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Effect of Chronic Exposure to Petroleum Hydrocarbon Pollution on Renal and Some Haematological Parameters of the Native Fowl (*Gallus domesticus*)

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Authors' contributions

This work was carried out in collaboration between both the authors. Author LAN designed the study, performed the experiment and wrote the first draft of the manuscript. Author GOCO supervised the experiment, assisted in manuscript preparation. Both authors performed the statistical analysis. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To investigate the effect of chronic exposure to petroleum hydrocarbon pollution on renal and some haematological parameters of the native fowl (*Gallus domesticus),* reared in an environment known to be polluted with petroleum hydrocarbon.
Study Design: Randomized controlled animal experiment using twenty-four male native fowls (12 each from the experimental areas).
Place and Duration of Study: Department of Biochemistry, Federal University of Technology, Owerri, Nigeria, between November 2009 and November 2010.
Methodology: Standard methods for haematological and renal function assessment were adopted.
Results: The study showed that there was no significant ($p>0.05$) difference in the electrolyte concentrations (K^+ and HCO_3^- ions) in the serum of fowls from both environments, but a significant decrease ($p>0.05$) in Na^+ and Cl^- ion concentrations in the serum of fowls from Mbaise when compared to those from Egbema. The effect of the pollution significantly ($p<0.05$) reduced serum concentrations of total bilirubin, urea and creatinine. The haematological parameters: [white blood cell (wbc) and red blood cell

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(rbc) counts were significantly ($p<0.05$) affected, percentage haemoglobin (Hb) and haemoglobin/ methaemoglobin (Hb/metHb) ratio] were not significantly ($p>0.05$) affected.
Conclusion: Petroleum hydrocarbon pollution is suggested to possess the potential to affect certain haematological parameters, total bilirubin and renal function.

Keywords: Gallus domesticus; electrolytes; haematological parameters; petroleum hydrocarbon pollution.

1. INTRODUCTION

The environmental impacts associated with the exploration and exploitation of petroleum hydrocarbon (crude oil, its refined products and gas flaring) have been a popular area of experimental research in Nigeria, especially in the Niger Delta Area. Some areas in the Niger Delta area have been exposed to crude oil contaminants, petroleum hydrocarbon, due to poor operational facilities and negligence on the part of regulatory agencies [1].

Petroleum hydrocarbons are organic constituent of crude oil, as a result of oil exploration and exploitation activities. Hydrocarbons from petroleum pollution are widely distributed over the environment (air, soil and water) over the world from oil spillage, purified petroleum products, gas flaring, and have directly or indirectly led to environmental health risks [2]. Petroleum hydrocarbon, as a pollutant, consists of complex mixtures of paraffinic alicyclic and aromatic hydrocarbons and a smaller proportion of non-hydrocarbon compounds such as naphthenic acid, phenols, thiols, heterocyclic nitrogen and sulphur compounds [3]. Atmospheric hydrocarbons, in themselves as primary pollutants, produce photochemical oxidants as secondary pollutants. The chief air pollutants are benzene, benzo pyrene and methane. Their sources are the motor vehicles, being emitted by evaporation of gasoline through carburetors and crank cases. About 40% of the vehicular exhaust hydrocarbons are unburnt fuel components, the rest are the product of combustion. Sources generally are located in areas of high population density, where maximum harm to humans and materials can occur [2]. Hydrocarbons and most other organic compounds in the atmosphere are thermodynamically unstable by oxidation through a series of steps. The oxidation process terminates with the formation of CO_2, solid organic particulate matter, that settles from the atmosphere, or water soluble products (e.g. acids, aldehydes) that are removed by rain [1]. Nigeria is a major world producer of crude oil. Petroleum hydrocarbon pollution of the environment has steadily increased as a result of oil activities. Crude oil production and export have brought tremendous financial benefits to the country, but have resulted in several negative environmental consequences due to numerous oil spillages, especially in the Niger Delta Area where the bulk of the oil exploration and exploitation are concentrated. In the Niger Delta Area alone, there have been over 550 reported cases of crude oil spillage since 1976, releasing over 2.8 million barrels of crude oil into the environment [4,5].

Petroleum hydrocarbon pollution has a significant impact on the natural resources upon which many poor Niger Delta communities and their domesticated animals depend. Drinking water is polluted, fishing and farming are significantly affected and ecosystems are degraded [6]. It affects the health and food security of rural people as well as their animals living near oil facilities. Nwilo and Badejo [7] maintained that petroleum hydrocarbon pollution (oil spillage and gas flaring) have serious impact on the biodiversity and environmental integrity of the Niger Delta. The collapse of local economies, induced by oil spillages, gas flaring and other activities of oil industries had displaced many from their occupations, without providing viable alternatives. The consequences are poverty, death of aquatic and terrestrial animals.

The roles played by the oil and gas industries in improving the quality of life in Nigeria cannot be overemphasized. Alongside the good things that brought an enhancement in the standard of living was the emergence of deleterious substances in the environment following activities of oil and gas industry. According to Celestine [8], petroleum hydrocarbon pollution can affect the environment in three different ways: by poisoning, after ingestion of affected agricultural yields, by direct contact and by destroying the habitats. Pollutants, when absorbed by living organisms, cause the release of reactive intermediates which induce changes in tissues of these organisms [9]. Several biochemical parameters have been studied by various researchers [9,10] to explain the organism's attempt to cope with the effects of the pollution.

Egbema is an area in the Niger Delta where oil and gas activities have gone on for over fifty years and organisms grown in the area are exposed to heavy pollution in the environment. The pollutants include spilled crude oil and or its refined products, effluents with traces of heavy metals, particulates and toxicants from gas flaring and green house gases [11]. The Energy Solution Conference of 2004 estimated that the Niger Delta region has about 123 gas flaring sites [12]. About 45.8 billion kilowatts of heat is released into the atmosphere from 1.8 billion cubic feet of gas burn daily in the Niger Delta region, leading to temperatures that render large areas non-habitable [12]. These pollutants (crude oil and their products) are considered recalcitrant to (natural) biodegradation, and persist in the ecosystem due to their hydrophobicity and low volatility [13].

The oil and gas activities in the Niger Delta have led also to the emergence of undesirable changes in the physical, chemical and biological characteristics of the land, water and air, which affect the ecosystem adversely [14,15]. Animals and plants growing in such environments have, over the years, exposed to large doses of harmful pollutants. These pollutants and any products of their degradation (no matter how small) can be carcinogenic, mutagenic, and are potent immunotoxicants [3]. Mbaise on the other hand, quite distant from Egbema, has no oil well or gas flaring site and as such may not have experienced petroleum hydrocarbon pollution.

The native fowl (*Gallus domesticus)* was chosen as the experimental species since it has a life span of several years. In exceptional cases, it can live for 10–15 years. It is free-living and grazes in search of food (and water) in the environment. It feeds on insects, earthworm and other worms, larvae, tender leaves of seedlings, grasses and other substances in the environment. Its free living/feeding habit permits it to ingest pollutants as part of the ecosystem food chain (in their free form or localised in its food). The experimental fowls had their nativity in Egbema in the Niger Delta Area and their ancestors had existed in that petroleum hydrocarbon carbon-polluted area for over fifty years. Control fowls were obtained from Mbaise that has neither oil well nor gas flaring facilities which is located far away from Egbema and as such may not have experienced petroleum hydrocarbon pollution. The aim of this study was to investigate the effect of chronic exposure to petroleum hydrocarbon pollution on renal and some haematological parameters in native fowl (*Gallus domesticus),* reared in an environment known to be polluted with petroleum hydrocarbon.

2. MATERIALS AND METHODS

2.1. Collection of Experimental Animals

The native fowls (*Gallus domesticus)* used for this study were reared and have their ancestary in Egbema for test and Mbaise for control respectively. Altogether, twelve apparently healthy male fowls (6-9 months old and weighed between 850-900g) from each sample area were examined for ectoparasites, identified and used.

2.2 Preparation of Blood Sample

The native fowls were allowed to acclimatize in the laboratory for 24 hours. The fowls from the environments were anaesthetized with dichloromethane and then sacrificed. Blood was obtained by puncture of the neck artery. Blood sample was collected from each fowl and allowed to stand for 2 hours for clotting to take place. The serum was collected into plain tubes after centrifugation at 1400rpm and stored at 4ºC until analyzed.

2.3 Chemicals and Kits

Thiobarbituric acid (TBA), Trichloro acetic acid (TCA), 2,4-dinitrophenyhydrazine (DNPH) and Sodium dodecyl sulphate were bought from Sigma-Aldrich Chemical Company St. Louis, Mo, USA. Other chemicals were from varied local sources and of analytical grade.

2.4 Renal Function and Electrolyte Indices

Serum sodium and potassium ions concentrations were determined by the use of ion selective electrode method using humalyte machine (Human Germany) described by Tietz [14]. Serum chloride concentration was determined by the titretric method. Serum bicarbonate (as total CO_2) concentration was determined by the use of a CO_2, gas electrode (ASTRA CO_2 apparatus, Beckem Instruments, USA). Serum urea and creatinine concentrations were determined by Jaffe's reaction and urease enzymatic method respectively described by Tietz [14]. Serum total bilirubin concentration was determination by Balistreri and Shaw [15] using standard kits (Human Laboratories, Germany).

2.5 Haematological Indices

The haematological indices involving haemoglobin (Hb)) concentration, haemoglobin/ methaemoglobin ratio, white blood cell (wbc) and red blood cell (rbc) counts were determined by using an automated haematology analyzer machine (Mindray BC 2300, USA) which performs blood cell count by Direct Current detection method, percentage haemoglobin and haemoglobin/ methaemoglobin ratio by non cyanide haemoglobin analysis method, after test running the auto-analyzer with a known sample.

2.6 Statistical Analysis

Each reading was taken in triplicate. All data were expressed as mean ± standard deviation and analysed for statistical significance using students' t-test. Values were considered significant at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

The results of the study showed a decrease ($p<0.05$) in concentrations of Na^+ and Cl^- for Mbaise (137±02 and 130±03) and (118±05 and 110±04) for Egbema respectively while apparent decline was observed in the concentrations of K^+ (11±01) and HCO_3^- (18±02) in the serum of fowls from Egbema when compared to those from Mbaise K^+ (15±01) and HCO_3^- (20±02) (Fig. 1). These results in parts indicate renal disorder according to Sood [16]. This is in contrast to the other biochemical parameters used in assessing renal function (total bilirubin and urea) in the serum of fowls from Egbema which showed significant ($p<0.05$) decrease when compared to those from Mbaise (Fig. 2). These results agrees with the reports of Azeez [17]. The kidneys are important organs that perform many complex functions to keep the blood clean and chemically balanced blood homeostasis.

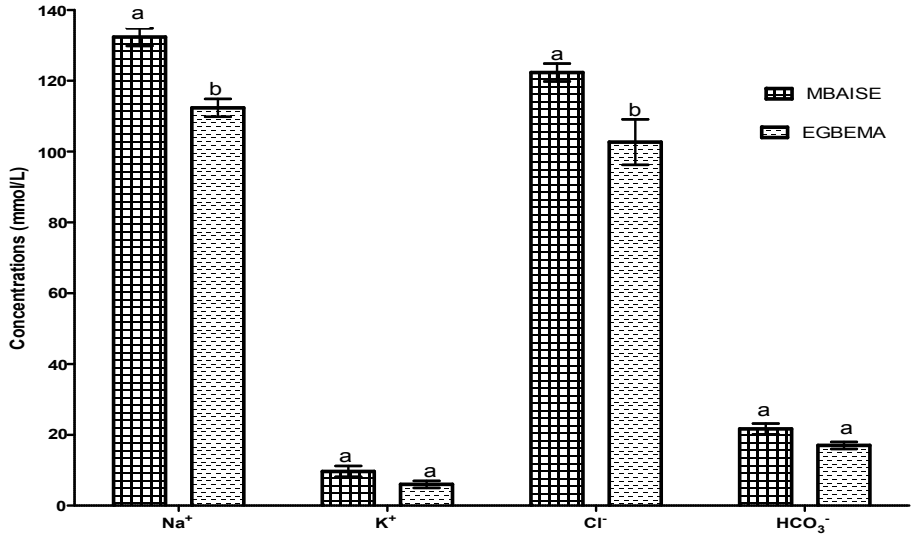

Fig. 1. Effect of petroleum hydrocarbon pollution on electrolyte parameters (n=12) of fowls from Mbaise and Egbema. Values with different alphabets per parameter are significantly ($p<0.05$) different

When the nephrons and their tubular cells are not functioning effectively, urea, creatinine and other metabolic products are retained within the blood stream of living organisms. Creatinine is used as a marker of kidney function. Creatinine is a waste product formed when muscle tissues use up energy sources. It is carried to the kidneys via the blood and cleared from the body [18]. Petroleum hydrocarbon-induced pollution in Egbema reduced ($p<0.05$) the mean concentrations of creatinine (0.50±0.03), urea (0.30±0.01) and total bilirubin (0.19±0.04) mmol/L of fowls reared in Egbema environment when compared to the values (0.60±0.1),(0.50±0.02) and (0.70±0.03) mmol/L obtained for creatinine, urea and total bilirubin respectively for fowls from Mbaise (Fig. 2). Sood [18] reported that decreased urea is associated with liver function and occasional nephritic syndrome while decreased creatinine levels indicate renal disorder.

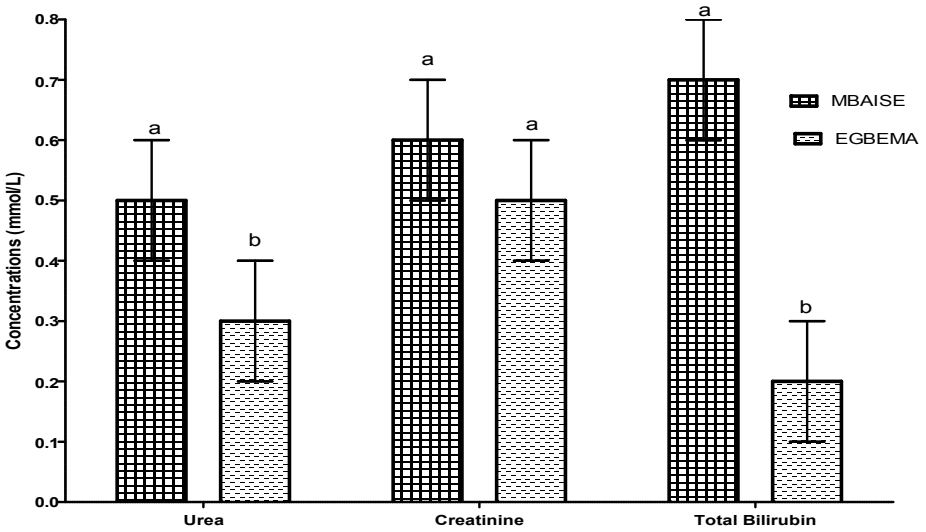

Fig. 2. Effect of petroleum hydrocarbon pollution on serum urea, creatinine and total bilirubin concentrations (n=12) of fowls from Mbaise and Egbema. Values with different alphabets per parameter are significantly (p<0.05) different

Also, the electrolytes: Na^+, K^+, Cl^- and HCO_3^- ions are similarly filtered but dynamically reabsorbed to maintain the body homeostasis [18]. The function of the kidney in the maintenance of the concentration of bicarbonate helps the body in maintaining acid-base equilibrium. This is because bicarbonate serves as the first line of buffer in controlling the normal physiological tendency towards acidification of the blood [18]. Thus, petroleum hydrocarbon pollution may cause harmful effects on the kidneys which may lead to ineffective filtration at the glomerulus and/or damage to the tubular cells thereby reducing their ability to secrete or absorb vital substances including water [18].

The results also revealed that the exposure caused a reduction ($p<0.05$) in white blood and red blood cell counts, $1.5x10^5$±15 and $0.5x10^5$±10 cell/mm^3 respectively of fowls from Egbema when compared to those from Mbaise with mean values as $3.2x10^5$ ±12 for wbc and 1.2 $x10^5$±20 for rbc in cell/mm^3)respectively (Fig. 3). The white blood cells are soldiers of the body, and their reduced counts may be due to decrease in the immune systems of the animals. There was a significant reduction in the wbc count in the serum of fowls from Egbema when compared to those from Mbaise. This indicates a marked reduction in the immune system of fowls from Egbema due to petroleum hydrocarbon exposure. This result agrees with the reports of Mckee and White [19]. The mean value of percent haemoglobin which gave 80±02 for fowls from Mbaise and 70±03 for those from Egbema (Fig. 4), indicates that there was a slight reduction in the percentage haemoglobin. The reduction may in part be due to the exposure of these fowls to petroleum hydrocarbon pollution from Egbema when compared to those from Mbaise.

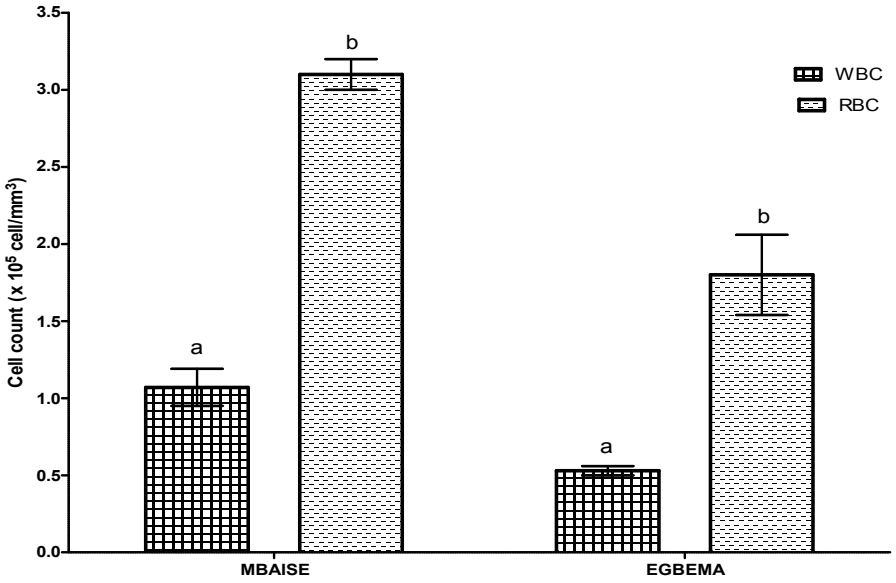

Fig. 3. Effect of petroleum hydrocarbon pollution on white blood cell (WBC) and red blood cell (RBC) counts (x 10^5 cell/mm^3) of fowls (n=12) from Mbaise and Egbema. Values with different alphabets per parameter are significantly ($p<0.05$) different

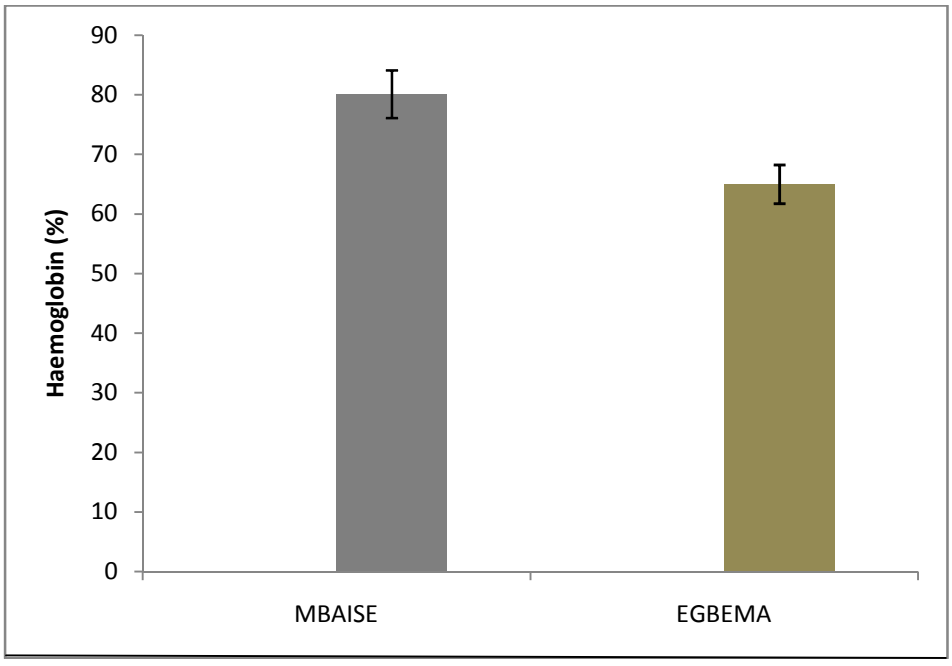

Fig. 4. Effect of Petroleum hydrocarbon pollution on haemoglobin concentrations of (n=12) from Mbaise and Egbema

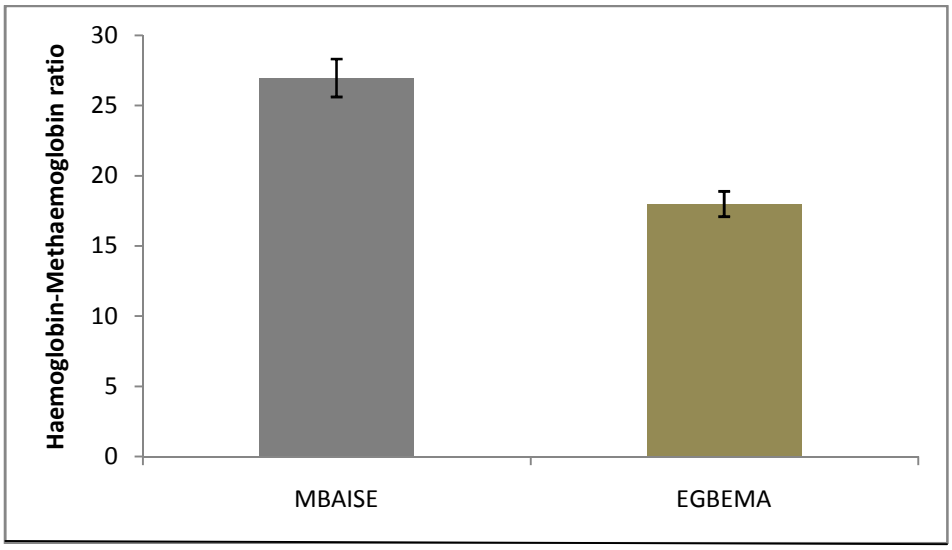

Fig. 5. Effect of Petroleum hydrocarbon pollution on haemoglobin/methaemoglobin concentrations of (n=12) fowls from Mbaise and Egbema

Haemoglobin is the major oxygen-carrying component of the red blood cell. It mediates the exchange of respiratory gases, oxygen and carbon dioxide.Haemoglobin contains iron (II) (Fe^{2+}) which is the reduced form of iron and the more stable. The mean value of haemoglobin/ methaemoglobin ratio from our results indicate that there was a slight reduction in the mean value of haemoglobin/methaemoglobin ratio in fowls from Egbema (17±01) when compared to those from Mbaise (28±02) (Fig. 5). Methaemoglobin results when haemoglobin becomes non-functional and unable to bind oxygen. Methaemoglobin contains iron (III) (Fe^{3+}), the oxidized form which is less stable. They were in unstable state, which implies that the reactive intermediates as a result of petroleum hydrocarbon pollution in Egbema slightly affected the haemoglobin/methaemoglobin ratio of fowls in that area.

4. CONCLUSION

The study revealed that petroleum hydrocarbon pollution possess the potential to affect haematological parameters, total bilirubin and renal function in fowls from Egbema when compared to those from Mbaise.

ETHICAL APPROVAL

This study was conducted in accordance with the national and international guidelines for the protection of human subjects and animal welfare and was approved by the Ethics committee of the Department of Biochemistry, Federal University of Technology, Owerri.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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