

Sub-chronic Effect of Methanol-Dichloromethane Stem Bark Extract of *Stemonocoleus micranthus* Harms. (Fabaceae) on Lipid Profile and Histology of Liver and Kidney of Rats

Florence N Mbaoji, Adaobi C Ezike, Chukwuemeka S Nworu, Collins A Onyeto, Bonaventure C Obi*, Theophine C Akunne, Chiamaka Okoli, Cynthia Okpalaeke and Peter A Akah

Department of pharmacology and toxicology, Faculty of pharmaceutical sciences, University of Nigeria, Nsukka, PMB 410001, Enugu state, Nigeria

*Corresponding author: Bonaventure Obi, Department of pharmacology and toxicology, Faculty of pharmaceutical sciences, University of Nigeria, Nsukka, PMB 410001, Enugu state, Nigeria, Tel: +2348035704228; E-mail: bonaventure.obi@unn.edu.ng

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Abstract

In Nigerian ethnomedicine, *Stemonocoleus micranthus* Harms (Fabaceae) is used in the management of heart related diseases. This study investigated the effect of sub-chronic administration of methanol-dichloromethane stem bark extract of *S. micranthus* (SME) on lipid profile and histology of liver and kidney of rats. Adult albino rats of both sexes were randomly divided into four groups (n=5) and received daily administration of SME (100, 200, and 400 mg/kg) and 2 ml/kg distilled water (control) respectively for 28 days per oral. Biochemical tests were performed pre-treatment and subsequently fortnightly as the treatment commenced. The results showed that the extract increased the serum high density lipoprotein cholesterol concentration while low density lipoprotein cholesterol, total cholesterol, triacylglycerol, and very low density lipoprotein cholesterol concentrations were reduced compared with the baseline values. However, the histopathological examination of the liver and kidney of the rats that received extract and solvent revealed normal portal area with bile ducts and hepatic artery as well as normal glomerulus and renal tubules without observable changes suggesting zero tendency of causing toxic effects. The preliminary phytochemical analysis showed that SME contained alkaloids, glycosides, flavonoids, reducing sugars, terpenoids, saponins, proteins and, fats oils. SME exhibited an oral LD₅₀ >5 g/kg in mice. These findings suggest that the stem bark extract of *S. micranthus* contains constituents that possess hypolipidemic activity in rats.

Keywords: *Stemonocoleus micranthus* extract (SME); Phytochemical screening; Lipid profile; Histopathology

Introduction

Atherosclerosis and coronary heart disease are the major health problems affecting both developed and developing countries [1,2]. Alterations in lipid metabolism may predispose to high levels of lipid in the system. Excessive storage of lipid causes cellular and tissue damage in the brain, peripheral nervous system, liver, spleen and bone marrow [3]. Many studies have shown that elevated concentration of low density lipoprotein (LDL) cholesterol and reduced high density lipoprotein (HDL) cholesterol level are powerful risk factors for atherosclerosis and coronary heart disease [4], whereas high concentration of high density lipoprotein (HDL) cholesterol or a low LDL to HDL cholesterol ratio may protect against coronary heart disease [5,6].

The use of herbs as medicine has played an important role in nearly every culture on earth [7]. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness. In Nigeria, herbal medicine is fast emerging as an alternative treatment for a wide range of ailments possibly, due to their lower costs, availability, fewer adverse effects and perceived effectiveness. Several Herbal extracts are often used to reduce high blood cholesterol concentrations; provide some protection against heart related diseases.

Stemonocoleus micranthus Harms (Fabaceae) is one of the wild African plants used for medicinal purposes. The plant is an ever green

tree endemic in West and Central Africa. The morphological characteristics of the plant have been described [8]. A bark decoction of the plant is used as treatment for a wide range of ailments including rheumatism, infertility in women, diarrhoea and dysentery, ulcers, hypertension, pyretic, emetic, helminthic and mental illnesses [8]. Additionally, it is well documented that the plant possessed analgesic, narrow spectrum antibacterial, CNS depression and local anaesthetic properties [9]. It is equally used in relieving pains, treating contaminated wound, enhancing conception and light construction work (non- medicinal uses) [10]. It has also been investigated to possess anti-ulcer [11], and antioxidant/hepatoprotective [12] activities among others. Hence in this study, we investigated the sub-chronic effect of the stem bark extract of *Stemonocoleus micranthus* Harms. (Fabaceae) on lipid profile and histology of liver and kidney of rats.

Materials and methods

Preparation of plant material

Fresh stem barks of *Stemonocoleus micranthus* were collected from a forest in Orba, Nsukka in April and May, 2016. The identity was established and authenticated at the International Centre for Ethnomedicine Drug Development (Inter-CEDD), Nsukka, Nigeria. The stem bark was carefully separated from the woody part, dried and then pulverized into fine powder. The powdered plant material (2 kg) was extracted with a 1:1 mixture of Methanol-dichloromethane by continuous extraction in a Soxhlet extractor. The resulting filtrate was concentrated under reduced pressure at a temperature of 40°C to obtain *S. micranthus* extract (SME) (106.28 g).

Preliminary phytochemical analysis

The test was carried out based on procedures outlined by Harborne [13]; Trease and Evans [14].

Experimental design

The study was performed on 20 healthy albino rats (150-200 g) of both sexes. The rats were purchased from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. All animal experiments were handled in compliance with the National Institute of Health Guidelines for Care and Use of Laboratory Animals (Pub No. 85-23, revised 1985), and in accordance with the University Ethical Committee on the use of Laboratory Animals. The animals were kept in standard laboratory conditions under a 12 h dark and 12 h light cycle, at 25°C, and were provided with a diet and water *ad libitum*. The rats were randomly divided into 4 groups: A, B, C, and D, 5 animals in each group. Groups A, B, C received SME at 100, 200, and 400 mg/kg BW p.o. once daily for 28 days and group D (control group) received distilled water (2 ml/kg BW p.o.).

Biochemical analysis

Blood samples were collected through ocular puncture thrice with the aid of non-heparinised capillary tube and transferred into clean sample bottles. The collected blood was allowed to clot and centrifuged at 3000 rpm for 10 min to obtain the serum component for further biochemical analysis. The blood was collected firstly at the beginning of the experiment (day 0) to assay for basal serum lipid levels. Subsequently, blood samples were recollected from the animals on days 14 and 28 post treatment to re-assay for serum lipid levels. The serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triacylglycerol (TAG) and very low density lipoprotein cholesterol (VLDL) concentrations were analyzed using the methods of Allain et al. [15], Albers et al. [16], Assman et al. [17]) and, Jacobs and Van Denmark [18] as contained in test kits by Quimica Clinica Aplicada (QCA), Spain.

Histopathological analysis

At the end of the experimental period, the kidneys and livers were obtained and fixed in 10% formalin solution for histological examination. The organs were each trimmed, processed, embedded in paraffin wax, sectioned, placed on glass microscope slides, and stained with hematoxylin and eosin (H.E.). The stained slides were viewed under the microscope in oil immersion with magnification of × 400.

Statistical analysis

Data obtained was analyzed using One-Way ANOVA and further subjected to Duncan post hoc tests and presented as Mean ± SEM. Differences between means were accepted significant at p<0.05.

Results and discussion

Hypolipidemic properties have been confirmed in many plant species and plant products in medicinal use [19,20]. As a result, there is need for continued search for such plants with hypolipidemic properties as their active phytoconstituents could make tremendous ameliorative impact in global health burden of dyslipidemia. In Nigerian ethnomedicine, documented and undocumented traditional evidence abounds of the use of *Stemonocoleus micranthus* Harms (Fabaceae) in the management of heart related diseases. The study therefore, investigated the sub-chronic effect of the stem bark extract of *Stemonocoleus micranthus* in an animal model.

Besides carrying out a qualitative phytochemical analysis of the plant extract, the changes in the lipid profile markers (TC, HDL, LDL, TAG and VLDL) of the different animal groups were observed as part of the design for this study, as well as the histology of the liver and kidney of the different animal groups. In Table 1, The TC concentration was not significantly (p>0.05) different as compared with the control group. Treatment with 400 mg/kg SME produced significant (p<0.05) reduction in the levels of serum TC on day 28 compared with the control group and day 0 values, respectively. However, relative to day 0 values, the changes (%) observed on days 14 and 28 in TC concentration for SME (100, 200, 400 mg/kg) were 29.10, 6.20, 15.90% and 6.90, 5.20, 24.50% respectively (Table 1).

Group	Treatment	Dose (mg/kg)	Day 0	Day 14	Day 28	Change (%) (Day 14)	Change (%) (Day 28)
A	SME	100	70.60 ± 5.37	91.20 ± 3.68	75.49 ± 3.66	-29.10	-6.90
B	-	200	80.94 ± 4.58	85.40 ± 7.04	76.74 ± 5.58	-6.20	5.20
C	-	400	81.42 ± 5.56	94.39 ± 5.54	61.48 ± 4.24*#	-15.90	24.50
D	Solvent	-	84.38 ± 5.45	84.56 ± 3.54	80.77 ± 4.48	-0.21	4.27

Values are mean ± SEM, n = 5 per group; *, #: p<0.05 compared with control and day 0 values respectively (One-Way Anova; Duncan test post hoc) SME - *Stemonocoleus micranthus* extract, solvent- distilled water. Change (%) calculated relative to day 0 values.

Table 1: Effect of SME on serum total cholesterol concentration.

Group	Treatment	Dose (mg/kg)	Day 0	Day 14	Day 28	Change (%) (Day 14)	Change (%) (Day 28)
A	SME	100	30.62 ± 2.84	46.42 ± 3.28	49.21 ± 5.55	-34.04	-37.78
B	-	200	32.88 ± 2.39	45.60 ± 2.29	49.47 ± 5.13	-24.17	-33.54

C	-	400	30.79 ± 4.39	40.79 ± 3.49	34.81 ± 4.58	-32.48	-11.55
D	Solvent	-	32.20 ± 3.87	40.79 ± 3.49	51.37 ± 3.68	-21.06	-37.32

Values are mean ± SEM, n=5 per group; *, #: p<0.05 compared with control and day 0 values respectively (One Way Anova; Duncan test post hoc). SME- *Stemonocoleus micranthus* extract, solvent- distilled water. Change (%) calculated relative to day 0 values.

Table 2: Effect of SME on serum HDL-cholesterol concentration.

In Tables 1 and 2, the changes in HDL-C and LDL-C of animals treated with the stem bark extract of *Stemonocoleus micranthus* were compared to those of the control group, as well as the baseline values (Day 0). No significant (p > 0.05) changes occurred in HDL-C and LDL-C measured compared with the control group. However,

compared to baseline values, the percentage change in HDL-C was 34.04, 24.17, 32.48% and 37.78, 33.54, 11.55% while LDL-C was 34.40, 12.30, 16.10% and 14.70, 29.90, 36.20% at days 14 and 28, respectively (Tables 2 and 3).

Group	Treatment	Dose (mg/kg)	Day 0	Day 14	Day 28	Change (%) (Day 14)	Change (%) (Day 28)
A	SME	100	22.70 ± 5.08	30.52 ± 2.99	19.36 ± 3.48	-34.40	14.70
B	-	200	27.69 ± 2.72	31.09 ± 5.89	19.40 ± 4.19	-12.30	29.90
C	-	400	29.26 ± 3.97	33.98 ± 6.19	18.67 ± 1.65	-16.10	36.20
D	Solvent	-	35.42 ± 4.65	30.13 ± 0.78	18.68 ± 6.89	14.90	47.30

Values are mean ± SEM, n = 5 per group; p<0.05 compared with control and day values respectively (One-Way Anova; Duncan test post hoc). SME- *Stemonocoleus micranthus* extract, solvent- distilled water. Change (%) calculated relative to day 0 values.

Table 3: Effect of SME on serum LDL-cholesterol concentration.

The result also showed that the serum concentrations of TAG and VLDL did not differ significantly (p>0.05) on day 14 post treatment as compared with the control and baseline values, respectively. However, on day 28 post treatment, SME (100, 200 and 400 mg/kg) was found to significantly (p<0.05) reduce serum concentrations of TAG and VLDL

compared to baseline values. The percentage change in TAG was 17.70, 46.20, 30.70% and 57.60, 61.40, 62.60% while VLDL was 18.30, 54.50, 31.10% and 60.20, 67.30, 62.80% at days 14 and 28 respectively (Tables 4 and 5).

Group	Treatment	Dose (mg/kg)	Day 0	Day 14	Day 28	Change (%) (Day 14)	Change (%) (Day 28)
A	SME	100	86.38 ± 7.76	71.11 ± 7.10	34.64 ± 2.24 [#]	17.70	57.60
B	-	200	101.80 ± 16.97	54.73 ± 7.57	39.33 ± 3.46 [#]	46.20	61.40
C	-	400	106.84 ± 12.72	74.07 ± 16.51	39.97 ± 3.05 [#]	30.70	62.60
D	Solvent	-	83.99 ± 13.35	68.19 ± 3.03	53.63 ± 4.16	18.80	36.10

Values are mean ± SEM, n = 5 per group; *, #: p<0.05 compared with control and base line values respectively (One-Way Anova; Duncan test post hoc). SME- *Stemonocoleus micranthus* extract, solvent- distilled water. Change (%) calculated relative to day 0 values.

Table 4: Effect of SME on serum triacylglycerol concentration.

The result showed that the extract at the different doses administered reduced the lipid profile markers (total cholesterol, triacylglycerol, LDL, VLDL) with concomitant increase in the HDL cholesterol concentration. It is well known that alterations in serum lipid profiles contribute to development of coronary heart disease [21]. Therefore, a reduction in serum lipids, particularly LDL and VLDL fractions and triacylglycerol concentration should be considered as beneficial in maintaining the normal physiological levels of these lipids thereby reducing the risk of dyslipidemia.

Moreover, it was suggested that HDL may play a protective role in atherogenesis by preventing the generation of an oxidative modified LDL. The mechanism of action of HDL may involve exchange of lipid peroxidation product between the lipoproteins [22] and its cardioprotective properties which is achieved by protecting LDL from oxidative modifications [23]. However, the mechanism by which *Stemonocoleus micranthus* extract exhibit its hypolipidemic role could be attributed to an increased cholesterol excretion and decreased cholesterol absorption through the gastro intestinal tract or as a result of rapid catabolism of LDL-cholesterol by the liver for final elimination

in form of bile acids [24]. The extract seemed to be more effective in reducing triacylglycerol, total cholesterol and VLDL; and increasing HDL than in reducing LDL in normoglycemic condition. Hyperlipidemia associated with lipid disorders are considered to cause

atherosclerotic cardiovascular disease [25]. Interestingly, the plant extract may be highly beneficial in preventing the development of atherosclerotic plaque formation considering its lipid lowering potentials especially in normal rats.

Group	Treatment	Dose (mg/kg)	Day 0	Day 14	Day 28	Change (%) (Day 14)	Change (%) (Day 28)
A	SME	100	17.40 ± 1.52	14.22 ± 1.51	6.93 ± 0.45 [#]	18.30	60.20
B	-	200	24.03 ± 2.83	10.94 ± 1.51	7.86 ± 0.69 [#]	54.50	67.30
C	-	400	21.50 ± 2.63	14.81 ± 3.30	7.99 ± 0.61 [#]	31.10	62.80
D	Solvent	-	23.44 ± 4.81	13.64 ± 0.61	10.73 ± 0.83	41.80	54.20

Values are mean ± SEM, n = 5 per group; *, #: p<0.05 compared with control and base line values respectively (One-Way ANOVA; Duncan test post hoc). SME - *Stemonocoleus micranthus* extract, solvent - distilled water. Change (%) calculated relative to day 0 values.

Table 5: Effect of SME on serum VLDL-cholesterol concentration.

Constituents	Relative abundance of constituents
Alkaloids	+
Carbohydrates	++++
Fats and oils	++
Flavonoids	++++
Glycosides	++++
Proteins	+++
Reducing sugars	+++
Resins	-
Saponin	++
Steroids	++
Tannins	++++
Terpenoids	+

Key: SME: *Stemonocoleus micranthus* extract; - = absent, + = present in small concentration; ++ present in moderately high concentration; +++ = present in very high concentration; ++++ = abundantly present.

Table 6: Phytochemical analysis.

The phytochemical analysis on the plant extract revealed that the extract tested positive to alkaloids, carbohydrates, fats and oil, flavonoids, glycosides, proteins, reducing sugars (Table 6). It equally gave positive reaction for saponins, steroids, tannins, and terpenoids. Several reports have documented the antiatherosclerotic effects of phenolic compounds (such as flavonoids, terpenoids tannins and saponins [26,27]). It is most likely that the hypolipidemic effect of this plant stem bark extract may largely be due to combined effects of carbohydrates, flavonoids, glycosides, protein, reducing sugars and tannins or may be ascribed to a single constituent.

The microscopic examination revealed that there were no significant changes in morphological or pathological lesions in the kidney (Figure 1) and liver (Figure 2) tissues of rats treated with various doses of *Stemonocoleus micranthus* extract compared with the control group. The organs had normal cellular architecture even in animals that

received higher doses of the extract, suggesting the safety of the extract at the tissue level. This could be attributed to its active principles being nephroprotective and hepatoprotective.

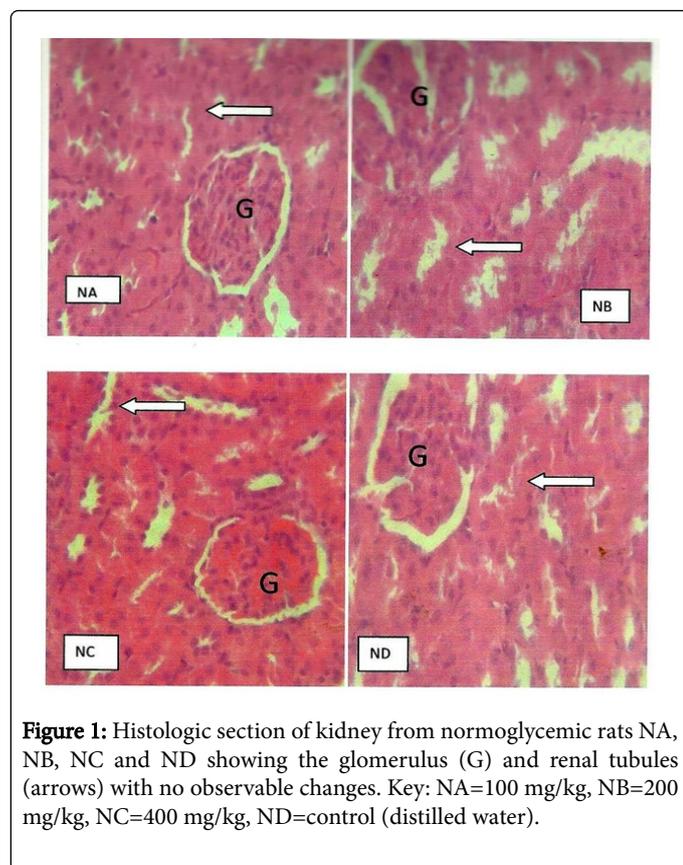


Figure 1: Histologic section of kidney from normoglycemic rats NA, NB, NC and ND showing the glomerulus (G) and renal tubules (arrows) with no observable changes. Key: NA=100 mg/kg, NB=200 mg/kg, NC=400 mg/kg, ND=control (distilled water).

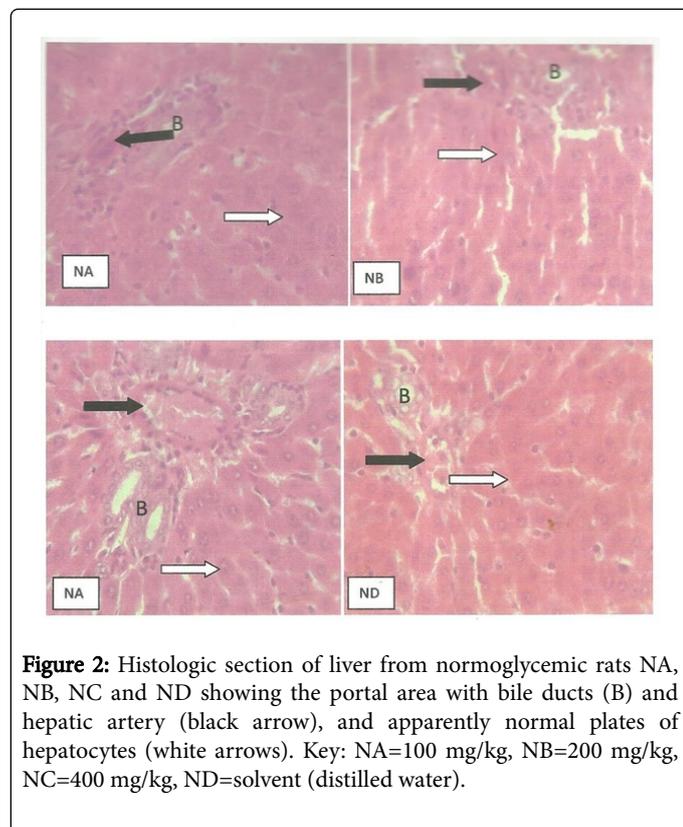


Figure 2: Histologic section of liver from normoglycemic rats NA, NB, NC and ND showing the portal area with bile ducts (B) and hepatic artery (black arrow), and apparently normal plates of hepatocytes (white arrows). Key: NA=100 mg/kg, NB=200 mg/kg, NC=400 mg/kg, ND=solvent (distilled water).

Conclusion

The results of the present study have shown that the stem bark extract of *Stemonocoleus micranthus* possesses hypolipidemic activity in rats. This activity is largely attributed to the combined effects of constituents present in the extract. Further studies on the isolation and characterization of the active phytoconstituents are ongoing.

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Authors Contribution

All authors contributed equally in designing, performing the experiment and writing up of the research work. All authors read and approved the work.

Conflict of Interest

The authors have declared that no competing interests exist.

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