HEPATOTOXIC EFFECT OF SUB-ACUTE EXPOSURE OF TREATED CARBANACEOUS EFFLUENT ON MICE

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ABSTRACT: The present study examined the hepatotoxic effects of carbonaceous wastewater in mice, the mice were exposed to five different concentrations of the waste water. Cyclophosphomide was used as the positive control and distilled water was used as a negative control, for a period of 35das. At post exposure, the activities of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and Alkaline phosphatase (ALP) in serum were measured as indicators of liver function. The weights of the animals were recorded weekly after which their liver were harvested. Organ weight was measured at post exposure and preserved afterwards for histology. The physical, chemical and heavy metal composition of the wastewater was also analysed. The liver weight of the exposed mice was however significantly different from that of the negative control in the 25% and 75% concentration of the wastewater administered at $p<0.05$. The activities of ALP, ALT, and AST in the serum of exposed mice were significantly increased compared to the negative control mice and this increase was concentration dependent at $P<0.05$. The histological lesions observed in the liver at various concentrations examined included Kupfer cell hyperplasia, severe portal congestion, portal and central venous congestion and mild hydrople degeneration of hepatocytes. The results of the study showed that the observed hepatotoxic effect in the exposed mice may be caused by the presence of heavy metal and other physical and chemical substances present in the wastewater. This suggests a higher risk to liver damage in humans and other organisms exposed to this wastewater and may also be deleterious to the surrounding environment.

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1.0 INTRODUCTION

The liver serves many vital functions in the body. It has a large capacity to hold blood and thus serves as a blood storage site (LeBlanc and Dauterman, 2001). The liver synthesizes and secretes many substances that are necessary for normal bodily function. It cleanses the blood of various endogenous and foreign molecules (Rankin, 1998). It biotransforms both endogenous and exogenous materials, reducing their bioreactivitiy and preparing them for elimination. It eliminates wastes and foreign materials through biliary excretion (Klaassen, 2001). Three of these functions occur in a manner that makes the liver a major organ of chemical elimination: chemical uptake from blood, chemical biotransformation, and biliary elimination of chemicals. This process is called entero-hepatic circulation. A chemical may undergo several cycles of entero-hepatic circulation resulting in significant increase in the retention time for the chemical in the body and increased toxicity. The liver functions to collect chemicals and other wastes from the body. Accordingly, chemicals may be retained in the liver at high concentrations resulting in toxicity. The biotransformation of chemicals that occur in the liver sometimes results in the generation of reactive compounds that are more toxic than the parent compound thus causing damage to the liver (Le Blanc and Dauterman, 2001)

Alanine Aminotransferase (ALT) is the enzyme produced within the cells of the liver. The level of ALT abnormality is increased in conditions where cells of the liver have been inflamed or undergone cell death (Cheesbrough, 2006). As the cells are damaged, the ALT leaks into the bloodstream, leading to a rise in serum levels (Cheesbrough, 2006). Any form of hepatic cell

damage can result in an elevation in the ALT. The ALT level may or may not correlate with the degree of cell death or inflammation. ALT is the most sensitive marker for liver or cell damage. This enzyme also reflects damage to the hepatic cell. It is less specific for liver disease. It may be elevated and lead to other conditions such as myocardial infarction (heart attack). The ratio between ALT and AST are useful to physicians in accessing the etiology of liver enzyme abnormalities.

Alkaline phosphates are an enzyme, which is associated with the biliary tract though it is not specific to it. It is also found in the bone or placenta. Renal or intestinal damage can also cause the alkaline phosphatise to rise. If the alkaline phosphatise is elevated, biliary tract damage and inflammation should be considered. However considering the above, other aetiologies must also be entertained. One way of assessing the aetiology of the alkaline phosphates is to perform a serological evaluation called isoenzyme. Another more common method to assess the aetiology of the elevated alkaline phoshatase is to determine whether the GGT is elevated or whether other function tests are abnormal (such as bilirubin). Alkaline phosphatise may be elevated in primary biliary cirrhosis, alcoholic hepatitis, PSC, gallstones in choledocholithiasis (Cheesbrough, 2006).

The aim of this study is to determine the hepatotoxic effect of wastewater discharged from a carbonaceous industry on mice (*Mus musculus*). The aim would be achieved through examination of liver function test. These will include the monitoring of the following parameters; AST, ALP, and ALT, histopathology of the liver, body and organ weight index. The waste water will also be analysed for the presence of some physical, chemical and heavy metals. The findings from this study may be useful in the assessment of the toxic effects of waste water samples on public health and environment.

2.0 MATERIALS AND METHODS

2.1. Sources of Experimental Materials and Treatment

2.1.1 Laboratory Mice

Forty two male albino mice were obtained from the animal breeding unit at the Institute for Advanced Medical Research and Training (IMRAT), College of Medicine, University of Ibadan, UCH, Ibadan, Nigeria. Mice were acquired and quarantined in a pathogen-free, well ventilated room in order to enable the animals acclimatize to their environment. During the period of acclimatization, the animals were supplied with food (pelleted foods) and drinking water on a daily basis. Their beddings were also changed daily (disinfected and discarded). The mice were maintained in the departmental animal and breeding unit at IMRAT where each cage contains six animals.

2.1.2. Waste water

The waste water sample was sourced from the drainage pipes of the Effluent Treatment Plant of a beverage producing company in Lagos State, Nigeria. This company is well known for the production of drink beverages which are consumed nationwide.

2.1.3. Storage of Effluent

The collected effluents were stored in plastic bottles and refrigerated at 4°C until when needed. They were then brought out and diluted to various concentrations at room temperatures. The various concentrations were in-turn stored in plastic bottles and refrigerated all through the experiment.

2.2. Preparation of Controls

2.2.1. Negative Control

Distilled water was used as a vehicular solvent for the dilution of the waste water used.

2.2.2. Positive Control

The drug cyclophosphamide was used. Administered dosage depends on the average body mass of the animal per kg, which is 40mg/kg. The value/information in mg provided by the manufacturer was taken into consideration when the calculation was made. Average body weight of animal for positive control per kg is 24.5g. 40mg ---→ 1000g, X ---→ 24.5g, X = 40 x 24.5/1000 = 0.98mg of cyclophosphamide dissolved in 1000ml distilled water and administered orally for 35 days.

2.3. Exposure of Animals to the Samples

The animals were randomly divided into seven groups. Each group was made up of six individuals. Five groups were each injected with a different dilution of the effluent. One group was administered a positive control and another group the negative control sample. The dosage that was administered depends on the average body weight. 0.3ml of the sample was administered orally to each animal for a period of 35 consecutive days.

2.4. Determination of Serum Aspartate Aminotransferase (AST), Alanine

Aminotransferase (ALT) and Alkaline Phosphatase (ALP) Activities

These were determined following the principle described and standardized by Reitman and Frankel (1957).

2.5. Tests and Methodology

pH determination, alkalinity, total sulphate, total chloride, total hardness, total dissolved solids, total suspended solids, biochemical oxygen demand (BOD), chemical oxygen demand (COD) and determination of metals.

2.6. Statistical Analysis

Results were analysed by the mean standard deviation and statistical analysis of variance (ANOVA).

3. RESULTS ANALYSIS

Table 1 shows the effects of industrial carbonaceous waste water on the body weights of the experimental animals.

Table 1: Effects of Industrial Carbonaceous Waste Water on the Body Weights of the Experimental Animals

Periods	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5
Positive Control	23.00 ± 2.68	25.83 ± 3.10	26.17 ± 2.71	26.83 ± 2.04	29.33 ± 1.03
Negative Control	25.50 ± 1.38	27.67 ± 1.37	28.00 ± 0.90	30.00 ± 1.10	29.00 ± 1.26
10%	24.33 ± 2.80	27.17 ± 2.71	28.20 ± 2.50	29.80 ± 2.50	30.80 ± 1.80
25%	24.33 ± 1.75	26.67 ± 2.00	27.67 ± 2.33	29.50 ± 2.66	30.50 ± 2.35
50%	24.17 ± 0.75	26.50 ± 2.26	28.00 ± 0.63	29.50 ± 1.05	31.00 ± 2.00
75%	25.17 ± 4.02	27.50 ± 4.08	29.17 ± 2.32	29.67 ± 3.08	30.00 ± 3.35
100%	24.00 ± 2.76	26.83 ± 3.76	27.33 ± 5.54	28.17 ± 4.88	30.67 ± 4.50

Keys: Results are expressed in mean ± S.D., positive control = administered cyclophosphamide, negative control = administered distilled water, n = 5, 10% = 10% waste water + 90% distilled water, 25% = 25% waste water + 75% distilled water, 50% = 50% waste water + 50% distilled water, 75% = 75% waste water + 25% distilled water, 100% = 100% waste water

Table 2 shows the effects of industrial carbonaceous waste water on the organ weights of the experimental animals.

Table 2: Effects of Industrial Carbonaceous Wastewater on the Liver Weights of the Experimental Animals.

	LIVER (g)
Positive Control	1.20 ± 0.17
Negative Control	1.20 ± 0.17
10%	1.63 ± 0.31
25%	1.80 ± 0.50^{a*b**}
50%	2.00 ± 0.05 ^{a*b**}
75%	1.57 ± 0.31
100%	1.43 ± 0.23

Keys: a = $p<0.05$ compared with positive control (administered cyclophosphamide), b = $p<0.05$ compared with negative control (administered distilled water). Results are expressed in mean ± S.D., n = 5, 10% = 10% waste water + 90% distilled water, 25% = 25% waste water + 75 distilled water, 50% = 50% waste water + distilled water, 75% = 75% waste water + 25% distilled water, 100% = 100% waste water, * = significant difference compared with positive control, ** = significant difference compared with negative control.

Table 3 shows the effects of industrial carbonaceous waste water on some liver enzymes in hepatoxicity.

Table 3: Effects of Industrial Carbonaceous Wastewater on Some Liver Enzymes in Hepatoxicity.

Liver Enzymes	Serum ALP Activity (IU/L)	Serum ALT Activity (IU/L)	Serum AST Activity (IU/L)
Positive Control	54.76 ± 2.62	107.10 ± 5.27 ^{b**}	230.46 ± 2.00
Negative Control	27.27 ± 0.92 ^{a*}	82 ± 2.73 ^{a*}	127.21 ± 2.66 ^{a*}
10%	34.92 ± 3.18 ^{a*b**}	93.48 ± 3.14 ^{a*b**}	147.79 ± 5.61 ^{a*b**}
25%	40.15 ± 1.51 ^{a*b**}	109.16 ± 2.24 ^{b**}	189.21 ± 3.00 ^{a*b**}
50%	52.38 ± 1.90 ^{a*b**}	117.67 ± 2.40 ^{a*b**}	233.59 ± 10.90 ^{b**}
75%	69.09 ± 5.80 ^{a*b**}	134.43 ± 6.40 ^{a*b**}	328.45 ± 24.39 ^{a*b**}
100%	82.05 ± 9.22 ^{a*b**}	198.33 ± 2.73 ^{a*b**}	511.46 ± 21.60 ^{a*b**}

Keys: a = $p<0.05$ compared with positive control (administered cyclophosphamide), b = $p<0.05$ compared with negative control (administered distilled water). Results are expressed in mean ± S.D., n = 3, 10% = 10% waste water + 90% distilled water, 25% = 25% waste water + 75% distilled water, 50% = 50% waste water + 50% distilled water, 75% = 75% waste water + 25% distilled water, 100% = 100% waste water, * = significant difference compared with positive control, ** = significant difference compared with the negative control.

Table 4 shows the physico-chemical parameters of the industrial carbonaceous waste water used in this study.

Table 4: The Physico-Chemical Parameters of the Industrial Carbonaceous Waste Water

Parameters	Value (%)
Lead	0.010
Nickel	0.062
Cadmium	0.003
Magnesium	24.061
Manganese	11.257
Nitrate	7.295
Sulphate	2.700
Chloride	3.000
pH	7.80
BOD	2.50
Suspended Solid	0.50
Dissolved Solid	0.01
Hardness/ $CaCO_3$	100.00
Alkalinity	3.20

4. DISCUSSION

Hepatotoxicity is a predominant effect of high concentrations of inhaled toxic substances in animals (ATSDR/USEPA, 1989). Hepatotoxicity of toxic substance has been described and investigated in numerous oral studies of acute, intermediate and chronic duration in several animal species (ATSDR/USEPA, 1989). Hepatotoxicity is the most prominent and characteristic systemic effect of toxic substances and heavy metals, resulting in centrilobular necrosis and hemorrhage often leading to hemorrhagic ascites (ATSDR/USEPA, 1989).

The liver is important organs for metabolism, detoxification, storage and excretion of these chemicals and their metabolites and thus is vulnerable to damage. The similarity in body weight of mice exposed to waste water and control may be due to the ability of the mice to feed even while being exposed. The increase in liver may be due to inflammation of these chemicals present in waste water. Such observations were also observed in rats exposed to cadmium and alcohol (Brzoska *et al.*, 2003).

The observed dose-dependent increase in the activity of ALP, AST and ALT can be traced to possible necrosis of the liver where these enzymes are naive. The histological examination of the organ showed it clearly. Omotuyi *et al.* (2008 cited by Daramola, 2010) also observed this in rats exposed to an overdose of artesunate drug.

The histological lesions observed in the liver at various concentrations examined included Kupfer cell hyperplasia, severe portal congestion, portal and central venous congestion and mild hydrople degeneration of hepatocytes. The lesions, like severe portal congestion, hydrople degeneration of hepatocyte, occlusion of the tubular lumen and tubular necrosis observed in mice may be due to the chemicals present in the waste water. These chemicals are higher than standard limit from Nigeria and World Health Organization.

In other related studies, four cases of liver disease in humans resulting from inhalation exposure to Nitrosodimethylamine (NDMA) have been described in the literature (ATSDR/USEPA, 1989). Of the subjects who did not die, one was a chemist who was exposed to unknown concentrations of fumes and experienced exhaustion, headache, cramps in the abdomen, soreness on the left side, nausea and vomiting for at least two years (Freund, 1937; ATSDR/USEPA, 1989). The second case was an automobile factory worker who was exposed to unknown levels of NDMA and became violently ill with jaundice and ascites (Hamilton and Hardy 1974; ATSDR/USEPA, 1989).

In another related study, pathologic examination of dogs following exposure to Nitrosodimethylamine (NDMA) for 4 hours showed marked necrosis and varying degrees of hemorrhage in the liver (Jacobson et al. 1955). Doolittle et al. (1984) reported that the only toxic signs observed in rats exposed to NDMA for 4 hours were reddened eyes and piloerection. The only additional information reported in this study pertained to genotoxic effects (ATSDR/USEPA, 1989).

In this study, no mortality was reported among the mice exposed to the wastewater discharged from a carbonaceous industry. The lack of mortality in mice may be attributable to the fact that the animals were killed immediately following exposure and consequently not observed for subsequent death. This is similar to what was reported in other related studies. The lack of mortality in rats at the higher concentrations of NDMA in the Doolittle et al. (1984) study may also be attributable to the fact that the animals were also killed immediately following exposure and consequently not observed for subsequent death. However, Petechial and larger hemorrhages were observed in the lungs of two people following lethal poisoning with NDMA (Kimbrough

1982; ATSDR/USEPA, 1989). Myocardial and endocardial bleeding was observed in a person following lethal poisoning with NDMA (Kimbrough 1982; ATSDR/USEPA, 1989). Gastrointestinal hemorrhage occurred in humans following lethal poisoning with NDMA (Kimbrough 1982, Pedal et al. 1982; ATSDR/USEPA, 1989).

Also in other previously related studies, five members of a family who consumed unknown quantities of NDMA in lemonade became ill with nausea and vomiting associated with acute liver disease, generalized bleeding and low platelet counts (Kimbrough 1982, Cooper and Kimbrough 1980). Two of these people died; the other three were released from a hospital 4-21 days after admission. Another fatality due to ingestion of NDMA was attributed to liver failure (Fussgaenger and Ditschuneit 1980, Pedal et al. 1982; ATSDR/USEPA, 1989). Autopsies of the subjects described above showed that the primary effects were hemorrhagic and cirrhotic changes in the liver and necrosis and hemorrhage in other internal organs (ATSDR/USEPA, 1989).

In this study, organ weight was measured at post exposure and preserved afterwards for histology. The physical, chemical and heavy metal composition of the wastewater was also analysed. The liver weight of the exposed mice was however significantly different from that of the negative control in the 25% and 75% concentration of the wastewater administered at $p<0.05$. The activities of ALP, ALT, and AST in the serum of exposed mice were significantly increased compared to the negative control mice and this increase was concentration dependent at $P<0.05$. In mammals, several sub-chronic exposures of rats, mice and monkeys to substances such as perfluorooctanesulfonate (PFOS) have resulted in effects on body weight gain in females and in males (Seacat *et al*., 2002, 2003; Thibodeaux *et al*., 2003; Luebker *et al*., 2005a, b; Du *et al*., 2008) as reported in this present study with carbonaceous effluent. Based on existing data, substances such as perfluorooctanesulfonate (PFOS) has been shown to influence membrane function and structure of hepatocytes, as assessed by increase in serum alanine aminotransferase (ALT) activity in carp (*Cyprinus carpio*) (Hoff *et al*., 2003) and hepatic PFOS concentration was significantly and positively related to serum ALT activity in both feral carp (*C. carpio*) and eel (*Anguilla anguilla*) (Hoff *et al*., 2005; Du *et al*., 2008).

In reproductive and developmental toxicity, it appeared that plasma androgens and estrogens can be affected after fathead minnow (*Pimephales promelas*) exposed to heavy metals and toxic substances (Oakes *et al*., 2004, 2005; Ankley *et al*.,

2005; Du *et al*., 2008). Substances such as PFOS exposure to zebrafish embryos resulted in developmental toxicity and altered certain gene expression (Shi *et al*., 2008; Du *et al*., 2008).

Recently, several studies showed that estrogenic properties of PFOA (induction of vitellogenin, VTG) in rare minnow (*Gobiocypris rarus*) (Wei *et al*., 2007; Du *et al*., 2008), induction of VTG in cultured male tilapia hepatocytes (Liu *et al*., 2007; Du *et al*., 2008) and in male medaka (*Oryzias latipes*) treated with fluorotelomer alcohol (FTOHs) (Ishibashi *et al*., 2008; Du *et al*., 2008). Ankely *et al*. (2005) reported that no significant adverse effects on growth were observed in developing fathead minnows exposed PFOS for 24 h. In a study by Du *et al*. (2008), histological examination revealed that the most pronounced morphological alternation is accumulation of lipid in the liver of male fish, suggesting the hepatic toxicity is gender specific.

Studies have also shown that administration of substances such as carbon tetrachloride (CCl_4) to rats inhibits endoplasmic reticulum calcium pump activity and reduces the amount of calcium associated with subsequently isolated microsomal subcellular fractions (Ray and Moore, 1986). Calcium released from an intracellular pool(s) may initiate hepatotoxic changes in liver (Ray and Moore, 1986).

Our results showed that the observed hepatotoxic effect in the exposed mice may be caused by the presence of heavy metal and other physical and chemical substances present in the wastewater. This suggests a higher risk to liver damage in humans and other organisms exposed to this wastewater and may also be deleterious to the surrounding environment.

5. CONCLUSION

The carbonaceous wastewater caused liver dysfunction in mice at various concentrations. This suggests that exposure to these waste may pose risk to human health and will pollute the aquatic environment, contaminating the source of water supply for both domestic and commercial uses. Therefore it becomes imperative that environmental policy makers in Nigeria to take stringent decision in order to avert pollution of waste water and prevent or reduce risk to human other organisms.

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