

Evaluation of heavy metal levels in serum of Wistar rats exposed to engine oil

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ABSTRACT

Purpose: Data are available that indicate there is an elaborate elemental constitution of petroleum products, with identified elements contained in the many products being additive (e.g. Ca, Zn and P) as well as wear metals (e.g. Ag, Al, Ba, Cd, Cr, Cu, Fe, Mg, Mo, Na, Ni, Pb and Sn). In addition, incessant deliberate exposure of engine oil to both human beings and farm animals for therapeutic reason has been reported. Therefore the objective of this study is to evaluate the levels of heavy metals in serum of engine oil-exposed rats.

Materials & Methods: Thirty adult female rats were divided equally into 5 groups. The first and second groups were treated with engine oil by oral route (as contaminant of feed) at dosage levels of 0.5 and 1.0 mL/kg body weight respectively. The third and forth groups received the test agent through the dermal route

at dosage levels of 0.5 and 1.0 mL/kg body weight while the fifth group served as the control. The duration of the study was 30 days, after which blood was obtained from each rat, centrifuged and the resultant serum used for the analysis of heavy metals by employing Atomic Absorption Spectrometry (AAS). Data were analyzed using analysis of variance (ANOVA), $p \leq 0.05$ was considered significant.

Results: Data obtained showed that there were significant differences in the levels of aluminium, silicon, cadmium, lead, arsenic, vanadium, and nickel.

Conclusions: These increases suggest that incessant exposure to engine oil may be dangerous and therefore constitute health hazard.

Keywords: Engine oil, oral and dermal routes, lead, cadmium, aluminium, silicon, vanadium, arsenic, nickel

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INTRODUCTION

Engine oil is obtained from crude oil or synthetic oil for lubrication of combustion engines [1,2]. It is known to inhibit corrosion, improve sealing, clean and cool the engine by conveying heat away from moving parts. The constituents of engine oil include a complex mixture of hydrocarbons that is between 80% and 90% by volume and performance enhancing additives that is between the range of 10% and 20% by volume [1,2]. The additives are usually metals that are incorporated to enhance the efficiency of the oil.

According to Chen and Jiang [3], fuel samples (petrol, diesel, engine oil) contain both mercury and lead. Virgin engine oil especially has been identified to contain iron, cadmium, chromium, lead, and other heavy metals, and that higher levels of these elements are found in used engine oils [4]. Data obtained from the study of Kim *et al.* [5] revealed a more elaborate elemental constitution of petroleum products, with identified elements contained in the products being additive (e.g. calcium, zinc and phosphorus) as well as wear metal elements (e.g. silver, aluminum, barium, cadmium, chromium, copper, iron, magnesium, molybdenum, sodium, nickel, lead and tin).

In addition, using wavelength-dispersive X-ray fluorescence spectrometry to measure elements of phosphorus, sulfur and calcium in 98 engine oil (different grades) samples from countries such as America, Germany and Japan, results revealed that there was a proportional relationship between zinc/phosphorus, sulfur/molybdenum and calcium/magnesium as illustrated by correlation between experimental data and additive contents [6].

Concentration levels of zinc, phosphorus, calcium, sulfur, magnesium and molybdenum in engine oil have been reported to be 0.09%-0.17%, 0.07%-0.15%, 0.13%-0.43%, 0.20%-0.47%, 0.04%-0.15% and 0.01%-0.05% respectively [6], of which the calcium, sulfur, phosphorus and zinc were the most abundant elements of engine oil. Moreover, they also observed that magnesium and molybdenum were found to exist in high quality level engine oil, whereas niobium, tungsten, thallium or sodium was identified in individual engine oil.

The presence of these elements in engine oil especially raises the possibility that incessant exposure may result in their increase levels in exposed-animals, and since the harmful effects of many of them are well described, it becomes expedient that this study be carried out to estimate the serum concentrations of heavy metals in Wistar rats treated with two different

doses of engine oil administered through different routes of exposure.

MATERIALS AND METHODS

Petroleum product

Engine oil (AP motor oil) was purchased from a filling station located in Osogbo, Osun State, Nigeria in December, 2011.

Experimental Animals

This study was carried out in compliance with national and international laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research Institutes of Health (revised 1985). Adult female albino rats with average weight of 218 g were obtained from the Animal House attached to the Department of Veterinary Physiology, University of Ibadan, Nigeria. The animals were left to acclimatize for two week prior to commencement of the experiment. Animals were housed in cages at ambient temperature of $24\pm 3^{\circ}\text{C}$ and a 12 h light, 12 h dark cycle. All the animals were given unrestrained access to their specific diets and water.

Thirty rats were divided into 5 groups comprising of 6 rats per group. The first and second groups were treated with engine oil by oral route (as contaminant of feed) at dosage levels of 0.5 and 1.0 mL/kg body weight respectively. The third and fourth groups received the test agent through the dermal route at dosage levels of 0.5 and 1.0 mL/kg body weight while the fifth group served as the control. The treatment groups were exposed to this product for a period of 30 days. Deliberate contamination of feed took place each morning shortly before the rats were supplied with the feed, in which engine oil was freshly and thoroughly mixed with feed. On the other hand, dermal exposure occurs each day through application of engine oil to the neck region to prevent oral contact from self grooming.

Preparation of serum samples & heavy metal estimation

On the 31st day the study was brought to an end, and blood samples were drawn from all the rats by retro-orbital bleeding. These were discharged into anticoagulant free bottles and centrifuged at 3000 g.

The resultant sera from the procedure were kept at -20°C until required for analysis. Serum levels of aluminium, silicon, cadmium, lead, arsenic, nickel and vanadium were carried out using the atomic absorption spectrometric method. Buck Scientific 205 Atomic Absorption supplied by Buck Scientific (East Norwalk, Connecticut, USA) was utilized for this purpose.

Statistical analysis

The mean values of the serum levels of the heavy metals for control and each of the treatment groups were compared using Student's t test while control, dermal and oral routes were compared using analysis of variance (ANOVA). Value of $p \leq 0.05$ was considered significant.

RESULTS

Administration of engine oil to Wistar rats through the oral route resulted in significant increases

($p < 0.05$) in the levels Si, Pb, As, V, Ni, Cd and Al at 0.5 mL/kg body weight when compared with control. On the other hand, only V was significantly increased ($p < 0.05$) in dermal exposed rats when compared with control (Table 1).

Also in Table 1 intergroup comparison of control, dermal and oral groups (using ANOVA) showed significant differences ($p < 0.05$) for all estimated elements.

Results of 1.0 mL/kg body weight presented in Table 2 showed significant increases ($p < 0.05$) for all elements determined, using not only Student's t test but analysis of variance as well.

Table 1. Serum levels of select heavy metals in engine oil administered rats (0.5mL/kg dosage level)

	Si ($\mu\text{g/L}$)**	Cd (mg/dl)**	Al ($\mu\text{g/dl}$)**	Ni ($\mu\text{g/L}$)**	V (ng/dL)**	Pb ($\mu\text{g/L}$)**	As (ng/dL)**
Controls	0.030 \pm 0.006	0.056 \pm 0.010	0.072 \pm 0.015	0.044 \pm 0.009	1.28 \pm 0.081	0.078 \pm 0.005	0.064 \pm 0.011
Oral route	0.041 \pm 0.012*	0.077 \pm 0.012*	0.091 \pm 0.021*	0.049 \pm 0.009*	1.34 \pm 0.046*	0.099 \pm 0.014*	0.074 \pm 0.010*
Dermal Route	0.032 \pm 0.009	0.061 \pm 0.017	0.078 \pm 0.020	0.047 \pm 0.006	1.80 \pm 0.054*	0.082 \pm 0.010	0.066 \pm 0.006

Results are expressed as mean \pm standard error of mean. * $p < 0.05$ is significant when compared with control using Student's t test. ** $p < 0.05$ is significant when control, oral and dermal groups were compared using ANOVA.

Table 2. Serum levels of select heavy metals in engine oil administered rats (1.0 mL/kg dosage level)

	Si ($\mu\text{g/L}$)**	Cd (mg/dl)**	Al ($\mu\text{g/dl}$)**	Ni ($\mu\text{g/L}$)**	V (ng/dL)**	Pb ($\mu\text{g/L}$)**	As (ng/dL)**
Controls	0.030 \pm 0.006	0.056 \pm 0.010	0.072 \pm 0.015	0.044 \pm 0.009	1.28 \pm 0.081	0.078 \pm 0.005	0.064 \pm 0.011
Oral route	0.053 \pm 0.018*	0.091 \pm 0.009*	0.099 \pm 0.026*	0.066 \pm 0.010*	1.80 \pm 0.071*	0.113 \pm 0.027*	0.094 \pm 0.011*
Dermal Route	0.036 \pm 0.013*	0.067 \pm 0.013*	0.084 \pm 0.018*	0.052 \pm 0.013*	1.98 \pm 0.062*	0.088 \pm 0.016*	0.069 \pm 0.014*

Results are expressed as mean \pm standard error of mean. * $p < 0.05$ is significant when compared with control using Student's t test. ** $p < 0.05$ is significant when control, oral and dermal groups were compared using ANOVA.

DISCUSSION

Normally for most newly introduced chemicals that humans or other living organisms may come in contact with, both the qualitative and quantitative evaluation of their harmful or toxic effects is essential to establish the potential hazard they pose to these animals [7]. On the other hand, some other chemicals that have been in use for decades may also

have evaluation of their toxicity revisited. This becomes necessary in many parts of the world where such chemicals are used for a variety of purposes for which they are not meant and for that reason, their toxicity through different routes are sometimes re-evaluated. Many of petroleum products fall into this category since it has been discovered that they are commonly used for treatment of a wide range of

medical conditions by the impoverished people of the developing countries [8-11].

Aside the natural biogeochemical cycles, human activities play an important role in the mobilization, transformation, and transportation of metals in the environment. Mining, dredging, construction, and manufacturing have been reported as known processes that involve removal of metals from the locations in which they naturally occur, and may incorporate them into the human economic sphere. Thus increasing the potential for both human and animal exposure as it is revealed through the results of this study in which significant increases were recorded for the elements, in engine oil exposed rats.

The toxic effects of a particular xenobiotic may be exerted through a myriad of ways: a direct interaction or reaction between a chemical and the specific target molecule though possible, sometimes it may not be the basis of toxicity for many xenobiotics. Rather very many different foreign agents have been reported to adversely influence the biological (micro) environment, thereby leading to molecular, organellar, cellular, or organ dysfunction which may manifest as one form of deleterious effect or another [12]. For many of the toxic elements contained in petroleum products e.g. engine oil, their basic concept of toxicity may be more complex and in most cases involves several steps.

For instance, ingestion of arsenic containing substances causes gastrointestinal irritation, peripheral neuropathy, vascular lesions, anemia, and various skin diseases. In addition, skin cancer has been reported from its high-level oral exposure from this element [12-14]. This means that careful attention has to be paid to the issue of the use of engine oil for therapeutic purpose. In addition, many case reports of death in humans due to intentional or unintentional ingestion of high doses of arsenic compounds have been established [13]. While there is insufficient information that link hepatic, renal, and dermal or ocular effects to arsenic exposure through inhalation, ingestion of arsenic containing substances may lead to severe cardiovascular effects, but this was not assessed in these rats. Other effects like anemia and leukopenia, also have been reported following acute, intermediate, and chronic oral exposures [14-16]. Many other heavy metals present in engine oil and identified in serum of the test animals are known for their characteristic toxic manifestations [17].

After the toxicant like Hg, V, Si, or Al is delivered to its target or targets, the ultimate toxicant may combine with endogenous target molecules, thereby initiating perturbations in cell function and/or structure. Although the first response after xenobiotic-exposure involves initiation of repair mechanisms at the molecular, cellular, and/or tissue levels, others

such as adaptive mechanisms that reduce delivery, boost repair capacity and/or compensate for dysfunction may occur. It is only after perturbations induced by the toxicant far exceed repair and adaptive capacity or when repair and adaptation are not effective or malfunctional, that toxicity occurs. The significant increases in the levels of these elements observed in the test groups compared with control may suggest that these adaptive mechanisms may be overwhelmed in Wistar rats, especially at 1.0 mL of engine oil /kg BW where all the elements were significantly increased.

The toxic effects of engine oil to mammals have been recognized in an earlier study [18] in which rats orally administered with engine oil at 1.0 mL/kg level of exposure featured the following abnormal histologic presentations (liver-diffuse vacuolar degeneration of hepatocytes and necrosis; kidney-protein casts in the lumen). In many instances, the degree of such toxicity is primarily determined by level and route of exposure as revealed by the results of this study. In which rats administered with a dose of 1.0 mL/kg had higher levels of Ni, Si, V, and As than those of 0.5 mL/kg level of exposure. Although when a product that is introduced to an animal is made up of many components as it is common with petroleum products, it may be difficult to postulate the individual role of each component in its possible toxic presentation.

The significant differences in the levels of many of the toxic metals estimated suggest that the level of exposure was sufficient to alter their serum levels in engine oil exposed rats. This also indicates that the significant increase in the serum level of Ni, Si, V, and As could have been one of the factors responsible for the earlier observation of tissue damage in Wistar rats treated with the same levels of engine oil. The significant differences in the levels of these heavy metals may be linked to their presence in the engine oil used for the study. It may also be appropriate to infer that this kind of presentation was one of the causes of tissue damage reported in an earlier study [18].

CONCLUSION

Data obtained from this study indicate that exposure to engine oil may result in significant presence of toxic metals in the serum of affected animals, a situation which may result in myriad of abnormal clinical presentations.

Toxic metal-induced organ toxicity for example is well described in literature.

Conflict of Interest

None

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