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Original Research Article

Renal Protective Effect of Antioxidant Vitamins C and E against Crude Oil-Induced Nephrotoxicity

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Abstract

The assessment of serum indices for renal function in male Wistar rats co-administered crude oil and antioxidant vitamins. A total of twenty (20) adult male Albino Wistar rats were randomly divided into four groups (group I, II, III and IV). Group I served as the control and was oral gavaged 3 ml/kg body weight of normal saline. Group II was oral gavaged 3 ml/kg body weight of Nigerian Bonnylight crude oil (NBLCO). This dose was calculated as 20% of the lethal dose (LD_{50}) of 14.14 ml/kg, while groups III and IV in addition to 3 ml/kg body weight of NBLCO, were supplemented with 1 ml/kg body weight of vitamins C and E respectively. The results showed that NBLCO ingestion significantly increased concentration of sodium, potassium, calcium, chloride and bicarbonate in serum with respect to the control group ($p<0.05$). Vitamin C or E supplementation on the other hand did not alter concentration of sodium, calcium and bicarbonate significantly with respect to control but were significantly lower than NBLCO-treated group ($p<0.05$). Similarly, vitamin C or E supplementation significantly reduced potassium concentration compared with NBLCO group ($p<0.05$), these vitamins did not alter chloride concentration significantly. The NBLCO administration significantly increased serum urea with respect to the control ($p<0.05$). Vitamin C or E supplementation was not significantly different from control group but was significantly lower than NBLCO treated group ($p<0.05$). The mean creatinine level in NBLCO group was significantly higher than control group ($p<0.05$). While vitamin C supplementation did not alter creatinine level significantly with respect to the control group, it significantly lowered creatinine level compared with NBLCO treated group ($p<0.05$). Vitamin E supplementation significantly elevated creatinine level compared with control and vitamin C supplemented groups ($p<0.05$), while it significantly reduced creatinine level with respect to NBLCO treated group ($p<0.05$). The histological sections of the kidney tissues showed NBLCO ingestion caused prominent damages to the cyto-architecture of the kidney.It is evidenced in this study that NBLCO have shown to cause significant impairment of kidney functions. This may results in derangement in normal homeostatic processes, and may ultimately affect the general health and survival of mankind. Interestingly, some of the dangerous effects of NBLCO can be ameliorated with adequate antioxidants supplementation.

Keywords: Antioxidants, Creatinine, Crude oil, Electrolytes, Kidney, Urea

INTRODUCTION

Water and electrolyte patterns of intracellular and extracellular compartments vary widely but the constancy within each compartment is closely controlled within a narrow physiological range, the maintenance of this constancy is vital homeostatic role of the kidney (Hill, 1990). Electrolytes may enter or leave the cell through specialized protein structures embedded in the plasma membrane ion channels, which depends specifically on

the integrity of the proteins forming these ion channels and enzymatic pumps. Interference of xenobiotic agents with the function of these channels and enzymatic pumps such as Na^+-K^+ ATPase which play an important role in the maintenance of cell volume, will ultimately prevent it from restoring the concentration of Na^+ and K^+ ions to their respective sides. Considering the importance of electrolyte in body physiology, evaluation of sodium, potassium, chloride and bicarbonate is often done for both diagnosis and management of renal, endocrine, acid-base, water balance and many other conditions (Gowda *et al*., 2010).

Injury to the kidney or impairment of its function therefore, has grave consequences for the body, because in such conditions wastes that ordinary would have been excreted would be accumulated and may become toxic. Furthermore, there could also be electrolyte imbalance leading to body fluid volume distortion, interference with activities of excitable tissues particularly the heart with associated life-threatening consequences such as heart failure, coma and tetany.

Petroleum hydrocarbons and other related carbon containing compounds during their normal process of oxidation are converted into activated metabolites in the cells (Hu and Well, 1994; Nwanjo and Ojiako, 2007), some of which may be very reactive thereby interacting in different ways with the excreting and metabolizing tissues like the liver and kidneys to elicit toxic effects (Nygren *et al*., 1994). Some composition of petroleum products such as volatile nitrates, benzene and lead have been reported to produce harmful effects on lymph nodes, bone marrow and spleen (Ovuru and Ekeozor, 2004) through such interactions, there is lipid peroxidation that injured the membrane (Onwurah, 1999). They may also react with enzymes to cause inactivation through protein oxidation (Stadtman, 1990) and/or DNA strand breaks (Birnbiom *et al*., 1985; Nwanjo and Ojiako, 2007).

Exposure to petroleum and its products therefore constitute a serious environmental health hazard. This has been reported to cause impairment of renal function as a result of derangement of serum electrolytes (Aryanpur, 1979; Orisakwe *et al*., 2004; Nwanjo and Ojiako, 2007; Uboh *et al*., 2009) manifesting in, blood disorders, renal damage, hepatic dysfunction and intoxication leading to serious psychotic problems, anaesthetic effects and dermatitis (Aryanpur, 1979; Nwanjo and Ojiako, 2007).

Kidney is the purifier (Ogbekhuemen, 2009) of the body so damage to it is indicated by the accumulation of wastes like urea and creatinine in the serum because of the kidneys' inability to excrete them, and accumulation of urea and creatinine in the serum are indicative of kidney impairment. This is associated with reduction in efficiency of the kidney as body purifier, causing accumulation of these nitrogenous products in the blood that is usually accompanied with fluid, electrolyte and acid-base disorders including hyperkalemia. Acute renal failure with oliguria after exposure to diesel oil has also been reported by Crisp *et al*., (1979). It is possible that these agents exert their effects by generation of oxidant radicals.

Biologic systems are therefore equipped with elaborate antioxidant mechanisms for protection against the toxicity of these oxidant radicals. Imbalances between biologic pro-oxidant and antioxidant processes present toxicity crises. 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; Dede and Nganwuchi, 2003; Dede *et al*., 2003). While vitamin C is an effective antioxidant in living organisms (Carr and Frei, 1999), that protects against harmful effects of free radicals generated as a result of exposure to xenobiotic agents. Vitamin E protects membranes and other fat-soluble parts of the body, such as low density lipoprotein cholesterol, against damage by radical agents. This study was designed to assess the serum indices for renal function in male Wistar rat co-administered crude oil and antioxidant vitamins by evaluating serum urea, creatinine, total protein, albumin, globulin and some electrolytes concentration.

MATERIALS AND METHODS

Chemicals and drugs

The crude petroleum used in this study was obtained from the Exxon Mobil laboratory, Ibeno, Nigeria. The vitamin C tablets and vitamin E capsules were products of Softgel Healthcare Private Ltd, India.

Experimental animals

Male Albino Wistar rats weighing between 150-180g were obtained from the Animal House of the Faculty of Basic Medical Sciences University of Calabar, Nigeria and were kept in a well-ventilated section of the Animal House in the Faculty of Basic Medical Science University of Uyo, Uyo Nigeria. They were allowed access to feed (Chow: vital feeds, Grand Cereals Ltd, Jos) and water *adlibitum*. The animals were allowed to acclimatize for a period of one week before commencement of studies.

Experimental design and treatment of animals

A total of twenty (20) adult male Albino Wistar rats were randomly divided into four groups (group I, II, III and IV). Group I served as the control and was oral gavaged 3 ml/kg body weight of normal saline. Group II 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 groups III and IV in addition to 3 ml/kg body weight of NBLCO, were supplemented with 1 ml/kg body weight of vitamins C and E respectively. 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 twenty eight (28) 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 twenty eight (28) 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 5 ml, 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.

Determination of serum urea

Urea kit from Dialab, (Austria) was used for the determination of urea in the serum according to method described by Veniamin and Vakirtzi (1970).

Evaluation of serum creatinine

Dialab diagnostic kit (France) was used for the determination of creatinine concentration in serum as described by Blass *et al*., (1974).

Evaluation of sodium and potassium ions in serum

TECO diagnostics kit (USA) for sodium and potassium were used to determine sodium and potassium respectively in the serum. These tests were carried out using spectrophotometry as described by Tietz (1990).

Evaluation of calcium ion in serum

Dialab diagnostics kit (Austria) for calcium was used to determine calcium in the rats' serum and urine. This test was carried out using spectrophotometry as described by Tietz (1990).

Evaluation of chloride and bicarbonate ions in serum

Agappe diagnostics kit (India) for chloride was used to determine chloride in the serum. TECO diagnostics kit (USA) for determination of carbon dioxide content in the serum. These tests were carried out using spectrophotometry as described by Tietz (1990).

Histology of kidney

The kidneys were harvested, weighed and fixed in 10% buffered formulae for 48 hours after the animals were sacrificed. The tissues were processed and embedded in paraffin wax. Sections of the tissues (5µm thick) cut were done using a rotary microtome (Micro 325, Thermo Scientific, Germany). The sections were stained by haematoxylin and eosin (H and E) method, these were examined and photographed by using a light microscope (Bran Scientific Company, England). Two histo-pathologists examined the sections, independently. The agreed opinion was taken where they differed in interpretation.

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 mean values for the electrolytes obtained in this study are shown on table 1.

As would be observed NBLCO ingestion significantly increased concentration of sodium, potassium, calcium, chloride and bicarbonate in serum with respect to the control group ($p<0.05$). Vitamin C or E supplementation on the other hand did not alter concentration of sodium, calcium and bicarbonate significantly with respect to control but were significantly lower than NBLCO-treated group ($p<0.05$). Similarly, vitamin C or E supplementation significantly reduced potassium concentration compared with NBLCO group ($p<0.05$), these vitamins did not alter chloride concentration significantly.

Table 1. Comparison of serum electrolyte concentrations in NBLCO-induced treated with vitamin C and E.

Groups	Na^+(mEq/L)	K^+(mEq/L)	Ca^{++}(mEq/L)	Cl^-(mEq/L)	HCO_3^-(mEq/L)
I	4.53± 0.10	4.39± 0.05	9.83± 0.35	91.51± 0.24	42.507± 0.79
II	5.69± 0.11[a]	6.27± 0.07[a]	12.19± 0.07[a]	94.58± 1.26[a]	47.56± 1.28[a]
III	4.64± 0.00[b]	4.86± 0.08[a,b]	10.17± 0.10[b]	96.12± 0.07[a]	42.067± 0.14[b]
IV	4.60± 0.00[b]	4.87± 0.11[a,b]	10.09± 0.15[b]	95.87± 0.13[a]	42.01± 0.28[b]

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

Table 2. Comparison of serum urea and creatinine in NBLCO-iducedWistar rats treated with vitamins C and E.

Groups	Urea(µmmol/L)	Creatinine(µmmol/L)	Urea/Creatinine
I	57.29± 0.98	72.80± 0.34	0.787± 0.016
II	61.44± 0.75a	83.20± 0.20a	0.738± 0.008a
III	57.20± 0.21b	72.04± 0.45b	0.794± 0.007b
IV	57.89± 0.33b	74.82± 0.32a,b,c	0.774± 0.005b

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 ($p<0.05$)

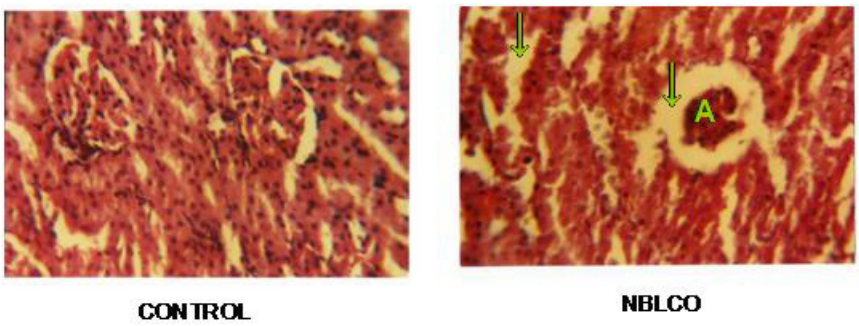

PLATE 1: **Photomicrograph of the sections of the kidney tissues of control and NBLCO-treated rat.**
Section of the control rat showing a distinct cortex and medullary region. Within the cortex capsules within a compact interstitium.
Section of NBLCO-treated rat showing oedematous glomerular (A), prominent cortical tub vessels and oedamatous interstitium (↓). The medullar tubules are compactly packed with scanty intervening interstitium. x400.

Serum creatinine levels in control and various experimental groups

The results of the mean values for the urea and creatinine obtained in this study are shown on table 2.
The mean urea level in NBLCO treated rats was significantly higher than control ($p<0.05$). Vitamin C or E supplementation was not significantly different from control group but was significantly lower than NBLCO treated group ($p<0.05$).

The mean creatinine level in NBLCO group was significantly higher than control group ($p<0.05$). While vitamin C supplementation did not alter creatinine level significantly with respect to the control group, it significantly lowered creatinine level compared with NBLCO treated group ($p<0.05$). Vitamin E supplementation significantly elevated creatinine level compared with control and vitamin C supplemented groups ($p<0.05$), while it significantly reduced creatinine level with respect to NBLCO treated group ($p<0.05$).

Histological sections of the kidney of rats gavaged NBLCO.

As would observe the histological sections of the kidney tissues showed distinct cortex and medullary regions for rats in the control and NBLCO treated groups are presented in plate 1. In the control group there was no sign of damage as prominent glomerular and Bowman's

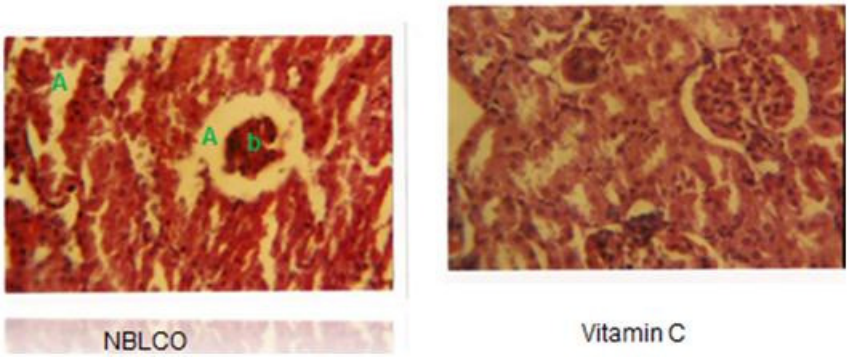

PLATE 2: Photomicrograph of the sections of the kidney tissues of NBLCO-treated and vitamin C co-treated rats. Section of NBLCO rat showing oedematous glomerular (b), prominent cortical tubules lined by oedematous cell with thickened cortical congested blood vessels and oedematous interstitium (A). The medullar tubules are compactly packed with scanty intervening interstitium. Section of the vitamin C co-treated rat showing a distinct cortex and medullary region; within the cortex, prominent glomerular and Bowman's capsules within a compact interstitium are seen.x400.

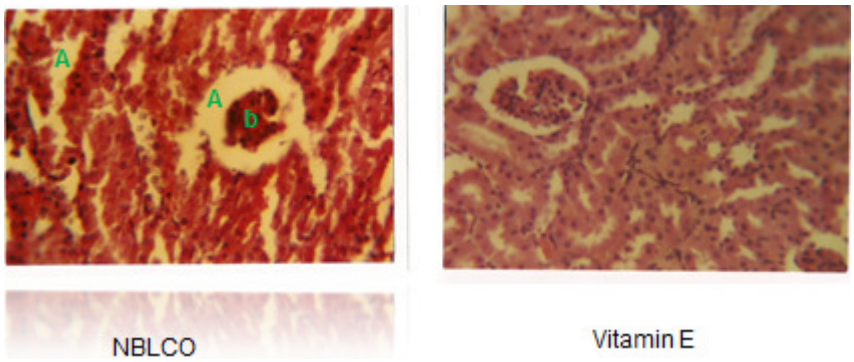

PLATE 3: Photomicrograph of the sections of the kidney tissues of NBLCO-treated and vitamin C co-treated rats. Section of NBLCO rat showing oedematous glomerular (b), prominent cortical tubules lined by oedematous cell with thickened cortical congested blood vessels and oedematous interstitium (A). The medullar tubules are compactly packed with scanty intervening interstitium. Section of the vitamin E co-treated rat showing a distinct cortex and medullary region; within the cortex, prominent glomerular and Bowman's capsules within a compact interstitium are seen . x400.

capsules were seen within the cortex and a compact interstitium. The collecting ducts and tubules are lined by cuboidal epithelium with prominent nuclei and eosinophilic cytoplasms. The intervening interstitium is compact. The medulla showed prominent ducts and tubules with distinct cell outline and lumen within a sparse interstitium. But section of NBLCO treated group showed oedematous glomerular indicated by the letter "A", prominent cortical tubular vessels and widespread oedematous interstitium. It also indicates compactly packed medullary tubules with scanty intervening interstitium.

The histological sections of the kidney tissues of the NBLCO-treated and vitamins C or E co-treated groups are presented in plates 2 and 3. The section of NBLCO treated group showed oedematous glomerular indicated by the letter "A", prominent cortical tubular vessels and widespread oedematous interstitium. It also indicates compactly packed medullary tubules with scanty intervening interstitium. The antioxidant vitamins C and E co-administration with NBLCO showed significant sign of ameliorating the associated damages.

DISCUSSION

Toxicological effects of crude petroleum and its refined product have been reported in literature to constitute serious health hazards and threat to life. This is demonstrated in the present findings where NBLCO not only cause serious perturbation in the electrolyte balance but adverse effect on the kidneys as reflected in the serum accumulation of wastes like urea and creatinine; and distortion in the cyto-architecture of the kidney.

The ingestion of NBLCO indicated electrolytes derangement as there were significant reductions in serum sodium and chloride concentrations and significant

elevation in serum potassium and calcium concentrations. Thus, indicating a possible distortion of the membrane integrity and functions by the toxicants in NBLCO. Increased serum potassium with corresponding decrease in serum sodium observed in the present study could be attributed to major disturbances or interference with plasma membrane homeostasis. Ingestion of crude oil has been reported to induce oxidative damage to trans-membrane ATPase activity, thereby inducing cell lysis (Brovelli *et al*., 1977; Ita *et al*., 2013). Furthermore, elevated lipid peroxidative activity may have interfered with the membrane architecture to increase membrane permeability leading to cell lysis. A similar report on erythrocyte membrane has been published by Ita and co-workers (2013), that ingestion of NBLCO causes erythrocyte haemolysis in rats. Brovelli *et al*, (1977) had reported that free radicals have the propensity to induce oxidative damage to membrane ATPase and in turn increase potassium efflux from the cell; this corroborates the findings of this study.

A significant increase in serum Ca^{2+} level was recorded when the NBLCO treated group was compared with the control group. It is not out of place to postulate that the NBLCO may have altered the structure of the membrane of endoplasmic reticulum (ER) and mitochondria which stored calcium considering its deleterious effect on the membrane as reported by Ita *et al*., (2013). The possible implication of such damages on erythrocyte membrane is that it altered ER/mitochondria Ca^{2+} sequestration which may have caused Ca^{2+} to leak out from ER and mitochondria (Oruambo and Jones, 2007) to increase cytoplasmic Ca^{2+} level which may in turn leak out into the extracellular compartment due to a similar damage to plasma membrane to increase extracellular Ca^{2+} level. There could be many yet to be investigated mechanism for this, as effect recorded here might be due to damaged renal tubules by NBLCO. Crude oil has been established to exert toxic effect on membrane architecture (Brovelli *et al*., 1997; Ita *et al*., 2013), it is likely that one of the major renal processes in the formation of urine, tubular re-absorption was impeded by NBLCO to cause reduction in tubular reabsorption (Duarte and Watson, 1967). Another possibility would be that NBLCO exerts some kind of natriuretic tendency on the renal tubules to reduce the tubular reabsorptive capacity of Na^+, which in turn could promotes high concentration of Na^+ and probably other electrolytes in urine.

On the epithelial cell of the renal tubules are the Na^+-K^+, Na^+-Ca^{++} pumps that play major roles in maintaining sodium, calcium and potassium ions between the tubular cells and the interstitium. Damage to the pump ATPase activity may likely have affected the reabsorption of the filtered ions making them to be excreted in urine, although analysis of these electrolytes in excreted urine was not part of the present study. High serum calcium level correlates with high serum sodium level as reduction in the tubular reabsorption of calcium for instance, has been shown to occur under circumstances which depress the renal reabsorption of sodium (Massry *et al*., 1967; Blythe *et al*., 1968).

Furthermore, increase in the serum level of urea as reported in this study is suggestive of renal impairment which might be mediated through oxidative stress (Traynor *et al*., 2006)

BLCO exerted deleterious effect which significantly caused derangements in the functions of the kidney as reflected by the histopathological studies documented in this study.

CONCLUSION

It is evidenced in this study that NBLCO have shown to cause significant impairment of kidney functions. If the results of this study are extrapolated to human, it is possible that direct and indirect consumption of crude oil contaminated foods and/or water may exert its deleterious effects on human through its interference with the integrity, stability and functionality of biological system and other vital organs in the body. This may result in derangement in normal homeostatic processes, and may ultimately affect the general health and survival of mankind. Interestingly, some of the dangerous effects of NBLCO can be ameliorated with adequate antioxidants supplementation.

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