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TOXICOLOGICAL ASSESSMENT OF CONSUMPTION OF BENZENE-POLLUTED WATER ON THE HEART AND BLOOD OF ALBINO RATS

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ABSTRACT: The long-term effects of daily consumption of benzene-contaminated water over a period of Sixty-five days on rats' cellular system were inspected. Haematological parameters such as haemoglobin, packed cell volume etc were determined. Standard enzyme assays were conducted on some enzymes (alkaline phosphatase, acid phosphatase and lactate dehydrogenase) activities of the heart and serum. Significantly lower ($P<0.05$) enzymes activities were observed in the heart of test rats relative to control. Conversely, enzymes activities in the serum significantly increased. The white blood cell of test rats was found to be 11.3±0.5 while that of control was 9.8±0.6 suggesting infection in the test rats. Haemoglobin, red blood cell count and packed cell volume of test rats were found to be significantly lower ($P<0.05$) than that of control which may be indicative of anaemia. It could be inferred therefore, that consumption of benzene-contaminated water could pose serious health problems such as heart failure and abnormal haematological parameters.

INTRODUCTION: Water pollution has a large set of adverse effects upon water bodies such as lakes, rivers, oceans, and underground water caused by human activities. The pollutants include benzene and may get into the underground water and even potable water by different ways. This could be from industrial discharge of chemical wastes, emissions from burning coal and oil, motor vehicle exhaust, and tobacco [1]. Co-exposure to benzene with ethanol can increase benzene toxicity. Benzene causes both structural and numerical chromosomal aberrations in humans.

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Several occupational studies suggest that benzene may impair fertility in women in high level exposure.

Adverse effects on the fetus, including low birth weights delayed bone formation, and bone marrow damage have been observed where pregnant animals were exposed to benzene by inhalation. The Rfl and RfD for benzene are under review by EPA [1].

Benzene has been seen to have neurologic effect, cause irritation of the upper respiratory tract, leukaemia, aplastic anaemia and increase cancer risk in human. Higher percent of diseases are water-borne and was observed to be as a result of some contaminants [2, 3]. As such, in the light of this study, it was deemed fit to examine the effects of benzene on heart and serum by the use of enzyme markers and haematological parameters.

MATERIALS AND METHODS: The experimental water for the study was collected from the water supply system of Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. Physiochemical properties of all water sample were determined in accordance with standard methods [4] and Atomic Absorption Spectrophotometer (Buck 210VGP) was used for the determination of heavy metals.

Experimental Design: Twenty Albino rats (*Rattus novergicus*) were obtained from the Animal Holding of the Department of Biochemistry of University of Ilorin, Nigeria. The experimental animals were assigned into two main groups of 10 animals each. Each group was further classified into two sub-groups detailed thus:

Group A: Rats placed on tap water (control).

Group B: Rats placed on water contaminated with benzene (0.015ml/l).

Group a: Treated as animals in Group A.

Group b: Treated as animals in Group B.

The feeding exercise lasted over a period of 65 days, a long term standard for rats [5]. All animals were kept in a wooden cage and fed *ad libitum* after 10 days acclimatization.

Collection of Blood and Enzyme Assays: The rats were anaesthetized by placing them in a jar containing cotton wool soaked with chloroform before being sacrificed by jugular puncture. The hearts of the animals were removed into a beaker containing ice cold 0.25M sucrose solution. The blood was obtained through their jugular veins. Each blood sample was then centrifuged at 3500rmp for 15 minutes using refrigerated centrifuge RC650s and the serum obtained was preserved as -8^{o}C.

Activity of alkaline phosphatase (ALP) and acid phosphatase (ACP) in the heart was determined by Bessey *et al* method [6] as modified by Wright *et al* method [7]. The activity of ALP in serum and tissues of experimental rats was determined following the method described by Bessey *et al* method as modified by Wright *et al* method. 0.1M carbonate buffer (2.2cm^3) was dispensed into test-tubes, 0.1cm^3 $MgSO_4.7H_2O$ was added and 0.2cm^3 of either the homogenate or serum (sample) obtained from the experimental animals was added to the test tubes. Distilled water (0.2cm^3) was added to the blank instead of the sample. The mixture was incubated at 37^{o}C for 10 minutes after which 0.5mls of 10mM PNPP was added. The mixture was further incubated at 37^{o}C for 10minutes in water bath. The reaction was stopped by adding 2ml of 1N NaOH solution and the absorbance was read at 400nm on a spectrophotometer.

The activity of ACP in serum and tissues of experimental rats was determined following the method described by Bessey *et al* method as modified by Wright *et al* method. 0.1M acetate buffer (1.3cm^3) was dispensed into two test-tubes labelled blank and sample. Sample (0.2cm^3) obtained from experimental rats was added to the sample test-tube while the same volume of distilled water was added to the blank instead of the sample. The mixture was incubated at 37^{o}C for 10 minutes after which 0.5cm^3 of 10mM PNPP was added. The mixture was further incubated at 37^{o}C for 30 minutes in water bath and the reaction was stopped by adding 2.0ml of 1N NaOH solution. The absorbance was thereafter read at 400nm on a spectrophotometer.

Activity of LDH in the heart was also determined based on Wroblewski and La Due method[8]. The reaction was started by addition of sodium pyruvate (42mM) in a final reaction volume of 3ml and the change in extinction of 340nm was followed for a further 5minutes. The blank was used to set the spectrophotometer. Serum lipids namely cholesterol, triglycerides, low density lipoprotein (LDL) and high density lipoprotein (HDL) were determined by the CHOP-PAP method.

Mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration of white blood cell (WBC), neutrophils, basophils, lymphocytes, monocytes and red blood cells (RBC) were observed in the research.

RESULTS: Table 1 shows the effects of benzene-contaminated water on the serum lipids of rats on a long-term exposure. Generally, there was no significant difference ($P<0.05$) in the serum lipids of the test group B compared with that of control group A.

TABLE 1: EFFECT OF BENZENE-CONTAMINATED WATER ON SERUM LIPIDS

Serum lipid (mg/dl)	A	B
Cholesterol	120±5	127±3
Triglycerides	131±2	133±3
LDL	127±6	130±5
HDL	48±2	44±3

Tabulated results are means of five determinations ± SD. *Values are significantly different ($p<0.05$).

Table 2 shows the effect of the contaminant on haematological parameters of the rats. Significant difference ($P<0.05$) exist in the RBC of the test group B relative to that of the control group. Conversely, there is no significant in the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) of the group B compared to that of the control rats.

TABLE 2: EFFECT OF BENZENE-CONTAMINATED WATER ON HAEMATOLOGICAL PARAMETERS

Haematological Parameters	A	B
RBC ($X10^5/mm^3$)	9.25±0.2	7.24±0.2*
Hb(g/dl)	13.5±0.5	10.2±0.5*
MCV (μ^3)	50.2±2	49.8±3
MCH (µg)	14.7±0.6	14.5±0.5
MCHC (%)	29.5±2	27.3±1
PCV (%)	48.1±3	42±2*
ESR (mm/hr)	0.88±0.01	1.4±0.1*
Platelets ($X10^3/mm^3$)	188±8	244±12*
WBC ($X10^3/mm^3$)	9.78±0.6	11.3±0.5*
Neutrophils ($X10^3/mm^3$)	2.44±0.2	3.75±0.2*
Eosinophils (%)	0.03±0.001	0.06±0.002*
Basophils ($X10^3/mm^3$)	0.02±0.001	0.06±0.001*
Lymphocytes ($X10^3/mm^3$)	6.88±0.2	8.23±0.4*
Monocytes ($X10^3/mm^3$)	0.02±0.001	0.04±0.001*

Tabulated results are means of five determinations± SD. *Values are significantly different ($p<0.05$).

Comparing the erythrocyte sedimentation rate (ESR), concentrations of white blood cell (WBC), neutrophils, basophils, lymphocytes and monocytes of group B relative to that of control group A, a significant difference ($P<0.05$) was observed. Also a significant difference ($P<0.05$) was observed in the platelet concentrations of test group B compared to that of control group A.

Figure 1 shows the growth of rats placed on benzene-contaminated water over a period of 65 days. There was no significant difference ($P<0.05$) in the body weight gain between the two groups of rats.

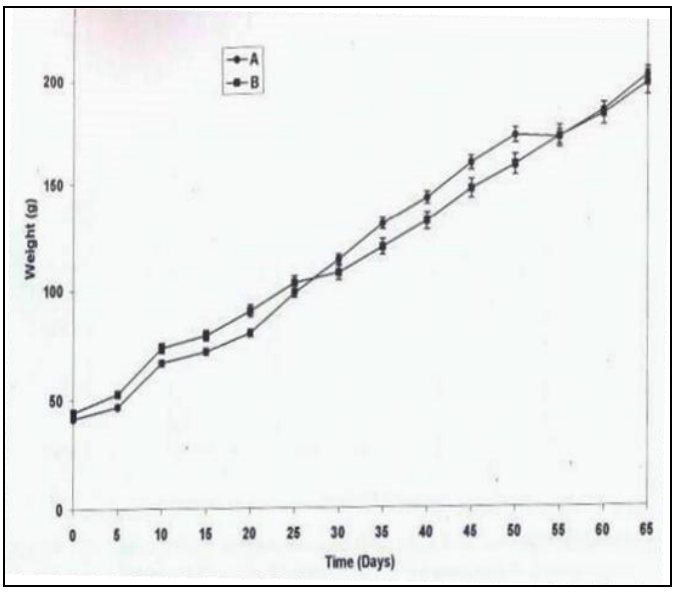

FIGURE 1: GROWTH PATTERN OF RATS PLACED ON BENZENE-CONTAMINATED WATER OVER A PERIOD OF 65 DAYS. Plotted results are means of five determinations ± SD.

Figure 2 shows heart/body weight ratio of rats in which there occurs no significant difference ($P<0.05$) between the test group B compared to that of control group A.

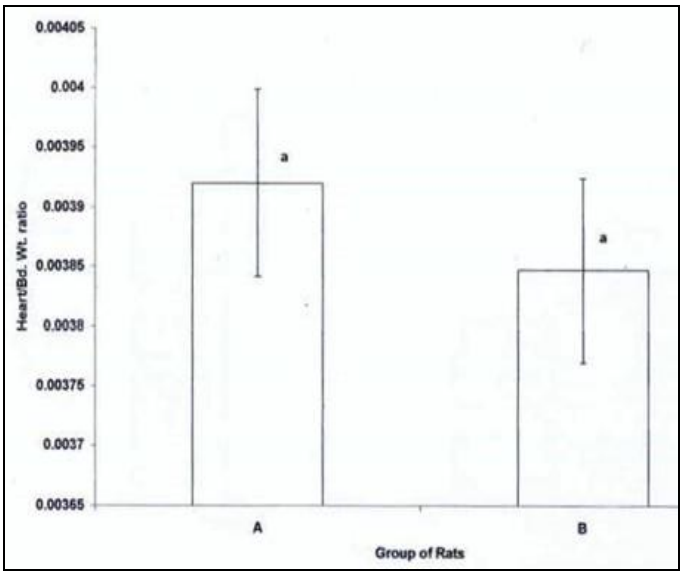

FIGURE 2: HEART/BODY WEIGHT RATIO OF RATS PLACED ON BENZENE-CONTAMINATED WATER OVER A PERIOD OF 65 DAYS. Plotted results are means of five determinations ±SD. Bars with the same notations are not significantly different ($p<0.05$).

Figure 3 displays the activity of lactate dehydrogenase (LDH), in the heart and serum of the rats placed on benzene-contaminated water and there is no significant difference ($P<0.05$) in the specific activity of LDH in the heart of the test group relative to the control group A.

In contrast, a significant difference (P<0.05) was observed in the specific activity of LDH in the serum of the test group B relative to the control group A.

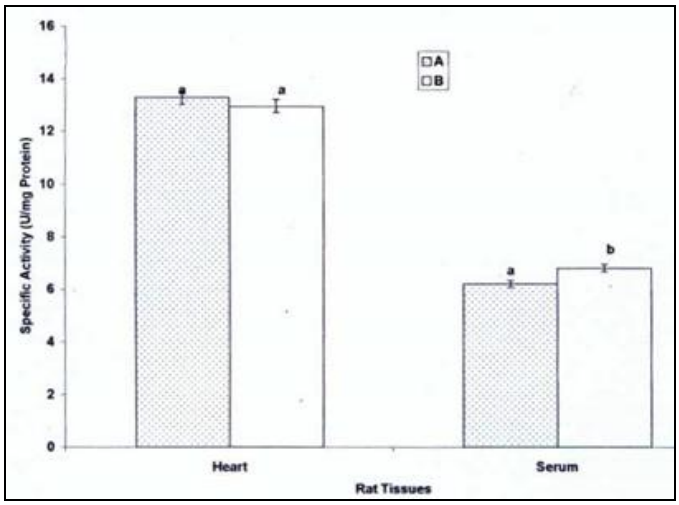

FIGURE 3: SPECIFIC ACTIVITY OF LDH OF HEART OF RATS PLACED ON BENZENE-CONTAMINATED WATER OVER A PERIOD OF 65 DAYS. Plotted results are means of five determinations ± SD. Bars with the same notations are not significantly different (p<0.05).

Figure 4 shows the specific activity of alkaline phosphate (ALP), in heart and serum of the rats. In the specific activity of ALP in heart of both test group B and the control group A, there was no significant difference (P<0.05) observed. In contrast, significant difference (P<0.05) occurred in ALP specific activity in serum of test group B relative to the control group A.

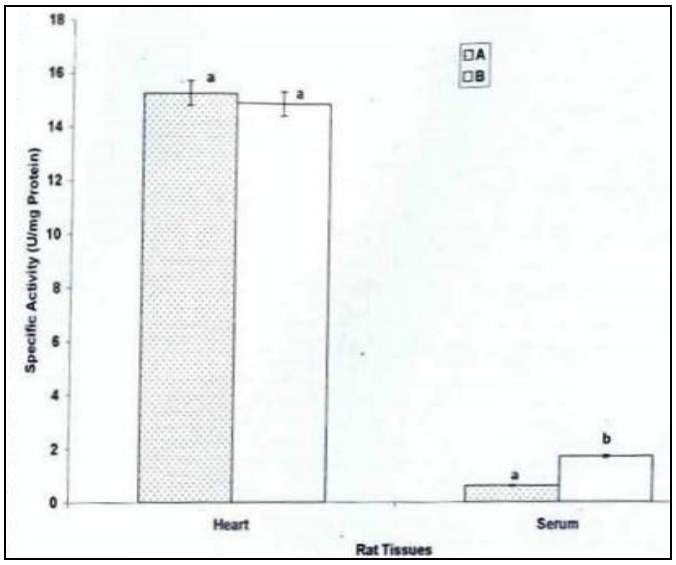

FIGURE 4: SPECIFIC ACTIVITY OF ALP OF HEART OF RATS PLACED ON BENZENE-CONTAMINATED WATER OVER A PERIOD OF 65 DAYS. Plotted results are means of five determinations± SD. Bars with same notations are not significantly different (p<0.05).

Figure 5 displays the specific activity of acid phosphate (ACP) in heart and serum of rats placed on benzene-contaminated water.

There exists no significant difference (P<0.05) in th e specific activity ACP in heart of test group B relat ive to the group A. Conversely, a significant differe nce (P<0.05) between the test rats and the control w as observed.

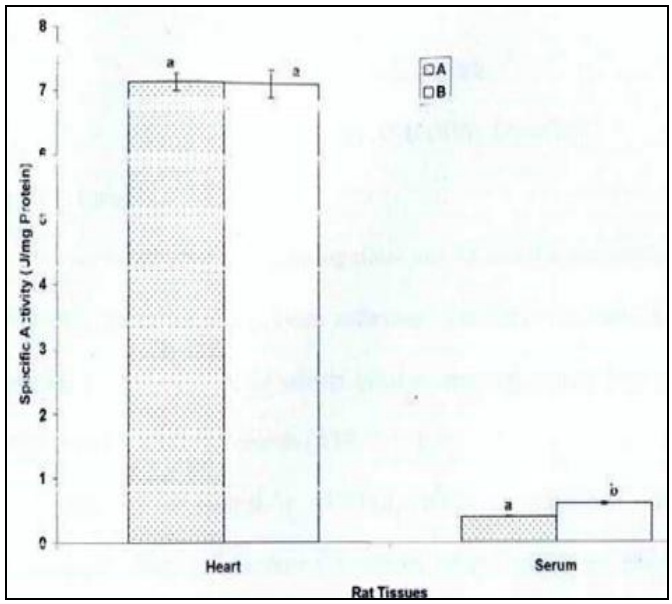

FIGURE 5: SPECIFIC ACTIVITY OF ACP OF HEART OF RATS PLACED ON BENZENE-CONTAMINATED WATER OVER A PERIOD OF 65 DAYS. Plotted results are means of five determinations ± SD. Bars with the same notations are not significantly different (p<0.05).

Statistical Analysis: The data of this research were analyzed using One-way analysis of variance (ANOVA) with SPSS version 18.

DISCUSSION: The observed unaffected serum lipids of the rats placed on benzene-contaminated water (table 1) may be due to the fact that within the period of the experiment, the concentration of benzene did not interfere with the lipid metabolism. Relatively, lower RBC, PCV and Hb (table 2) observed in rats placed on benzene-contaminated water implies loss of blood (anaemia) and may result from RBC lysis.

It was reported that lowered PCV implies significant hemorrhage. This suggestion is supported by the unaffected MCV, MCH, and MCHC that the type of anaemia is normocytic. Reduced RBC and aplastic anaemia have also been reported on a long term exposure to benzene [9].

Neutrophils are matured leucocytes that control many biochemical infections. The observed elevated concentration of neutrophils was caused by the presence of infections due to the benzene-contaminated water. This suggestion is supported by significant increase in the WBC, basophils, lymphocytes and monocytes of the test rats. WBC fights infections therefore the observed increase in the WBC of the test rats may be as a result of infection arising from ingesting benzene-contaminated water. This is further supported by the increased level of the ESR of the test rats and it was reported that when there is infection in the body, ESR becomes high [10, 11]. The disease condition associated with increased WBC on exposure to benzene is leukaemia [12].

Platelet is responsible for blood clotting. The increased platelet concentration observed in the blood of the test rats placed on benzene-contaminated water may be attributed to the presence of benzene in the water. It was reported that elevated platelet concentration is associated with coagulation problem known as thrombocytosis and myeloproliferative disease [13, 14].

The unretarded growth pattern (figure 1) observed in the test rats results from the concentration of benzene in the water during the experimental period which is not capable of altering the rate at which cells cleave to bring growth and also did not affect the daily water and food intake. It was reported the benzene at biomagnification level causes both structural and numerical chromosomal aberrations in humans, and also retards growth[15].

LDH is an intracellular enzyme found particularly in the kidney, liver, lungs, skeletal muscle and heart. The significantly higher serum level of LDH activity observed in test rats placed on benzene contaminated water (figure 3) may be due to leakage of the enzymes into the serum organ. It was reported the LDH is a marker for myocardial infarction, RBC disease like haemolytic anaemia, kidney disease, testicular tumours, lung disease such as pneumonia, congestive heart failure, pancreatitis, liver disease such as muscular dystrophy and muscular trauma [16, 17].

Relatively higher ALP activity in the serum of test rats (figure 4) observed, may result from the interaction of the benzene with the components of the membrane leading to leakage of the enzyme from the cell into the serum since ALP is a membrane bound enzyme which was reported to be the symptoms of disease conditions like bone cancer, osteomalacia, paget's disease, renal disease and primary hypothyrodism. Also, the increase may be as a result of pregnancy, healing, bone and normal growth in children [18, 19].

The observed increase (figure 5) in the serum of rats placed on benzene-contaminated water may be due to the overproduction and leakage of the enzyme from the lysosome into the serum being a lysosomal enzyme. It is used as enzyme marker for carcinogenesis [20]. ACP testing is done to diagnose whether prostate cancer has metastasized and this occurs only at the elevated level of ACP. Therefore, the serum enzyme level is used as an index of prostate cancer [21]. The observed unaffected heart body weight ratio of rats placed on benzene-contaminated water (figure 2) showed that within the period of the experiment, the concentration of benzene in the water may not be enough to have affected the heart body weight ratio of the test rats.

CONCLUSION: The data generated from this study suggests that:

1. Benzene-contaminated water may possibly cause reduced Hb, PCV, RBC and increased ESR, platelets, WBC, neutrophils level as evident in the test rat.

2. The contaminant (benzene) may be impairing heart function by the elevated activity of ACP, ALP, and LDH in serum as portrayed by the test rat.

However, to improve the quality of this research it is better to include the histopathological aspect.

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