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Research Article
Protective Effect of Ascorbic Acid Against Bonny Light Crude Oil Induced Atherosclerosis in Rats

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Abstract

Background and Objective: Crude oil has been reported to cause various toxic effects in the body. And vitamins C and E are among the antioxidants known to play important role in attenuating chemical toxicants-induced toxicities in the biological systems. This study aimed at assessing the protective effect of vitamin C against crude oil induced atherosclerosis in rats. **Materials and Methods:** Twenty four male albino Wistar rats, weighing 150-180 g, used in this study were distributed into four groups (1, 2, 3 and 4), with 6 rats each. Group 3 animals were orally administered 60 mg of NBLCO kg^{-1} b.wt., while group 4 animals were orally administered 60 mg NBLCO kg^{-1} b.wt. and co-administered 200.0 mg of vitamin C kg^{-1} b.wt., of the rats daily for 30 days. The animals in groups 1 and 2 served as the controls receiving only distilled water and vitamin C, respectively. At end of the experimental period, the animals were sacrificed and blood samples collected through cardiac puncture for plasma lipid profile analyses. Total plasma cholesterol (TC), triacylglycerol (TG), low density lipoprotein (LDL), very low density lipoprotein (VLDL), high density lipoprotein (HDL) levels and atherogenic indices were determined in rats orally exposed to Nigerian Bonny light crude oil (NBLCO) and co-administered vitamin C. **Results:** The results of the study showed that exposure to crude oil is a risk factor for atherosclerosis by producing a significant ($p<0.05$) increase in plasma TC, TG, LDL and VLDL levels and the evaluated atherogenic indices (Group 3), compared to the control (Group 1). The plasma TC, TG, LDL, VLDL levels and the atherogenic indices recorded for rats exposed to NBLCO and co-administered vitamin C (Group 4) were significantly ($p<0.05$) lower, compared to the rats exposed to NBLCO only (Group 3). **Conclusion:** This study demonstrated that vitamin C is capable of providing protection against crude oil induced atherosclerotic conditions by regulating serum lipids profiles and atherogenic indices in rats. It may be concluded that vitamin C is potent in preventing cardiovascular disorders associated with crude oil induced atherosclerotic conditions.

Key words: Crude oil, plasma lipids, atherogenic indices, atherosclerosis, albino Wistar rats

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Crude oil is known the major source of revenue in most of the oil producing areas. Pollution from crude oil is one of the major environmental problems in most of these oil producing areas, including Nigeria[1-4]. Environmental pollution accruing from crude oil may pose significant biochemical and physiological stress and dysfunction on animals, humans and plants in the area. According to Ali and Mai[5], some of the chemical components of crude oil that are absorbed into the living cells, on exposure, are metabolized into such metabolites that may be reactive. It was earlier reported that such compounds like xylene, naphthalene, benzene and toluene are among the toxic components that are present in crude oil. Saeed and Al-Mutairi[6] also reported that the water-soluble fraction of crude oil and their metabolic derivatives/products contain a mixture of polycyclic aromatic and monoaromatic hydrocarbons such as benzene, toluene, ethylbenzene and xylenes, phenols and heterocyclic compounds containing nitrogen and sulfur.

Generally, most of the chemical constituents of crude oil are known to be lipophilic and the toxicity of a petroleum fraction have been reported to be related to its hydrophobicity[7]. It has been demonstrated that some petroleum derived hydrocarbons express their toxic by preferentially accumulating in lipid-rich compartments like cellular membrane and disturb various physicochemical and physiological membrane properties in a wide marine animals[8,9]. These imply that lipid solubility is an important factor in the absorption of petroleum components through the plasma membrane of cells, as well as the degree of membrane disruption. According to Patrick-Iwuanyanwu *et al*.[10], ingestion of water-soluble fraction of Bonny Light crude oil caused hepatotoxicity in rats, using conventional indicators of liver injury. In the earlier studies, atherogenic implication was reported in male and female rats following exposure to gasoline vapour[11-13]. Also, Sese *et al*.[14] reported increased serum total cholesterol in rabbits fed with crude oil contaminated feeds.

Increased serum LDL cholesterol and decreased HDL cholesterol levels are known to be important independent risk factors for the development of atherosclerosis and cardiovascular diseases[15]. Hence, any treatment mechanism that is capable of reducing serum LDL cholesterol and elevates HDL cholesterol levels is considered to play important role in the prevention of atherosclerosis and treatment of cardiovascular diseases. In our previous, vitamins A and E were reported to provide protective effect against gasoline vapour induced atherosclerosis in rats[13]. Information on whether vitamin C is capable of providing protection against crude oil induced atherosclerosis is grossly inadequate and it is therefore a subject for research investigation. However, there are several reports on the hepatoprotective effect vitamin C against various chemical induced toxicities[16,17]. Also, our previous studies have earlier reported the protective potential of vitamin C against gasoline induced alterations in sex hormonal levels in rats[13,18,19].

Vitamin C is one of the widely distributed vitamins in most of the tissues in the body. However, higher levels of vitamin C are found in the adrenal glands, pituitary and retina, while lower levels are found in the kidney and muscle tissues. Vitamin C is hydrophilic in nature and is one of the important known free radical scavenger in extracellular fluids. It scavenges radicals, thereby protecting the biomembranes from peroxide damages. It has also been reported that vitamin C is an effective scavenger of singlet oxygen, superoxide, hydroxyl, water soluble peroxyl radical and hypochlorous acid[20]. According to Bendich[21], vitamin C, as an excellent source of electrons can donate electrons to several free radicals thereby quenching their activities. The protective property of vitamin C against chemical agents induced toxicities is therefore likely to be said to be associated with it antioxidant property[22,23]. This study assessed the protective potential of vitamin C against Bonny light crude oil induced atherosclerosis in rat model.

MATERIALS AND METHODS

Animals handling: The study was conducted within one month; between September to October, 2017. Twenty four albino Wistar rats weighing 150-180 g were obtained from the animal house of the Department of Biochemistry, University of Calabar, Calabar, Nigeria and used for this study. The rats were housed in well-ventilated plastic bottom and wire mesh top cages (North Kent Co. Ltd.) maintained at normal prevailing tropical room temperature, on a 12 h light-dark cycle and allowed free access to standard rat chow (Guinea Feeds Product) purchased from the High Quality Livestock Feeds stores, Calabar, Nigeria and tap water *ad libitum*. They were acclimatized for 2 weeks before the commencement of experimental administration. The rats were randomly distributed into four groups (1, 2, 3 and 4), with 6 rats each. Group 3 animals were orally administered 60 mg of NBLCO kg^{-1} b.wt., while group 4 animals were orally administered 60 mg NBLCO kg^{-1} b.wt. and co-administered 200.0 mg of vitamin C kg^{-1} b.wt., of the rats daily for 30 days. The animals in groups 1 and 2 served as the controls receiving distilled water and vitamin C, respectively, only. Vitamin C and

NBLCO were suspended in water as vehicle for administration. All the procedures involving care and handling of the animals were performed in accordance with the guidelines of the Animal Ethics Committee of the Institution and the recommendations of the National Institutes of Health (NIH).

Collection and analysis of blood: All the animals were anaesthetized with chloroform vapour, 24 h after last day of experimental treatments and dissected for blood collection. Blood samples were collected by cardiac puncture into a set of EDTA sample bottles and spun in a bench top centrifuge (MSE, England) to obtain the plasma. The plasma samples were separated into a set of plain sample tubes and were stored in a refrigerator until required for the assay of lipid profile. All assays were done within 36 h of the sample collection. Reagents kits obtained from the Randox Chemical Company, UK were used for lipid profile assays.

The "high performance" enzymatic colorimetric, CHOD-PAP method described by Richmond[24] was used to determine total plasma cholesterol. The principle is based on the proportionate formation of hydrogen peroxide (following oxidation of free cholesterol), which is quantified when treated with chromogem, 4-aminoantipyrine. A coloured compound whose intensity was in proportion to the evolved peroxide and thus total cholesterol concentration in the sample, was formed and assayed spectrophotometrically, using HAICH, DR3000, Germany, model of spectrophotometer.

Plasma triacylglycerols were estimated by the modified enzymatic colorimetric test, according to the glycerol phosphate oxidase (GPO) method described by Trinider[25]. It is based on the action of L-d-glycerol phosphate oxidase on glycerol-3-phosphate and glycerol, obtained from the lipase action on triacylglycerols in plasma. Hydrogen peroxide, a by-product of the GPO reaction is estimated as already described for total cholesterol.

The HDL-cholesterol estimation employed the method of Richmond[24]. The principle entails the separation of HDL-cholesterol from chylomicrons, VLDL- C and LDL- C with a suitable precipitant and then estimation of cholesterol by the method described for total plasma cholesterol.

The VLDL and LDL cholesterols were obtained by calculations using the empirical relationships of Friedewald *et al.*[26]:

$$\text{LDL-Cholesterol} = \text{Total cholesterol-(HDL-C+TG/5)}$$

$$\text{VLDL-C (mg dL}^{-1}\text{)} = \text{TG/5}$$

Atherogenic index (AI) and atherogenic index of plasma (AIP) were calculated using the following equations as described by Dobiasova and Frohlich[27] i.e.:

$$AI = \frac{(TC\text{-}HDL)}{HDL}$$

While:

$$AIP = Log\left(\frac{TG}{HDL\text{-}C}\right)$$

Statistical analysis: The results obtained from this study were analyzed by one-way analysis of variance (ANOVA), followed by Student's t-test to evaluate the significance of the difference between the mean value of the measured parameters in the respective test and control groups. A significant change was considered acceptable at $p<0.05$.

RESULTS

The results of the plasma lipid (TC, TG, LDL and HDL) profile of male rats exposed to crude petroleum and treated with vitamin C were presented in Table 1, while the calculated lipid ratios and atherogenic index were presented in Fig. 1-4. The results of this study showed that oral exposure to crude oil induced a significant ($p<0.05$) increase in plasma TC, TG, LDL and VLDL levels and decrease in HDL level, compared, respectively with the values obtained for rats in the control group (Table 1). The lipid ratios and atherogenic indices recorded in this study indicated that exposure to crude oil induced hyperlipidaemia, which may be a risk factor for

Table 1: Plasma lipid profile of male rats exposed to crude oil and treated with vitamins C

Groups	Treatments	TC (mmol dL^{-1})	TG (mmol dL^{-1})	LDL (mmol dL^{-1})	VLDL (mmol dL^{-1})	HDL (mmol dL^{-1})
1	None (control)	1.26±0.16	1.32±0.14	1.34±0.41	0.26±0.11	2.34±0.79
2	Vitamin C	1.22±0.15	1.28±0.20	1.36±0.34	0.26±0.12	2.32±0.88
3	NBLCO	3.26±0.86*	3.32±0.78*	1.43±0.20*	0.66±0.07*	1.17±0.11*
4	NBLCO+vitamin C	1.51±0.09	1.58±0.87	1.09±0.22	0.32±0.15	2.28±0.84

Values are expressed as "Mean±SEM", n = 6. TC: Total cholesterol, TG: Triacylglycerol, LDL: Low density lipoprotein cholesterol, VLDL: Very low density lipoprotein cholesterol. *Significantly different from groups 1 and 4, respectively, at $p<0.05$

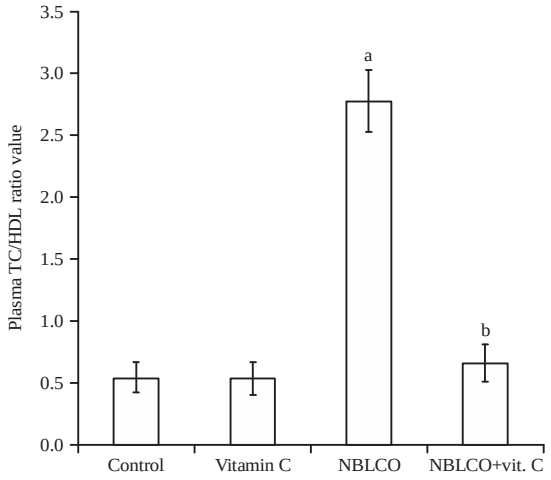

Fig. 1: Plasma TC/HDL ratio of male rats exposed to crude oil and treated with vitamins C. n = 6, ap<0.05 compared to other groups, bp<0.05 compared to NBLCO group

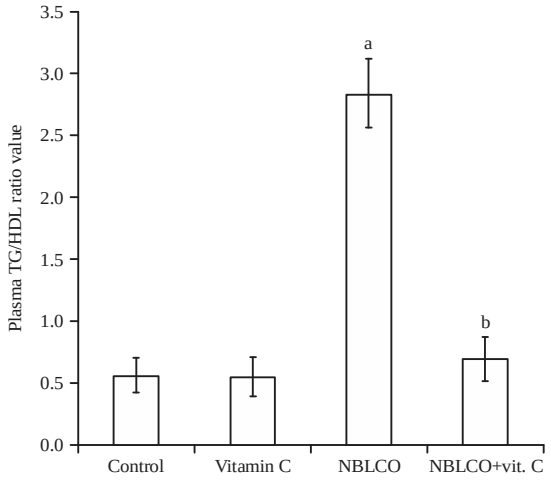

Fig. 2: Plasma TG/HDL ratio of male rats exposed to crude oil and treated with vitamins C. n = 6, ap<0.05 compared to other groups, bp<0.05 compared to NBLCO group

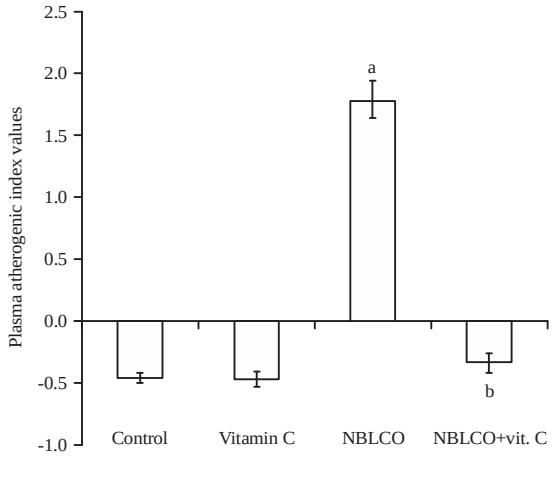

Fig. 3: Plasma atherogenic index (AI) of male rats exposed to crude oil and treated with vitamins C. n = 6, ap<0.05 compared to other groups, bp<0.05 compared to NBLCO group

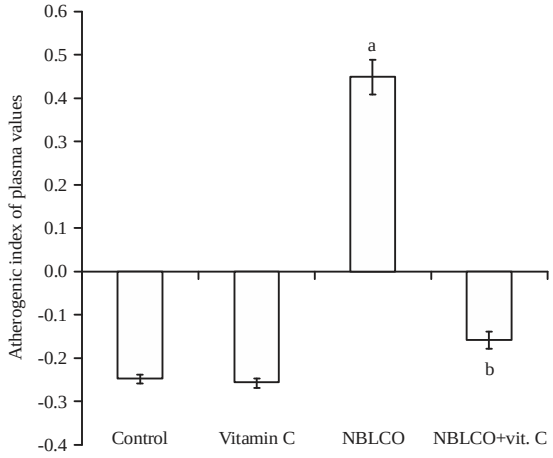

Fig. 4: Atherogenic index of plasma (AIP) of male rats exposed to crude oil and treated with vitamins C. ap<0.05 compared to other groups, bp<0.05 compared to NBLCO group

atherosclerosis in rats. It was also observed from the results of this study that the levels of the plasma TC, TG, LDL and VLDL recorded for rats exposed to crude oil and treated with vitamin C were significantly ($p<0.05$) lower, while HDL levels were significantly higher, compared, respectively with the levels recorded for rats exposed to crude oil only (Table 1). However, the plasma lipid profile recorded for male rats orally exposed to crude oil and treated with vitamin C, were insignificantly ($p>0.05$) higher, compared, respectively with the profile recorded for the control rats (Table 1).

Moreover, the results showed that the calculated lipid ratios, including TC/HDL and TG/HDL ratios (Fig. 1, 2, respectively) and atherogenic indices, including (TC-HDL)/HDL and Log (TG/HDL) (Fig. 3, 4), of male rats exposed to crude oil only were significantly ($p<0.05$) higher, compared to the values recorded for the control rats (Fig. 1-4). This indicated that exposure to crude oil has potential high risk of atherosclerosis in male rats. However, administration of vitamin C was observed to produce a significant ($p<0.05$) reduction in crude oil induced increase in atherogenic indices in male rats (Fig. 1-4). The observations made from the

results of this study suggested that vitamin C is potent in providing protective measures against crude oil induced hyperlipidaemia, hence atherosclerosis, in male rats.

DISCUSSION

Lipid profile is one of the groups of biochemical assays that are often used for monitoring, predicting, diagnosing and treating such lipid-related diseases as atherosclerosis[28]. In this study, significant increase in plasma TC, TG, LDL and atherogenic indices, as well as low plasma HDL were recorded for male rats exposed to crude oil. This agreed with the report of Uboh *et al.*[11,12] that exposure to gasoline is capable of causing atherosclerosis in male and female rats. Increase in blood lipid levels (hyperlipidaemia) is one of the predisposing factors for various cardiac related disorders. According to Goldberg[29], hyperlipidaemic conditions have long been established to be among the risk factors for ischaemic heart disease and peripheral vascular disease. Also, the existence of strong relationship between blood lipid "total cholesterol and triacylglycerols" profile, their associated blood transporting lipoproteins "HDL-C, LDL-C and VLDL-C" and the incidence of atherosclerosis and coronary artery disease (CAD) have been reported[30].

Moreover, the existence of the relationship between the risk of CADs and increased LDL-C, as well as decreased HDL-C levels in the blood has been documented[31,32]. Increase in blood triacylglycerol levels has also been reported to be common in with coronary artery disorders as well as increased levels of small dense LDL-C particles[33-35]. The ratios of TC and TG to HDL-C, often considered as atherogenic indices, have been considered to be strong predictors of atherosclerosis and myocardial infarction[27,36-38]. It is therefore clear from the results of this study that exposure to crude oil may produce a high cardiovascular risk, considering the relationship between atherogenic index values and cardiovascular risk defined by Dobiasova[38].

According to Brunzel *et al.*[39], increased blood LDL-cholesterol level is one of the indications of oxidative stress, supporting that the significant increase in LDL-cholesterol concentrations recorded in this study may be attributed to crude oil induced oxidative stress, as earlier reported by Uboh *et al.*[11] for gasoline vapour in rat model. It has also been demonstrated that high concentration of cholesterol in the serum could lead to such conditions like arthrosclerosis and other cardiovascular diseases, which may be implicated in stroke[40]. There is therefore a strong indication that exposure to crude oil predisposed the rats to atherosclerotic conditions, as one of the expressions of the activities of oxidative stress.

This study also recorded that administration of vitamin C to rats exposed to crude oil was observed to produce a marked decrease in serum TC, TG, LDL, lipid ratios and AIP, as well as increase in HDL levels. There are literature reports supporting the potencies of vitamin C against xenobiotics-induced oxidative stress in different species of animals[41-43]. The results obtained from this study suggested that vitamin C is potent in protecting the tissues against crude oil induced hyperlipidaemia in male rats.

CONCLUSION

From the results of this present study, the ameliorating effect of vitamin C, as antioxidant, against crude oil induced atherosclerosis is reported. This signifies that vitamin C has a potential in improving the cardiovascular disorders associated with exposure to crude oil in rats.

SIGNIFICANCE STATEMENT

The present study found the ameliorating effect of vit C as antioxidant against the arthrosclerosis induced by crude oil. Hence, it signifies signify Vit C has potential in improving the cardiovascular disorders. This would help the researchers to determine its biochemical mechanism in deterring the arthrosclerosis caused by crude oil.

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