ASSESSMENT OF HISTOPATHOLOGICAL DAMAGES IN SWISS ALBINO MALE MICE INDUCED BY AUTOMOBILE WASTE LEACHATE

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ABSTRACT

The activities of auto-mechanic workshops in Nigeria are known to adversely affect the environment and pose threat to its biodiversity. This present study examined the testes, liver and kidney histopathological damages in Swiss albino male mice caused by automobile waste leachate from Ipata-Oloje, Ilorin, Kwara State, Nigeria. Six treatment groups of 0, 10, 15, 20 and 25% leachate and a positive control each containing four mice per replicate were intraperitoneally administered 0.5 mL leachate sample as well as negative and positive control for 5 consecutive days. The result of heavy metal analysis of the sample presented elements such as Pb, Cd, As, Hg, Cr, Cu, Fe and Zn at concentrations above the permissible limits. The histology of kidney, liver and testes show gross structural abnormalities compared to the negative control. Testes histopathological damage includes shrinking of the tubular epithelium, slight vacuolation, degeneration of the tubular epithelium and atrophy of the germinal epithelium. Liver damages include distorted liver tissue architecture with infiltration, unevenly distributed sinusoids around the hepatic and portal tracts. This study showed organ toxicity arising from automobile waste and will help in proper management and disposal of this waste.

Keywords: Mice, Automobile waste, Histopathological damages, Vacuolation, Tubular epithelium, Sinusoids, Heavy metals

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INTRODUCTION

Groups of small-scale auto-mechanic businesses and mechanic villages are common practice in Nigeria. In many cases, their workshops are located in yards, roadsides or hectares of land designated for mechanic village. It has been presumed that there are environmental impacts and biological threats related to this practice on the host community (Adelekan and Abegunde, 2011). Automobile wastes appear in different form such as spent oil, metal scraps and parts of automobiles, plastic and rubber components. The gaseous form include, emission of carbon dioxide from large number of vehicle exhaust and nitrogen (in diesel exhaust), soot (mostly in diesel exhaust), a large number of hydrocarbons with nitrogen and sulphur dioxide (in diesel exhaust). These groups of hazardous pollutants unavoidably pose a serious threat and concern to man and his environment.

According to Bakare *et al.* (2013a), the contaminant rich aqueous solution of pollutants is called leachates and contain chemicals and element like lead (Pb), iron (Fe) benzene(C_6H_6), sulphuric acid (H_2SO_4) and nitrogen dioxide (NO_2) (European Union, 2002; Oshode *et al.,* 2008). The heavy metals found in waste have toxic effect on animals and can undergo bioaccumulation and biomagnifications (European Union, 2002). The high concentration of these chemicals brings about occupational

hazards and can cause severe damages especially to the households located close to the dumpsites (Bakare *et al.,* 2013b).

This study examined the histopathological damages in Swiss albino male mice caused by simulated automobile waste leachate from Ipata-Oloje, Ilorin, Kwara State, Nigeria.

MATERIALS AND METHODS

Sampled Site: The sampled site was situated at Ipata-Oloje, Jengbe in Ilorin West Local Government Area of Kwara State (N8°509'E4°518'), Nigeria. It is a notable area in Ilorin known to accommodate all types of abandon vehicle parts and car waste. It has been in existence for some considerable time, but the documentation of its existence began in 1992.

Leachate and Test Samples: The soil samples at the site were collected randomly at 0 – 10 cm depth. Leachate was simulated from the soil according to the American Society for Testing and Materials (Category A) extraction procedure as modified by Bakare *et al*. (2007). 200 gram of the sample was soaked in 2000ml of distilled water for 48 hours, sieved and filtered to remove debris. From the resultant stock solution (simulated leachate) of the automobile waste, 0.0, 10.0, 15.0, 20.0 and 25.0 % (v/v, simulant/distilled water) solutions were prepared and refrigerated at 4^0C for 20 hours before being administered intraperitoneally (IP) to mice. The highest concentration of 25% was chosen based on the research carried out by Alimba *et al.* (2009).

Experimental Animal: Seventy-two pathogen-free male Swiss albino mice (*Mus musculus*) of 8 – 10 weeks old, weighing between 16 – 25 g were obtained from an inbred colony of the Animal Breeding Unit, Department of Physiology, University of Ibadan, Ibadan. They were acclimatized for 2 weeks in well-ventilated plastic cages in the animal house of Department of Zoology, University of Ilorin, Ilorin. Under the same laboratory condition, commercial feed pellets purchased from Aromokeye Pharmacy, Ilorin, Kwara State, Nigeria and drinking water was made available *ad libitum* through the 35 day period of the experiment. Animals were cared for according to the standard guidelines (CIOMS, 1985) and the policy laid down by the Animal Ethics Committee of the University of Ilorin, Ilorin, Nigeria.

Physicochemical and Heavy Metals Assay: pH, electrical conductivity (EC) and total dissolved solids (TDS) were measured in triplicates using Hanna portable pH/ EC/ TDS/ Temperature combined waterproof meter (model HI 98129). Total alkalinity, dissolved oxygen, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solute (TSS), salinity, chlorides, nitrates, ammonia, phosphates and heavy metals were analyzed and determined in the leachate using Hanna multiparameter bench photometer (model HI 83200).

Experimental Design: The experimental design used was complexly randomized block design of six treatments replicated thrice. Each replicate had four mice (n = 4) of an average weight of 23 ± 2 grams. Group 1, negative control received intra peritoneal injection of distilled water (0.5 ml/mouse) for 5 consecutive days. Group 6 received intra peritoneal injection of cyclophosphamide (20mg/kg body weight) for 5 consecutive days which served as the positive control. The remaining four groups serve as the test groups and were exposed to 10, 15, 20 and 25 % (convert to mg/kg for uniformity) of to the test samples concentrations respectively. The test animals were exposed via intraperitoneal injection of 0.5 ml/day per mouse of each concentration for five consecutive days. The mice were observed for 30 days and the animals were sacrificed by cervical dislocation (Bartke *et al.*, 1974).

Histopathology Analysis: The testes, livers and kidneys were surgically removed (one per replicate) and examined for histopathological changes under the microscope. Testes were transferred into Bouin's fixative while liver and kidney transferred into formalin and kept at room temperature. The tissues were taken to

the histopathology laboratory for further analysis. The organs were dehydrated in ascending grades of 10, 20, 90 and 100% ethanol. They were cleared in xylene, infiltrated and embedded in molten paraffin wax to enable a good sectioning. Sections of 5 µm thickness were cut, stained using Haematoxylin and Eosin (H & E), and examined under light microscope (Bancroft *et al.,* 1994). Slides were examined with Olympus light microscope at x 400 magnification. Photomicrographs of representative organ were taken using Motic camera.

Data Analysis: The data on physicochemical and heavy metal characteristics of leachate sampled from dumping sites were analysed using analysis of variance (ANOVA) and presented as means ± standard error of means (SEM). The Statistical Package for Social Sciences (SPSS) version 20.0 was used for all analyses.

RESULTS

Physicochemical and Heavy Metals: The result of the physicochemical parameters and heavy metals in the leachate are presented in Table 1. The simulated sample was dark brown in appearance and the pH value of the leachate was within the stipulated standard limit by USEPA and NESREA. The alkalinity, BOD and COD of the leachate sample was higher than standard limit set for waste sample. Likewise, Pb, Cr, Cd, Cu, Fe and Hg concentrations were also higher than the standard limit for the wastewater.

Histopathology of Organs: The histopathology of the testes indicated that the section through the seminiferous tubules of the negative control mice had normal regular histological features namely: compactly organized germinal epithelium consistent with the description of the normal histology of testis (Figure 1a). The cross section of the testes from 10 % concentration group showed shrink tubular epithelium which resulted in reduced testicular lumen as well as slight fusion of adjacent seminiferous tubules due to reduced interstitial compartment.

Table 1: Physicochemical and heavy metal characteristics of leachate sampled from municipal solid waste (MSW) dumping sites at Ipata-Oloje spare parts Ilorin, Kwara State, Nigeria

Parameter	Leachate samples	NESREA	USEPA
pH value	8.34 ± 0.21	6.0-9.0	6.5-8.5
DO	3.00 ± 0.11	ND	ND
Nitrates	16.80 ± 0.15	10.00	10.00
NH^-_4N	6.18 ± 0.42	10.00	0.02
Alkalinity	152.00 ± 0.10	150	20.00
BOD	110.00 ± 0.51	50.00	ND
COD	130.00 ± 0.11	90.00	410.00
TDS	85.60 ± 0.95	500	500.00
Salinity	164.20 ± 0.12	ND	ND
TSS	104.00 ± 0.26	ND	ND
EC	184.00 ± 1.21	ND	ND
TS	196.00 ± 1.45	ND	ND
Hg	0.209 ± 0.01	ND	ND
As	0.256± 0.05	ND	0.01
Pb	0.219 ± 0.02	0.05	0.015
Cd	0.702 ± 0.21	0.2	0.05
Cr	0.855 ± 0.10	0.05	0.1
Zn	0.649 ± 0.07	ND	ND
Fe	0.904 ± 0.11	ND	0.30
Cu	0.608 ± 0.10	0.5	1.3

Units of the parameters are in mg/L except for pH which has no unit and EC in uScm3, ND: not detected; USEPA: United State Environmental Protection Agency (2005); NESREA: National Environmental Standards and Regulation Enforcement Agency (2009)

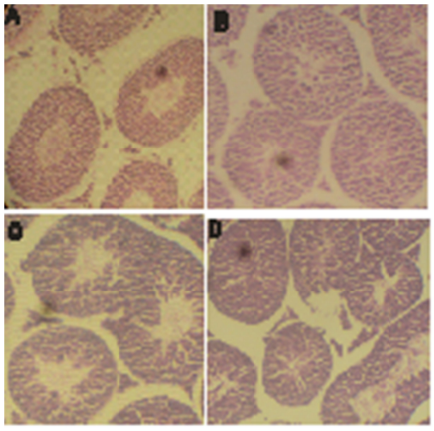

Figure 1: Histopathology of the seminiferous tubule staining with H&E (x 400). *(A) Negative control group, (B) 10 % concentration group, (C) 25 % concentration group, (D) Positive control group*

The tubules also showed slight vacuolation of the tubule germinal epithelium (Figure 1b). The testes from 25 % concentration showed degeneration of the tubular epithelium

accompanied by acute vacuolation and atrophy of the germinal epithelium as well as fusion of adjacent seminiferous tubules (Figure 1c), while the positive control group showed similar indented histological organisation like 10 and 20 % concentration groups; degeneration of the tubular epithelium, fusion of adjacent tubules and shrinking of the germinal epithelium of the tubule (Figure 1d).

Histopathological changes in the liver of the test group and of the control group showed that the negative control micrograph had normal liver architecture with vacuolation (Figure 2). The positive control, 10 and 25 % micrograph showed distorted liver tissue architecture with infiltration. Unevenly distributed sinusoids around the hepatic and portal tracts were recorded in 25 % concentration.

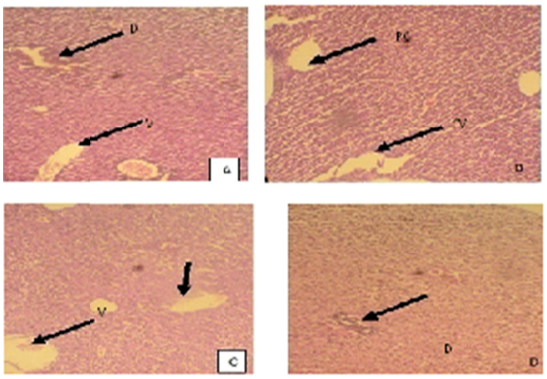

Figure 2: Histopathology of the mice liver tissue after a period of 35 days exposure to lecheate (H&E, x 1000). *(A) Positive control (abnormal liver with vacuolation) (B) Negative control (normal liver architecture). (C) 25 % leachate (irregular liver architecture with unevenly distributed sinusoids around the hepatic and portal tracts) and (D) 10 % leachate (fairly distorted liver architecture). V: Vacuolation; D: Distortion; PC: Portal canal; CV: Centre vein*

The kidney histopathology of 10 % concentration micrograph showed parts of the helium and renal cortex with irregular tubules (tubular damage) and the 25 % kidney histopathology showed a grossly distorted renal architecture, while the kidney histopathology of the positive control revealed interstitial haemorrhage. The abnormalities observed in the kidneys are given in Figure 3.

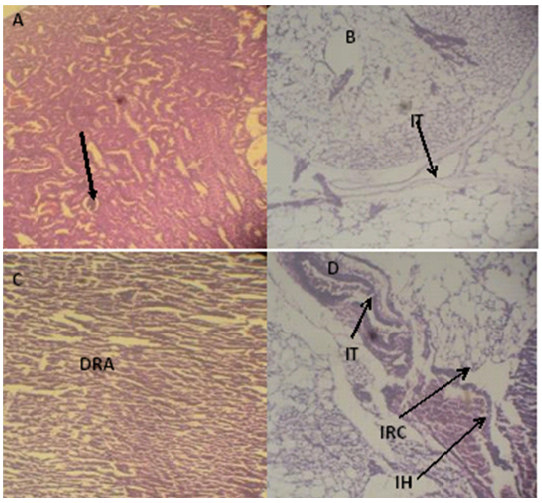

Figure 3: Histopathology of the mice kidneys exposed for the period of 35 days of experiment (H&E, x 1000). *(A) Negative control. Micrograph shows a grossly normal renal architecture. (B) 10 % concentration of leachate. Micrograph shows parts of the helium and renal cortex with irregular tubules (IT). (C) 25 % concentration of leachate. Micrograph shows grossly distorted renal architecture (DRA) and (D) Positive control (cyclophosphamide). Micrograph shows infiltrated renal cortex (IRC) with irregular tubule and interstitial haemorrhage (IH)*

DISCUSSION

The organic and inorganic elements in the leachate can react with each other (Sang and Li, 2004). It was observed that in the physical and chemical analyses of the leachate, that the biological oxygen demand, chemical oxygen demand, alkalinity and the heavy metals values were high and above environmentally acceptable limit. The pH value of 8.34was within the permissible limit and agreed with the study of Kanmani and Gandhimathi (2013) that reported pH range of 6.96-8.33 of leachate from an open dumping site. The high concentration of heavy metals analyzed in the leachate can be attributed to its stabilized state (Kjeldsen *et al.,* 2002; Kanmani and Gandhimathi, 2013) which is in agreement with the study carried out by Alimba *et al.* (2006). Furthermore, the heavy metals content of the leachate may be because of chemicals from disposed batteries, older lead-based paints and disposed lead pipes.

Histopathological examination of tissues is a tool used in identifying the type of abnormal tissue caused by xenobiotic and is acknowledge as the most sensitive method for detecting organ toxicity (CIOMS, 1985). The histological presentation of the hepatic section showed abnormal liver with vacuolation in the positive control compared to the negative control having normal liver architecture. The other histological presentation of the exposed mice showed different level of dose dependent vacuolation that indicated the likelihood of abnormal tissue formation due to exposure.

The kidney histology of the exposed group showed gross structural abnormalities compared to the negative control. These observed structural abnormalities showed that this simulated leachate sample has the potential to induce kidney dysfunction. The kidney showed proximal tubular damage and degeneration which may be traced to the presence of heavy metals especially cadmium and lead corresponded to the observation made by Oshode *et al*. (2008) on *Clarias gariepinus*. The presence of ammonia, nitrogen dioxide (NO_2) and other nitrogenous compounds in car waste leachate especially battery and other dissolved gaseous substances also has toxic effect on animals and can lead to behavioural changes and induce damage to the histology of kidney.

Conclusion: This study give a report of histological damages of testes, liver and kidney from automobile wastes sites which can be of direct or indirect effect on both workers and people living around the workshop. This study is relevant for the management and control of human-automobile waste interaction community and the nation at large calling for separation of automobile workshop from the residential homes. Further studies are required so as to provide the mechanism of action through the pathway of chemical present in the waste to ascertain the effect of critical exposure to automobile waste in the biological system.

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