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RESEARCH ARTICLE

EVALUATION OF SERUM URIC ACID, CREATININE AND BLOOD UREA NITROGEN AS BIOMARKERS OF RENAL FUNCTION OF RATS ADMINISTERED NIGERIAN BONNY LIGHT CRUDE OIL (NBLCO) AND 50% BEE HONEY

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ABSTRACT

The nephrotoxic effect of Nigerian Bonny-light crude oil (NBLCO) was assessed using serum uric acid, creatinine, and blood urea nitrogen as indicators of kidney function. Eighteen Wistar rats were divided into three groups, Groups I, II, and III. Group I was administered 3ml/kg of 0.9 % saline and served as control. Group II was administered 3 ml/kg of crude oil (NBLCO) whereas group III was co-administered 3 ml/kg of crude oil and 3 ml/kg of 50 % bee honey for of twenty-one (21) days. The result obtained showed that NBLCO significantly increase serum uric acid, creatinine and blood urea nitrogen levels compared to control ($P<0.05$). But, co-administration of bee honey with NBLCO in group III significantly increase serum values of the aforementioned parameters ($P<0.05$) compared to both the control and NBLCO groups ($P<0.05$). This research concludes that chronic ingestion of crude oil has the potential to depress kidney function with propensity to cause kidney damage and has also demonstrated that co-administration of 50% bee honey is unable to prevent the hazardous effects of NBLCO on the kidney.

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INTRODUCTION

There is a widespread and frequent contamination of the environment with petroleum hydrocarbon likewise animal and human is exposed to crude oil and its refined products. In addition, it is reported that crude petroleum are taken orally against toxic venom such as snakebites, and in the treatment of gastrointestinal disorders, convulsions, ulcers etc, (Chilcott and Chapd, 2007) with neglect to the hazardous effect it poses to health which may result from accumulation of toxic substances in the body. Studies also show that ingestion of aquatic species exposed to spillages poses risk of possible bioaccumulation and bio-concentration of toxic components of crude oil (Eyong *et al*., 2004). Crude oil has been described as a complex mixture of over 6000 potentially different hydrocarbons and metals (Edwards, 1989). Aliphatic and aromatic hydrocarbons which are the major components of crude petroleum and petroleum products and other xenobiotics lead to the generation of free radicals in various tissues during the metabolism in the body (Achuba and Orisakwe, 2003).

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The free radicals in turn cause destruction of various cellular membranes leading to development of hematopoietic, hepatic, renal, and pulmonary abnormalities (Mark *et al*., 2014). The toxicity of crude petroleum and some of its products have been widely reported in literature. These include the observation that a relatively short exposure to crude oil led to the inhibition of growth in weaned rabbits (Berepubo and Johnson, 1994). A similar observation was reported in juvenile pink salmor concorhyncus gorbuscha (Wang *et al*., 1994). Conclusions from a recent study suggest that exposure to NBLCO, such as its use in ailment management, might induce kidney damage particularly by way of induction of oxidative stress **(**Orisakwe *et al*., 2004). In addition, Ita and Udofia reported that crude oil adversely affects the hematopoietic system resulting in anemia (Ita and Udofia, 2011). Nephrotoxic effect of crude oil has also been reported to include marked degeneration of the kidney evidenced in cortical hemorrhage, tubular necrosis, protein casts, and cellular infiltration in Wistar rats (Adedara, 2011; Ita and Edagha, 2016). The underlying mechanism of action of petroleum toxicity might be induction of oxidative damages mediated by oxidant radicals. Elsewhere in a study of effect of crude oil on erythrocyte demonstrated that ingestion of crude oil induces oxidative damage to trans-membrane ATPase activity, thereby inducing cell lysis (Brovelli *et al*., 1977; Ita *et al*., 2013). Renal dysfunction is gradually becoming a public

health concern with rising incidence and prevalence globally. Despite the fact that Nigeria appears to be badly hit by this epidemic, there is paucity of data on renal dysfunction prevalence in the region. In a recent study done by Egbi *et al*., the prevalence of chronic kidney diseases (CKD, a form of renal dysfunction) among Bayelsans, that is residence of Bayelsa State in Niger Delta of Nigeria was fairly high (7.8%) compared to other regions of the country (Egbi *et al*., 2014). Chronic exposure of humans to bio-toxics content of crude oil may account for this high incidence in the region. In another study carried out by Okafor and co-workers, the incidence of CKD in the Niger Delta community was also reported to be high (Okafor *et al*., 2015). In this circumstance, substance(s) with potential to reduce these hazardous effects of NBLCO will suffice. This study was therefore designed to investigate the efficacy of a 50% concentration bee honey, a known reservoir of antioxidants to ameliorate the NBLCO-induced nephrotoxicity.

MATERIALS AND METHODS

Crude oil and bee honey

Nigerian Bonny-light crude oil was obtained from Exxon/Mobil laboratory Eket, Nigeria. Honey was purchased from the local market in Uyo, Nigeria. The 50% honey was prepared by dissolving 50 ml of honey in 50 ml of distilled water.

Experimental animals

Twenty five mature mice and eighteen mature Wistar albino rats were purchased from the animal facility center of the Faculty of Basic Medical Sciences, University of Uyo, Nigeria. The animals were kept in a well-ventilated experimental room in the Animal House for two weeks to acclimatize. The animals were kept at a room temperature of about 23 ± 3°C and exposed to a 12 hour day 12 hour dark cycle and were allowed food and water *ad libitum*.

Acute toxicity test

Acute toxicity study (LD_{50}) was estimated using Lorke's method (Lorke, 1993). The acute toxicity test for the NBLCO 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, 20ml/kg, 25ml/kg, 30ml/kg and 40ml/kg of body weight respectively.

- **Group I**–10ml/kg
- **Group II**–20ml/kg
- **Group III**–25ml/kg
- **Group IV**–30ml/kg
- **Group V**–40ml/kg

The mice were starved for an hour after the administration to enhance bioavailability. Twenty-four hours following the administration, only group II mice were alive. The median lethal dose of the NBLCO 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:

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

Experimental design and treatment of animals

Eighteen (18) Wistar albino rats of both sexes were randomly divided into three of six (6) rats each. Group I received 3ml/kg of 0.9 % saline and served as control. Group II received 3 ml/kg of crude oil (NBLCO) whereas group III were co-administered 3 ml/kg of crude oil and 3 ml/kg of 50 % bee honey. Rats in all groups (both control and test groups) received gavage for twenty-one (21) 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

At the end of twenty-one days exposure period, the animals were weighed and sacrificed under chloroform anesthesia. A 5 ml syringe was used to collect blood from the heart by puncture. Collected blood samples were transferred into plain sample bottles for serum and the samples were allowed to stand for two hours to clot. The clotted blood was spun using tabletop centrifuge (Rm – 12 – micro centrifuge, REM, England) at 4,000 RPM for ten (10) minutes. The serum was separated greatly using a polypropylene pasture pipette and stored in a well-labeled set of plain sample bottles, which was then used for biochemical analysis of uric acid, creatinine and blood urea nitrogen (BUN).

Determination of uric acid

Serum uric acid was determined using isotope dilution-mass spectrometry. In this method, a definite amount of isotopically labeled uric acid is added to a serum sample containing an unknown amount of the non-labeled substance, both of which are purified by ion exchange chromatography and then converted to the trimethylsilyl derivates. The ratio of non-labeled to labeled substance is measured by combined gas chromatography-mass spectrometry using the selected ion monitoring technique. The unknown amount of uric acid in the serum sample is calculated from the isotope ratio. The concentration of uric acid in the serum samples is calculated from the isotope ratios measured by selected ion recording.

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).

Statistical analysis

Statistical analysis was carried using window SPSS package (SPSS22.00 version). Data were analyzed using analysis of variance (ANOVA) followed by post hoc Least significant difference (LSO). The data were expressed as mean ± standard error and values of $P < 0.05$ were considered significant.

RESULTS AND DICUSSION

The results of the effect of Bonny light crude oil and co-administration of bee honey on serum uric acid, creatinine, and blood urea nitrogen after twenty-one days of exposure are shown on table 1. As would be observed NBLCO ingestion significantly increased concentration of uric acid, creatinine and blood urea nitrogen with respect to the control group ($p<0.05$). Bee honey supplementation on the other hand did not reverse the trend, rather it significantly elevated the three parameters with respect to NBLCO-treated group ($p<0.05$).

Table 1. Effect of NBLCO and bee honey on serum uric acid, creatinine, and blood urea nitrogen (BUN) after 21 days of exposure

Treatment dose (ml/kg)	Uric acid (mg/dl)	Creatinine (mg/dl)	Blood urea nitrogen (mg/dl)
Group I (control)	0.41 ± 0.06	0.47 ± 0.02	8.08 ± 0.12
Group II (NBLCO)	0.53 ± 0.05a	0.72 ± 0.00a	14.32 ± 0.21a
Group III (NBLCO + 50% honey)	0.66 ± 0.02a,b	0.84 ± 0.00a,b	16.17 ± 0.24a,b

N = 6, a=significantly different from group I ($p<0.05$), b=significantly different from group II ($p<0.05$)

Serum uric acid, creatinine, and blood urea nitrogen are parameters used for evaluation of kidney functions. Increase in these parameters indicates renal impairment. Hence, the increase in these parameters seen in the present study is indicative of NBLCO-induced impairment of renal functions.

Exposure of humans and animals to crude oil, which is becoming very common in terms of environmental pollution and abuse, as well as application to the body, could be toxic. Ingestion of Nigerian Bonny-light crude oil within the period of administration of this study caused a significant increase in serum uric acid, creatinine, and blood urea nitrogen. These are parameters often used in the assessment and evaluation of kidney function.

The significant increase in serum uric acid in both test groups (group II and III) compared to control indicates the kidney has suffered considerable damage. According to Orisakwe, approximately 50% or more of renal capacity can be lost to alter the normal clinical indication of renal disease such as serum uric acid and creatinine (Longman-Adman, 1997). However the significant increase in both serum uric acid and serum creatinine in NBLCO-treated group when compared with the control is suggestive of renal pathology. This in part is in agreement with Kluwe definition of renal toxicity (Kluwe, 1981). In an earlier study, Orisakwe *et al*., (2000) observed that exposure to NBLCO caused a significant reduction in kidney weight adding that kidney weight is sensitive indicator of nephrotoxicity (Shilpa *et al*., 2015). This suggests that NBLCO is a potent nephrotoxicant. In another study done by Uboh and coworkers in 2009, it was observed that exposure of rats to some refined products of crude oil like gasoline vapor and diesel caused elevated levels of serum uric acid, creatinine, and blood urea nitrogen, and glucose, which implies renal impairment (Orisakwe *et al*., 2004). Appel reported that a persistent increase in serum creatinine is indicative of chronic kidney disease, which could result in renal failure (Appel *et al*., 2003). Although histopathological examination was not carried out, the result of this study suggests renal impairment induced by NBLCO. Recent study by Ita and Edagha (2016) demonstrated that NBLCO is injurious to the kidney as exposure to it causes histopathological distortions in cyto-architecture of the kidney. A large portion of crude oil components is lipophilic in nature making biological membranes the main target of their adverse actions. Crude oil causes an increase in the production of free radicals within the body; these free radicals may induce renal injury by causing peroxidation of membrane lipids of the glomerulo-tubular cells (Orisakwe *et al*., 2004). Co-administration with 50% honey caused a significant increase in these parameters compared to both control and group II. Honey, generally, is known to contain a variety of antioxidants agents (Gheldo *et al*., 2002). Moreover, NBLCO induced toxicity is reportedly due to the generation of free radicals. Hence, one would expect co-administration of honey to reduce NBLCO nephrotoxicity by reducing serum uric acid, creatinine, and blood urea nitrogen. However, from result obtained from this study, honey was ineffective in this direction.

Considering the antioxidant potential of bee honey, perhaps a higher concentration of honey or even 100% concentrated bee honey may present a different result. Mounting evidence suggest that peroxidative damage to the renal tubular cells plasma membrane is likely to impair the basic renal processes – ultrafiltration, tubular reabsorption, and tubular secretion – thus, leading to the onset of renal impairment. The increase in these parameters found in this study is in agreement with the results of other studies that submitted that NBLCO is nephrotoxic. A previous study by Orisakwe indicates that oral administration NBLCO may cause a dose-dependent nephrotoxicity secondary to induction of oxidative stress in the kidney cells (Orisakwe *et al*., 2004). This study also suggested that oxidative stress might be a common pathway linking diverse mechanisms for the pathogenesis of nephrotoxicity. Numerous studies suggest that oxidative stress participates in the progression of NBLCO-induced nephrotoxicity. In another study done by Adedara *et al*. (2012) NBLCO has been implicated as a potent kidney toxicant and therefore has been suggested to be responsible for at least some components of nephrotoxicity in the Niger Delta human population. Exposure of humans to toxic chemicals contained in the NBLCO appears to be related to various components of acute kidney injury and various forms of nephrotoxicity – such as acute tubular necrosis, acute interstitial nephritis, and glomerular injury – however, evidence is inconclusive.

Conclusion

In study of evaluation of serum uric acid, creatinine and blood urea nitrogen as biomarkers of renal function of rats administered Nigerian Bonny-light crude oil (NBLCO), it is concluded that NBLCO increased levels of serum uric acid, creatinine, and BUN to indicate impairment of renal functions. Although mounting evidence show that free radical mediate the damage during the initial stage of the pathogenesis of renal failure, co-administration of honey, in this study, was ineffective in combating the NBLCO-induced renal impairment.

REFERENCES

Achuba, F. I., Osakwe, S. A. 2003. Petroleum Induced Free radical toxicity in African catfish. *Fish Physiol Biochem*, 29:97-103.

Adedara, I., Teberen, R., Ebokaiwe, A., Ehwerhemuepha, T., Faroubi, E. 2012. Induction of oxidative stress in liver and

kidney of rats exposed to Nigerian Bonny-light crude oil. *Environmental Toxicology*. 27(6):372-379.

Appel, L. J., Middleton, J., Lopokowit, M., Normis, K., Agoda, L., Bakri, G., Douglas, J., 2003. The National and Design of Aask. *Journal American Society of nephrology*. 14 166-172

Berepubo, N. A., Johnson N. C., Sese, B. T. 1994. Growth potentials and organ weights of weaner rabbits exposed to crude oil contaminated feed. *International Journal of Animal Science*. 9:73-76

Blass, K. G., Thiebert, R. J., Lam, L. K. 1974. Study of mechanism of JAFE reactions. *Clin. Chem*., 12(7) :336-343.

Brovelli, A., Suhail, M., Sinigaglia, F., Baldrini, C. 1977. Self-digestion of human erythrocyte membranes: role of adenosine triphosphatase and glutathione. *Biochemical Journal*, 164: 469-472.

Chilcott, R. P., Chapd, H. Q. 2007. Summary of Health Effects of diesel. *Science of the Total Environment*. 20:129-38.

Edwards, C. W. 1989. Toxicology of oil field waste hazards to livestock associated with the petroleum industry. *Vet. Clin. N. Amer*., 5:363-374

Egbi, O. G., Okafor, U. H., Miebodei, K. E., Kasia, B. E., Kunle-Olowu, O. E., Unuigbe, E. I. 2014. Prevalence and correlates of chronic kidney disease among civil servants in Bayelsa state, Nigeria. *Nigerian Journal of Clinical Practice*, 17(5):602-607.

Eyong, E. U., Umoh, I. B., Ebong, P. E. Eteng, M. U. Antai, A. B., Akpa, A. O. 2004. Haematoxic effects following ingestion of Nigerian crude oil and crude oil polluted shellfish by rats. *Nigerian Journal of Physiological Sciences*, 19(1-2):1-6.

Gheldof, N., Engeseth, N. 2002. Antioxidant capacity of honeys from various floral source based on the determination of oxygen radical absorbance capacity and inhibition of *in vitro* lipoprotein oxidation in human serum samples. *Journal of Agriculture and food chemistry*. 50:3050-3055

Ita, S. O., Aluko, E. O., Atang, D. E., Antai, A. B., Osim, E. E. 2013. Vitamin C or E Supplementation Ameliorates Nigerian Bonny Light Crude Oil-induced Erythrocyte Haemolysis in Male Wistar Rats. *Biochemistry & Molecular Biology*, 1(3): 44-51

Ita, S. O., Edagha, I. A. 2016. Renal Protective Effect of Antioxidant Vitamins C and E against Crude Oil-Induced Nephrotoxicity. *Merit Res. J. Med. Med. Sci.,* 4(9):425-431.

Ita, S. O., Udofia, U. A. 2011. Comparative study of some hematological parameters in rats following ingestion of crude oil (Nigerian Bonny light), petrol, kerosene, and diesel. *Asian Journal of Biological Sciences*, 4:498-505

Kluwe, W. M. 1981. Renal function tests as indicators of kidney sub-acute toxicity studies. *Toxicol. Appl. Pharmacol..*, 57: 414-424.

Longman-Adman, N., Renal effects of Environmental and occupational lead exposure. *Environ. Health.Perspect*, 105:938.

Lorke, D. 1993. A New Approach to Practical Acute Toxicity Testing. *Arch Toxicol*., 54: 275-287.

Mark, A., D'Andrea, M., Kesava, R. 2014. Health risks associated with crude oil spill exposure. *The American Journal of Medicine*. 27: 886.e9-886.e13.

Okafor, U. H., Ahmed, S., Unuigbe, E. I. 2015. Screening for risk factors of chronic kidney disease in a community in Niger Delta Nigeria. *Ann Afr Med*., 14:137-42

Orisakwe, O. E., Akumka D. D., Afonne O. J., Gamaniel K. S., 2000. Investigation into the pharmacological basis for folkloric use of Bonny light crude oil in Nigeria. *Ind. J. Pharmac*., 32:231-34.

Orisakwe, O. E., Njan, A. A., Afonne, J. O., Akumka, D. D., Orish, N., Udemezue, O. O . 2004. Investigation into the nephrotoxicity of Nigerian bonny light crude. *Nigerian Journal of Clinical Practice*. 17(5):602-7.

Shilpa, S., Harpreet, K., Anita, Y., Rashmi, D. 2015. Status of Antioxidant and Lipid Peroxidation in Patients with renal failure with DM. *International Journal of Current Medical Research* 4 (10): 381-383.

Uboh, F. E., Akpanabiatu, M. I., Ndem, J. I., Alozie, Y., Ebong, P. E. 2009. Comparative nephrotoxic effect associated with exposure to diesel and gasoline vapours in rats, *J. ToxicolEnviron Health Sci*., 1:68-74.

Veniamin, M. P., Varkirtzi, C. 1970. Chemical basis of the carbamidodi-acetyl micro-method for estimation of urea, citrulline and carbamyl derivatives. *Clinical Chemistry*, 16: 3-6.

Wang, S. Y., Lum, J. L., Carls, M. G., Rice, S. D. 1994. The relationship between growth and total nucleic acids in juvenile pink salmon (Oncorhynchus gorhuscha) fed crude oil contaminated food. *Can. J. Fish. Aquat. Sci.* 50: 996-1001.

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