

Toxicological Effects Of Effluent From Steel Construction Industry On *Rattus Novergicus*

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ABSTRACT

Environmental degradation is on the high increase as a result of constant discharge of industrial effluent into ecosystem. One of the major industries are industries where high level concentration of heavy metal is being generated. Steel construction companies have been implicated in production of effluents with high concentration of heavy metals. It is of utmost importance to evaluate the impact of the effluent on the environment and human who depend directly or indirectly on this environment. This work focused on the investigation of different concentration of effluent on the haematological, biochemical and histopathological parameters of *Rattus novегicus* over a period of 30 days. Thirty – six adult male rats were put into six groups with six rats per group. The effluent at 100% was diluted to 20 %, 40 %, 60 % and 80 % concentrations and each rat was gavaged with 0.5 ml of the prepared effluent assigned to the group twice a day. The toxic potential of the effluent was evaluated and analysed by using Analysis of Variance followed by Duncan multiple range test at 0.05 level of significance. There was significant difference in the mean values of RBC, Hb and WBC ($p < 0.05$) compared with the control. A none dose-dependent significant reduction in biochemical parameters of treated rats especially Glucose with highest values of 58.33 ± 2.93 , Sodium of 140.33 ± 0.21 and creatinine of 1.20 ± 0.07 was observed. Liver and Kidney dysfunction of test organisms was observed by the increased concentration of Aspartate amino transferase on every group considered relatively to the control ranges from 470.17 ± 9.67 to 499.17 ± 0.40 . It can be deduced from this study that aberrations observed were becoming more severe as the concentrations increases in the treated rats.

Key words: Effluent, *Rattus novегicus*, Haematology, Biochemical, Histopathology

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I. Introduction

Industries have been equated to a large extent, as one of the major contributing factor to the economic growth of any nation. However, the effects of pollution resulting from operation of these industries on surface and ground water cannot be overemphasized. In fact, many living organisms have been brought on the verge of extinction as a result of the introduction of certain contaminants and pollutants (Galadima *et al.*, 2010). The discharges of industrial effluents into municipal drains in residential and commercial environment stand to pose deleterious environmental threat. Wang and Chen (2006) identify that, effluents discharged from industries (fertilizer, refineries and pesticide, photography, leather working, electric appliance manufacturing, iron and steel, metallurgy, metal surface treating and wastewater treatment plants) directly cause heavy metal pollution in water and soil. It may also occur indirectly through contaminants that enter the water supply from soil/ground water systems and from the atmosphere through rain water (Galadima *et al.*, 2010).

Similarly, waste substances generated by industries are the main causes of soil, water and air contamination which causes damage or injury to both humans in relation to their health and environments. Heavy metals, sulphur dioxide, particulate matters, aluminium, carbon monoxide benzene, nitrogen dioxide, dioxins, effluents from industries and agricultural runoff have been identified as the main environmental pollutants (Yahaya and Okpuzor(2012). The main cause of acute and chronic health problems in animals and humans which involve several organs and systems are pollutants. High mortality and short life expectancy is as a result of short– and long- term exposures to pollutants (Marilena and Elias (2008). Industries keep expanding due to high demand for finished products and advancement in technology despite the health consequences.

Steel industry is among the industries that make use of high temperature, starting from cooking and sintering processes to the furnaces, steel plants and steel mills. Besides, they also make use of excessive amount of water either for cooling or washing (Caneghem *et al.*, 2010).

II. Materials and Methods

Sample Collection and procedure

Effluents discharged from the industry were collected into clean bottles that had been pre-washed. A total number of thirty six healthy male rats (*Rattus novegicus*) that weighed 140 ± 20 g were procured. The rats were fed with the standard rat pellet feed and water given to them *ad libitum*. The rats were kept in six well aerated plastic cages and acclimatized for two weeks. The animals were grouped into six per group. The rats in groups 1, 2, 3, 4 and 5 were administered with the effluents at 20, 40, 60, 80 and 100% concentrations. Each rat was gavaged with 0.5ml of the effluent twice a day at intervals of 12 hours for thirty days. Group 6 rats were gavaged with distilled water as control group.

Haematological Procedure

Each group were sacrificed and the blood was collected immediately by the use of sterilized disposable 2ml needle and syringe, the blood was stored in vials containing anticoagulant Ethylene diamine tetra acetic (EDTA) for evaluation of haematological parameter namely red blood cells count (RBC), packed cell volume (PCV), White blood cells count (WBC) and haemoglobin concentration (Hb) with their indices Mean Corpuscle Haemoglobin Concentration (MCHC), Mean Corpuscle Haemoglobin (MCH), Mean Corpuscle Volume (MCV) were estimated by the standard methods as described (Dacie and Lewis (1977); Lee *et al.*, 1999). Blood samples were withdrawn and left to clot in clear dry centrifuge tubes without the EDTA. Thereafter, they were centrifuged at 3500 rpm for 15 minutes.

Biochemical Procedure

A small portion of the clear supernatant serum was used immediately to determine the glucose level using the enzymatic colorimetric method described by Trinder (1969). The remaining serum was frozen at -20°C for other analysis like Aspartate amino transferase (AST) and Alanine amino transferase (ALT) activities were estimated using the method described by Varley (1969). Serum alkaline phosphate was examined adopting the method of Belfield and Golderg (1971). The total cholesterol contents of the serum were determined according to Allain *et al.*, (1974). The albumin levels and serum total protein were estimated using the methods described by Doumas (1975, Doumas *et al.*, 1971). Serum globulin was determined using the method of Latner (1975). Urea and creatinine level of the serum were determined adopting the method of Bartels and Bohmer (1972).

Histopathological Procedure

The liver and kidney were surgically removed from the treated rats. The organs were preserved in 10% formalin prepared by dissolving 10 ml into 90 ml of distilled water, for histological studies to detect any pathological changes. Tissue was dehydrated through increased grades of alcohol (70%, 80%, 90% and absolute). The preparation was cleared in Xylene for 30 minutes, the tissues were treated by wax impregnated and finally embedded in paraffin wax. They were sectioned at $4 \mu\text{m}$ on a rotary microtome, then, stained with Mayer's haematoxylin for 15 minutes. Rinsed in mixture of alcohol and acid (addition of 1 ml of hydrochloric acid in 99 ml of 70% alcohol) according to Baker *et al.*, (1985). The tissues preparations were washed in running tap water for 5-10 minutes, counterstained with 1% aqueous eosin for 2 minutes. Rinsed in water until excess eosin was removed, dehydrated, cleared and mounted in neutral balsam method for microscopic observation. Any evidence of histopathological changes was reported using the bright field Leitz microscope. The data were subjected to statistical analysis SPSS (Statistical Packaging for Social Sciences) version 22.0. Data were subjected to one way analysis of variance (ANOVA) followed by the Duncan multiple range test. Level of significance was set at $p < 0.5$.

III. Results

The mean values obtained for the PCV of *Rattus novegicus* were shown in Table 1, in treated rats at 20ppm, there was no significant difference ($p < 0.05$), likewise at the 40, 60, 80, 100 and the control; the lowest value of PCV of 38.17 ± 2.26 was observed at 20ppm while the highest value of 44.67 ± 2.53 was obtained at 80ppm, 44.33 ± 3.16 at 40ppm, 44.33 ± 2.43 at 60ppm and 39.00 ± 2.85 at 100ppm respectively. RBC at different concentration showing significant difference ($p < 0.05$), none dose dependent reduction observed compared with that of control, with highest value of $2.36b \pm 0.31$ at 40ppm, lowest value at $1.87b \pm 0.17$ at 80ppm, $2.19b \pm 0.31$ at 20ppm, $1.95b \pm 0.17$ at 60ppm and $2.24b \pm 0.30$ at 100ppm. Haemoglobin (Hb) obtained at different concentrations were significantly difference ($p < 0.05$), none dose dependent reduction observed compared with the control with highest value of $9.60b \pm 0.75$ at 40ppm, the lowest value of $7.28c \pm 0.54$ at 100ppm, $8.85bc \pm 0.64$ at 20ppm, $8.66bc \pm 0.41$ at 60ppm and $7.68bc \pm 0.30$ at 80ppm respectively.

White Blood Cell (WBC) obtained in control has higher value compared with the treated rats at different concentrations hence there were significant difference with none dependent reduction observed when compared with the control and the highest value of $9.92ab \pm 0.54$ was observed at 40ppm, lowest value of $7.98b \pm 0.30$ at 80ppm, $8.70b \pm 0.26$ at 20ppm, $9.33ab \pm 1.48$ at 60ppm and $8.00b \pm 0.54$ at 100ppm respectively. MCV obtained as presented in Table 1, there were significant difference ($p < 0.05$) in the concentrations compared with the control. The value obtained in the control is lesser than the values obtained at different concentrations of the treated rats with variation in values obtained in all the concentrations and the highest value of $2.44a \pm 0.16$ was observed in 80ppm, $2.03ab \pm 0.41$, $1.96ab \pm 0.40$. The mean values of MCV obtained are as shown in Table 1., $2.36a \pm 0.25$ and $1.90ab \pm 0.26$ at 20ppm, 40 ppm, 60 ppm and 100 ppm respectively.

There was variation in mean value of MCH obtained in all the different concentrations. However, there was no significant difference ($p < 0.05$) in the MCH values. The value of 6.86 ± 3.23 obtained in the control is higher compared to the values obtained in treated rats at different concentration of 4.40 ± 0.54 , 4.30 ± 0.36 , 4.53 ± 0.24 , 4.23 ± 0.39 and 3.37 ± 0.37 at 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm respectively as shown in Table 1. The control and all the different concentrations in *Rattus novegicus* treated shows that there were variation in mean values of MCHC and there was no significant difference ($p < 0.05$) with none dependent reduction observed, with the highest mean value of 29.47 ± 2.74 in 80ppm, lowest value of 19.53 ± 3.17 in 100ppm, 23.70 ± 2.39 , 25.07 ± 3.46 and 19.86 ± 1.41 at 20 ppm, 40 ppm and 60 ppm respectively.

The mean values of glucose obtained for *Rattus novegicus* as shown in Table 2 shows that there was decrease in values in the treatment compared to control hence, there were significantly different $p < 0.05$ in the mean of glucose in all different concentrations with the highest value of $58.33ab \pm 2.93$ at 60ppm, $41.17ab \pm 6.96$ at 20 ppm, $47.00bc \pm 7.30$ at 40 ppm, 42.50 ± 6.36 at 80 ppm and 35.5 ± 8.05 at 100 ppm, Potassium shows that there was decrease in values of the treated rats compared with the control of 12.20 ± 0.20 hence, there was significant difference ($p < 0.05$) in the mean of Potassium in all the concentrations and as the concentrations increases the mean values obtained decreases with the highest values of 11.72 ± 0.04 at 20ppm and 40ppm and lowest value of 11.03 ± 0.34 at 100ppm, Sodium shows there was decrease in mean values of untreated rats compared to the control.

There significant difference $p < 0.05$ with the highest mean value of $140.33bc \pm 0.21$ observed at 60ppm, lowest value of 125.50 ± 6.63 at 100ppm, 139.17 ± 0.48 at 20 ppm, 137.17 ± 1.19 at 40 ppm and 127.33 ± 6.44 at 80 ppm, Chloride showed no significant difference although variations were observed. 92.83 ± 0.92 obtained at the control decreases in all the different concentrations with the highest mean value of 96.00 ± 0.37 observed at 60ppm and lowest value of 91.83 ± 2.77 at 100ppm, 93.17 ± 1.38 , 95.67 ± 0.92 and 93.67 ± 1.33 at 20 ppm, 40 ppm and 80 ppm respectively, bicarbonate shows that there were variations in the values and shows that there were significant difference $p < 0.05$ with the highest mean value of $22.00a \pm 0.037$ observed at 20ppm, lowest value of $20.50b \pm 0.02$ at 60ppm, $20.83ab \pm 0.31$ at 40 ppm, $21.50ab \pm 0.56$ at 80 ppm and $21.33ab \pm 0.42$ at 100ppm respectively, total cholesterol showed variations in the values and there was significant difference ($p < 0.05$) with the highest mean value of $102.83ab \pm 1.11$ observed at 60ppm, lowest value of $93.33ab \pm 4.01$ at 80ppm.

Urea shows variation in increment compared with the control and also shows there were no significant difference with the highest mean value of 300.00 ± 16.53 obtained at 100ppm, lowest value of 168.83 ± 44.33 at 40ppm, Creatinine mean values decreases as the concentrations increases with significant difference ($p < 0.05$) compared with the control with $1.20ab \pm 0.07$ at 20ppm, $1.17ab \pm 0.07$ at 40 ppm, $1.15ab \pm 0.04$ at 60 ppm, $1.13b \pm 8.12$ at 80 ppm and $1.03ab \pm 0.02$ at 100ppm, The mean values of Total protein showed variation and there were significant difference with highest mean value of $6.43ab \pm 0.13$ at 80ppm, lowest value of $5.47c \pm 0.30$ at 100ppm, $6.63a \pm 0.88$ at 20 ppm, $5.97bc \pm 0.16$ at 40 ppm and $5.82c \pm 0.14$ at 60 ppm., There were decreased in mean values of Albumin as the concentration decreases compared with the control. However, there was significant difference with the highest mean value of $3.28ab \pm 0.09$ obtained at 20ppm while lowest value of $3.13ab \pm 0.12$ was obtained at 100 ppm.

There were variations in the mean values of AST with the least value of $470.17b \pm 9.67$ observed at 60ppm, $499.17a \pm 0.40$ at 20 ppm, $476.83ab \pm 11.61$ at 40 ppm, $470.83ab \pm 33.21$ at 80 ppm and $498.50ab \pm 0.34$ at 100 ppm. However, there was significant difference ($p < 0.05$) in reduction compared to the control, The mean values of ALT for control and the treated rats with different concentration varies in the mean values and were significant difference $p < 0.05$ with the least value of $212.67b \pm 10.27$ observed at 60ppm, highest value of $306.50a \pm 44.77$ at 20ppm, $273.67ab \pm 2.22$ at 40 ppm, $282.83ab \pm 33.21$ at 80 ppm and 252.17 ± 11.25 at 100 ppm, There were variations in values of alkaline phosphate obtained for the treated rats at different concentrations compared with the control. The mean value showed that there was significant difference $p < 0.05$ in all the different concentrations with the highest value of $237.67ab \pm 32.61$ in 40ppm, lowest value of $145.67ab \pm 25.87$ in 60ppm. Histological changes were noticed in liver and kidney sections prepared from treated rats at different concentrations.

IV. Discussion

The assessment of haematological parameters and complete blood counts could help to determine the physiological status of an organism; these factors could be considered stress indicators and a valuable tool for evaluating the harm caused by certain substances (Flaiban *et al.*, 2008). The blood parameters investigated for the haematological test for all the treated groups and the control group were PCV, WBC, Hb, and RBC as show in Table 1. During the experiment, *Rattusnovegicus* in the control group and the ones treated with different concentration of the effluents from the industry does not show toxicity in their appearance and activities, however, it was revealed that there was no significant difference in MCH, PCV and MCHC, compared to Hb, RBC and WBC were significant none dose dependent reduction was observed ($P < 0.05$).

MCV values revealed significant difference with value obtained for control lower than values for treated rats. Similar reduction in haematological parameters had been reported for rats treated with Dimethoate (Khogali *et al.*, 2005; Mohammad *et al.*, 2013) and there was no other significant clinical manifestation that were observed from the treated rats. Decrease or increase in cell counts and depletion of plasma constituent or their elevation above the reference range could demonstrate haematotoxicity. In the present study, there was significant difference in the haematological parameters of all the treated animals relative to the control group, there were non obvious haemolytic changes in the plasma of the rats treated with different concentration of the effluent on RBC, Hb, PCV, MCV, MCH and MCHC. These indices are well known to determine the haemolytic damage on red blood cells. The changes on these indices suggest that the effluent possess anaemic condition in treated rats.

Although, there were no behavioural responses observed in the control and the ones treated with different concentration of the effluents, Amara *et al.* (2012) reported that the haematological effect of the presence of metals are sensitive index of the physiological changes of an animal to any environmental pollutants and its nature of toxicity shows little or no significant changes in the blood of *Rattusnovegicus*. However, the stability in PCV level in the blood parameters may indicate that presence of metals does not have negative effect on the flow of blood on the animal. Due to chemical toxicity the destruction of erythrocytes leading to decreases in the RBC in the blood causes the distruption action of the metals on the erythropoietic tissue which in turn affects the cell viability as presented by Mohammed *et al.* (2013). Jyostana *et al.* (2003) supported that the traces metals decreases RBC counts and the heamoglobin levels.

The little decrease in haemoglobin value in the treated rats compared with the control is as a result of increase in the rate at which haemoglobin is destroyed; iron is obtained from stored ferritin and a dietary source which is essential for synthesis of haemoglobin as reported by Khogali *et al.* (2005). This present study showed that hemoglobin content and blood cell counts were significantly reduced in treated rats with different concentrations in comparison to control group. This results suggests that the presence of metals in the effluent suppresses bone marrow ability to produce new ones, resulting in lowering of blood cells counts resulting in decrease of Hb percentage in the blood resulting in alteration in liver and kidney functions by modulating liver enzymes as presented by Davila *et al.* (1989) and Abraham *et al.* (2007). However, the activity of this enzyme is not limited only to the liver as it is also present in the red blood cells as reported by Ballantyne (1988).

The haemocrit value of PCV, Neutrophil investigated and showed no significant difference in the treated rats when compared with control rats. The values obtained in this study compared to the values presented in previous studies in increased values may be as a result of differences in species of the test organism used, geographical locations and exposure period. Blood toxicity is usually followed with significant changes in the values of hematological parameters in Hb, RBC and PCV indices causing the likely suppression of erythropoietic processes or haemolysis of the available RBC and resulting in liver necrosis and anemia with changes in blood biochemical parameters (Uhmacher *et al.*, 2010). Since little decrease in RBC values was observed following the concentrations of the effluent which means the effluent does not affect the production of the RBC.

The decrease in WBC counts suggest likely immune suppression since the WBCs are known to be the important actors in immune responses as they form the first line of defense against invasion of microorganism and the lowering of WBC values usually indicates fall in immune strength (Sembulinggam and Prema, 2010). Daily oral intake of the effluent of different concentrations administered to the rats did not result in any obvious clinical abnormalities or death to the treated rats after exposure to the concentrations. The appraisal of some biochemical parameters such as the activities of enzymes in tissues plays an important role in disease investigation, diagnosis and liver toxicity (Malomo, 2000; Larrey, 2002).

The non-significant increase or decrease in the haematological values is a signal that the effluent does not affect the haemopoetic system when administered orally at the concentrations used in this research work. The biochemical parameters in the studied groups were to a large extent similar to the control at all the

concentrations only that the AST has a very high concentration on each group considered and this may result to liver and kidney dysfunction of the test organisms as reported by Jens and Hanne (2002). It is plausible to infer that the effluent when administered orally at lower concentrations in rats doesn't have much toxic effect. However, daily oral intake of higher concentrations of effluent can cause liver and kidney damage if it is continually administered for a longer period.

ALT is a more specific enzyme of damage and known to increase when there is liver cell damage. According to Bush (1991), ALT has been employed as a tool for measuring hepatic necrosis. It is also located in the liver and increase in its concentration suggests that there is liver damage which can cause enzyme leakage from liver into the blood stream. To Wright and Plummer (1974) and Shajahan *et al.* (2004), ALP is a marker enzyme for plasma membrane and endoplasmic reticulum, hence it is employed to evaluate the integrity of plasma membrane (Akanji *et al.*, 1993). However, increase in ALP will therefore result in the loss of the enzyme from the tissues into the serum (Shajahan *et al.*, 2004, Aboyade *et al.*, 2009). Such loss from tissue may seriously affect adequate transportation of ions across the membrane (Akanji *et al.*, 1993) and other metabolic processes.

The total plasma proteins and albumin increased compared with the control concentrations were slightly affected, these indicate that the effluent can cause liver damage most especially at higher concentration over a long period of time as studied. Protein and creatinine are indicators of the liver and kidney malfunction and the amount of creatinine in the blood also depends on the ability of the kidney to excrete creatinine causing kidney failure or impaired protein synthesis as a result of liver disorders causing reduction of creatinine in liver as reported by Steinberg and Steinberg, 1991.

Mehta *et al.* (1989) reported that glucose is an energy yielding component that comprises the main source of food materials and the cholesterol is the animal sterol which can be found in free state or as fatty esters and it is a major component of some cell membrane and plasma protein, in control serum value is reduced compared with the treated rats. Histopathological changes examined in the liver and the kidney of the rats treated with the effluents in the form of dilations, vacuolization, nuclear fragmentation are in conformity with the observations of Bhushan *et al.* (2013). The liver is likely to be affected because of direct exposure to toxic products due to its impact in the detoxification of metabolic by-products and xenobiotics. The changes occurred in liver and kidney due to presence of metals in the effluent on *Rattusnovegicus* which may also suggest that the metals might be modulating the hepatic and renal functions by acting directly or indirectly on these organs and these effects on the concentration and duration dependant.

The microscopic intensity degenerative changes in the concentration increases as the pathological changes observed with higher concentration is an indication of toxicity. The toxicological evaluation of the effluent indicates that if consumption increases with time, it will pose toxicological risks as evident by the elevation of serum, liver and kidney and pathological changes. Sections from the control group show radically arranged hepatic cords around the central vein showing normal histoarchitectural features arranged around tributulations of the hepatic vein, cells were large in size contained homogenous cytoplasmic material with prominent nuclei as reported by Mohammed *et al.* (2013).

He also reported that the changes in the morphology of liver cells might be due to chemicals induced from metals that can express itself through different ways which may cause acute effect and resulting in the death of cells. Thangavel *et al.* (1994) revealed that the histopathological changes in the destruction of hepatoocytes and extrusion of nuclei in the liver is caused by the exposure or inhalation of toxic metals and Abd Rabou, 1996 also investigated on the histological changes of the liver which showed changes in hepatic cells. The changes in liver at higher concentration are similar to findings of Choudhary *et al.* (2003), who reported that treatment of rats with endosulfan causes liver damages, Jens and Hanne (2002) revealed that rats treated with effluents from battery production company has negative effect on the liver in hepatocytes and lymphocytes infiltration in the central vein, in relating to this findings, there were changes in distruption of hepatic architecture, dilated congested blood vessels and increase in lymphocytes infiltration also reported by Yahya *et al.* (2012).

The significant reduction observed in the blood parameters of rats and various degrees of lesions observed in liver and kidney especially at higher concentrations of effluent treated rat further indicates the potential danger of the effluent. With the results obtained in this study, the higher the concentration of the effluent, the higher the risk, hence, there is the need to educate inhabitants of the environment on the likely consequences of the operational impacts of the company in order to minimize the danger.

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