Journal of Medical and Biological Science Research
Vol. 1 (7), pp. 71-77, September, 2015
ISSN: 2449-1810
Research Paper
http://pearlresearchjournals.org/journals/jmbsr/index.html

Effects of Nigerian Bonny light Crude Oil on Some Immunological Parameters: The Role of Antioxidant Vitamins C and E and Honey in Male Wistar Rats

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Accepted 31 August,2015

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ABSTRACT

The effect of Nigerian Bonny light crude oil (NBLCO) on some immunological parameters: the role of some antioxidants supplementation was the focus of this study. 48 adult male Wistar rats were randomly divided into six groups of 8 per group. Group I (control) oral gavaged 3 ml/kg of normal saline; group II received 3 ml/kg of NBLCO, groups III, IV and V received in addition to 3 ml/kg of NBLCO 1 ml/kg vitamin C, vitamin E and 3 ml/kg of honey, respectively; group VI received 3 ml/kg of honey. After 28 days of treatment, animals were sacrificed and blood collected by cardiac puncture. Results showed that NBLCO did not alter WBC, eosinophil and monocyte count significantly but significantly increased neutrophil, neutrophil-lymphocyte count ratio, globulin, IgG and IgM ($p<0.05$); while it significantly reduced lymphocyte compared to the control group ($p<0.05$). Vitamins C and E supplementation significantly increased WBC count ($p<0.05$) but did not alter neutrophil level significantly compared to group II. Interestingly, vitamins supplementation significantly increased lymphocyte level ($p<0.05$) while significantly reducing neutrophil-lymphocyte count ratio, IgG and IgM levels compared to group II ($p<0.05$). Honey supplementation significantly increased WBC, neutrophil and neutrophil-lymphocyte ratio while it significantly reduced lymphocyte, IgG and IgM compared to group II ($p<0.05$). Vitamin C supplementation did not alter globulin level while vitamin E and honey significantly reduced globulin level ($p<0.05$). It is concluded that ingestion of NBLCO substantially interfere with functions of the immune system with high risks of inflammatory response, and supplementation with antioxidants could ameliorate the aforementioned effects.

Key words: Antioxidants, Crude Oil, Honey, Immunoglobulins, Rats and White Blood Cells.

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INTRODUCTION

Petroleum hydrocarbon in its crude or refined forms has negative impact on both, human, animal and plant species (Odu, 1997). The toxicity of hydrocarbons is directly related to their physical properties, specifically the viscosity, volatility, surface tension, and chemical activity of the side chains. Crude oil's toxic ingredients can damage every body organ and system including the pulmonary, neurologic, gastrointestinal, hepatic, renal, dermatologic, haematologic and immune systems; and can also cause disruption of normal metabolism (Golet et al., 2002; Achuba and Osakwe, 2003; Suzanne, 2003; Ubani et al., 2009). The rural dwellers that indulge in the unorthodox folklore medicine with unverified claims of its therapeutic potency actually ingest crude oil directly and in most cases, in combination with other substances as a therapeutic agent. Another form of contact is its indirect incorporation into food chain by way of contamination from spillages and other sources (Dede and Kagbo,

2001). This can results in spectrum of toxic effects which include inflammation and inflammation related disorders (IARC, 1989; Yadau and Seth, 2001; Golet et al., 2002).
Inflammatory effects can be minimized or prevented or even eliminated by certain active compounds serving as valuable antioxidants, or substances that possess antioxidant activity (Achuba and Osakwe, 2003). Toxicants in crude oil can accumulate in foods and get to reach organisms through food chain where it disrupt physiological activities in living organisms and humans, thus causing inflammation (Sweet and Hume, 1996). Since crude oil toxicities have been reported to affect almost all organ system in the body including the hematologic system (Ita and Uyai, 2011; Ita et al., 2011; Sunday et al., 2013), the immune system might be one of the primary targets. This may account for the high susceptibility of organisms to other debilitating agents resulting in disease conditions. Leucocytes are the cells of immune system that are involved in defending the body against both infectious disease and foreign invaders (Lafleur-Brooks, 2008). Antibody production is one of the specific functions of the immune system associated with a fraction of white cell, B-lymphocytes. Pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL- 6), interleukin-8 (IL-8) as well as C-reactive protein (CRP) are potent inducers of leukocyte proliferation which plays a pivotal role in body's innate immune system (Kapoor et al., 2011).
In view of the increasing rate of crude oil ingestion and its refined products directly or indirectly, it is pertinent to study the accumulated effects on the body systems. It is on the strength of this that evaluation of the status of immunoglobulins particularly immunoglobulin G and M in the phase of NBLCO administration was investigated. Since antioxidants are useful in mopping up free radicals to prevent oxidation, the role of vitamins C, E and honey supplementations were also investigated in this study.

MATERIALS AND METHODS

Chemicals and Drugs

The crude petroleum used in this study was obtained from the EXXON/MOBIL laboratory, Ibeno, Nigeria. The bee honey was purchased from a local market situated in Uyo, Akwa Ibom State, Nigeria. The vitamin C tablet and vitamin E capsule (Softgel Healthcare Private Limited, India).

Experimental Animals

Mature male Wister rats weighing between 150 to 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 Animal House in the Faculty of Basic Medical Science University of Uyo Animal House for two days to acclimatize. The animals were allowed free access to feed and water *ad libitum* (Chow: vital feeds, Grand Cereals Limited, Jos).

Experimental Design and Treatment of Animals

A total of forty-eight (48) adult male Wister rats were randomly divided into six groups (group I, II, III, IV, V, and VI). 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, according to the dose described by Eyong et al., (2004), while group 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. Group V in addition to 3 ml/kg body weight of NBLCO were supplemented with 3 ml/kg body weight of honey. Group VI was oral gavaged 3 ml/kg body weight of honey only. 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 again weighed and anaesthetized with chloroform soaked in swap of cotton wool in a transparent killing chamber. Blood was collected from the heart by cardiac puncture with a 5 ml sterile syringe and needle. The total volume of blood collected was 10 ml, which was divided into portions of 5 ml each. One portion was collected into EDTA sample bottle for determination of white blood cells and differential white cell count using automated hematology analyzer (BC-2300, Mindray, Germany). The second portion was collected into plain sample bottles. This was allowed to stand for 2 hours to clot after which the blood sample were then spun with a table top centrifuge (RM-12 micro centrifuge, REMI, England) at 4000 rpm for 10 min. The serum was then carefully separated with the help of Pasteur pipette into clean labeled sample bottles and preserved at -20^0C until assayed for serum chemistry.

Determination of Immunoglobulins G and M

Centronic protein diagnostic kit from Germany was used for the determination of IgG and IgM by turbid metric immunoassay method as described by Etzel et al. (1997).

Determination of Total Protein

Randox kit (USA) for total protein determination was

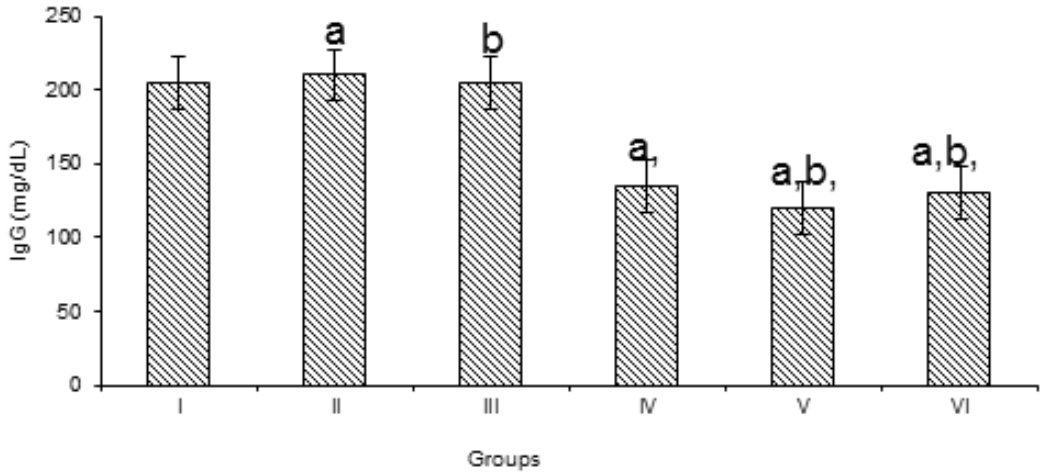

Figure 1. Comparison of the IgG levels between the groups. Values are mean ± SEM, n =8. a=p<0.05 vs group I, b=p<0.05 vs group II, c=p<0.05 vs group III, d=p<0.05 vs group IV, e=p<0.05 vs group V.
LEGEND a=significantly different from group I (p<0.05). b=significantly different from group II (p< 0.05).

adopted following Biuret method (Henry et al., 1974).

Determination of Albumin

Albumin was estimated with albumin reagent from Dialab, France as described by Tietz (1994).

Determination of Globulin

Serum globulin concentration = total protein - serum albumin as described by Tietz (1995).

Statistical Analysis

Statistical analysis was carried out using window SPSS package (SPSS 22.0.0.0 version). Data were analyzed using one way analysis of variance (ANOVA), results obtained were further subjected to test for least significant difference (LSD). Data were expressed as the mean ± standard error of the mean and values of P<0.05 were considered significant.

RESULTS AND DISCUSSION

Immunoglobulin G and immunoglobulin M levels were significantly elevated following NBLCO ingestion compared to the control group (p<0.05), these values were significantly reduced by vitamins C and E supplementations (Figures 1 and 2). The value of IgM was significantly lower in group VI (oral gavaged honey) compared to groups II, III, IV and V (p<0.05) but did not change significantly from group I. The significant elevations in serum IgG and IgM levels recorded in this study following ingestion of NBLCO to male Wistar rats may be associated with inflammation, since elevated immunoglobulins has been reported to be indicative of inflammation or infection (Loh et al., 2013), these workers in a study on quantitative immunoglobulin's testing, concluded that an increase in IgG and IgM can be a strong indicator for a range of diseases such as chronic inflammation or infection. Although, the immunotoxic effect most often reported following exposure to poly aromatic hydrocarbon (PAH) is immunosuppression.
A few reports also deal with immune-potentiation either *in vitro* or following inhalation or topical exposure. Such immune-potentiation can actually compromised immune system resulting in increased production of cytokines by immune cells, thus leading to inflammation which could under specific circumstances facilitates tumor development or expression of hypersensitivity or auto immunity (Burchiel and Luster, 2001). A similar result of a follow-up study in our laboratory shows that NBLCO significantly elevated C-reactive protein (CRP) level confirming our hypothesis that NBLCO induces inflammation and perhaps oxidative processes to interfere with normal physiological processes of the immune system. CRP being an acute-phase protein produced by hepatocytes upon stimulation by the pro-inflammatory cytokines plays a vital role in dual capacity to both enhance the immune system and to protect against tissue damage (Ridker et al., 2000). Relatively, an elevated CRP suggests a covert inflammatory response as well as an overt challenge of the immune system. This in turn alarms the immune system resulting in the significantly higher IgG, IgM as well as the globulin levels. However, in this investigation, there was a

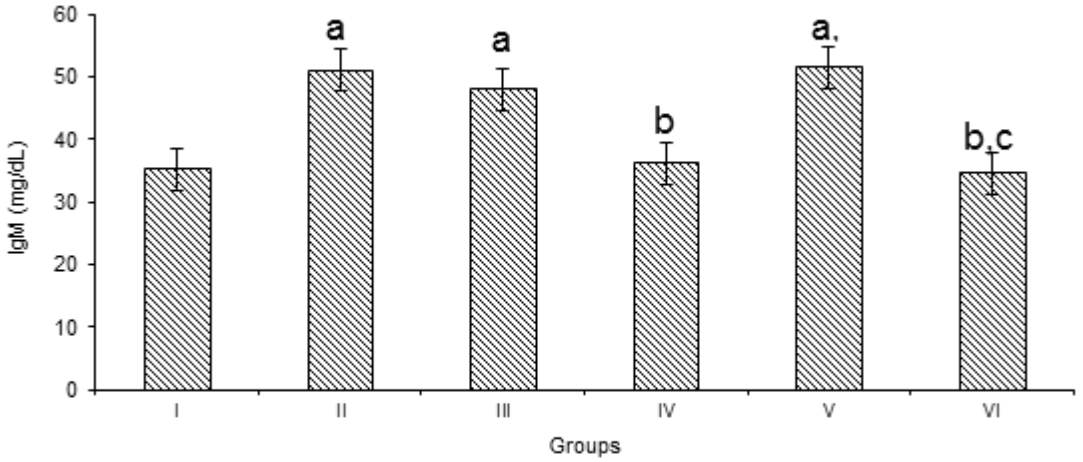

Figure 2. Comparison of the IgM levels between the groups. Values are mean ± SEM, n =8. a=p<0.05 vs group I, b=p<0.05 vs group II, c=p<0.05 vs group III, d=p<0.05 vs group IV, e=p<0.05 vs group V.
Legend a=significantly different from group I (p<0.05), b=significantly different from group II (p< 0.05) and **c=**significantly different from group III (p< 0.05).

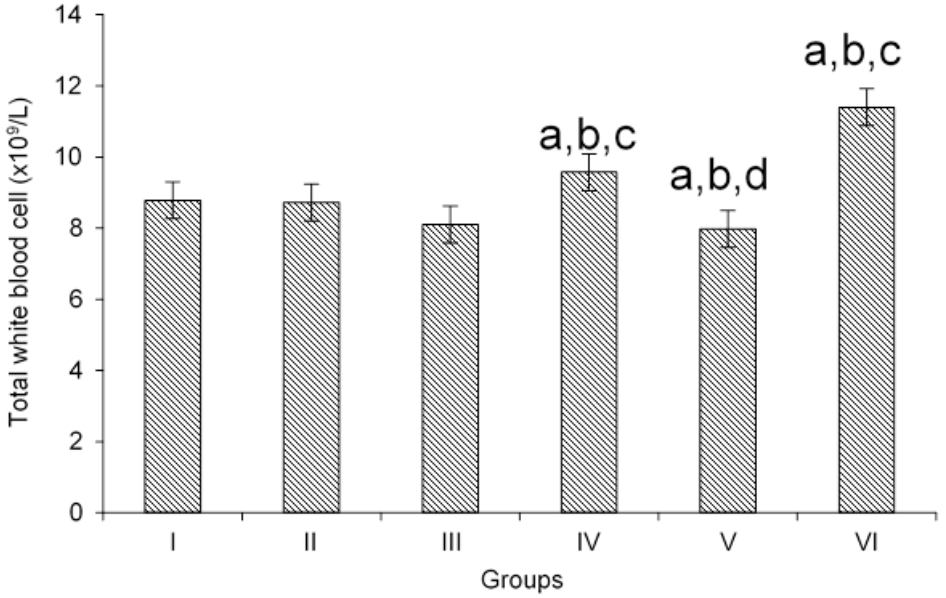

Figure 3. Comparison of WBC counts between the groups. Values are mean ± SEM, n =8. a=p<0.05 vs group I, b=p<0.05 vs group II, c=p<0.05 vs group III, d=p<0.05 vs group IV, e=p<0.05 vs group V.
LEGEND a=significantly different from group I (p<0.05). b=significantly different from group II (p< 0.05), **c=**significantly different from group III (p< 0.05) and d =significantly different from group IV (P<0.05).

significant reduction in IgG and IgM values in the groups supplemented with antioxidant agents.

The protective effect of vitamin C, E and honey against oxidative stress generated by crude oil corroborates previous report that vitamin C and E are free radical scavengers that protect the body against inflammation (Farriss, 1991; Ogujanovic et al., 2003). The values of total white blood cells count in groups II and III were not significantly different from group I. The value of total white blood cells in groups IV supplemented with vitamin E was significantly higher than groups I, II and III (p<0.05). Administration of honey to group VI significantly elevated total white blood cells count when compared with the other five groups (p<0.05) (Figure 3).

Table 1. The mean total protein, albumin and globulin in control and treated rats after 28 days of treatment.

Groups	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
I (Control)	44.20 ± 0.97	33.00 ± 0.55	11.20 ± 0.58
Ii (NBLCO)	42.60 ± 0.87	22.40 ± 0.93[a]	20.20 ± 0.37[a]
III (NBLCO and Vitamin C)	56.00 ± 0.71[a,b]	36.75 ± 1.11[b]	19.25 ± 0.48[a]
IV (NBLCO and Vitamin E)	44.80 ± 0.80[c]	34.80 ± 1.20[b]	10.00 ± 0.55[b,c]
V (NBLCO and Honey)	44.50 ± 0.65[c]	28.50 ± 0.96[a,b,c,d]	16.25 ± 0.25[a,b,c,d]
VI (Honey)	44.00 ± 1.00[c]	36.50 ± 2.96[a,e]	7.25 ± 2.29[a,b,c,e]

a = significantly different from group I ($p<0.05$), b = significantly different from group II ($p<0.05$), c = significantly different from group III ($p<0.05$), d = significantly different from group IV ($p<0.05$) and e = significantly different from group V ($p<0.05$).

Table 2. Comparison of the differential white cell count in rats after 28 days of treatment.

Groups	Neutrophil (%)	Eosinophil (%)	Lymphocyte (%)	Monocyte (%)	N-L
I (Control)	40.50 ± 0.65	1.75 ± 0.48	53.75 ± 1.03	4.50 ± 0.68	0.76 ±0.03
II (NBLCO)	51.80 ± 0.97[a]	1.80 ± 0.49	42.40 ± 0.51[a]	4.00 ± 1.05	1.24 ±0.03[a]
III (NBLCO plus Vitamin C)	51.50 ± 0.63[a]	0.75 ± 0.48[a,b]	46.00 ± 0.91[a]	1.75 ± 0.63[a,b]	1.12 ±0.10[a]
IV (NBLCO plus Vitamin E)	50.33 ± 0.88[a]	1.00 ± 0.58[a,b]	45.67 ± 0.88[a]	3.00 ± 0.00	1.10 ±0.04
V (NBLCO plus Honey)	62.25± 1.11[a,b,c,d]	2.00 ± 0.71[a,b,c,d]	32.00 ± 3.85[a,b,c,d]	3.75 ±0.85	2.03 ± 0.25[a,b,c,d]
VI (Honey)	69.33±1.20[a,b,c,d,e]	0.33± 0.33[a,b,c,d,e]	28.67 ± 0.88[a,b,c,d,e]	1.67 ± 0.88[a,b]	2.42 ± 0.01[a,b,c,d,e]

a = significant different from group I ($P < 0.05$), b = significant different from group II ($P < 0.05$), c = significant different from group III ($P < 0.05$), d = significant different from group IV ($P < 0.05$) and e = significant different from group V ($P < 0.05$).

The observed reduction in white blood cells by NBLCO in this study agrees with earlier report of Ngodigha et al. (1999), who reported a leukocytopenia in goats following crude oil contamination; also reported in humans exposed to petroleum fumes (Okoro et al., 2006).

This reduction was attributed to stress imposed by crude oil hydrocarbons which presumably may have affected the overall immune system. Benzene, an important component of crude oil is reported to produce hematological changes, ranging from pancytopenia to total bone marrow aplasia affected through its mylototic action (D'Azevedo et al., 1996). Honey is an antioxidant which potentially interacts with free radical and terminates the chain reaction produced by NBLCO before vital molecules are damaged in the body as supported by Frankel et al. (1998). It is possible that honey supplementation may have increase WBC by reducing oxidative damage and modulating the antioxidant enzyme activities; this observation is in accordance with the findings of Yao et al. (2011). The effect of vitamin E supplementation could also be attributed to improved immune system and a sign of optimum response to infection or foreign invaders. Even though oxidative stress markers were not evaluated in the present study, the fact that vitamin E significantly increase WBC count is suggestive of oxidative potential of NBLCO, which could probably be responsible for the leukemic tendency of the vitamins supplementation recorded.

Comparatively, NBLCO did not significantly alter total protein except in group III where vitamin supplementation recorded a significantly higher value ($p<0.05$) (Table 1). But its administration significantly reduced albumin level compared to control and other groups ($p<0.05$). This effect was reversed by antioxidants supplementations which recorded significantly higher albumin level in groups III, IV, V as well as group VI that had only honey ($p<0.05$). It significantly elevated globulin level when compared to control which was significantly ($p<0.05$) reduced by antioxidants supplementations except group III, which was supplemented with vitamin C. The NBLCO ingestion may have induced the production of globulin in an unprecedented fashion as globulins are known to be larger proteins that play a pivotal role in immunological response (Tietz, 1986). Ingestion of NBLCO could induce free radicals generation leading to myriad of consequences including damage to biological membranes (Ita et al., 2011; Sunday et al., 2013), lipid peroxidation etc. Moreover, it is also possible to infer that the protective capacity of vitamin E and its complex antioxidants defense system could have been overshadowed by the hazardous effects of NBLCO, on the account that imbalance between free radical production as well as pro-oxidants and antioxidant level leads to oxidative stress.

A reduction trend was also recorded for albumin following ingestion of NBLCO suggestive of liver dysfunction that could interfere with its synthetic function accounting for the low production of these glycoproteins. The mean values of the differential white blood cell count obtained after treatment are presented in Table 2. The results showed significantly higher neutrophil levels and neutrophil-lymphocyte count ratio in groups II, III, IV, V

and VI compared to group I (control group) ($p<0.05$). Supplementation with antioxidant vitamins C and E did not alter these values significantly compared to group II (NBLCO) but honey supplementation in group V and VI administered honey only showed significant alteration ($p<0.05$). The lymphocyte levels obtained in groups II to VI were significantly lower than group I ($p<0.05$). Vitamins C and E supplementation to group III and IV, respectively however were significantly higher than group II (NBLCO). Group V supplemented with honey and group VI that had honey only recorded significant reduction in lymphocyte level compared with groups I, II, III and IV ($p<0.05$).

The NBLCO ingestion caused a marked increase in neutrophils level as well as the neutrophil-lymphocyte count ratio while significantly reducing lymphocyte level in rats. This result corroborates the findings of Ita and Uyai (2011) who submitted that administration of petrol increases neutrophil count. While antioxidant vitamins C or E supplementation could not alter neutrophil level significantly, honey supplementation significantly reversed effect of NBLCO on neutrophil level. The ameliorative effect of honey reported in this study is supported by Johnson et al. (2007), who highlighted similar ameliorative properties of honey in metabolic and cardiovascular disease. Lymphocytopenia was recorded in all groups compared to the control. The antioxidant vitamins C or E supplementation significantly ameliorated effect of NBLCO by increasing the lymphocyte levels, these correlated positively with lower neutrophil-lymphocyte count ratio recorded for these groups. The groups supplemented with honey showed a rather higher level of lymphocytopenia when compared to other groups. From the results, it could be suggested that NBLCO administration compromise the endogenous immune capacity thereby increasing risk of infection to injurious antigens.

CONCLUSION

It can therefore be concluded that ingestion of NBLCO could substantially interfere with immune function with high risks of inflammatory response, and that supplementation with antioxidants could ameliorate the aforementioned effects.

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