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Cessation of contact altered toxic effects of diesel on heavy metal metabolism but not on renal dysfunction

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Abstract
Although petroleum products are known toxicants, there is dearth of information on how effective the different physiological mechanisms in mammals are, in correcting altered heavy metal metabolism and renal dysfunction commonly associated with diesel exposure. The objective of the study therefore is to explore that possibility. Forty female rats (180 - 210 g) were divided into 4 groups of 10 rats each. 3 mL/kg was administered daily to rats in GROUPS 2, 3, and 4 for 21 days, GROUP 1 constituted the control. Rats in GROUPS 2 and 4 received diesel orally as constituent of feed but diesel was administered by dermal route to GROUP 3. Blood samples were obtained and rats were euthanized on 22nd [GROUPS 1-3] and 50th day [GROUP 4]. Serum levels of heavy metals were determined by Atomic Absorption Spectrometry. Evaluation of renal damage was by estimation of serum levels of creatinine and urea. Histologic examination of kidney was by hematoxylin and eosin. Statistical analysis of the data was by analysis of variance (ANOVA). $p \leq 0.05$ was considered significant. Pd, Cd, Al, Ni, and As were significantly higher in GROUPS 2 and 3 compared with Group 1. As, Cd, and Pb were significantly lower in GROUP 4 compared with GROUP 2 or GROUP 3, but they were not significantly different compared with GROUP 1. 10% death rate was observed among GROUP 4 rats. No visible lesion (GROUP 1); but various degree of distortion in renal histo-architecture in GROUPS 2-4 were also observed as well as abnormal urea and creatinine levels. In conclusion, data obtained reveals that altered heavy metal metabolism as well as renal dysfunction occurred from both oral and dermal contact and that its nephrotoxic effects lasted well beyond the immediate period of contact.

Keywords: irreversible nephrotoxicity, heavy metals, diesel

Introduction
Analysis of diesel for its metal content using atomic absorption spectrophotometric technique showed detectable amounts of both essential (Cu, Zn, Cr) and non-essential elements (Cd, Pb) [1]. Although Cd, Cr and Zn levels fell within permissible range, both Cu and Pb were observed to be above acceptable limit [1]. An indication that Cu and Pb content in diesel is pronounced. Even though Cu and Zn are essential elements, at above physiologic levels they may provoke deleterious effects. Moreover both Cd and Pb have been implicated as agents that are capable of inducing various pathological presentations [2-4].

Exposure to various petroleum products have been linked with alteration in morphology of renal cells [5]. Yet it is worthwhile to indicate that it is not in all instances that the toxic effects of an agent on renal cells are permanent. This is because the kidney possesses some regenerative ability [6,7]. Moreover, various mechanisms have also been identified through which abnormal heavy metal levels can be corrected. The objective of the study therefore is to evaluate whether altered renal function and abnormal heavy metal metabolism are reversed upon cessation of contact to diesel.

Materials and Methods
Experimental design
Adult female albino rats weighing between 180 - 210 g were obtained from the Department of Physiology, University of Ibadan, Nigeria. Before the commencement of the study, the rats were kept for a 2-week acclimatization period. The experimental animals were given unrestricted access to both feed and water. The experimental protocol consisted of 4 groups of rats, with each group consisting of 10 rats. The first group [GROUP 1] was designated as the control group. The second [GROUP 2] and third [GROUP 3] groups were treated daily with diesel by oral and dermal routes respectively, at dosage level of 3 mL/kg. The fourth group [GROUP 4] also received the treatment for the same period of time and route as GROUPS 2 but was not sacrificed for another four weeks. GROUP 4 was used for determine whether abnormal effects of diesel is reversible upon cessation of contact.

Oral route exposure was planned by intentional adulteration of feed daily, each morning prior to the supply of feed to rats. Adulteration of feed took place by thoroughly mixing feed and diesel. For the rats in oral exposure group, to preclude the possibility of self-grooming, dermal exposure occurs each day through application of diesel to the neck region to prevent oral contact. The experimental period was for 3 weeks. The petroleum product used for the study was purchased from a filling station located in Ibadan, Oyo State, Nigeria. The study was conducted in compliance with National and International Laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research Institutes of Health (revised 1985).

Preparation of serum samples & heavy metal estimation
Samples (blood) were obtained from each animal at the end of the experiment (on the 22nd day for GROUPS 2 AND 3; 50th day for GROUP 4) by retro-orbital bleeding after which they were sacrificed and sections of kidney obtained. The obtained blood samples were dispensed into anticoagulant free bottles and centrifuged at 2000 g. Sera obtained after centrifugation was stored at -20 ºC until required for analysis. Atomic absorption spectrometric procedure was employed for the estimation of serum concentrations of aluminium, silicon, cadmium, lead, arsenic, and nickel. Buck Scientific 205 Atomic Absorption supplied by Buck Scientific (East Norwalk, Connecticut, USA) was employed for these analyses.

Clinical Chemistry and Histopathology
The serum levels of creatinine, and urea were assessed using Jaffé reaction, and diacetyl monoxime oxidase method respectively. Hitachi® 902 automated machines (Roche Diagnostic, Germany) was used for the assessment. Histologic examination of kidney was undertaken on collected sections of the kidney, which were fixed in 10% neutral buffered formalin, and were dehydrated in ascending concentration of ethanol, cleared in xylene and embedded in paraffin. Sections 4-5 µm in thickness were prepared and stained with Hematoxylin and Eosin (H & E).

Statistical Analysis
Data obtained from the study were subjected to statistical analysis using Statistical Package for Social Sciences (version 15). The means of serum concentrations of renal markers as well as those of estimated heavy metals were determined and compared using Student's t test and analysis of variance (ANOVA). Value of $p \leq 0.05$ was considered significant.

Results
The results of the study are presented in Table 1 and Figure 1 below. When compared with control (GROUP 1), Pd, Cd, V, Si, Al, Ni, and As were significantly higher in GROUPS 2 and 3 ($P<0.05$). Silicon, Ni and Cd, and Pb were significantly lower in GROUP 4 compared with GROUP 2 or GROUP 3, but they were not significantly different compared with GROUP 1 (Table 1). Both urea and creatinine were significantly higher in diesel treated rats compared with control, much higher values were observed in GROUP 4 (Table 2). While all the rats in GROUPS 1, 2, and 3 survived till the end of the experiment, 10% death rate was observed in GROUP 4. The results of renal histology are presented in the photomicrographs (Figure 1).

Table 1: Serum concentrations of select toxic metals in diesel-exposed wistar rats.

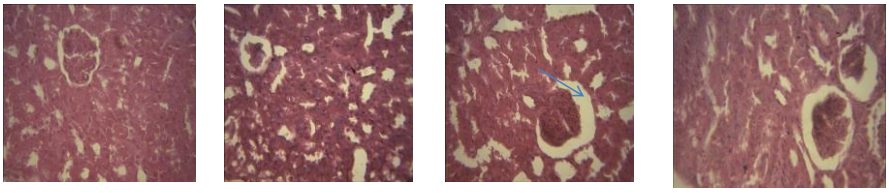
	Ni (µg/L) †§	Al (µg/dL) †§	Cd (mg/dL) §	Si (µg/L)	As (µg/dL) §	Pb (µg/L) §
Group 1	0.022±0.009	0.016±0.006	0.008±0.003	0.006±0.003	0.009±0.003	0.023±0.004
group 2	0.040±0.024*	0.027±0.011*	0.019±0.011*	0.010±0.005*	0.016±0.005*	0.035±0.007*
Group 3	0.034±0.012*	0.029±0.008*	0.014±0.007*	0.007±0.002	0.012±0.003*	0.030±0.010*
Group 4	0.032±0.009*	0.022±0.008*	0.010±0.004	0.006±0.001	0.009±0.004	0.025±0.008

Results are expressed as mean ± standard deviation. *p <0.05 is significant when compared with GROUP 2, 3, or 4 is compared with control (GROUP 1) using Student's t-test. †p <0.05 is significant using ANOVA when GROUPS 1, 2 and 3 were compared. ‡p <0.05 is significant using ANOVA when GROUPS 1, 2 and 4 were compared. N = 10.

Table 2: Serum levels of urea and creatinine in diesel-treated wistar rats.

Groups	Urea (mmol/L) †§	Creatinine (µmol/L) †§
1	7.51±1.22	67.44±20.61
2	14.07±3.95*	89.10±19.27*
3	10.78±2.67*	114.73±11.20*
4	16.30±4.42*	132.82±38.06*

Results are expressed as mean ± standard deviation. *p <0.05 is significant when compared with GROUP 2, 3, or 4 is compared with control (GROUP 1) using Student's t-test. †p <0.05 is significant using ANOVA when GROUPS 1, 2 and 3 were compared. ‡p <0.05 is significant using ANOVA when GROUPS 1, 2 and 4 were compared. N = 10.



GROUP 1- No visible lesion seen

GROUP 2- Congestion of the cortical vessels.

GROUP 3- Mild congestion of the cortical vessels.

GROUP 4- Tubular epithelial degenerati on (mild).

Fig 1: The Photomicrographs of kidney of experimental animals. (Group 1-control; Group 2- Oral route; Group 3-Dermal route; Group 4- Oral Route with 4 weeks cessation of contact). Max. X 400

Discussion

The results of the study that revealed significant higher levels of various heavy metals in wistar rats treated with diesel compared with control rats suggest that the quantity of heavy metals in diesel being sold in Nigeria is profound enough to alter the serum levels of toxic metals in a mammalian species in a sub-acute setting. Although it is understandable to assume that considerable pollution of petroleum products with toxic metals may not be intentional, the occurrence of heavy metals in diesel may arise for a number of reasons or causes.

While the significant increase in the level of Pb in diesel exposed rats (GROUPS 2, 3) suggests the presence of Pb in diesel. It was addition of tetra-ethyl lead to petrol that was a well known phenomenon. Pb was incorporated into petrol to serve as anti-knock agent. Because of the negative impact of Pb (contained in petroleum products) on the environment; calls for exclusion of Pb in petrol were made and subsequently headed in many quarters. While deliberate contamination has been addressed by and large, the presence of heavy metals in diesel or other related products cannot be over-ruled. Crude oil from which diesel is derived has been reported to contain several potentially toxic elements. That their presence in this natural product can be considered significant can be adduced from reports obtained through several studies.

According to Akpoveta and Osakwe [1] soil in oil-producing areas in Nigeria has been reported to be heavily contaminated in Pb, Cd, and other related toxic metals, to the extent that edible and non-edible plant products, grown on the soil in oil-producing areas contain significant amount of toxic metals compared with the same species of plants grown in non-oil producing areas.

Iderah and colleagues [8] opined that these metals are natural components of rocks and sediments. And that while anthropogenic activities are capable of causing heavy metal contamination of the environment, heavy metal contamination of diesel and other petroleum products can equally arise from leaching or absorption of the metals from supply vessels and storage tanks. Although the possibility of elevated levels occurring from natural sources especially-source rock from which the crude was obtained was not discounted.

The significant increases in renal markers of Groups 2 and 3 compared to Group 1, suggest the nephrotoxic effect of diesel. The high sensitivity of xenobiotics to very many different cell types is a well known event; examples of such cells include hepatocytes, enterocytes, renal cells etc. The renal cells especially have been identified to be prone to the damaging effects of foreign substances [9, 10]. Aside the fact that the kidney is also rich in enzymes responsible for xenobiotic metabolism and therefore plays a role in detoxification of foreign and sometimes dangerous substances, its functional integrity is vital to total body homeostasis since the kidney plays a major role in the excretion of metabolic wastes and in the regulation of extracellular fluid volume, electrolyte composition, and acid–base balance. Moreover, the renal cells produces and releases hormones, such as renin and erythropoietin, and metabolizes vitamin D3 to the active 1,25-dihydroxy vitamin D3 form. This means that exposure of renal cells to a toxic substance could disrupt any or all of these functions and have significant effects on overall body metabolism. To prevent such devastating outcome, the kidney is fashioned with a variety of detoxification mechanisms and has considerable functional reserve and regenerative capacities [6, 7], but when such reserve is overwhelmed kidney injury ensues.

Even after cessation of contact to diesel for 4 weeks (GROUP 4), the results of the study indicate significant renal dysfunction. Serum levels of many of the heavy metals though were not significantly different in rats in GROUP 4 compared with control. This means that renal pathology that occurs from contact with diesel may be permanent. Adenine has also been identified to cause permanent renal damage when administered to rats for 6 weeks at 0.75% level, although when duration of exposure was for 2 weeks at the same dosage level, its harmful effects were reversible [11]. That renal dysfunction that arose from diesel toxicity is permanent can be deduced from 10% death rate in rats in the group left within the 4 weeks in which cessation of contact occurred. On the other hand, the persistent or uncorrected renal damage even after cessation of contact with diesel may be linked with heavy metal effects. Chamberlain *et al.* [12] and Ajayi *et al.* [13] reported of accumulation of a heavy metal- Pb in tissues with devastating consequences. Lead accumulation from pollution has been reported to occur in non-human living organisms also [14].

Among other things, the study is limited by the absence of data on tissue distribution of the heavy metals detected in the serum of rats treated with diesel. Serum is usually not the most ideal biologic specimen to assess heavy metal burden in mammals, there may be the need to assess trace elements levels in kidney, to confirm that metals sequestrated in kidney are not responsible for continual nephrotoxicity even after cessation of contact.

In conclusion, this study indicates that oral and dermal contact with diesel resulted in abnormal heavy metal metabolism as well as renal dysfunction. In addition, it was observed that its nephrotoxic effects lasted well beyond the period of contact. Since the total metal body burden was not assessed, it was impossible to establish if high metal content sequestrated in renal cells was responsible for persistent damage. It is for this same reason that the seemingly normal serum heavy metal levels could not be taken at face value.

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