



Effects of Leaf Extract of *Cnidoscolus aconitifolius* on Serum Lipids and Oxidative Stress Markers of Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author Chibuike Obiandu designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author Ijeoma Ezebuoro and author Saronee Friday managed the analysis of the study. Author Adesua C. Obiandu managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: *Cnidoscolus aconitifolius* is considered to be an important and effective medicinal plant in folklore remedies where it has been applied as an alternative therapy for the treatment of various ailments.

Aim: The present study aims to determine the effects of *Cnidoscolus aconitifolius* on lipid profile and some oxidative stress markers of male Wistar rats.

Methodology: A total of 15 male wistar rats were procured for the study and randomly assigned into three groups of 5 rats each. Group 1 served as control and received distilled water only. Group 2 received 200 mg/kg and group 3 received 400mg/kg of the hydromethanolic (1:4) extract of

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Cnidoscopus aconitifolius which was administered as single daily dose using oral cannula. On completion of treatment, blood samples were collected by cardiac puncture for determination of some serum lipid parameters and oxidative stress markers.

Results: Results showed that there were no significant difference in the serum level of total cholesterol, triglyceride, low density lipoprotein cholesterol and high density lipoprotein cholesterol at both doses of the extract, compared to control. However, compared to control, there was a significant ($p < 0.05$) increase in the activity of superoxide dismutase and glutathione reductase but significant reduction in malondialdehyde level. The catalase enzyme activity was not significant.

Conclusion: The result obtained suggest that the extract may be useful in reducing oxidative stress by improving some antioxidant enzyme activities and may also prevent cell death due to lipid peroxidation.

Keywords: *Cnidoscopus aconitifolius*; hydromethanolic; antioxidant; lipid profile; Wistar rats.

1. INTRODUCTION

Diseases account for one half of all mortality in the tropical countries [1]. As a result, people of all continents have longed used poultice and imbibed infusions of indigenous plants products far back in history for the purposes of good health [2,3]. In addition, herbs, fruits and some vegetables have been appreciated as natural sources of various essential bioactive constituents [4], which could be due to the presence of important biologically active components including phenolic compounds, flavonoids, vitamins, anthocyanin, dietary fiber and carotenoids present in them [5].

The lipid profile is traditionally used as a screening tool in different populations to identify subjects with high risk of developing cardiovascular event. This is usually indicated by highly sensitive and specific positive lipid profile examination.

Antioxidants play important roles aimed to terminate chain reactions characterizing lipid peroxidation by removing the free radical and inhibiting other oxidation reactions [6]. The body's antioxidant mechanisms may not sufficiently neutralize all the free radical, thereby, increasing the need for intake of antioxidants to boost the system in the maintenance of health and prevention of diseases.

Cnidoscopus aconitifolius is a perennial shrub belonging to the family Euphorbiaceae. Propagation is normally by stem cuttings of about 6-12 inches long [7]. The edible parts of the plant, serve as important nutritional source of the macromolecules (protein, vitamin and minerals) [7,8]. Different parts of *Cnidoscopus aconitifolius* offers an array of medicinal applications including: the shoots and leaves which serves as

laxative, heretic and circulating stimulant to improve diabetes, digestion, stimulate lactation and harden fingernails; they have high fiber content and antibacterial activities [9]. There are anecdotal reports suggesting that *Cnidoscopus aconitifolius* may boost antioxidant activities and reduce the development of dyslipidemia. The aim of the present study is to evaluate the effects of *Cnidoscopus aconitifolius* on serum lipids and oxidative stress markers in Wistar rats.

2. MATERIALS AND METHODS

2.1 Plant Materials and Extraction

Fresh leaves of *Cnidoscopus aconitifolius* were obtained from the University of Port-Harcourt Botanical Garden and subsequently identified by the taxonomist in the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria. Voucher specimen of the plant was deposited in the herbarium. The leaves were air dried for a minimum of 2 weeks and extracted using soxhlet extractor. The dried leaves were pulverized with a grinding machine into pieces. Extraction was carried out in the percolation section of the extractor using hydromethanol (80% methanol) as solvent. The solution was filtered using Whatman filter paper. The filtrate was concentrated under reduced pressure in vacuum at 45°C using rotary evaporator. The extract yield was transferred to a hot oven where they were dried to a constant weight at 45°C and stored at 4°C. The extract was resuspended in distilled water before administration.

2.2 Experimental Animals

A total of 15 male wistar rats weighing between 100-250g were procured and housed at the animal house, Department of Human Physiology,

Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria under the laboratory conditions following National and Institutional guidelines for animal usage in experimental purposes. The animals were fed with normal rat pellet and tap water and allowed to acclimatize for 14 days.

2.3 Experimental Design

The 15 male rats were distributed into 3 groups of 5 rats each; as follows:

Group 1: Control group; animals in this group received distilled water only.

Group 2: Low dose group; rats in this group received 200mg/kg body weight of *Cnidoscopus aconitifolius*.

Group 3: High dose group; rats in this group received 400mg/kg body weight of *Cnidoscopus aconitifolius*.

Extract was administered daily using an oral cannula. On completion of treatment, the animals were made unconscious with chloroform inhalation (cotton wool soaked in 3.5% chloroform) and blood samples were collected by direct cardiac puncture using a 5-ml syringe attached to a needle (21 SWG); the blood was collected into appropriate sample bottles for the determination of some lipid parameters and oxidative stress markers.

2.4 Determination of Lipid Profile and Antioxidants

Serum lipid parameters were determined using Randox kits of Randox Biosciences, Randox Korea Ltd. 415, Heungan-daero, Dongan-gu,

Anyang-si, Gyeonggi-do, Republic of Korea. Serum total cholesterol was estimated according to the method described by Stein [10]. Serum triglycerides were determined using the method described by Chawla [11]. The low and high density lipoproteins (LDL-c/HDL-c) were evaluated according to the method described by Mire and Snow [12]. The concentration of Malondialdehyde (MDA) was calculated using the molar extinction coefficient of the chromospheres [13; determination of catalase activity was based on the splitting of hydrogen peroxide (H₂O₂) by the enzyme in the sample preparation which was then measured spectrophotometrically at 240nm as previously described [14]. The glutathione reductase (GSH) was measured by the method described by Tietz [15] and Akerboom & Sies [16].

2.5 Statistical Analysis

Results are expressed as mean \pm standard error of mean. Significant differences were determined using one way analysis of variance (ANOVA). A p-value of less than 0.05 was considered statistically significant.

3. RESULTS

The results for the study are presented in Tables 1-3.

Table 1 shows the result of *Cnidoscopus aconitifolius* administration on serum lipid parameters. There were no statistically significant ($p < 0.05$) differences in the level of serum total cholesterol, triglyceride, LDL-c, and HDL-c concentrations at both low and high doses of the extract compared to control.

Table 1. Effects of the leaf extract of *Cnidoscopus aconitifolius* on serum lipids

Groups/Extracts (mg/kg)	Serum lipid profile (mg/dl)			
	Total cholesterol	Triglyceride	LDL-c	HDL-c
Control	2.45 \pm 0.14	1.47 \pm 0.12	0.88 \pm 0.23	0.59 \pm 0.16
200	2.79 \pm 0.18	1.64 \pm 0.12	0.83 \pm 0.17	0.73 \pm 0.12
400	2.77 \pm 0.16	1.53 \pm 0.14	1.00 \pm 0.18	0.65 \pm 0.13

Values expressed as Mean \pm SEM. n=5

Table 2. Mean level of Glutathione and Superoxide dismutase activity

Groups/Extracts (mg/kg)	Oxidative stress markers			
	Glutathione (μ g/min/mg.protein)	% Change	SOD (Ug/mg.protein)	% Change
Control	0.96 \pm 0.06	0	0.44 \pm 0.06	0
200	1.10 \pm 0.07	14.58	0.46 \pm 0.09	4.55
400	1.48 \pm 0.21*	54.17	0.71 \pm 0.04*	61.36

Values expressed as mean \pm SEM. n=5. Significant at [$* (P < 0.05)$] when compared to control group

Table 3. Mean level of malondialdehyde and catalase activity

Groups/Extracts (mg/kg)	Oxidative stress markers			
	MDA (Umol/mg.protein)	% Change	Catalase (Units/mg.protein)	% Change
Control	0.51±0.08	0	1.63±0.40	0
200	0.52±0.09	1.96	2.06±0.16	26.38
400	0.22±0.02*	-56.86	2.31±0.32	41.92

Values expressed as Mean ± SEM. n=5. Significant at [* (P<0.05)] when compared to control group

Compared to control, there was a significant (p<0.05) increase in the activities of superoxide dismutase and glutathione reductase (Table 2); this significant increase was only observed in group 3 which received high dose (400mg/kg) of the plant extract.

There was a significant (p<0.05) reduction in the level of malondialdehyde (MDA) (Table 3); which was observed in the group treated with 400mg/kg of the extract. The catalase enzyme activity was not significantly affected at both extract doses.

4. DISCUSSION

The present study investigated the effects of the leaf extract of *Cnidoscopus aconitifolius* on serum lipid profile and some oxidative stress markers. Lipid parameters serve as a medical screening tool for lipid abnormalities and these parameters are usually considered in the evaluation of dyslipidemia with much emphasis on LDL-c which has been reported to be a bad lipoprotein [17,18]. There are incontrovertible reports that an increased LDL-c concentration is atherogenic whereas, a high HDL-c is cardioprotective [19,20,21]. In this study, there was no statistically significant (p<0.05) differences in the level of serum total cholesterol, triglyceride, LDL-c, and HDL-c concentrations at both low and high doses of the extract compared to control. This finding suggests that the leaf extract of *Cnidoscopus aconitifolius* may have no effect on serum lipids or its metabolism. The findings in the present study is at variance with the reported findings in some other previous studies [22,23,24]. The findings in these studies showed that the extracts of *Cnidoscopus aconitifolius* demonstrated a significant anti hypercholesterolemic and anti-hypertriglyceridemic potentials. These differences may be due to the higher dose (500mg/kg bw) of extract used, duration of administration and mode of extraction of plant materials.

There was a significant (p<0.05) increase in glutathione reductase and superoxide dismutase enzyme activities for the groups

treated with 400mg/kg of the extract, with a 54.17% and 61.36% increase respectively. A significant (p<0.05) reduction (-56.86%) in malondialdehyde was observed in group 3 treated with high dose of the extract. The result obtained in the present study is in agreement with the result in previous studies [24,25]; despite differences in the mode of plant extraction. The most prevalent phytochemicals in this plant are its phenolic compounds, and their antioxidant capacity is responsible for many of its health benefits [24,25]. Malondialdehyde is a by-product of lipid peroxidation. When lipid peroxidation becomes extensive, there would be a decrease in membrane fluidity which may result in cell death. This complication arise due to the peroxidation of unsaturated fatty acids and imbalance in the ratio of polyunsaturated to other fatty acids [26]. The ability of the leaf extract of *Cnidoscopus aconitifolius* to prevent lipid peroxidation may lead to a decrease in the build-up of free radicals minimizing its tendency to initiate cell damage. The observable changes in these enzymatic and non-enzymatic processes suggest that the extract of *Cnidoscopus aconitifolius* may be capable of boosting the antioxidant production in Wistar rats.

5. CONCLUSION

The leaf extract of *Cnidoscopus aconitifolius* was observed to have no significant effect on serum lipid concentrations. Furthermore, the extract exhibited antioxidant potential due to its ability to improve antioxidative enzyme activities and prevent the production of malondialdehyde which is a by-product of lipid peroxidation.

ETHICAL APPROVAL

The research protocol for the study was approved by the ethics committee of the institution. The present study was carried out in accordance with the guidelines for the care and use of laboratory animals issued by the United States Institute for Laboratory and Animal Research [27].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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