An Evaluation of the Anti-Atherogenic Property of *Ageratum conyzoides* against Nigerian Bonnylight Crude Oil-Induced Dyslipidaemia in Female Rats

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ABSTRACT

This study was designed to evaluate the ameliorating efficiency of the ethanol leaf-extract of Ageratum conyzoides on Nigerian Bonnylight crude oil-induced dyslipidemia. Twenty female Wistar rats (120-150g body weight) were divided into four groups of five rats each. The rats in group I served as the control group and were oral gavaged 3ml/kg body weight of normal saline; group II gavaged 748.33mg/kg body weight of the extract of A. conyzoides, which was 20% of the LD_{50} (3741.66mg/kg). Group III was oral gavaged 3 ml/kg body weight of NBLCO. This dose was calculated as 20% of the lethal dose (LD_{50}) of 14.14 ml/kg, while group IV animals were gavaged 748.33mg/kg body weight of the extract of A. conyzoides, and 3ml/kg body weight of Nigerian Bonnylight crude oil (NBLCO). In all cases, doses were applied daily for 31 days according to animal's most recent body weight.The results showed that while the NBLCO administration to group III animals significantly reduced high density lipoprotein (HDL-C), it significantly increase levels of total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), total lipid (TL) an atherogenic index (AI) compared with control and extract groups (I and II) ($p<0.05$). In group IV that A. conyzoides was co-administered with NBLCO, there was a significant increase in HDL-C level, while TC, TG, LDL-C, VLDL-C, TL, and AI were all significantly reduced ($p<0.05$). It is evidently demonstrated in this study that NBLCO administration induced dyslipidaemia by increasing the atherogenic indices with concomitant reduction in HDL-C levels that were ameliorated by co-administration of ethanol leaf extract of Ageratum conyzoides.

Keywords: Ageratum conyzoides, Nigerian Bonnylight crude oil, lipid profile, atherogenic index, cardiovascular disorder

INTRODUCTION

The bioaccumulation of petroleum hydrocarbon which may be due to unrestricted exposure could alter the physiology and biochemical activities of tissues and vital organs in the body resulting in disease conditions such as atherosclerosis. Atherosclerosis by the way is a condition associated with dyslipidemia[1] directly linked with lipid peroxidation and micro-inflammation,[2,3] where cholesterol fractions distribution play prominent role. Cholesterol fractions are very important factors in the development of cardiovascular disease (CVD), thus the ratio of triglyceride, LDL and HDL are of immense importance. There is supportive evidence in literature that crude petroleum has atherogenic dyslipidemic tendency in mammals. The synergy between LDL-C, particularly oxidized LDL with some fractions of WBC in the phase of inadequate HDL can be harmful to the micro-structure of wall of arteries. This is because when LDL-C particles get oxidized, the endothelial cells respond by attracting monocytes and causing them to leave the bloodstream and enter the artery where they developed into macrophages. The macrophages in turn ingest oxidized LDL particles to triggers a cascade of immune responses leading to accumulation of fat which thicken the wall of artery. This can in turn result in the development of atherosclerosis, considered the leading cause of death globally.[4] The major known risk factors of cardiovascular disorders are unnecessarily high LDL cholesterol and concomitant inadequate HDL cholesterol, hypertension and non-insulin dependent diabetes mellitus.[5] So protocol aimed at lowering serum LDL cholesterol and enhancing HDL cholesterol fractions is considered a strategy that can delay the on-set of hyperlipidemic disorders.[6] Considering the important of medicinal plants which have continued to provide valuable therapeutic agents in traditional and modern medicine practices, *Ageratum conyzoides,* an herbal plant with enormous beneficial effects is

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one of such herbal plants. This common annual herbaceous weed with history of traditional medicinal benefits in folk medicine in many countries including Nigeria is used as purgative, analgesic,[7] to cure wounds and burns,[8] treatment of high blood pressure, fever, diabetes, pneumonia and numerous infectious diseases,[9] as a blood booster[10] and hepato-protective effect.[11] The present study therefore focused on the possibility of using ethanol leaf extract of *Ageratum conyzoides* to improve dyslipidemic effect of NBLCO with a view to preventing associated cardiovascular disorders in rats.

MATERIALS AND METHODS

Crude oil

The crude petroleum used in this study was obtained from the Exxon Mobil laboratory, Ibeno, Nigeria.

Collection of plant material

The whole plant was obtained from the Botanical farm of the Department of Pharmacognosy and Natural Medicine, University of Uyo, Uyo, Nigeria. Specimen of the leaves was authenticated in the Department of Botany and Ecological Studies, University of Uyo, Uyo. A voucher specimen (UUH 3517) was deposited at the Herbarium.

Preparation of leave extract

The leaves of *Ageratum conyzoides* were rinsed with distilled water and dried under shade. The dried leaves were ground into powder with an electric blender. Four hundred grammes of the blended leaves sample was macerated in 700ml 70% ethanol, agitated for 10 minutes with an electric blender and left overnight in a refrigerator at 4°C. The mixture was filtered with a cheese cloth and the filtrate obtained concentrated under reduced pressure using a rotary evaporator (at 37°C) to about 10% of its original volume. The concentrate was then allowed in a water bath at 37°C for complete evaporation to dryness yielding 40.64g (10.15%) of the extract.

Acute toxicity test

Acute toxicity study (LD_{50}) was estimated using Lorke's method.[12] A total of 25 mice weighing between 15-22g were divided into five groups with five mice per group. Mice in the five groups were administered 3000mg/kg, 3500mg/kg, 4000mg/kg, 4500mg/kg and 5000ml/kg of body weight respectively (intraperitoneally). All experimental animals were observed for physical signs of toxicity such as gasping, palpitation, writhing, decreased respiratory rate, body limb and death after 24 hours.

The median lethal dose of *Ageratum conyzoides* was calculated as geometrical means of the maximum (most tolerable) dose producing 0% mortality (a) and the minimum (least tolerable) dose producing 100% mortality (b) using the formula:

$$LD_{50} = \sqrt{ab}$$
$$LD_{50} = \sqrt{3500 \times 4000}$$
$$= 3741.66 mg/kg$$

The acute toxicity test for the NBLCO also involved 25 mice weighing between 15-22g were divided into five groups with five mice per group. Mice in the five groups were administered intraperitoneally 10ml/kg, 15ml/kg, 20ml/kg, 25ml/kg and 30ml/kg of body weight respectively.

$$LD_{50} = \sqrt{10 \times 20}$$
$$= 14.14 ml/kg$$

Experimental design and treatment of animals

Female Albino Wistar rats weighing between 120-150g were obtained from the Animal House of the Faculty of Basic Medical Sciences University of Uyo, Uyo, Nigeria and were kept in a well-ventilated section of the Animal House. They were allowed access to feed (Chow: vital feeds, Grand Cereals Ltd, Jos) and water *adlibitum*. The animals were kept in separate experimental room and allowed to acclimatize for a period of one week before commencement of studies. These rats were randomly divided into four groups (group I, II, III and IV) of five (5) rats each. Group I served as the control and was oral gavaged 3 ml/kg body weight of normal saline. Group II was oral gavaged 748.33mg/kg body weight of ethanol leaf extract of *Ageratum conyzoides*, this dose was calculated as 20% of the lethal dose (LD_{50}) of

3741.66 mg/kg. Group III was oral gavaged 3 ml/kg body weight of NBLCO. This dose was calculated as 20% of the lethal dose (LD_{50}) of 14.14 ml/kg, while group IV in addition to 3 ml/kg body weight of NBLCO, were supplemented with 748.33 mg/kg body weight of ethanol leaf extract of *Ageratum conyzoides*. In all cases, the doses were based on the rat's most recently recorded body weight. The calculated volume in milliliter (ml) was applied daily for thirty one (31) days. The experimental procedures involving the animals and their care were conducted in conformity with the approved guidelines by the Research and Ethical Committee of the Faculty of Basic Medical Sciences, University of Uyo, Uyo. Nigeria.

Collection of blood sample for analysis

After the thirty one (31) days of administration, the rats were anaesthetized with chloroform soaked in swap of cotton wool in a killing chamber. Blood was collected by cardiac puncture with a 5ml sterile syringe and needle. The total volume of blood collected was 4ml, which was transferred into plain sample bottles. This was allowed to stand for 2 hours to clot after which the serum was separated by centrifugation (RM-12 micro centrifuge, REMI, England) at 4000 rpm for 10 minutes. The serum obtained was stored at -20^{o}C until required for analysis.

Lipid profile test

The lipid components such as total cholesterol were estimated using standard kits from Randox (USA), while triglyceride, HDL-C and LDL-C were estimated in serum using standard kits supplied by Randox (UK), Dialab (France) and Sunlong Biotech co limited (China) respectively.

The rat total lipid assay was determined spectrophotometrically using standard kits from Randox (UK) according to the method of Fringsand Dunn (1970).[13]

Calculation of very low density lipoprotein (VLDL)

The value of VLDL was obtained mathematically using the formula as described by Friedewald *et al*.[14].

$$\text{VLDL (mg/dL)} = \frac{\text{TG (mg/dL)}}{5}$$

Atherogenic index (AI) was calculated as the ratio of serum levels of cholesterol to serum levels HDL-cholesterol.

AI = Total cholesterol/HDL-C

Statistical analysis

Data were expressed as the mean $\pm$ standard error of the mean. Statistical analysis was carried out using window SPSS package (SPSS 22.00 version). Data were analyzed using one way analysis of variance (ANOVA), results obtained were further subjected to test for least significant difference (LSD). Values of $P<0.05$ were considered significant.

RESULTS

The results of the lipid profile obtained after 31 days administration are shown in Table 1. The administration of the leaf extract of *Ageratum conyzoides* to group II did not alter total cholesterol, LDL-C, total lipid and atherogenic index significantly compared with group I (control), but significantly increased triglyceride, HDL-C and VLDL-C compared with the control group ($p<0.05$). The administration of Nigerian Bonnylight crude oil to group III animals significantly increased the total cholesterol, triglyceride, LDL-C, VLDL-C, total lipid and the atherogenic index, but significantly reduced HDL-C. The co-administration of extract of *Ageratum conyzoides* to group IV animals caused significant reductions in total cholesterol, triglyceride, LDL-C, VLDL-C, total lipid and the atherogenic index, while it significantly increased HDL-C comparatively ($p<0.05$). The co-administration of *Ageratum conyzoides* with NBLCO to group IV rats did not alter LDL-C significantly compared with NBLCO group III.

Table 1: Comparison of lipid profile and the atherogenic index in rats following exposure to NBLCO and ethanolic leaf extract of *Ageratum conyzoides* for 31 days.

Groups	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	Total Lipid (mg/dL)	Atherogenic index
I	30.50 ± 1.48	25.67 ± 1.82	26.17 ± 2.96	21.83 ± 1.97	5.03 ± 0.35	59.67 ± 3.43	1.02 ± 0.10
II	37.83 ± 3.30	37.50 ± 1.72[a]	33.33 ± 2.12[a]	29.33 ± 1.69	7.50 ± 0.35[a]	67.00 ± 2.48	1.13 ± 0.02
III	50.83 ± 4.41[a,b]	56.50 ± 2.23[a,b]	19.50 ± 0.76[a,b]	44.63 ± 2.12[a,b]	11.30 ± 0.45[a,b]	142.83 ± 9.45[a,b]	2.62 ± 0.08[a,b]
IV	46.00 ± 2.25[a,c]	40.00 ± 1.93[a,c]	52.33 ± 1.93[a,b,c]	44.33 ± 1.02[a,b]	0.80 ± 0.39[a,c]	109.33 ± 8.27[a,b,c]	0.78 ± 0.06[a,b,c]

Legend:
a = significantly different from group I ($p<0.05$)
b = significantly different from group II ($p<0.05$)
c = significantly different from group III ($p<0.05$)

DISCUSSION

This study was designed to evaluate the ameliorating efficiency of the ethanol leaf extract of *Ageratum conyzoides* on Nigerian Bonny light crude oil-induced dyslipidaemia. The oral administration of Nigerian Bonny light crude oil (NBLCO) to the rats significantly elevated the serum concentrations of total cholesterol, triglyceride (TG), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), total lipid, and the atherogenic index (AI), while it significantly reduced high density lipoprotein cholesterol (HDL-C) of female Wistar rats. These results with particular reference to TG and VLDL-C are contrary to similar reports on male Wistar rats, where NBLCO was reported to have significantly reduced TG and VLDL-C.[15,16] This finding agrees with documented evidence that continuous exposure to crude oil by ingestion, inhalation and skin contact causes increase in lipid profile,[17] and consequently increase atherogenic index[15,16] which could expose the rats to the risk of developing cardiovascular disorder. It has been reported that an increase in TC, LDL-C and concomitant HDL-C reduction are predisposing risk factors for the development of cardiovascular disorder[15,16] such as atherosclerosis among others.[18] The toxicant present in crude oil could probably have induced inflammation; this can in turn accelerate atherogenic activities including recruitment of macrophages, cytokines and other cells.[19] High concentration of LDL-C in the plasma with corresponding low HDL-C concentration exposes LDL-C to oxidation process, this in turn raises the atherogenic potential of the body.[20] Atherogenic index could be used to diagnose the atherosclerotic risk which is estimated by the ratio of TG to HDL.[21] The co-administration of *Ageratum conyzoides* with NBLCO significantly reversed the results including atherogenic index indicating its possible usefulness in management of associated cardiovascular disease of dyslipidaemia origin.

It appears the efficiency with which the crude ethanol leaf extract of *Ageratum conyzoides* exert the significant reversal in the levels of the aforementioned lipoproteins and the atherogenic index can be attributed to its anti-oxidative properties.[22] Substances with efficient anti-oxidative tendencies can effectively mitigate oxidant activities by reducing the energy of the already formed oxidant radicals to stop further production of free radicals[23] in a cascading fashion. Although the anti-oxidative properties of the medicinal plant was not investigated in this present study, but information available in literature indicates that the medicinal plant discussed is very rich in antioxidants[22] and could exert its ameliorative effects through this

mechanism. The medicinal efficacy of *Ageratum conyzoides* is associated with its many bioactive chemical compounds which have enhanced its value in medicinal field, it is reported that these chemical compounds constitute the secondary metabolites, which are responsible for most pharmacological activities of the plant.[23] Other than the antioxidant property of the plant, the metabolite, saponins in particular prevent excessive intestinal cholesterol uptake and thus reduce the risk of cardiovascular diseases such as hypertension.[24]

CONCLUSION

It is evidently demonstrated in this study that NBLCO administration induced dyslipidaemia by increasing the atherogenic indices with concomitant reduction in HDL-C levels that were ameliorated by co-administration of ethanol leaf extract of *Ageratum conyzoides*.

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