E3 Journal of Medical Research Vol. 2(1). pp.0117-0122, April, 2013
Available online @ http://www.e3journals.org
ISSN 2276-9900 © E3 Journals 2013

Full length research paper

Anti-atherogenic potentials of vitamin C or E administration against ingested Nigerian Bonny Light Crude Oil (NBLCO) in male Wistar rats

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Accepted February 22, 2013

Evaluations of anti-atherogenic potentials of vitamin C or E administration against the hazardous effects of ingested Nigerian Bonny Light Crude Oil (NBLCO) in rats were made. Forty adult male Wistar rats (120g – 165g body weight) were randomly divided into four groups of 10 rats each. Group I was control and oral gavaged 6ml/kg normal saline. Group II oral gavaged 6ml/kg body weight NBLCO. Groups III and IV were in addition to 6ml/kg NBLCO supplemented with 1mg/kg and 9mg/kg of vitamins C and E respectively. The results showed that NBLCO significantly ($p<0.001$) increased total cholesterol (TC), low density lipoprotein (LDL-C) compared with control, while it significantly ($p<0.001$) reduced triglyceride and very low density lipoprotein (VLDL-C). Vitamin supplementations significantly ($p<0.001$) lower TC, TG, LDL-C, as well as VLDL-C at $p<0.05$ than NBLCO group. In contrast, NBLCO significantly ($p<0.001$) reduced HDL-C level compared with the control, while vitamins supplementation recorded significantly ($p<0.001$) higher values than NBLCO group. The atherogenic index (AI) ratio was significantly ($p<0.001$) higher in NBLCO-treated rats than control, this was significantly ($p<0.001$) reversed by vitamin C or E supplementation. A significantly ($p<0.001$) higher platelet, neutrophil, eosinophil and neutrophil-lymphocyte ratio (N/L) were also recorded compared with control. Vitamins supplementation did not significantly change their values when compared with NBLCO-treated rats. While the monocyte level was significantly ($p<0.001$) raised in the NBLCO, neither of the vitamins cause any significant change compared with NBLCO group. The fact that NBLCO significantly increases TC, LDL-C and the atherogenic index coupled with significantly high level of circulating platelets, neutrophils, monocytes and high neutrophil-lymphocyte ratio in rats in this study underscores the inherent danger associated with NBLCO ingestion. Interestingly, such harmful effects can be ameliorated with adequate antioxidant vitamin C or E supplementation.

Keywords: Crude oil; anti-atherogenic potentials; antioxidant vitamins; platelets; neutrophil.

Introduction

Human and industrial activities such as drilling, manufacturing, storing, transporting, waste management of oil and vandalizing of oil pipe lines in the petroleum sector result in extensive pollution of the entire aquatic ecosystem with petroleum hydrocarbons. These constitute serious socioeconomic and public health hazards. The Niger Delta region of Nigeria where oil is produced in large quantity is a purely agrarian society where the people depend on farming and fishing for survival. When the aquatic ecosystem is polluted with oil and petroleum hydrocarbons, these dangerous substances may accumulate in fish and other sea foods and farm produce from the adjoining farmlands and eventually get to man and animals probably through the food chain as observed by Jessup and Leighton, (1996). Furthermore, crude petroleum is seriously abused, by

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being ingested as an antidote to poisons and as laxative (Orisakwe *et al*, 2000). Reports from various researchers have established the toxic effect of crude oil on tissues and organs which include anaemia in experimental animals (Suzanne 2003; Becki, 2007, Ita and Udofia, 2011, Ita *et al*, 2011), damage to plasma membrane (Prescott *et al*, 1996).Bioaccumulation of petroleum hydrocarbon has been reported to mediate its hazardous effects by inducing oxidative stress to generate free radicals (Val and Almeida-Val, 1999; Achuba and Osakwe, 2003), which in turn cause lipid peroxidation (Halliwell, 1994) that damages critical cellular macromolecules including DNA, lipids and proteins (Romero *et al*., 1998; Souza *et al*., 1999) and disruption in functions of tissues and vital organs resulting in disease conditions such as atherosclerosis. Atherosclerosis is associated with deranged lipid metabolism (Wang and Briggs, 2004) and micro-inflammation (Danesh *et al*, 2004; Gotsman *et al*, 2008). 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. The classical concept of the pathogenesis of CVD, based on lipid and lipoproteins, has implicated elevated LDL-C, as the central atherogenic lipoprotein class (Graaf *et al*, 2007). It is important to note that there is a positive correlation between improper distribution of lipid, lipoprotein fractions and some predisposing factors such as genetic and environmental insults (Dansky *et al*, 2002) including crude oil (Ita *et al*, 2011), such insults can trigger off the cascade of events leading to atherosclerosis. At high concentration, LDL particles could invade the endothelium and become oxidized forming the required stimulus to trigger formation of atherosclerotic plaque and in the process generate excessive free radical concentrations. The oxidized LDL can also promote atherosclerosis by attracting other cells such as circulating mononuclear cells (lymphocyte and macrophages) (Krishnaswamy *et al*, 1998; Powell, 1998). These mononuclear cells have the potentials to produce inflammatory chemical cytokines, this, coupled with the capacity of macrophages to take up LDL and transform into foam cells (Morrow and Ridker, 1999) further promotes plaque progression. Another blood element that is implicated in the development and progression of atherosclerosis is platelets, as inflammation is reported to play a pivotal role in this regard (Ross, 1999). Inflammatory cytokines promote platelet adhesion to other platelets, the endothelium, leukocytes and progression of plaque growth.

The concentration of oxidants in the body can be elevated by xenobiotic insults such as crude oil (Di Toro *et al*, 2000). The oxidation is associated with inflammatory responses that are implicated in the pathogenesis of various cardiovascular diseases, neurodegenerative disorders, autoimmune diseases, diabetes and cancer (Mates *et al*, 1999; Kayode *et al*, 2009; Vaghasiya and Chanda, 2010). Since generation of free radicals is the underlying mechanism responsible for lipid peroxidation, antioxidant vitamins C and E could possibly play important role of ameliorating some of these effects. Vitamin C, a known water soluble antioxidant is reported to reacts with peroxyl radicals formed in the cytoplasm before they reach the membrane (Khoja and Marzouki, 1994) thereby preventing injury to the membrane; it also has a sparing effect on vitamin E (Tanaka *et al.,* 1997). While Vitamin E, a lipid soluble free radical scavenger is reported to protect the membrane from lipid peroxyl radical (Buttner and Burns, 1996). Similarly, a good number of studies have established the effectiveness of antioxidant vitamins against oxidative stress (Verma and Nair, 2001; Ognjanovic *et al.,* 2003). The study was therefore designed to investigate the ameliorating effects of antioxidant vitamin C or E on the atherogenic influence of NBLCO *vis-à-vis* the role of the circulating platelet and some of the white blood cell fractions.

Materials and methods

The Crude petroleum was obtained from the EXXON/MOBIL laboratory Eket, Nigeria.

Experimental animals

Mature male albino Wister rats weighing between 120-165g were obtained from the animal house of the Faculty of Pharmacy, University of Uyo, Nigeria and were kept in a well-ventilated experimental section in the animal house for fourteen (14) days to acclimatize. The animals were allowed food and water *ad libitum*.

Experimental design and treatment of animals

A total of forty (40) male adult Wister albino rats that were divided randomly into four groups. The rats in control group were gavaged with 6ml/kg of normal saline as control vehicle for 28 days. Group II was gavaged 6ml/kg body weight of NBLCO according to dose described Eyong *et al*, 2004). The remaining groups IV and V were in addition to 6ml/kg of Nigerian Bonny Light Crude Oil (NBLCO) supplemented with 1ml/Kg body weight of vitamin C and 9mg/Kg body weight of vitamin E respectively. In all cases, the dose, which was based on the rat's most recently recorded body weight, was applied daily for 28 days. The experimental procedures involving the animals and their care were conducted in conformity with the approved guidelines by the Local Research and Ethical Committee.

Table 1: Comparative lipid profile and the atherogenic index of control, NBLCO, vitamins C or E supplemented groups after 28 days of treatment.

Groups	TC (mg/dL)	TG (mg/dL)	HDL(mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	Atherogenic index (AI)
Control	36.79 ± 0.38	73.11 ± 0.92	21.11 ± 0.52	0.81 ± 0.18	14.73 ± 0.18	1.71 ± 0.03
NBLCO	40.97 ± 0.36[a]	60.28 ± 0.34[a]	19.91 ± 0.22[a]	8.61 ± 0.40[a]	12.01 ± 0.08[a]	2.06 ± 0.03[a]
COC	35.53 ± 0.24[b]	56.94 ± 0.20[a,b]	23.67 ± 0.16[b]	1.15 ± 0.13[b]	11.40 ± 0.03[a,c]	1.51 ± 0.01[b]
COE	37.77 ± 0.11[b]	58.65 ± 0.20[a,b]	22.71 ± 0.34[b]	2.60 ± 0.70[b]	11.71 ± 0.04[a,c]	1.66 ± 0.03[b]

a = p<0.001 vs Control, b = p<0.001 vs CO group, c = p<0.05 vs CO group.

Collection of blood sample for analysis

After twenty eight (28) days of administration, the rats were kept fasting overnight and anaesthetized in chloroform chamber and immediately sacrificed. Blood was collected from the heart by cardiac puncture with 5ml sterile syringe with needle, into plain sample bottles and kept for 2hours for clot formation. The blood samples were then centrifuged at 3000 rpm with a table top centrifuge (RM-12 Micro centrifuge, REMI, England) for 10 minutes. Then the serum was separated gently with the help of Pasteur pipette into clean labelled bottles and preserved at -20 ^{o}C until required. Part of the blood samples were collected in EDTA sample bottles for the determination of platelet count, WBC and the differential white cell counts. The analysis was done with the aid of an automatic haematology analyzer (BC-2300,Mindray, Germany).

Lipid profile test

The lipid components such as triglyceride and HDL-C were estimated in serum using standard kits supplied by Randox (UK), and Dialab (France) respectively. Total cholesterol, on the other hand was estimated using standard kits from Randox (USA).Calculation of low density lipoproteins (LDL) and very low density lipoprotein (VLDL).The values of LDL and VLDL were obtained mathematically using the formulae below respectively (Friedewald *et al*, 1972):

$$\text{LDL (mmol/L)} = \text{Total Cholesterol} - \text{HDL (mmol/L)} - \frac{\text{TG (mmol/L)}}{2.2}$$

$$\text{VLDL (mmol/L)} = \frac{\text{TG (mmol/L)}}{2.2}.$$

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

$$\text{AI} = \frac{\text{Total cholesterol (mmol/L)}}{\text{HDL-C (mmol/L)}}.$$

Statistical analysis

Values of the biochemical assays were expressed as mean (±) standard error (SE) and were statistically analysed with SPSS 15.0 evaluation version using one way analysis of variance and results were further subjected to post hoc test using Least Square Deviation (LSD). P<0.001 was considered to be significant.

Results

The results showed that NBLCO significantly (p<0.001) increased TC, LDL-C and atherogenic index (AI) when compared with control. NBLCO, on the other hand significantly (p<0.001) reduced triglyceride and VLDL-C. Vitamin C or E supplementation did not significantly altered TC, LDL-L and AI when compared with the control but significantly (p<0.001) lower these parameters than NBLCO group. In like manner, vitamin C or E supplementation significantly (p<0.001) reduced TG level than the control and NBLCO groups. Similarly, vitamin C or E supplementation significantly (p<0.001 and p<0.05) lowered VLDL-C than control and NBLCO groups respectively. NBLCO significantly (p<0.001) reduced HDL-C level when compared with the control. Vitamin C or E supplementation did not alter HDL-C level significantly when compared with the control but recorded significantly (p<0.001) higher values than NBLCO group; these results are presented in table 1.

The results obtained for the haemtological parameters (platelet level white blood cell fractions) are shown in table 2. The results showed significant (p<0.001) increase in platelet, neutrophil, eosinophil values as well as the neutrophil-lymphocyte ratio when compared with the control. Vitamins C or E did not show any significant change when compared with NBLCO-treated rats. The lymphocyte value obtained was significantly (p<0.001) lower in NBLCO group and those supplemented with either vitamin C or E when compared with the control.

Table 2:The platelet values and differential white cell counts in the control and different experimental rats
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Haematological parameters	GROUPS			
	Control	NBLCO	COC	COE
Platelets(x10^6/ µL)	976.30 ± 3.42	995.10 ± 2.89[a]	999.30 ± 0.60[a]	999.50 ± 0.34[a]
Lymphocytes(%)	81.60 ± 0.45	72.70 ± 0.34[a]	77.10 ± 0.28[a]	73.20 ± 0.25[a]
Monocytes(%)	2.70 ± 0.15	4.70 ± 0.21[a]	3.00 ± 0.15	4.20 ± 0.25[a]
Neutrophils(%)	15.50 ± 0.27	19.50 ± 0.27[a]	21.00 ± 0.45[a]	21.50 ± 0.40[a]
Eosinophils(%)	2.44 ± 0.17	3.89 ±0.19[a]	4.22 ± 0.21[a]	4.22 ± 0.14[a]
Neutrophil-lymphocyte ratio (N/L)	0.19 ± 0.00	0.27 ±0.00[a]	0.28 ± 0.01[a]	0.30 ± 0.01[a]

a = significantly different from control ($p<0.001$)

Either vitamin C or E supplementation did show any significant change when compared NBLCO group. While the monocyte level was significantly ($p<0.001$) raised in the NBLCO and vitamin E supplemented groups compared with the control, vitamin C supplementation did not show any significant different. Again vitamin C or E supplementation did not show any significant different compared with NBLCO group.

Discussion

The Nigerian Bonny Light Crude oil (NBLCO) ingestion increased serum lipoprotein fractions of TC and LDL. An elevated serum TC and LDL with corresponding decrease in HDL recorded in this study corroborated the results of earlier studies, where other petroleum products such as distillate aromatic extract (Feuston *et al*, 1996), and gasoline (Ubani *et al*, 2009) were established to interfere with lipid metabolism resulting in high serum TC, LDL-C and decreased HDL-C levels. These results are suggestive of the risk of developing cardiovascular disease (CVD) as earlier postulated by Mckee and Mckee (1999) when crude oil is ingested or crude oil contaminated foods are consumed. At the present time, cardiovascular disease (CVD) is recognized as a chronic inflammatory condition of the vessel wall that results from the trans-endothelial passage of cholesterol-rich atherogenic apo-B lipoproteins (VLDL, IDL and LDL) from the plasma into the intima. The retention of cholesterol and LDL in the sub-endothelial space could attract infiltration of platelets and macrophages that ultimately interact with each other and perhaps other chemical agents with the cells of the arterial wall (Williams and Tabas, 2005). It is likely the toxicants in the crude oil induced inflammation that could accelerate atherogenic activities in the rats of this study. The report of this study agreed with the reasoning of Benditt (1974) that injury to large blood vessels caused by toxic insult to vascular wall could trigger inflammatory response as inflammatory response is an essential component in the initiation and evolution of atherosclerosis. The possibility of such inflammatory reaction in this study is confirmed by significantly high neutrophil-lymphocyte ratio recorded; this possibility is not out of place as inflammation caused by high neutrophil-lymphocyte ratio could result in atherosclerotic lesion (Kayode *et al*, 2009; Vaghasiya and Chanda, 2010). Specific leukocyte fractions have been reported to have higher predictive value in assessing cardiovascular risk and such predictive value is even higher when the neutrophil-lymphocyte ratio is used (Horne *et al*, 2005). The development of atherosclerotic lesions, regardless of other risk factors is characterized by the disruption of the normal function of the endothelial cells, which may be due to high level of LDL with corresponding reduction in HDL level recorded in this study which could probably results in high yield of free radicals to cause damages. Chronic inflammation is directly associated with atherosclerosis, alongside other markers of inflammation and coagulation which might have been up-regulated by high level of circulating platelet, which may possibly explain the significantly high values of neutrophils and platelets recorded in this study following NBLCO ingestion; since exposure to environmental xenobiotics like polycyclic aromatic hydrocarbons (PAHs) have been reported to be among the risk factors for atherosclerosis (Binkova *et al*, 2001; Izzotti *et al*, 2001). The recorded high platelet reactivity due to NBLCO ingestion is indicative of high prothrombotic effects, a contributing activity in the promotion of atherosclerosis. Although high plasma HDL-C levels prevent the deposition of vascular cholesterol and development of atherosclerosis, the elevation of LDL level which is the major carrier of cholesterol of all fractions leads to early development of atherosclerosis. This is because elevated LDL level is known to confer susceptibility to lipid peroxidation which in turn increases its atherogenic potential (Ita *et al*, 2011).

Biologic systems are equipped with elaborate protective mechanisms against the toxicity of reactive

oxygen species (ROS). Oxidative stress toxicity crises may arise from imbalances between biologic pro-oxidant and antioxidant processes. There are various plant-based antioxidants which are among the many elaborate, redundant and overlapping mechanisms for combating oxidant hazards; these antioxidants include ascorbic acid, tocopherol, carotenoids and polyphenols (Halliwell and Gutteridge, 1999). In addition to their important role in regulating the redox state of cells, vitamins C and E have been reported to reverse endothelial dysfunction in patients with coronary artery disease (Padayatty and Levine, 2000; Levine and Gresham, 2009) and cultured human aortic endothelial cells exposed to oxidized LDL (oxLDL) (Keaney *et al*, 1996). It is not surprising that the increased TC and LDL-C recorded in this study was completely reversed by antioxidant vitamins C or E supplementation, is in agreement with other studies that have reported the capacity of the antioxidant vitamins to retard development of atherosclerosis by preventing lipid oxidation and oxidative damage (Gaziano and Hennekens, 1992).

Results of this study may have several striking health implications, ingestion of NBLCO significantly raised firstly total cholesterol and LDL while it reduced HDL, secondly, platelet count, thirdly leukocyte fractions (neutrophil and monocyte counts), therefore, significantly raised neutrophil-lymphocyte ratio and fourthly that administration of vitamin C or E ameliorates some of its hazardous effects. This implies that adequate administration of vitamins with anti-oxidative properties like vitamins C and E are able to minimize or prevent damage of vascular wall (Levine and Gresham, 2009) from oxidant injury of NBLCO and reduced risk of cardiovascular disease. The fact that NBLCO significantly increases TC, LDL-C and the atherogenic index coupled with significantly high level of circulating platelets, neutrophils, monocytes and significantly high neutrophil-lymphocyte ratio in rats reported in this study underscores the inherent danger associated with ingestion of crude oil or crude oil-contaminated foods and drinks. Even though, the total white blood cell count was not significantly different from control, the neutropilia reported in this study may not only mirror the exacerbated inflammatory response in the rats, but also ascertain the association of NBLCO in the pathogenesis of CVD.Although the hypoglycaemic effect of NBLCO is not capture in this study but the unpublished data showed that NBLCO indeed caused reduction in blood glucose supported by the work of Ben-David *et al* (2001), these workers reported ingestion of petroleum caused reduction in blood glucose. This may possibly explain the significant reduction in triglyceride and VLDL levels recorded in this study, since lipid, triglyceride in particular is reported to be an alternative energy substrate in the in phase low blood glucose. It is not also surprising that NBLCO significantly reduced VLDL since it is derived from triglyceride.

List of abbreviations

COC - Crude oil plus vitamin C; COE - Crude oil plus vitamin E;CVD - Cardiovascular disease; DNA - Deoxyribonucleic acid; EDTA - Ethylene diamine tetracetic acid; HDL - High density lipoprotein; HDL-C - High density lipoprotein cholesterol; LDL - Low density lipoprotein; LDL-C - Low density lipoprotein cholesterol; LSD - Least significant difference; NBLCO - Nigerian Bonny Light Crude oil; ox-LDL - Oxidized Low density lipoprotein; PAHs - Polycyclic aromatic hydrocarbons; ROS - Reactive oxygen species; TC - Total cholesterol; TG – Triglycerides; UK - United Kingdom; USA - United States of America; VLDL - Very low density lipoprotein; VLDL-C- Very low density lipoprotein cholesterol; WBC - White blood cell

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