LABORATORY STUDY

Alterations in morphology and hepatorenal indices in rats subacutely exposed to bitumen extract

Chiagoziem A. Otuecherea, Oluseyi Adesanya, Precious Otsupius and Nathaniel Seyitan

Division of Biochemistry, Department of Chemical Sciences, Redeemer’s University, Ede, Osun State, Nigeria; Department of Biological Sciences, Redeemer’s University, Ede, Osun State, Nigeria

ABSTRACT

Bitumen is a complex mixture of dense and extremely viscous organic liquids produced by distillation of crude oil during petroleum refining. Nigeria has a large deposit of natural bitumen, yet to be fully exploited. Discharges of petroleum hydrocarbons and other petroleum-derived products have caused environmental pollution and adverse human health effects in several oil-rich communities. In this study, bitumen obtained from a seepage source in Agbabu, the town of first discovery, was used in sub-acute toxicity studies in a rat experimental model, in order to assess potential health risks posed to local populace sequel to full exploitation of bitumen. Dosages were chosen to accommodate low to high cases of environmental exposures. Male Wistar rats were administered, per os, dosages of bitumen extract at 5, 3, 2, and 1 mg/kg body weight. Following euthanasia 28 days later, histological findings revealed severe portal congestion and cellular infiltration in the liver, while in the kidney there were protein casts in the tubular lumen. The relative liver and kidney weights in the 5 mg/kg groups were 34% and 40% higher than in the controls, with a concomitant decrease in food and water consumption. Furthermore, plasma clinical analyses revealed marked elevation in aspartate aminotransferase and triglycerides levels in bitumen extract-intoxicated rats. The results indicate the potential hepatorenal toxicity in adult rats following repeated exposure to bitumen extract.

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Introduction

Bitumens are produced by distillation of crude oil during petroleum refining, and also occur naturally. The main use of bitumen is for road paving; other uses include roofing, waterproofing, sealing, and painting.1 The Nigerian bitumen is believed to have similar composition as the light crude oil which includes hydrogen, carbon, and minor amounts of sulfur and oxygen and is believed to have formed from biodegradable and water washing of light crude.2 Crude oil and bitumen contain petrogenic polycyclic aromatic hydrocarbons (PAHs) which are introduced into the environment through accidental oil spills, discharge from routine tanker operations, municipal and urban run-offs, and so on. The combustion of bitumen could also be a source of pyrogenic PAHs, which are released in the form of exhaust and solid residues. A significant accumulation of PAHs in soil, plants and water bodies occur when the release of PAHs into the environment exceeds their degradation capacity. Soil or water contamination originates mainly from PAH emissions to the atmospheres, which reach the soil or water bodies via precipitation.3 Since these contaminated soil and water represent a long-term source of bitumen exposure to the indigenous people, it is imperative to characterize the toxicity of this compound in order to access its potential health risks.

The probable reserve of bitumen and heavy oil in the entire Nigerian belt is about 120 x 4 km, spreading along the bitumen belt encompassing Lagos, Ogun, Ondo and Edo States. Agbabu and Temidire villages, in the Nigerian bitumen deposit area, are farm settlements in Odigbo local government area of Ondo State, South West Nigeria. Agbabu has coordinates of E004 48–49’ and N06 34–36’ and is approximately 210 km to Lagos, while Temidire, a smaller settlement, is located in the coordinates E004 49–50’ and N06 36–37’, about 2 km to Agbabu.4,5

Although physical biodegradation mechanisms for bitumen have not been well investigated, but there has
been a report investigating the ability of some selected bacteria to degrade aliphatic and polycyclic aromatic hydrocarbon fractions of Agbabu natural bitumen. However, the complete remediation and reclamation of seepage sources by indigenous microbial communities are challenging and proceed slowly. This protracted process is mainly due to the low degradation efficiency of organic contaminants under anaerobic condition typically encountered in seepage sources.6,7

The principal toxicological concern has been the potential for bitumen to cause cancer, based in part on reports that bitumen fume condensate was carcinogenic when repeatedly applied to mouse skin.8 In mammalian cells, exposure to bitumen emissions or their condensates produced mutagenic intermediates and DNA adducts, and also caused cellular stress and disruption of cellular defense programs. Compared with control populations, blood or urine from road pavers working with bitumen showed higher levels of mutagenic urine, higher levels of reactive oxygen species, increased DNA damage, cytogenetic alterations and chromosomal aberrations in lymphocytes.9–11 To the authors’ knowledge, studies on hepatorenal toxicity testing in rodents involving repeated oral dosing of bitumen have not been reported previously. The objective of this current study was to determine the health risk to indigenous populace as a result of exposure to bitumen in their environment. Wistar rats were used to test the sub-acute toxicity of bitumen. Animals were gavaged repeated doses of bitumen extract. Effects on body weight, water and food consumption, and other clinical signs of toxicosis were monitored. Effects of bitumen on plasma biochemical parameters were also evaluated, followed by the histopathological examination of the liver and kidney.

Materials and methods

Chemicals and reagents

The bitumen sample used for this study was collected from a seepage source in Temidire camp, in Agbabu, Ondo State. Reagents were of analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO). Kits for blood biochemistry were purchased from Randox Laboratory Limited, Crumlin, UK.

Animals and animal husbandry

All thirty animals used in this study were outbred Wistar rats, obtained from the primate colony of the Department of Veterinary Anatomy, University of Ibadan. The rats were 10 weeks old at the beginning of treatment. Animals were housed in polypropylene cages lined with hard wood bedding. They were fed on commercial pelleted diet (Ladokun Feeds Ibadan, Nigeria) and drinking water ad libitum. Animals were maintained under a light:dark schedule 12 h:12 h at a temperature of 25°C. Animals were allowed a 14-day acclimatization period prior to the toxicity testing. They were subsequently assigned randomly into five groups of six rats each, including a control group and four experimental groups. The dosages delivered, by gavage, to treatment groups were 5, 3, 2 and 1 mg/kg body weight of bitumen extract (BE 5, BE 3, BE 2 and BE 1, respectively) 3 days per week for 28 days. Control rats were administered 0.6 ml distilled water by gavage for equivalent number of days. Doses and extraction procedure were based on modifications of earlier reports by Rogers et al.12 Fractions of the high dose group (60 mg/kg/day), used in their study, were selected to maintain the classification of low dose, medium dose and high dose. All experiments conformed to guidelines governing the handling of laboratory animals as outlined by the Redeemer’s University Committee on Ethics for Scientific Research. Throughout the duration of the test, animals body weight, food and water consumption, and clinical signs were monitored daily. Rats were sacrificed by cervical dislocation and blood samples were collected by cardiac puncture into clean EDTA bottles and centrifuged at 4000 g for 10 min (Heraeus Labofuge 300, Thermo Scientific, Hampshire UK). Plasma was carefully separated out and stored frozen until required for analysis. Liver and kidneys of rats were excised, homogenized in phosphate buffer (0.1 M, pH 7.4), centrifuged at 4500 g and the supernatants used for the other biochemical analysis. Selected organs were removed, fixed, sectioned, and stained for histopathological examination.

Biochemical analyses

Biochemical analyses on biochemical markers were carried out to determine the plasma concentrations of alanine and aspartate aminotransferases, alkaline phosphatase, albumin, total bilirubin, total cholesterol, triglycerides, uric acid, urea and creatinine using diagnostic kits (Randox Laboratories Limited) as reported in our previous studies.13–15

Histopathology

Liver and kidney tissues obtained from all experimental groups were fixed in 10% formaldehyde, dehydrated in graded alcohol and embedded in paraffin. The tissues were subsequently cut into 4–5 mm sections by a
microtome, fixed on the slides and stained with hematoxylin and eosin for light microscopic analyses. The slides were coded and were examined by a histopathologist who was blinded to the treatment groups as reported in our previous studies.16

**Statistical analyses**

All data were expressed as mean ± standard error of the mean (SEM). Differences between the groups were determined by one-way analysis of variance (ANOVA) and post hoc testing was performed using Tukey’s multiple comparison tests (Graph Pad Prism software, Inc., San Diego, CA). Values were regarded as significantly different at \( p < .05 \).

**Results**

**Clinical signs and mortality patterns**

Rats administered Bitumen extract at the highest dose of 5 mg/kg (BE 5) experienced seizures especially at the time of intubation. These seizures were accompanied by brief tetanic contractions and about 5 min period of immobility. During the four weeks of treatment, dose-dependent mortalities of 33.3 and 14.2% occurred in the BE 5 and BE 3 groups, respectively. Small portion of rats in other groups presented with mild episodes of muscle twitching but no other adverse clinical manifestations (e.g. diarrhea, hematuria, restlessness) were seen in the experimental animals during the dosing period.

**Body weight gain and relative organ weights**

The control rats gained weight throughout the duration of the treatment. Weight losses were observed in rats fed BE 5, BE 2, and BE 1 after 7 days of treatment. However, there were compensatory gains in weight among treatment groups as the experiment progressed. These body weights were not significantly different from control (Figure 1). Liver weights of animals administered BE 5 were 40% heavier, and significantly increased than controls \( (p < .01) \). Although the kidney weight was 15% heavier than the control, this was not statistically significant. There were no significant changes in the relative weights of the liver and kidney of treated rats in relation to control groups (Table 1).

**Food and water consumption trends**

Weekly and mean food and water consumption patterns are shown in Figure 2. Food consumption was less in high-dose rats especially after 21 and 28 days of treatment. However, the average food consumption of all the groups was similar. Compared with controls, water consumption by high-dose rats was significantly \( (p < .01) \) depressed during the 4th week of the treatment at the doses of BE5 and BE3, but these changes were not significantly expressed in the mean water consumption across all treatment groups.

**Effect of BE on biochemical parameters**

BE administration did not influence the basal levels of ALT, ALP, albumin, bilirubin, uric acid, urea, and creatinine (Table 2 and Figure 3). Administration of BE to animals across all treatment doses significantly elevated AST activity when compared with control values. Furthermore, the triglyceride level was significantly elevated in comparison with basal value. The total protein level in the control group was 0.23 ± 0.02 mg/dL, but
Table 1. Effect of Bitumen extract on body parameters in experimental rats.

| Variable                  | Control      | BE 5          | BE 3          | BE 2          | BE 1          |
|---------------------------|--------------|---------------|---------------|---------------|---------------|
| Liver weight (g)          | 4.93 ± 0.11  | 6.90 ± 0.75\(^a\) | 5.78 ± 0.21  | 5.44 ± 0.17  | 5.28 ± 0.15  |
| Liver weight/Body weight  | 0.027 ± 0.001| 0.033 ± 0.005 | 0.026 ± 0.001| 0.027 ± 0.001| 0.026 ± 0.001|
| Kidney weight (g)         | 1.08 ± 0.03  | 1.24 ± 0.07   | 1.26 ± 0.06   | 1.10 ± 0.03   | 1.16 ± 0.04   |
| Kidney weight/Body weight | 0.0060 ± 0.0001 | 0.0058 ± 0.0007 | 0.0056 ± 0.0003 | 0.0052 ± 0.0001 | 0.0056 ± 0.0002 |

BE 5, 5 mg/kg Bitumen extract; BE 3, 3 mg/kg Bitumen extract; BE 2, 2 mg/kg Bitumen extract and BE 1, 1 mg/kg Bitumen extract. Values are expressed as mean ± SEM of 6 rats per group after 28 d treatment period.

\(^a\)Significantly different from control (\(p < .01\)).

Figure 2. (A) Weekly food consumption, (B) total food intake, (C) weekly water consumption, and (D) total water intake in experimental rats. BE 5, 5 mg/kg Bitumen extract; BE 3, 3 mg/kg Bitumen extract; BE 2, 2 mg/kg Bitumen extract and BE 1, 1 mg/kg Bitumen extract. Values are expressed as mean ± SEM of six rats per group after 4 weeks treatment period. **Significantly different from control (\(p < .01\)).

Table 2. Effect of Bitumen extract on the level of plasma functional parameters in experimental rats.

| Variable            | Control       | BE 5          | BE 3          | BE 2          | BE 1          |
|---------------------|---------------|---------------|---------------|---------------|---------------|
| Albumin (g/dl)      | 7.59 ± 0.28\(^a\) | 6.56 ± 0.64\(^a\) | 6.31 ± 0.25\(^a\) | 6.52 ± 0.21\(^a\) | 6.05 ± 0.30\(^b\) |
| Total Protein (mg/dl)| 0.23 ± 0.02\(^a\) | 0.12 ± 0.02\(^b\) | 0.13 ± 0.01\(^a\) | 0.15 ± 0.01\(^a\) | 0.17 ± 0.03\(^a\) |
| Total Cholesterol (mg/dl)| 30.28 ± 3.24\(^a\) | 47.84 ± 6.69\(^b\) | 25.46 ± 5.32\(^a\) | 23.23 ± 4.39\(^a\) | 22.56 ± 8.29\(^a\) |
| Triglycerides (mg/dl) | 47.40 ± 4.81\(^a\) | 64.19 ± 6.25\(^a\) | 83.81 ± 10.42\(^a\) | 61.87 ± 7.15\(^a\) | 89.42 ± 6.48\(^b\) |
| Uric acid (mg/dl)    | 2.64 ± 0.28\(^a\) | 3.50 ± 0.37\(^a\) | 3.13 ± 0.28\(^a\) | 2.15 ± 0.19\(^a\) | 2.17 ± 0.31\(^a\) |
| Urea (mg/dl)         | 36.02 ± 1.25\(^a\) | 38.86 ± 4.45\(^a\) | 32.59 ± 1.34\(^a\) | 35.57 ± 2.62\(^a\) | 34.7 ± 1.70\(^a\) |
| Creatinine (mg/dl)   | 14.86 ± 1.50\(^a\) | 18.67 ± 1.76\(^a\) | 19.14 ± 2.46\(^a\) | 18.00 ± 2.07\(^a\) | 18.66 ± 2.61\(^a\) |

BE 5, 5 mg/kg Bitumen extract; BE 3, 3 mg/kg Bitumen extract; BE 2, 2 mg/kg Bitumen extract and BE 1, 1 mg/kg Bitumen extract. Values are expressed as mean ± SEM of 6 rats per group after 28 d treatment period. Values in each row with different superscript are significantly different from control group (\(p < .05\)).
significantly decreased by 47.8%, 43.5%, 34.8% and 26.1% following treatment with BE 5, BE 3, BE 2 and BE 1, respectively.

**Histopathology of rat liver and kidney**

There were no discernible alterations in the control groups as they appeared structurally and functionally normal. However, dose-related histological changes were observed in tissues sub-acutely treated with Bitumen Extract. In the liver of BE-treated rats, severe portal congestion, mild periportal fibrosis and cellular infiltration by mononuclear cells were observed (Figure 4). The kidneys of rats exposed to BE at doses of 5 mg/kg and 3 mg/kg showed some degenerated tubular lumen with several protein casts as well as mild congestion of the cortex (Figure 5).

**Discussion**

Nigerian bitumen possesses relatively large quality of naphthenes, aromatics and asphaltenes that are similar to the conventional oil. This makes the Nigerian bitumen a very useful alternative source of petroleum hydrocarbon and a potential feedstock for petrochemical industries. Our study investigated the potential hepatorenal toxicity in rats exposed to sub-acute oral dosing of bitumen extract (BE).

The high mortality rate and seizures observed in high-dose experimental animals are probably indications that the dose of 5 mg/kg is toxic and could cause respiratory problems. Intoxication of animals to graded doses of BE elicited some treatment-related changes, especially in the 5 mg/kg and 3 mg/kg doses. The administration of BE to rats produced a significant increase in the weight of the liver, but not in the kidney. Although BE did not affect the mean weight of the rats, the organ weights were significantly increased, and this may indicate its early toxic potential. According to Maronpot and co-workers, the increased liver weight of the high-dose rats could be in response to hepatic enzyme induction, which is typically associated with hepatocellular hypertrophy and transient hepatocyte hyperplasia. The kidney weights in the 5 mg/kg and 3 mg/kg groups were 15% and 18% higher than the basal group, however these differences were not significant and may just reflect the slightly lower body weights of the animals in the high-dose treatment groups.
Repeated dosing of rats to BE affected both food and water consumption. Food consumption of all the treatment groups was similar up till the third week, with a non-significant suppression observed in the high-dose groups in the fourth week. As expected, there was a correlating depression in water consumption in the high dose-rats as the days of exposure extended. Specifically, water consumption decreased significantly in the final week of administration. It is plausible the bitumen extract reduced the sense of taste and appetite of the animals.19 Our data is further corroborated by an earlier cross-sectional study which provided evidence suggesting a potentially protective effect of higher total water intake on the kidney. By extension, the authors reported an association between low intake of plain water and chronic kidney disease.20

Plasma biochemical analyses indicated that the liver, and to a lesser extent, the kidney are target organs for toxicity induced by the bitumen extract. Sero-clinical markers, including ALT, ALP, bilirubin, total cholesterol, uric acid, urea and creatinine, were not significantly affected across all treatment groups. Our results agree with another study which reported that rats gavaged with Alberta crude oil, at doses of 1.2–5 mL/kg, did not

Figure 4. Representative photomicrograph of hematoxylin and eosin-stained sections of liver from the experimental groups. The livers of control rats showed normal histology but severe portal congestion (arrow) and mild periportal cellular infiltration by mononuclear cells (arrow head) were observed in livers of rats exposed to Bitumen extract. BE 5, 5 mg/kg Bitumen extract; BE 3, 3 mg/kg Bitumen extract; BE 2, 2 mg/kg Bitumen extract and BE 1, 1 mg/kg Bitumen extract. Original magnification: × 400.
significantly affect body weight gain, alanine amino-transferase, gamma glutamyltransferase, blood urea nitrogen and creatinine. It is plausible that the tissue damage was not sufficient to allow the leakage of these marker molecules from the tissues into the blood. In contrast to our findings, Olabemiwo et al. reported an increase in ALP activity in rats fed diets formulated with simulated bitumen leachate.

Interestingly, AST and triglycerides were significantly increased in BE-exposed rats. AST is found in the mitochondria and appears in high concentration in the liver, kidney and heart, so it could serve as an index of hepatorenal damage. The increased level of AST, as observed in this study, is also an indication of increased permeability and necrosis of hepatocytes by BE. This finding is consistent with previous study on organ toxicity in rats treated with landfill leachate.

Experimental and clinical studies have suggested a correlation between the progression of renal disease and dyslipidemia. High cholesterol and triglyceride plasma levels have been demonstrated to be independent risk factors for progression of renal disease in humans. The underlying pathophysiologic mechanisms for the relationship between lipid levels and progression of renal disease are not yet fully understood, although there are data that oxidative stress and insulin...
resistance may mediate the lipid-induced renal damage.\textsuperscript{25} It has also been suggested that plasma cholesterol and triglycerides assays may be early and reliable indicators of hepatotoxicity in the rat experimental model.\textsuperscript{26,27} In the present study, dose-related, and statistically significant increases were seen in the levels of cholesterol and triglycerides, which rose by 60\% and 35\%, respectively, at the 5 mg/kg dose. In agreement with our data, a 5-day administration of rifampicin was shown to cause hypertriglyceridemia possibly by inducing the activity of regulatory enzymes involved in the biosynthesis of triglyceride. Similarly, male rats sub-chronically treated with ethyl tertiary-butyl ether, an additive of gasoline, at a dose of 400 mg/kg, showed an increase in the level of total cholesterol, suggesting a possible induction of cytochrome P450.\textsuperscript{28,29}

Furthermore, our study showed that albumin and total proteins levels were slightly statistically reduced. Reduction in albumin levels as observed in the present study is in agreement with an earlier report of decrease in the albumin levels of catfish fed with crude oil-contaminated diet.\textsuperscript{30} Decrease in the levels of total protein is possibly a reflection of the decreased albumin levels. According to Ramaiah,\textsuperscript{31} impaired liver functions are generally reflected by decreased protein synthesis and depleted plasma levels of albumin.

The examination of tissue sections for histological changes supplements evidence from biochemical analyses. The histopathological observations associated with repeated BE dosing included portal congestion and appearance of tubular protein casts in the liver and kidney, respectively. In addition, the incidence of cellular degeneration and infiltration by mononuclear cells may likely indicate cellular toxicity. It might be due to the formation of highly reactive radicals and subsequent lipid peroxidation induced by BE.\textsuperscript{22,32}

In conclusion, significant sero-clinical and pathological observations were associated with the sub-acute exposure of BE to rats especially at the doses of 5 mg/ kg and 3 mg/kg. Extrapolation of research findings from the laboratory to the bedside involves variables such as age, season, diet, general health, contaminant interaction, and interspecies differences in metabolism, but there exists the possibility that indigenous human population could be sensitive to BE.\textsuperscript{12} Hence, this study will be a valuable reference for other scientists involved in similar researches.

Disclosure statement
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References
1. Lauby-Secretan B, Baan R, Grosse Y, et al. Bitumens and bitumen emissions, and some heterocyclic polycyclic aromatic hydrocarbons. \textit{Lancet Oncol}. 2011;12:1190–1191.
2. Akinmosin A, Osinowo OO, Oladundoye MA. Radiogenic components of the Nigerian Tar Sand Deposits. \textit{Earth Sci Res J}. 2009;13:64–73.
3. Olajire AA, Alade AO, Adeniyi AA, Olabemwo OM. Distribution of polycyclic aromatic hydrocarbons in surface soils and water from the vicinity of Agbabu bitumen field of Southwestern Nigeria. \textit{J Environ Sci Health Part A}. 2007;42:1043–1049.
4. Oboh OB, Ilori MO, Akinyemi JO, Adebusoye SA. Hydrocarbon degrading potentials of bacteria isolated from a Nigerian bitumen (Tarsand) deposit. \textit{Nature Sci}. 2006;4:51–57.
5. Fagbote E, Olanipekun EO, Uyi HS. Water quality index of the ground water of bitumen deposit impacted farm settlements using entropy weighted method. \textit{Int. J. Environ Sci Technol}. 2014;11:127–138.
6. Olabemwo OM, Adeniran GO, Adekola FA, Adelowo OO, Olajire AA. Biodegradation of hydrocarbon compounds in Agbabu natural bitumen. \textit{African J Biotechnol}. 2014;13:1257–1264.
7. Phillips LA, Armstrong SA, Headley JV, Greer CW, Germida JJ. Shifts in root-associated microbial communities of \textit{Typha latifolia} growing in naphthenic acids and relationship to plant health. \textit{Int J Phytotherapy}. 2010;12:745–760.
8. Niemeier R, Thayer P, Menzies K, Von Thuna P, Moss C, Burg JA. Comparison of the skin carcinogenicity of condensed roofing asphalt and coal tar pitch fumes. In: \textit{Polynuclear Aromatic Hydrocarbons: A Decade of Progress. Tenth International Symposium Columbus, Ohio: Battelle Press, 1988:609–647.
9. Wang JJ, Frazer DG, Stone S, et al. Urinary benzo[a]pyrene and its metabolites as molecular biomarkers of asphalt fume exposure characterized by microflow LC coupled to hybrid quadrupole time-of-flight mass spectrometry. \textit{Anal Chem}. 2003;75:5953–5960.
10. Zhao HW, Yin XJ, Frazer D, et al. Effects of paving asphalt fume exposure on genotoxic and mutagenic activities in the rat lung. \textit{Mutat Res}. 2004;557:137–149.
11. Lindberg HK, Vaananen V, Jarventaus H, et al. Genotoxic effects of fumes from asphalt modified with waste plastic and tall oil pitch. \textit{Mutat Res}. 2008;653:82–90.
12. Rogers V, Wickstrom M, Liber K, MacKinnon M. Acute and subchronic mammalian toxicity of naphthenic acids from oil sands tailings. \textit{Toxicol Sci}. 2002;66:347–355.
13. Abarikwu SO, Adebayo OL, Otuechere CA, Iserhienrhien BO, Badejo TA. Selenium and rutin alone or in combination do not have stronger protective effects than their separate effects against cadmium-induced renal damage. \textit{Pharm Biol}. 2016;54:896–904.
14. Adewuyi A, Otuechere CA, Oteglolade ZO, Bankole O, Unuabonah EI. Evaluation of the safety profile and antioxidant activity of fatty hydroxamic acid from
underutilized seed oil of *Cyperus esculentus*. J Acute Dis. 2015;4:230–235.

15. Otuechere CA, Madarikan G, Simisola T, Bankole O, Osho A. Virgin coconut oil protects against liver damage in albino rats challenged with the anti-folate combination, trimethoprim-sulfamethoxazole. *J Basic Clin Physiol Pharmacol*. 2014;25:249–253.

16. Otuechere CA, Abarikwu SO, Ekor MN, Rufai AM, Oshoto EA, Farombi EO. Protective effects of Vitamin C against Propanil induced hepatotoxicity in Wistar rats. *Asian Pac J Trop Dis*. 2012;5212–5217.

17. Adegoke OS, Omatola ME, Coker JL. The Geology of the Nigerian Tar-Sands. *Heavy crude and tar sands hydrocarbons for the 21st Century*. Proc. 5th UNITAR International Conference 1991; 369–835.

18. Maronpot RR, Yoshizawa K, Nyska A, et al. Hepatic enzyme induction: Histopathology. *Toxicol Pathol*. 2010;38:776–795.

19. Yakubu MT, Musa IF. Effects of post-coital administration of alkaloids from *Senna alata* (Linn. Roxb) leaves on some fetal and maternal outcomes of pregnant rats. *J Reprod Infantil*. 2012;13:211–217.

20. Sontrop JM, Dixon SN, Garg AX, et al. Association between water intake, chronic kidney disease, and cardiovascular disease: A cross-sectional analysis of NHANES data. *Am J Nephrol*. 2013;37:434–442.

21. Khan AA, Coppock RW, Schuler MM, Sharma AK, Lillie LE. Induction of hepatic cytochrome p450 and xenobiotic metabolizing enzymes in rats gavaged with an alberta crude oil. *J Toxicol. Environ Health*. 1989;28:297–307.

22. Olabemiwo OM, Adeniran GO, Adekola FA, Adelowo OO, Olajire AA, Adeleke OS. Impacts of simulated Abgabu bitumen leachate on hematological and biochemical parameters of Wistar Albino rats. *Res J Environ Toxicol*. 2011;5:213–221.

23. Otuechere CA, Abarikwu SO, Olateju VI, Animashaun AL, Kale OE. Protective effect of curcumin against the liver toxicity caused by propanil in rats. *Int Scholar Res Notices*. 2014;2012. doi:10.1155/2014/853697.

24. Farombi EO, Akintunde JK, Nzute N, Adedara IA, Arojoyojo O. Municipal landfill leachate induces hepatotoxicity and oxidative stress in rats. *Toxicol Ind Health*. 2012;28:532–541.

25. Trevisan R, Dodesini AR, Lepore G. Lipids and renal disease. *J Am Soc Nephrol*. 2006;17:145–147.

26. Mitruka DM, Rawnsley HM. *Clinical, Biochemical and Hematological References Values in Normal Experimental Animals and Normal Humans*, 2nd ed. New York: Massons Pub, 1981:413.

27. Provost JP, Hanton JJ, Le Net JL. Plasma triglycerides: An overlooked biomarker of hepatotoxicity in the rat. *Comp Clin Path*. 2003;12:95–101.

28. Farombi EO, Akinloye O, Akinmoladum CO, Emerole GO. Hepatic drug metabolizing enzyme induction and serum triacylglycerol elevation in rats treated with chlorodiazepoxide, griseofulvin, rifampicin and phenytoin. *Clin Chim Acta*. 1999;289:1–10.

29. Miyata K, Koga T, Aso S, Hoshuyama S, Ajimi S, Furukawa K. A subchronic (180-day) oral toxicity study of ethyl tertiary-butyl ether, a bioethanol, in rats. *Drug Chem Toxicol*. 2014;37:303–310.

30. Sunmonu TO, Oloyede OB. Biochemical assessment of the effects of crude oil contaminated catfish (*Clarias gariepinus*) on the hepatocytes and performance of rat. *African J. Biochem Res*. 2007;1:83–89.

31. Ramaiah SK. A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. *Food Chem Toxicol*. 2007;45:1551–1557.

32. Adedara IA, Abolaji AO, Odion BE, Okwudi IJ, Omoloja AA, Farombi EO. Impairment of hepatic and renal functions by 2,5-hexanediol is accompanied by oxidative stress in rats. *J Toxicol*. 2014;2014. doi: 10.1155/2014/239240.