ISSN 1996-3351

Asian Journal of Biological Sciences

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Asian Journal of Biological Sciences 4 (6): 498-505, 2011
ISSN 1996-3351 / DOI: 10.3923/ajbs.2011.498.505

Comparative Study of Some Haematological Parameters in Rats Following Ingestion of Crude Oil (Nigerian Bonny Light), Petrol, Kerosene and Diesel

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ABSTRACT

The exposure of Nigerians to crude oil and its refined products is in the increase following incessant oil spills and proliferation of sales outlet. Against this backdrop, comparative effects of Nigerian Bonny Light Crude Oil and some of its refined products (gasoline, kerosene and diesel) on some haematological parameters of male rats of Wister strain were investigated. A total of twenty-five male rats were grouped into five groups, with group 1 serving as control. The remaining four groups were oral gavaged with 6 mL kg^{-1} body weight of petrol, kerosene, diesel and crude petroleum, respectively. The result obtained showed that Red Blood Cell (RBC), Packed Cell Volume (PCV) and Haemoglobin (Hb) content were significantly lower in petrol ($p<0.001$), kerosene ($p<0.01$) and diesel ($p<0.05$) than the control group. Crude oil also recorded lower values for these parameters which were not statistically different from the control values. Significantly ($p<0.05$) Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) higher values were recorded for petrol and kerosene groups. Platelets values were significantly higher in all the test groups ($p<0.001$), than control. White Blood Cell (WBC) was significantly higher in petrol group ($p<0.05$) but significantly ($p<0.01$), lower in kerosene diesel and crude petroleum groups. Lymphocytes was significantly ($p<0.001$) lower in petrol group and recorded marginal reduction in kerosene and diesel but marginal increased in crude petroleum. Neutrophils and eosinophil values were significantly higher ($p<0.001$) in petrol group and marginally higher in kerosene and diesel groups but marginally lower in crude petroleum group than control group. Values obtained for monocytes in the test groups were not statistically different from the control value. It is, therefore concluded that crude petroleum and some of its products are highly toxic and are potential damaging agents to the haematopoietic system and could cause anaemia, particularly petrol and kerosene when ingested.

Key words: Crude petroleum, petrol, kerosene, diesel, haematopoietic system, anemia

INTRODUCTION

Contamination of the environment by petroleum hydrocarbons is widespread and frequent. Similarly exposure of animals including humans to crude oil and its refined products are widespread and frequent. Petroleum products account for a large fraction of the contamination at hazardous waste sites. Road side automobile mechanics have formed wrong habits such as sucking of petrol with their mouth to wash their hands after work. Also, patients with sore throat

are wrongly advised to lick sugar soaked in kerosene because it is claimed to cure sore throat. It is reported that crude petroleum are taken orally against toxic venom such as snakebites (Chilcott and Chapd, 2007). Such acclaimed uses occur with little or no care at all about its hazardous effects on health, which may result from accumulation of toxic substances in them. According to Eyong *et al.* (2004), ingestion of aquatic species exposed to spillage poses the risk of possible bioaccumulation and bio-concentration of toxic components of crude oil.

The metabolisms of aliphatic and aromatic hydrocarbons which are the major components of crude petroleum and petroleum products and even other xenobiotics result in generation of free radical species in various tissues (Achuba and Osakwe, 2003). In red blood cell, free radicals are known to alter erythrocyte membrane as well as other cell membranes as a consequence of oxidative stress by Shakirov and Farkhutdinov (2000).

A large proportion of crude oil and other petroleum products are lipophilic in nature and biological membrane may, therefore be the target sites where their adverse effects occur (Anozie and Onwurah, 2001). Many compounds exert considerable dangerous influence on the membrane integrity by the direct chemical contact with biomolecules particularly the protein that constitute the architectural structure of plasma membrane (Anozie and Onwurah, 2001; Khan *et al.*, 2001).

Perturbations of this architectural assembly by exogenous insult such as cigarette smoking, carbon monoxide and polycyclic aromatic hydrocarbons lead to various altered membrane material properties and altered red blood cell behavior in the circulation. Cellular deformability is one of the major parameters that determine red cell life span and certain xenobiotic have been known to compromised the capacity of red blood cell to withstand osmotic stress (Ojo *et al.*, 2006), in this regard.

Anaemia has been reported to occur in birds following ingestion of crude oil (Suzanne, 2003; Becki, 2007) due to massive blood cell destruction. Udonwa *et al.* (2009) reported that a high level of met haemoglobin concentration was observed in station attendants and auto-mechanics exposed to premium motor spirit fumes. In fact, anaemia is said to occur in experimental animals few days after oil ingestion, as a result of oxidant chemical damage to haemoglobin, resulting in haemoglolysis (Suzanne, 2003). This is seen more commonly with crude oil and less evidence with diesel.

This research was designed to study comparative effects of crude oil (Nigerian Bonny Light) and some of its refined products (gasoline, kerosene and diesel) on some haematological parameters of male rats of Wister strain.

MATERIALS AND METHODS

Chemicals: This study was conducted in year 2010. Diesel, kerosene and petrol were purchased from the NNPC MEGA station Itam, Uyo; the Crude petroleum was obtained from EXXON Mobil Laboratory Ibeno, all in Akwa Ibom State, Nigeria.

Experimental animals: Mature male albino Wister rats were obtained from the animal house of the department of Physiology, University of Calabar, Nigeria and were kept in a well-ventilated experimental section in the animal house of the Faculty of Pharmacy, University of Uyo, Nigeria for fourteen days to acclimatize. After the acclimatization period, the animals were weighed and their weight ranges between 150-170 g. These animals were kept in wooden cages of 50×30 cm dimension. They were fed with rat chow from vital feed and they were equally allowed free access to drinking water while the experiment lasted.

Experimental design and treatment of animals: A total of twenty five adult male Wister rats with weight range of 150-170 g were randomly assigned to five groups, group I as the control and II-V as test groups. It was experimentally designed thus; each of the test group (II-V) was administered 6 mL kg^{-1} of body weight of petrol, kerosene, diesel and crude petroleum, respectively. In order to know the actual dose to be administered to each animal in a group, calculation was done according to the body weight before administration. The weight which was in grams was first converted to kilogram by dividing through by 1000, then multiply by the dose of the substances (6 mL kg^{-1}) to be administered.

The animals in groups II-V were repeatedly exposed by oral gavage to 6 mL kg^{-1} of petrol, kerosene, diesel and crude petroleum for 21 days. Rats in the control group were similarly oral gavaged with 6 mL kg^{-1} of normal saline as control vehicle for the same 21 days.

Determination of haematological parameters: After twenty one days of administration, the rats were suffocated with chloroform soaked in swap of cotton wool in a desiccator. A 5 mL sterile syringe with needle was used for collection of blood from the heart, by a process known as cardiac puncture. The blood sample was transferred into properly labelled, EDTA sample bottles. The anti-coagulated blood was used for the determination of erythrocyte count, Packed Cell Volume (PCV), Haemoglobin (HB), White Blood Cell (WBC) and haematological indices with the aid of an automatic haematology analyzer (Mindray Hematology analyzer, BC-2300).

Statistical analysis: Statistical analysis was carried out using Window SPSS. One way analysis of variance was adopted for comparison and results were subjected to post hoc test using Least Square Deviation (LSD). The data were expressed as Mean±Standard Error and values of $p<0.05$ were considered significant.

RESULTS

The results for the mean values of RBC in ($\times10^6$ μL^{-1}) are 7.72±0.21 in control, 6.21±0.04 in petrol, 6.61±0.10 in kerosene, 6.81±0.15 in diesel and 7.12±0.30 in crude oil. The RBC values were significantly lower in petrol ($p<0.001$), kerosene ($p<0.01$) and diesel ($p<0.05$) groups when compared to the control value. The mean PCV values in % are 46.38±1.37 control, 40.63±0.85 petrol, 40.62±0.72 in kerosene, 42.58±2.24 in diesel and 41.92±1.49 in crude oil. These values were significantly ($p<0.05$) lower in petrol and kerosene groups. The mean Hb values in g dL^{-1} are 13.62±0.31 control, 11.83±0.28 petrol, 11.64±0.14 in kerosene, 11.95±0.30 in diesel and 12. 38±0.55 in crude oil. In petrol and diesel groups, these values were significantly ($p<0.05$) lower than control group and significantly ($p<0.01$) lower in kerosene group than the control group. Crude oil group recorded values that were not statistically different from the control values. The mean MCV values in fL are 59.90±1.08 control, 65.47±1.59 petrol, 61.46±0.99 in kerosene, 62.43±2.00 in diesel and 58.95±0.73 in crude oil. The mean MCH values in pg are 17.66±0.33 control, 19.10±0.49 petrol, 17.60±0.08 in kerosene, 17.60±0.08 in diesel and 17.40±0.17 in crude oil. While MCV and MCH recorded significantly ($p<0.05$) higher values for the petrol group MCH recorded significantly ($p<0.05$) lower value in diesel group than the control group. The mean MCHC values in g dL^{-1} are 29.38±0.24 control, 29.10±0.58 petrol, 28.70±0.44 in kerosene, 28.20±0.87 in diesel and 29.80±0.29 in crude oil, these values were not significantly different from the control value as shown in Table 1.

The mean platelet counts in ($\times10^3$ μL^{-1}) are 867.80±2.67 control, 1444.50±1.50 petrol, 986.20±2.15 in kerosene, 983.30±2.40 in diesel and 995.40±0.40 in crude oil. These values were

Table 1: Total RBC: Red Blood Cell counts, PVC: Packed cell volume, Hb: Haemoglobin content and red cell indices responses (Mean±SEM) of Wister male rats after ingestion of crude petroleum, refined petrol, kerosene and diesel after 21 days

				Red blood indices		
Groups	RBC ($\times 10^6$ μL^{-1})	PCV (%)	Hb (g dL^{-1})	MCV (fL)	MCH (pg)	MCHC (g dL^{-1})
Control	7.72±0.21	46.38±1.37	13.62±0.31	59.90±1.08	17.66±0.33	29.38±0.24
Petrol (6 mL kg^{-1})	6.21±0.04^c	40.63±0.85^a	11.83±0.28^a	65.47±1.59^a	19.10±0.49^a	29.10±0.58
Kerosine (6 mL kg^{-1})	6.61±0.10^b	40.62±0.76^a	11.64±0.14^b	61.46±0.99	17.60±0.08^d	28.70±0.44
Diesel (6 mL kg^{-1})	6.81±0.15^a	42.58±2.24	11.95±0.30^a	62.43±2.00	17.60±0.08^a	28.20±0.87
Crude petroleum (6 mL kg^{-1})	7.12±0.30^d	41.92±1.49	12.38±0.55	58.95±0.73^d	17.40±0.17^e	29.80±0.29

a: significantly different from control ($p<0.05$), b: significantly different from control ($p<0.01$), c: significantly different from control ($p<0.001$), d: significantly different from petrol ($p<0.05$), e: significantly different from petrol ($p<0.01$)

Table 2: Platelets, total white blood cell counts and differential counts responses (Mean±SEM) of Wister male rats after ingestion of crude petroleum, refined petrol, kerosene and diesel after 21 days

			Differential counts			
Groups	Platelets ($\times 10^3$ μL^{-1})	WBC ($\times 10^3$ μL^{-1})	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)	Eosinophils (%)
Control	867.80±2.67	15.54±2.28	75.40±1.83	2.00±0.58	20.40±1.44	2.75±0.48
Petrol (6 mL kg^{-1})	1444.50±1.50^c	24.90±0.16^a	48.50±2.10^c	1.33±0.33	45.50±1.19^c	7.30±0.88^b
Kerosine (6 mL kg^{-1})	986.20±2.15^{c,f}	14.32±2.20^e	70.80±1.74^{f,} **	3.00±0.00	22.80±2.82^f	3.25±0.25^e
Diesel (6 mL kg^{-1})	983.30±2.14^{c,f}	14.00±1.85^e	71.25±2.69^{f,} *	3.33±1.20	23.50±2.73^f	3.50±0.96^d
Crude petroleum (6 mL kg^{-1})	995.40±0.40^{c,f}	11.32±1.24^f	81.60±1.23^f	2.00±0.58	15.20±1.20^f	2.67±0.67^f

a: significantly different from control ($p<0.05$), b: significantly different from control ($p<0.01$), c: significantly different from control ($p<0.001$), d: significantly different from petrol ($p<0.05$), e: significantly different from petrol ($p<0.01$), f: significantly different from petrol ($p<0.001$), *: significantly different from crude petroleum ($p<0.05$), **: significantly different form crude petroleum ($p<0.01$)

significantly higher in all the test groups ($p<0.001$), than control (Table 2). The mean WBC counts in ($\times 10^3$ μL^{-1}) are 15.54±2.28 control, 24.90±0.16 petrol, 14.32±2.20 in kerosene, 14.00±1.85 in diesel and 11.32±1.24 in crude oil. Only the petrol group recorded significantly ($p<0.05$) higher value than control group. The mean lymphocytes counts in % are 75.40±1.83 control, 48.50±2.10 petrol, 70.80±1.74 in kerosene, 71.25±2.69 in diesel and 81.60±1.23 in crude oil. Again only the petrol group recorded a significantly ($p<0.001$) lower value than the control group. Lymphocyte values in kerosene, diesel and crude oil were significantly ($p<0.001$) higher than petrol group. The mean monocytes counts in % are 2.00±0.58 control, 1.33±0.33 petrol, 3.00±0.00 in kerosene, 3.33±1.20 in diesel and 2.00±0.58 in crude oil. Values obtained for monocytes in the test groups were not statistically different from the control value. The mean neutrophils counts in % are 20.40±1.44 control, 45.50±1.19 petrol, 22.80±2.82 in kerosene, 23.50±2.73 in diesel and 15.20±1.20 in crude oil. Neutrophils recorded significantly ($p<0.001$) higher value only in petrol group. The mean eosinophils counts in % are 2.75±0.48 control, 7.30±0.88 petrol, 3.25±0.25 in kerosene, 3.50±0.96 in diesel and 2.67±0.67 in crude oil. This value was significantly ($p<0.01$) higher in petrol group than control group, these results are as shown Table 2.

DISCUSSION

Alteration in some haematological parameters and indices were recorded in male albino rats of Wister strain that were oral gavaged with Nigerian Bonny light Crude Oil (NBLCO) and some of its refined products (petrol, kerosene and diesel). In the light of this, consideration of the analysis

of haematological parameters and red cell indices provide useful information on the general state of the blood after such exposure to exogenous insult.

The mean RBC values showed significant reduction in the refined product groups compared with the control. The RBC reduction was significant in the following descending order; petrol, kerosene and diesel groups. These results are in agreement with previous reports by Eyong *et al.* (2004) and Ovuru and Ekweozor (2004), these researchers observed a similar haematoxic effects in rats and rabbits, respectively following ingestion of crude oil.

The PCV was significantly lower in both petrol and kerosene groups but with marginal reduction in the diesel and crude petroleum groups.

Like the RBC, haemoglobin concentration showed significant reduction in the refined product groups but with marginal reduction in crude petroleum group. The result is consistent with the work of Suzanne (2003), who reported that chronic ingestion of petroleum oil by birds caused oxidative chemical damage to haemoglobin causing reduction haemoglobin level in birds.

MCV value was significantly higher in petrol group, significantly lower in crude petroleum group but marginally higher in both kerosene and diesel groups than the control. The mean MCH value was significantly higher in petrol group than the control and other test groups. The values obtained in kerosene, diesel and crude petroleum were significantly lower than petrol group.

The observed reduction in RBC may be attributed to the cytotoxic effects of compounds present in petrol, kerosene and diesel. Oxidative stress may be induced by petroleum products with its attendant effect on red cell membrane, this could possibly have accounted for the susceptibility of the red cell membrane to oxidative attack giving way to haemolysis. In some other studies carried out by Shakirov and Farkhutdinov (2000), exposure or contact with chemicals in oil-refining industry have been established to caused changes in the red cell adenyl and blood monooxygenase system. They suggested that such effect could alter the integrity of the red cell membrane to cause cellular haemolysis. Therefore, the result of this study particularly with petrol and kerosene agreed with their claim.

The observed decrease in PCV is believed to be as a result of the decreased RBC which is consistent with the findings of Eyong *et al.* (2004). The MCV and MCH served to indicate variations in erythrocyte shape, size and haemoglobin content. The increase in MCV values obtained in this study could have been due to the presence of larger number of reticulocytes in the circulating blood than the mature red blood cells. Even though reticulocyte counts was not estimated in this study, elevated reticulocyte count is an indication of an extensive presence of immature RBCs in circulation to replace destroyed RBCs (Bernard *et al.*, 2006). Reticulocytes are known to be larger than the mature erythrocytes and present larger volume. The number of circulating reticulocytes spontaneously increased to carry sufficient oxygen to meet cellular demands where substantial number of mature red blood cells were destroyed, this perhaps may have accounted for the MCV result recorded in this study. Reduction in the values of RBC, PCV and Hb content as recorded in this study is suggestive of anaemic conditions which agrees with the report of Eyong *et al.* (2004) on the haematoxicity of crude oil. The haemopoietic system, in response to this likely anaemic condition may have flooded the system with reticulocytes which has the ability to carry oxygen to meet the body's demand as earlier stated.

Most constituents of petroleum are highly toxic to biological membranes and proteins, naphthalene for example, has been reported to cause haemoglobin denaturation and is one of the compound groups responsible for the development of haemolytic anaemia in oiled wild life (Debra, 2003). This may possibly explains the low values of haematological parameters recorded in petrol

and kerosene groups in this study; these refined products are known to contain high level of naphthalene. It also amongst other toxicants, suppresses the immune system, causes disruption or suspension of haematopoiesis (Hodgson and Smart, 2001); which collaborate the results in this study (Table 1, 2).

It has also been established that; the toxic constituents of petroleum such as benzene and lead are activated in the bone marrow, where these substances exert cytotoxic effects that could be mediated through disturbance in DNA function (Okoro *et al*., 2006). The resultant bone marrow depression is characterized by inadequate production of red cell and other formed elements. This is in line with the findings in this study, as each of the refined products showed a significant reduction of RBC from the control value. The crude petroleum also fell in line with this assertion, even though the decrease in this group was only marginal.

The mean platelet and WBC count were significantly higher in the petrol group than control group.

The mean values of lymphocytes recorded in kerosene, diesel and crude oil groups were within the normal lymphocytes range for rats (69-86%). Lymphocytopenia was observed in the group that ingested petrol (gasoline) with a mean value (48.5%) and this was accompanied by high value of WBC ($24.9\pm0.16\times10^{3}$ μL^{-1}) suggestive of leukemia. In this study, it is possible that the membranes of these lymphocytes were oxidized as the rats were subjected to petrol ingestion as a low normal to low absolute lymphocyte concentration is associated with increased rates of infection after trauma (Abbas and Lichtman, 2003). This is likely due to the fact that high percentage of deadly benzene vapours 1.77%w/w and toluene 1.63%w/w amongst other toxic elements is found in petrol.

There was neither increase nor decrease in the percentage of monocytes in this study. This might be due to short circulation period of monocytes in blood stream; monocytes circulate for only about one to three days and then typically move into tissues throughout the body, it is likely most of the monocyte would have moved into tissue before the blood was obtained for analysis.

The mean percentage neutrophils obtained in this study was significantly higher in petrol group than the control and other test groups. This is suggestive of high degree of infection. The normal to almost normal neutrophils levels in the control, kerosene, diesel and crude petroleum groups suggest a low or no damage/inflammation, as neutrophils are the first-responders to inflammation and cell damage. Ingestion of crude petroleum and some of its refined products, as a matter of fact, may have induced an increased in the metabolic rate, with the resultant increased in the generation of free radicals with the attendant cellular damage. The immune system responds to this damages caused by production of oxidants during stressful conditions. During such responses, free radicals are produced by the neutrophils the first-responders to inflammatory cells to remove damage cells. Being highly mobile, neutrophils quickly congregate at a focus of infection, attracted by cytokines expressed by activated endothelium, mast cells and macrophages (Ear and McDonald, 2008). Neutrophils also recruit and activate other cells of the immune system.

The mean eosinophils obtained in this study were significantly higher in petrol group than control and other test groups. The values were marginally higher in kerosene, diesel and crude petroleum groups than control but significantly lower than petrol groups. Eosinophils primarily are associated with parasitic infections and an increase in their number may indicate such (Alberts, 2005). Eosinophils along with basophils and mast cells are important mediators of allergic responses and associative pathogenesis in the development of asthma (Rothenberg and Hogan, 2006). The increased in eosinophils percentage (7.3 ± 0.88), in petrol ingested group above normal range in this study is suggestive of a high level of infection the rats might have been exposed to coupled with depressed immune system.

CONCLUSION

The present study has provided insight into the possible toxicity of petroleum and some of its refined products (Petrol, kerosene and diesel) and the degree to which they can alter the integrity of haematological responses by the effects of the toxic constituent of these products on the haematopoietic system.

It has been observed that chronic ingestion of crude petroleum and some of its refined product might result in anaemia, as the haemapoietic system is the major target of challenge.

Also reduced lymphocytes percentage in petrol group suggests that some constituents in this product can suppress the immune system following chronic digestion therefore practice of ingesting petrol for whatever reason might render the subject susceptible to infection. However, the increase in the WBC counts following exposure to gasoline, observed in this study, may be one of the mechanisms devised to defend the body against the toxicity of the gasoline constituents. It is, therefore, concluded that crude petroleum and some of its products are highly toxic and damaging to haematopoietic system.

ACKNOWLEDGMENTS

The authors express appreciation to Mrs. Comfort Umoh and Mrs. Eme Abia, both technologists in Physiology laboratory as well as Mr. Nsikan Malachy of department of Pharmacology and Toxicology, University of Uyo, Uyo, Nigeria, for technical assistance.

REFERENCES

Abbas, A.K. and A.H. Lichtman, 2003. Cellular and Molecular Immunology. 5th Edn., Saunders, Philadelphia.

Achuba, F.I. and S.A. Osakwe, 2003. Petroleum-induced free radical toxicity in African catfish (*Clarias gariepinus*). Fish Physiol. Biochem., 29: 97-103.

Alberts, B., 2005. Leukocyte functions and percentage breakdown. Mol. Biol. Cell, 4: 41-43.

Anozie, O.I. and I.N. Onwurah, 2001. Toxic effect of bonny light crude oil in rat after ingestion of contaminated diet. Nig. J. Biochem. Mol., 16: 103-108.

Becki, L., 2007. General chemical information and toxicity of crude petroleum. Chemicals, 26: 6-19.

Bernard, A.W., A. Venkat and M.S. Lyons, 2006. Best evidence topic report. Full blood count and reticulocyte count in painful sickle crisis. Emerg. Med. J., 23: 302-303.

Chilcott, R.P. and H.Q. Chapd, 2007. Summary of health effects of diesel. Sci. Total Environ., 20: 129-138.

Debra, B.M., 2003. The rapentic and toxicity information of crude oil. J. Nutri., 68: 297-400.

Ear, T. and P.P. McDonald, 2008. Cytokine generation, promoter activation and oxidant-independent NF-kappaB activation in a transfectable human neutrophilic cellular model. BMC Immunol., 9: 14-14.

Eyong, E.U., I.B. Umoh, P.E. Ebong, M.U. Eteng, A.B. Antai and A.O. Akpa, 2004. Haematoxic effects following ingestion of Nigerian crude oil and crude oil polluted shellfish by rats. Nig. J. Physiol. Sci., 19: 1-6.

Hodgson, E. and R.C. Smart, 2001. Introduction to Biochemical Toxicology. 3rd Edn., Wiley, New York, pp: 199-220.

Khan, A.A., R.W. Coppock and M.M. Schuler, 2001. Effect of multiple exposure of small dose of *Pembina cardium* crude oil and diesel in rat. Arch. Environ. Contam. Toxicol., 40: 418-424.

Ojo, O.O., F.R. Kabutu, M. Bell and U. Babayo, 2006. Inhibition of paracetamol-induced oxidative stress in rats by extracts of lemongrass (*Cymbropogon citrates*) and green tea (*Camellia sinensis*) in rats. Afri. J. Biotechnol., 5: 1227-1232.

Okoro, A.M., E.J. Ani, J.O. Ibu and B.A. Akpogomeh, 2006. Effect of petroleum products inhalation on some haematological indices of fuel attendants in Calabar metropolis, Nigeria. Nig. J. Physiol. Sci., 21: 71-75.

Ovuru, S.S. and I.K.E. Ekweozor, 2004. Haematological changes associated with crude oil ingestion in experimental rabbits. Afr. J. Biotechnol., 3: 346-348.

Rothenberg, M.E. and S.P. Hogan, 2006. The eosinophil. Annu. Rev. Immunol., 24: 147-174.

Shakirov, D.F. and R.R. Farkhutdinov, 2000. Identification of high-risk groups at the examination of workers engaged in oil- refining industry. Gig. Sanit., 15: 33-36.

Suzanne, I.B., 2003. Therapeutic and nutritional information on crude oil. Chemicals, 6: 4-11.

Udonwa, N.E., E.K. Uko, B.M. Ikpeme, I.A. Ibanga and B.O. Okon, 2009. Exposure of petrol station attendants and auto mechanics to premium motor sprit fumes in Calabar, Nigeria. J. Environ. Public Health, 2009: 1-5.