, and 600 mg/kg b.w ME-CH when compared to the normal control. This possibly demonstrates the toxicological safety of *Cola hispida* leaf at these doses. However, the presence of mild congestion of blood vessels and haemorrhage observed in the histopathology of rats treated with 800 mg/kg b.w of ME-CH possibly indicates a potential unfavorable effect on long-term administration of higher doses.

Conclusion

The methanol leaf extract of *Cola hispida* is relatively safe in rats up to the dose of 5000 mg/kg and may not cause any severe short term treatment-related toxicity. However, the toxicological effect of prolonged administration of higher doses still needs to be confirmed.

Conflict of Interest

The Authors declares no conflict of interest.

Authors' Declaration

The author hereby declares that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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