Assessment of liver function status of chickens (Gallus gallus domestica) exposed to factory sites in Nnewi, Anambra State, South Eastern Nigeria

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Abstract
Objective: Nnewi is a growing industrial town in Nigeria and the industries are sited very close to human habitation. Exposure to industrial effluents come with deleterious consequences. The purpose of this study was to investigate the liver function status of chickens exposed to these factory sites in Nnewi Nigeria.

Materials and Methods: A total of twenty-nine (29) chicks were allowed to feed from the surrounding homeland of the factory until they aged between four (4) and five (5) months as adult birds (chickens) for the study. The chickens were sacrificed to obtain the blood for analyses. Five (5) ml of blood sample was collected from the heart into plain containers and the serum obtained was used for the estimation of biochemical parameters. Total protein (TP), albumin (ALB), total bilirubin (TB), direct bilirubin (DB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were assayed using standard laboratory procedures. The data obtained was subjected to statistical analysis using Students t-test using Statistical Package for Social Sciences (SPSS) (version 16) software. Statistical significance was tested at p<0.05.

Results: The mean serum TP, ALB, TB and DB levels as well as ALP activity in chickens reared around factory sites were not significantly different when compared with the control chickens (p>0.05), however, the mean serum activities of AST and ALT were significantly elevated in the factory exposed chickens than those of the control chicken (p<0.05). In addition, a significant reduction in serum bicarbonate level in chickens aged more than five (5) months were observed.

Conclusion: This study found a possible compromise between the liver enzymes and the factory environment which could result in poor growth of the chickens. It is recommended that factories be sited away from the reach of the chickens.

Keywords: Industrialization, Factory site, Liver, Protein, Bilirubin, Aspartate aminotransferase, Alanine aminotransferase, Alkaline phosphatase, Chickens (Gallus gallus domestica), Nnewi.

Introduction
Industrialization of the modern world has been found to generate compounds which are deleterious to life especially to those who work in such industries. Nnewi is one of such growing industrial towns in South Eastern Nigeria and it is the second largest city in Anambra State. Several industries in Nnewi are manufacturing such products as motor car and motor cycle spare parts, electrical appliances, cooking utensils, lead acid batteries, plastics, lead ingots etc. Many of such industries are located near residential areas with the attendant poor enforcement of environmental laws1. One of the major effluents of some of these industries is ‘heavy metals’. Heavy metals are those components of the earth’s crust that cannot be degraded or destroyed. Heavy metals have relatively high density and may be toxic or poisonous even at low concentration2,3. They have atomic density greater than 4g/cm³ with a specific gravity that is about 5 times the specific gravity of water4. Heavy metals are among the contaminants in the environment and they can bio-accumulate. Bio-accumulation may result due to an increase in the concentration heavy metals within the environment over a long period of time5. Threatening levels of such metal pollutants have been detected in meat6, chickens7,8 and vegetables9,10 and soil11 around such sites.

In Nigeria, the growing rate of industrialization is gradually leading to contamination and deterioration of the environment12. Heavy metal toxicity in the liver bring degenerative changes in enzymes of lipid peroxidation, glutathione, glutathione peroxidase, catalase, and superoxide dismutase - biomarkers of acute hepatic damage13. Industrial effluents have been known to compromise liver and kidney functions14-16 and chronic effects of copper can damage the liver and kidneys17. Previously, Okpogba et al.18 had earlier reported a significant reduction in serum bicarbonate level in chickens exposed to factory sites in Nnewi. Also, metals such as lead have been shown to be toxic to humans and animals by causing liver cells to enlarge leading to hyperplasia and initiating the formation of tumors in liver cells19. The intake of nickel in concentrations above the normal recommended reference range either in food or water have been reported to produce lung disease in dogs and rats and affects the stomach, blood, liver, kidneys and immune system in rats and mice, as well as their reproduction and development20,21. Therefore, this study assessed the liver function status of chickens (Gallus gallus domestica) exposed to factory sites in Nnewi, Anambra State, South Eastern Nigeria.
Table 1: Liver function status of the factory chickens (Gallus gallus domesticus)

| Chickens          | TP (g/dl) | ALB (g/dl) | TB(μmol/L) | DB (μmol/L) | AST (U/L) | ALT (U/L) | ALP(U/L) |
|-------------------|-----------|------------|------------|-------------|------------|-----------|----------|
| Control (n=13)    | 3.68±0.50 | 17.77±0.26 | 8.23±1.30  | 2.68±0.32   | 2.69±0.63  | 3.38±0.77 | 114.85±13.22|
| Factory (n=16)    | 3.87±0.53 | 15.19±0.49 | 8.69±1.49  | 2.64±0.27   | 6.19±2.29  | 5.50±2.39 | 118.38±7.34 |
| p-value           | 0.373     | 0.135      | 0.394      | 0.722       | *0.000     | *0.005    | 0.370     |
| t-value           | -0.905    | 1.540      | -0.867     | 0.360       | -4.742     | -3.051    | -0.911    |

*Statistically significant at p<0.05; Results are Mean±SD.

Materials and Methods

Experimental Site

This study was carried out around four factories including lead acid battery manufacturing factory, cable manufacturing factory, metal fabricating factory and metal forging factory, all located within Nnewi.

Experimental Design, Research Procedure and Parameters Measured

This is a cross-sectional study designed to assess the liver function status of chickens (*Gallus gallus domesticus*) exposed to factory sites in Nnewi, Anambra State, South Eastern, Nigeria. A total of twenty-nine (29) chicks comprising of sixteen (16) chicks exposed to factory sites and thirteen (13) non-exposed chicks were grown to adult birds (chickens) for the study. The chicks in the exposed group were obtained from the surrounding households, about 250m, to these factories under study (lead acid battery manufacturing factory, cable manufacturing factory, metal fabricating factory and metal forging factory) while the chicks to serve as control were obtained in Elele. They were aged between four (4) and five (5) months. They were allowed to feed from the surrounding homeland until they were due for the experiment. Control chickens of the same age group were obtained from environments outside Nnewi. The chickens were sacrificed to obtain the blood for analyses.

At the end of the study period that lasted eighteen (18) weeks, the (birds) chickens were each anaesthetized with ether soaked in absorbent cotton wool and kept in a dessicator with the lid firmly put in place to prevent evaporation. 5mls of blood sample was collected from the heart into plain containers for estimation of biochemical parameters (Total protein, albumin, total bilirubin (TB), direct bilirubin (DB), Aspartate aminotransferase, Alanine aminotransferase and Alkaline phosphatase). Serum total protein (TP) was determined according to the method of Gornall et al.22. Albumin (ALB) by using the method of Doumas and Watson23. Bilirubin was concentration was determined using the colorimetric method as described by Jendrassik and Grof.24 Aspartate aminotransferase activity (AST) and Alanine aminotransferase activity (ALT) were estimated according to the method of Reitman and Frankel25 while Alkaline phosphatase activity (ALP) was assayed according to the method as described by Bessey et al.26.

Ethical Consideration

Ethical approval for the research was obtained from Ethical Committee, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria (NAUTH/CS/66/Vol.2/149).

Statistical Analysis

The data were presented as mean±SD and the mean values of the control and test group were compared by Student’s t-test using Statistical package for social sciences (SPSS) (Version 16) software. Statistical significance was tested at P<0.05.

Results

Table 1 presents the liver function status of control and factory chickens. There was no significant difference between the mean serum TP, ALB, TB and DB levels of control and factory chickens although TP and TB levels of the factory chickens were higher while ALB and DB concentration were lower compared with the control (p>0.05).

However, the mean serum AST and ALT activities were significantly elevated in the factory chickens than in control (p<0.05), but there was no significant difference between the mean serum ALP activity of the factory chickens and the control chickens (p>0.05).

Discussion

The results of the liver function status of the chickens reared around the factory sites have shown that the mean serum TP, ALB, TB and DB levels were not statistically significantly different when compared with the control chickens. This may imply that the synthetic role of the liver was not compromised. Increased protein concentration in the blood could be caused by among other things; dehydration and chronic infections while decreased plasma protein may be caused by insufficient protein in the diet and decreased albumin formation in the liver27. According to Nsirim28, serum proteins act as transport medium for bilirubin and hormones.

In the present study, the liver enzymes AST and ALT were significantly higher in the factory chickens compared with the control. In a work reported by Yacoub and Gad29 that assessed the accumulation of some heavy metals and biochemical alteration in muscles of the *Oreochromis niloticus* from the Nile River in Egypt, they reported a marked decrease in the muscle ALT and AST activities which they attributed to disturbance in the structure and
integrity of cell organelles like the endoplasmic reticulum and membrane transport system while Marie attributed reduction in AST and ALT to high accumulation of heavy metals in fish tissues. Similar reports by and showed reduced AST and ALT levels in fish exposed to various pollutants such as heavy metals. These results are in contrast with our findings.

In this study, there was no significant difference between the mean serum ALP activity of the factory chickens and the control chickens. Organ congestion and biliary stasis can lead to loss of membrane enzymes such as ALP which is a marker enzyme for plasma membrane and endoplasmic reticulum. Therefore, this study revealed the harmful effects of industrial effluents on the liver and this poses serious public health implication for the general human population.

Conclusion
The results of the liver function status of the chickens reared around the factory sites have shown that the mean serum TP, ALB, TB and DB levels were not statistically significantly different when compared with the control chickens while the liver enzymes AST and ALT were significantly different when compared with the control TP, ALB, TB and DB levels were not statistically significantly higher. Therefore, this study revealed the harmful effects of industrial effluents on the liver and this poses serious public health implication for the general human population.

Conflicts of Interest: None.

References
1. Oritsakwe, OE., Asomugha, R., Afonne, OJ., Chilaka, KC. and Dioka, C. Effect of industrial effluents on water and soil qualities in Nnewi Nigeria. J Health Sci 1999;45(4):177-183.
2. Lide, D. (2002). Heavy metals. CRC handbook of Chemistry and Physics. 73rd edn. Boca. Raton. FL:CRC Press. Pp. 63-5.
3. Singh, M.R. Impurities-heavy metals: IR perspective. Indian pharmacopoeia commission, 2009.
4. Jarup, L. Hazards of heavy metal contamination. Br Med Bull 2003;68:167-182.
5. Njar, G.N., Iwara, A.L., Offiong, R.A. and Deekor, T.D. Assessment of heavy metal status of boresholes in Calabar South Local Government, Cross River State, Nigeria. Ethiopian J Environ Bull Management 2012;5(1):86-90.
6. Akan, J.C., Abdulrahman, F.I., Sodipo, O.A. and Chiroma, Y.A. Distribution of heavy metals in the liver, kidney and meat of beef, mutton, caprine and chicken from Kasuwan Shaku market in Maiduguri metropolis, Borno State, Nigeria. Res J Appl Sci, Eng Technol 2010;2(7):43-8.
7. Okoye, C.O.B, Ibeto, C.N. and Ihedioha, J.N. Assessment of heavy metals in chicken feeds sold in south Eastern Nigeria. Adv Appl Sci Res 2011;2(3):63-8.
8. Okpogba, A.N., Ogbo, E.C., Ugwu, E.C., Oguya, V.N., Dike, C.C. and Ujowundu, F.N. Comparative assessment of heavy metal levels in chickens (Gallus gallus domesticus) in rural (Elele) and urban (Nnewi) Areas. Asian J Sci Technol 2018;9(11):9056-9.
9. Harmanescu, M., Alda, L.M., Bordean, D.M., Gogosa, I. and Gergen, I. Heavy metals health risk assessment for population via consumption of vegetables grown in old mining area; a case study L Banat County, Romania. Chem Central J 2011;5:64.
10. Suruchi and Pankaj Khanna. Assessment of heavy metal contaminants in different vegetables grown in and around urban areas. Research J. Environ. Toxicol. 2011;5:162-79.
11. Nduka, J.K.C., Oritsakwe, O.E., Ezenwaka, O.L., Abiaiam, C.O., Nwanguma, C.K. and Madubuchi, J.M.U. Metal contamination and infiltration into the soil at refuse dumpsites in Awka, Nigeria. Arch. Environ. Occup. Health 2006;61:197-204.
12. Ibeto, C.N. and Okoye, C.O.B. High Levels of Heavy Metals in Blood of the Urban Population in Nigeria. Res J Environ Sci, 2010;4:371-82.
13. Parthiban, P. and Muniyan, M. Effect of heavy metal nickel on aminotransferase activities in liver tissue of Cirrhinus mirigala (HAM). Int. J. Current Research 2011;2(1):55-60.
14. Draghi, Ch., C., Mman, G., Jelescu, C., Dim, C. and Chira, E. (2010). Heavy metals determination in environmental and biological samples. In: Environmental heavy metal pollution and effects on child mental development-risk assessment and prevention strategies, NATO Advanced Research Workshop, Sofia, Bulgaria 28th April 2010.
15. Vieira, C., Morais, S., Ramos, S., Delerue-Matos, C. and Oliveira, M.B.P. Mercury-Cadmium, lead and arsenic levels in three pelagic fish species from the Atlantic Ocean: intra- and inter-specific variability and human health risks for consumption. Food chemical Toxicol 2011;49:923-32.
16. Wu, Z., Du, Y., Xue, H., Wu, Y. and Zhou, B. Aluminum induces neuro-degeneration and its toxicity arises from increased iron accumulation and reactive oxygen species (ROS) production. Neurobiol. Aging 2012;33(1):199-212.
17. Neebor, E., Thomassen, Y., Romano, M., Nikonor, A., Odland, J.O. and Chaschin, V. Multi-component assessment of worker exposures in a copper refinery: Part 2, Biological exposure indices for copper, nickel and cobalt. J Environ Monit 2007;9:695-700.
18. Okpogba, A.N., Ogbo, E.C., Okpogba, J.C., Analike, R.A., Amah, A.K., Odgehe, B.O., Ujowundu, F.N., Oguka, V.N and Onyeneye, C.E. Assessment of kidney function status in chickens (Gallus gallus domesticus) in rural (Elele) and urban (Nnewi) Areas. J Med Clin Res 2018;6(12):1048-52.
19. Durgut, R., Koc, A., Gonenc, R., Bal, R., Celik, S., Guzel, M., Atug, M. and Atesolu, O. Effects of High Dose Lead Toxication on Liver, Kidneys, Heart, Brain and Blood in Rabbits: an Experimental Study. J Appl Biol Sci 2008;2(2):11-18.
20. Davies, J.R. ( ). Uses of Nickel. ASM Specialty Handbook: Nickel, Cobalt and their Alloys. SM International, 2000;7-13.
21. Tejion, C., Olmo, R., Blanco, D., Romeo, A., and Teijon, J.M. Low doses of lead: effects on reproduction and development in rats. Biol Trace Elem Res 2006;11:51-165.
22. Gornall, A.G., Bardawill, J. and David, M.M. Determination of serum proteins by means of the biuret method. J Biol Chem 1949;171:751.
23. Doumas, B. and Watson, W. The determination of serum albumin using Bromocresol Green (BCG). Clin. Chimica Acta 1971;31:87-97.
24. Jandrasslik, S. and Grof, P. Bilirubin estimation. Biochem. 1938;297:81-84.
25. Reittman, S. and Frankel, S. A colorimetric method for aspartate and alanine aminotransferases in serum. Am J Clin Pathol 1957;28:56-63.
26. Bessey O.A., Lowry, O.H. and Brock, M.J. One point colorimetric method of determining alkaline phosphatase in serum or plasma. J Biol Chem 1946;164:321.
27. Ochei, J. and Kolhatkar, A. (2007) Liver Function Tests. In Medical Laboratory Science Theory and Practice. Tata McGraw-Hill Publishing Company Ltd, New Delhi. Pp152-162.
28. Nsirim, N. (1999). Clinical Biochemistry for Students of Pathology. Longman Nigeria Plc, 52 Oba Akran Avenue, P.M.B. 21036, Ikeja Lagos.

29. Yacoub, A.M. and Gad, N.S. Accumulation of some heavy metals and biochemical alterations in muscles of Oreochromis niloticus from River Nile in Egypt. *Int J Environ Sci Engr* 2012;3:1-10.

30. Humtsoe, N., Dawodi, R., Kulkami, B. and Chayan, B. Effect of arsenic on the enzymes of the Rohn carp. *Labeorohita Hamilton, 1822*. Raffles Bull Zool 2007;14:17-19.

31. Marie, M.A. Toxic effects of aluminum on blood parameters and liver function of Nil cat fish. *Clarias Lazer* 1994;13:279-94.

32. Rao, J. Biochemical alterations in euryhaline fish, Oreochromis mosambicus exposed to sublethal concentrations of organophosphorous insecticides monocotophos. *Chemosphere* 2006;65:1814-20.

33. Mohamed, F.A. and Gad, N.S. Environmental pollution induced biochemical changes in tissues of Tilapia zilli, Solea vulgaris and Mugil cephalus from Lake Qarun, Egypt. *Global Veterinaria* 2008;2(6):327-36.

34. Wright, P.J. and Plummer, D.T. The use of urinary enzyme measurement to detect renal damage caused by nephritic compounds. *Biochem Pharmacol* 1974;23:65-73.

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