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Petroleum Hydrocarbon-induced Changes in Tissues of the Native Fowl (*Gallus domesticus*) following chronic exposure.

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ABSTRACT

The effect of pollution on the native fowl (*Gallus domesticus)* from the petroleum hydrocarbon (PHC)-polluted area of Etekwuru in Ohaji Egbema was studied. Identical fowls from an unpolluted area of (Okwunakuwa-Uvuru) Mbaise served as the control.The concentrations of albumin, bilirubin, cholesterol, liver protein, ascorbic acid (AA) and glutathione (GSH), and the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in the serum of the native fowls from the two different environments in Imo State were determined/assayed . Results of serum albumin, bilirubin, cholesterol and liver protein concentrations obtained for fowls reared at Ohaji Egbema were not significantly different ($p<0.05$) from those obtained for fowls from Mbaise. However, mean values obtained for ascorbic acid (AA) and glutathione (GSH) for test and control animals were significantly different ($p<0.05$). The activities of ALT and AST in the fowls from both environments were essentially similar. The mean activity of ALP was substantially lower in the fowls from Ohaji Egbema (43.32±4.44) when compared to that in fowls from Mbaise (54.98±3.30). Thus, only the concentrations of ascorbic acid and glutathione, and the activity of alkaline phosphatase proved responsive to pollution due to the petroleum hydrocarbon in Ohaji Egbema.

Key words: Petroleum hydrocarbon, pollution, *Gallus domesticus,* ascorbic acid, glutathione,liver enzymes.

INTRODUCTION

Nigeria is the 6th world's major producer of crude oil. Imo State is located in the Niger Delta area of Nigeria where oil exploration and mining activitties have gone on for over four decades. Over the years, an increased demand for petroleum and its products as a source of energy and primary raw material for petrochemical industries has led to siting of a number of oil and gas drilling and processing centres. Earnings from gas and oil operations evidently lifted Nigeria's economic status and brought a substantial improvement in the standard of living of Nigerians. Alongside these good things of life was the emergence of deleterious substances in the environment. These pollutants include spilled crude oil and or its refined products, effluents with traces of heavy metals, particulates and toxicants from gas flaring and greenhouse gases. 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. 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. The changes affected the ecosystem adversely (Ademoroti, 1996; Holdgate,1979).

Ohaji Egbema is located in the Niger Delta area where oil exploration and gas flaring have gone on continuously for over 30 years, and have contributed to the pollution level in the locality. Its ecosystem, definitely, has recieved the impact of the pollution. Animals and plants growing in such environments have, over the years, taken in 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 (Boonchan *et al*, 2000).

The effects of the PHC pollution on plants could manifest as (i) die-back of the more susceptible trees, (ii) low germinability of seeds, (iii) poor growth of plants and (iv) diminished crop yield (Dickson *et al*, 1998;Odu,1996). Though Achuba and Osakwe (2003) reported petroleum -induced toxicity in the cat fish (*Clarias gariepinus*), there is scanty or no information on the effect of PHC pollution on farm (terrestial) animals.

The objective of this study was to investigate the effect of chronic exposure on the blood/liver tissue parameters of the native fowl reared in an environment known to be polluted with petroleum hydrocarbon.

MATERIALS AND METHODS

Experimental Animals and Locations.

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 - 15years. 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/grass and other substances in the environment. Its free-living/ feeding habit permits it to ingest pollutants (in their free form or localised in its food). The experimental fowls had their nativity in Etekwuru in Ohaji Egbema in the Niger Delta, and their ancestors had existed in that PHC-polluted area for over four decades. Identical fowls from non-polluted area of Mbaise served as control. Altogether, 12 healthy male fowls (6-9months old) from each sample area were identified and used.

Chemicals/Reagents

All chemicals used in this study were of analytical grade. Total protein kit (Biosystem), ALT and AST kits (Randox Laboratories) were purchased and used for the various determinations and assays.

Methods

Preparation of blood and liver samples

The native fowls were acclimatized in the laboratory for 24 hours 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, collected by centrifugation,was used for the (i) determination of the concentrations of the blood parameters, and (ii) assay of the activities of the serum enzymes (ALT,AST&ALP). Each fowl was then dissected, the liver collected and stored in the refrigerator at 4°C. Chilled liver tissues were later homogenised and homogenate centrifuged. The homogenate was reserved for determination of liver protein, ascorbic acid, and glutathione concentrations.

Determination of concentrations of blood parameters/assay of activities of enzymes

The concentration of serum albumin was determined according to Doumas *et al* (1971); that of serum bilirubin was determined according to Pearlman and Lee (1974). Cholesterol concentration was ascertained according to Allain *et al* (1974) and liver protein concentration was determined according to the method of Gornall *et al (*1949). AA and GSH concentrations were determined according to Roe and Kuether(1961, 1943) and Jollow (1974) respectively. The activities of the liver enzymes ALT and AST were assayed using Reitman and Frankel (1957) method and that of ALP according to Tietz (1991).

This study spanned a three– year period; and the results presented reflect data generated within the time frame.

Statistics

One way analysis of variance (ANOVA) was employed for the analysis of the results.

RESULTS

The effect of chronic exposure on liver and blood tissue parameters of the native fowls reared in a PHC- polluted environment was studied using fowls from a non-polluted area as control.The results obtained from this study reveal that there were no significant differences (p<0.05) in the mean values obtained for serum albumin, bilirubin, cholesterol and liver protein for the test and control animals (Figs. 1,2,3 and 4).

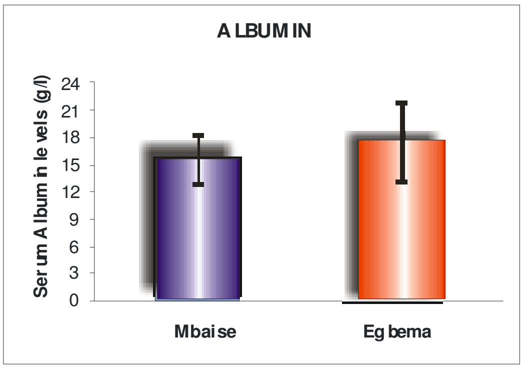

Figure 1: Serum albumin concentrations in the domestic fowls from Mbaise and Egbema .

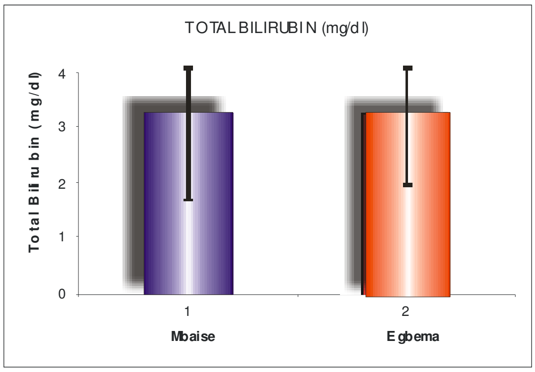

Figure 2: Serum bilirubin concentrations in the domestic fowls from Mbaise and Egbema

However, mean values obtained for ascorbic acid (AA) and glutathione (GSH) for test and control animals were significantly different (p<0.05) (Figs.5 and 6). The mean concentrations of AA obtained for fowls reared in Ohaji Egbema and

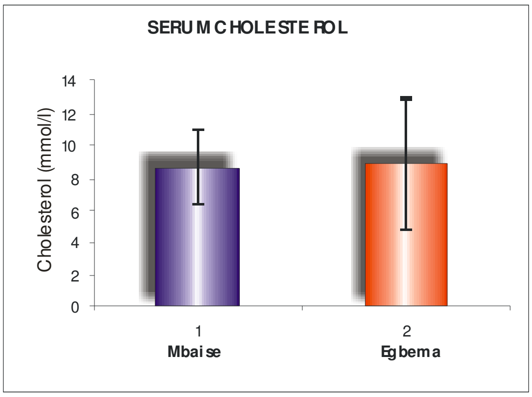

Figure 3: Serum cholesterol concentrations in the domestic fowls from Mbaise and Egbema.

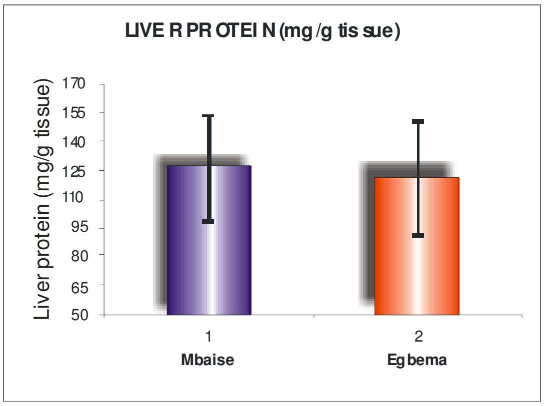

Figure 4: Liver protein concentrations in the domestic fowls from Mbaise and Egbema.

Mbaise were 6.11±2.14 and 16.91±4.99 µg/ml respectively; while the mean concentrations of GSH were 1368.16 ±217.76 and 1847.80±485.50 µg/g tissue respectively.

The activities of ALT and AST in both sample fowls were similar (Figs 7 and 8).This result shows that the enzymes did not leak out into the blood stream, since the liver was not diseased. On the otherhand, ALP activity was significantly lower ($p>0.0001$) in the test than in the control animals (Fig.9).This marked reduction in ALP activity of fowls from Ohaji Egbema only is attributable, wholly, to the PHC pollution in that environment.

DISCUSSION

The results obtained in this study reveal the extent to which the parameters measured were affected (or not) by the pollution in the Ohaji Egbema area. The fact that the mean values obtained for serum albumin, bilirubin, cholesterol and liver tissue protein for fowls from Ohaji Egbema did not differ from those of control fowls from Mbaise (figs.1-4) would mean that the pollution did not affect those parameters. This observation would imply that the liver of fowls from Ohaji Egbema maintained its functional integrity in the face of the level of pollution in that environment. Iriruaga (2004) observed increased serum glucose concentration in native fowls grown in Warri (an industrialised and PHC-polluted area) as against values in identical fowls grown in Abraka (an unpolluted area). Silbergeld (1974) reported that blood glucose level could be a sensitive indicator of environmental stress in fish. In as much as blood glucose concentration was not determined in the present study, the blood parameters indicated above did not give any response to the pollution in Ohaji Egbema.Ascorbic acid (AA) is a (water -soluble) potent antioxidant molecule which scavenges free radicals in the soluble cytoplasm and intercellular environment (McKee and McKee, 1999). As a powerful cellular reductant, it readily donates electrons to reduce free radicals in the cellular compartment and is converted to dehydroascorbic acid (DHAA), its oxidized form. Petroleum hydrocarbon-induced pollution in Ohaji Egbema significantly ($p< 0.05$) reduced the mean concentration of ascorbic acid

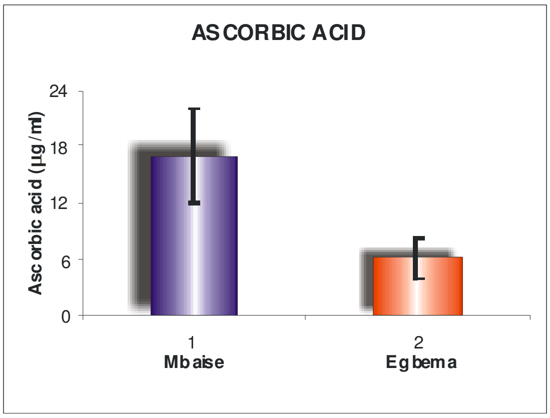

Figure 5: Liver ascorbic acid concentration in the domestic fowls from Mbaise and Egbema

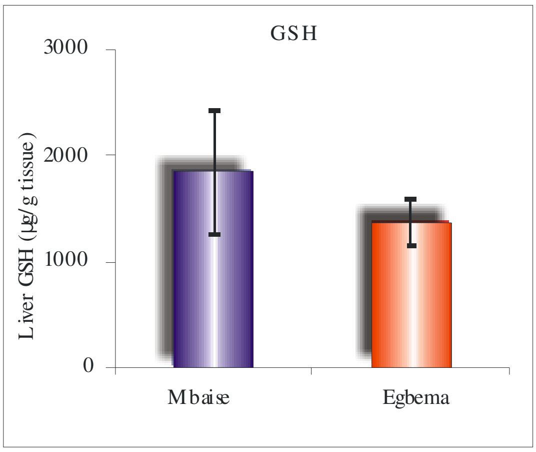

Figure 6: Liver glutathione concentrations in the domestic fowls from Mbaise and Egbema

in native fowls reared in that environment when compared to the mean value obtained for fowls from Mbaise (fig.5). This finding agrees with that of Iriruaga (2004), who reported a substantial reduction in liver ascorbic acid concentration in fowls from polluted Warri area in contrast to that of fowls from un-polluted Abraka environment. This observed marked reduction in ascorbic acid concentration due to pollution in the environment could be attributed to the involvement of ascorbic acid in reactions with reactive species, thereby reducing its concentration.

Glutathione (GSH) is abundant in the cytosol, nuclei and mitochondria and is the major soluble antioxidant in these cell compartments (Masella *et al*, 2005). The oxidized form of glutathione, GSSG, accumulates inside the cells and the ratio of GSH/GSSG can be a good indicator of oxidative stress in an organism (Nogueira *et al;* 2004). According to Knight *et al* (2001), oxidant stress and early increase in the GSSG/GSH ratio *in vivo* all precede cell injury in acetaminophen-induced situation in mice liver. In this study, the result obtained, as shown in fig. 6, has revealed that liver GSH of fowls from the PHC-polluted Ohaji Egbema area was responsive to the pollution in the environment. The effect of the pollution manifested as a marked reduction in GSH concentration. A build-up of reactive intermediates in tissues of the native fowls, caused by this exposure to PHC pollution, might have resulted in the significant ($p<0.05$) reduction in GSH concentration, since GSH in the native fowls reared in Ohaji Egbema might have been employed to mop up various reactive intermediates induced by the pollution.

ALT and AST are members of the transaminase family of enzymes produced mainly in the liver. These enzymes are capable of leaking out into the bloodstream in cases of liver damage or loss of integrity. Hence their use as diagnostic enzymes The results obtained in this study on these enzymes (ALT and AST) (figs. 7 and 8) from fowls from the PHC- polluted area of Ohaji Egbema have shown that their liver tissues were healthy; hence no leakage of any of the enzymes.

Alkaline phosphatase, another liver enzyme, is also capable of leaking out into the bloodstream when the liver is damaged; and a substantial increase in its activity in the blood can be a sign of liver dysfunction. In this study, the activity of ALP was substantially lower in the fowls from the PHC-polluted Ohaji Egbema as against that in fowls from the non-polluted area of Mbaise. Substantial decrease in ALP activity is not a common phenomenon in metabolism. However, it has been observed in severely malnourished children and in hypophosphatasia-an uncommon inborn error of metabolism (Henry, 1984). Increase in alkaline phosphatase activity in serum has been known to be associated with bone disorders or obstructive liver diseases (Devlin, 2006). In this study, this observed decrease in ALP activity is not linked in any way to liver disease and consequent leakage; rather it is attributable to the pollution. Meyers *et al.* (1988) reported that one effect of reactive metabolite formation is mitochondrial dysfunction, which results in ATP depletion and oxidant stress (Jaeschke, 1990). It might be that the PHC pollution in Ohaji Egbema resulted in decreased concentration of ATP (and other phosphorylated compounds) in the fowls, and that this observed decrease in ALP activity could be a reflection of diminished concentrations of various cellular phospho-compounds.

Altogether, the marked decrease in the concentration of ascorbic acid or glutathione, or the remarkable decrease in the ALP activity observed in fowls from Ohaji Egbema area as

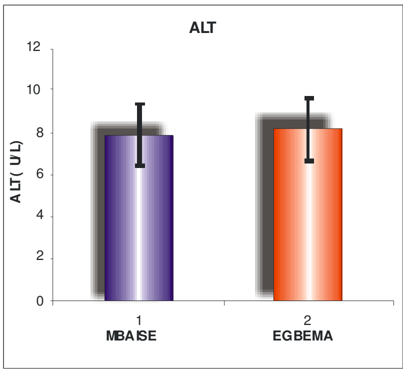

Fig. 7: Serum ALT activities of the fowls from Mbaise and Egbema

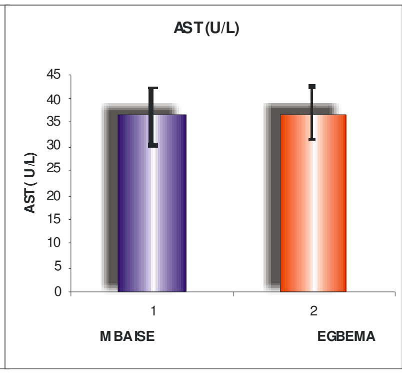

Fig. 8: Serum AST activities of the fowls from Mbaise and Egbema

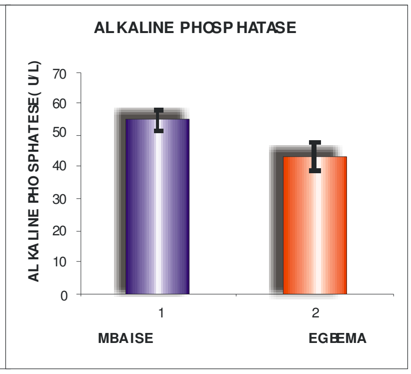

Fig. 9: Serum ALP activities of the fowls from Mbaise and Egbema

against that observed in fowls from the unpolluted Mbaise area shows, in clear terms, that the antioxidant molecules and liver enzyme (ALP) proved responsive to the PHC pollution in the former. It would follow that a significant decrease ($p<0.05$) in AA or GSH concentration, or a significant decrease ($p<0.001$) in ALP activity in native fowls can be an indicator of oxidant/ metabolic stress due to PHC pollution in the place of their nativity.

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