The Hepatotoxic Effects Of The Water-Soluble Fraction Of Spent Lubricating Oil In Wistar Albino Rats

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Citation

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Abstract

This aim of this study was to evaluate the hepatotoxic effects associated with pollution caused by spent lubricating oil, a major pollutant in Nigeria on terrestrial organisms. The hepatotoxic effects of three concentrations (10%, 50% and 100%) representing low, medium and high concentration of the water-soluble fraction (WSF) of spent lubricating oil in wistar albino rats was investigated. The range-finding test of WSF of spent lubricating oil was determined to be higher than 100% concentration after 48 hours. Serum L-alanine aminotransferase (L-ALT), L-aspartate amino transferase (L-AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) increased significantly ($p \leq 0.05$) after the administration of 2mL WSF of spent lubricating oil orally for 28 days. The order of increase was 100% >50% >10%. The WSF of spent lubricating oil was slightly acidic with a pH value of 6.7. Histopathological examination of the liver tissues revealed obliteration of sinusoids, macrovesicular fatty change, disorganized cords and enlargement of hepatocytes. The results suggest that oral administration of varying concentration of WSF of spent lubricating oil may cause an adverse effect on the function of the liver.

INTRODUCTION

The water-soluble fraction (WSF) constituents are dispersed particulate oil, dissolved hydrocarbon and soluble contaminants such as metallic ions¬¬[1].The components of spent lubricating oil that go into solution make up the WSF. They are taken up by living cells and are metabolized[2]. This is ecologically important because in event of an oil spill into aquatic habitat, this is absorbed by living organisms with serious effects on the ecosystem. Large amounts of spent lubricating oil are liberated into the environment when the motor oil is changed and disposed into gutters, water drains, open vacant plots and farmlands, a common practice by motor mechanics and generator mechanics[3]. The ubiquitous and pervasive nature of petroleum-derived hydrocarbons and the magnitude of their input to aquatic ecosystems are the two main motivating factors for research focused on their toxicity to terrestrial organisms. The fraction of oil that is most bioavailable to marine biota such as teleosts is the dissolved hydrocarbons, which include the polycyclic aromatic hydrocarbons (PAHs). The general importance of PAHs as a toxic component of petroleum-derived hydrocarbons is well established. Predicting the toxicity of these compounds is fraught with challenges, including dynamic chemical profiles, nonspecific and chemical-specific mechanisms of action, and variable intra- and inter species sensitivities. Moreover, because hydrocarbons from oil spills can persist in near-shore sediments for decades or longer[4], investigations into toxicity need to incorporate long-term exposure regimes.

The spent lubricating oil, otherwise called waste-lubricating oil or waste crankcase oil (WCO) obtained after servicing and subsequent draining from automobile, generators and industrial machines is disposed off indiscriminately, and adequate attention has not been given to its disposal[5]. Analytical procedures commonly used to assess contamination by petroleum products are determination of hydrocarbon fractions, total hydrocarbon and heavy metal contents. Edebiri and Nwanokwale[6], reported that metals present in spent lubricating oil are not necessarily the same as those present in the unused lubricants. It has also been observed that most heavy metals like Va, Pb, Al, Ni and Fe that are below detection in unused lubricants oil gave high concentration values in used oil[7]. The disposal of spent lubricating oil into open vacant plots and farms, gutters and water drains is an environmental risk considering the water table in the South-South Region of Nigeria and shallow bore-holes dug to get water for domestic use [8]. The suspected major soil contaminant/pollutant was spent lubricating oil from engines and other machinery. Oil in soil makes the soil condition become unsatisfactory for plant

growth[9], due to the reduction in the level of available plant nutrient or a rise in toxic levels of certain elements such as iron and zinc[10]. Various contaminants such as spent engine oil have also been found to alter soil biochemistry, which includes alteration in soil microbial properties, pH, oxygen and nutrient availability[3, 10, 11, 12]. There are relatively large amounts of hydrocarbons in the spent lubricating oil including the highly toxic polycyclic aromatic hydrocarbons (PAHs)[13]. The concentration of PAHs in lubricating oil increases with time of usage and those with two and three rings accumulate rapidly in used lubricating oil to very high levels[14]. Since Nigeria was reported to account for more than 87 million litres of spent oil waste annually [15], the need to evaluate the risk posed by this pollutant becomes imperative. Spills arising from disposal of spent lubricating oil are becoming a visible problem especially in developing countries such as Nigeria. Therefore the usual improper disposal of used lubricating oil generated by service stations and other users now demands attention in order to protect the terrestrial organisms which depend directly or indirectly on water bodies polluted by the indiscriminate disposal of spent lubricating oil.

MATERIALS AND METHODS

COLLECTION OF SAMPLES

Spent lubricating oil was collected from various mechanic workshops in Obio- Akpor Local Government Area of Rivers State Nigeria.

PREPARATION AND PRESERVATION OF THE WATER-SOLUBLE FRACTION (WSF).

The water-soluble fraction was prepared according to the method of Anderson et. al.,[16] with slight modification by Ogali et al.,[17]. Briefly, some spent lubricating oil (150 ml) was slowly mixed with distilled H_2O (450 ml) in a 1000- ml conical flask. The flask was covered with Aluminum foil and held tightly with a rubber band. The flask was fastened to an electric stirrer, and shaken for 24 h, as recommended by Parker et al.[18]. Then, the mixture was left standing for 3 h to obtain a clear phase separation between oil and H_2O. The mixture was then poured into a separating funnel (with glass stopper) and allowed to settle overnight. The next day, most of the oil droplets in the mixture had settled in the upper layer, the pure and clear WSF being obtained at the lower part of the funnel. The WSF was thereafter siphoned into a dark-colored, screw-capped Winchester bottle, and stored in a refrigerator (0–4°C) until required for use.

PH MEASUREMENT

The pH of the WSF of the spent oil was determined on a Jenway 3015 pH meter. The instrument was calibrated with buffers at pH 4.0, 7.0, and 9.0. The electrode of the pH meter was immersed into the WSF and allowed to stabilize before final reading was taken.

RANGE FINDING TESTS

Range finding tests to determine the lowest dose of WSF of spent lubricating oil capable of eliminating 50% of the test animals and the highest concentration that will not have any effect on the animals were first carried out. Five different concentrations (100, 30, 9, 2.7 and 0.81) of the WSF of the Spent lubricating oil were used based on a dilution factor of 0.3. Animals were closely monitored for 48 hours for observational changes such as discharges from the eyes, nose, hair loss, tremors, changes in respiratory rate and movement within the cage.

ANIMALS

Twenty four matured Wistar albino rats weighing between (175g – 225g) used in this experiment were obtained from the animal house of the Department of Biochemistry, University of Port Harcourt. The rats were housed and kept under laboratory conditions with free access to a standard diet and water. After seven (7) days of acclimatization, the rats were assigned to four groups comprising of six animals each. The experiments were performed after the experimental protocol was approved by the institutional animal ethics committee

EXPERIMENTAL PROTOCOL

The experimental animals were arranged into four groups, each group comprising six animals.

Group 1

Normal control rats (feed only) for 28 days

Group 2:

Received normal feed and 2mL 10% WSF orally daily for 28 days

Group 3:

Received normal feed and 2mL 50% WSF orally daily for 28 days

Group 4:

Received normal feed and 2mL 100% WSF orally daily for

28 days

PREPARATION OF SAMPLES

28 days after the administration of WSF of spent lubricating oil, the rats were anaesthetized in a chloroform-saturated chamber. Blood was collected from the jugular vein with the aid of a 2mL hypodermic syringe and needle into an anti-coagulant-free bottle. The animals were dissected from anus to thorax using a surgical blade. The liver was excised immediately and fixed in 10% formalin for histological assessment of hepatic damage. Serum was separated by centrifugation and stored in a refrigerator. The levels of serum L-alanine aminotransferase (L-ALT), L-aspartate amino transferase (L-AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were performed using the Humazym M UV colorimetric-test kits.

ANALYSIS OF DATA

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's t-test. P values ≤0.05 were considered to be significant.

RESULTS

Oral administration of WSF of spent lubricating oil at doses of 10, 50 and 100% was found to significantly ($p \leq 0.05$) alter hepatic marker enzymes as shown in Table 1. The range-finding test of WSF of spent lubricating oil was determined to be higher than 100% concentration after 48 hours. However, results show that rats treated with 100% WSF recorded severe hepatic damage when compared with control rats. This was evidenced by a marked elevation in the levels of serum marker enzymes: (AST, ALT, ALP) and LDH. The order of increase in experimental rats administered WSF of spent lubricating oil was 10% <50% < 100%.

Histopathological examination of the livers of the control group and the treated groups of rats is recorded in photomicrographs in Figs. 1. Liver sections of rats in control and treated groups administered with different concentrations (10%, 50% and 100%) of WSF of spent lubricating oil are shown respectively in Figs. 1a - d. Results of Histopathological examination showed defects ranging from disorganized cords of hepatocytes with sinusoids, obliteration of sinusoids, fatty change, macrovesicular fatty change and enlargement of hepatocytes in rats treated with different concentrations of WSF of spent lubricating oil. However, normal cords of hepatocytes with sinusoids were observed in the control group. The pH value of the WSF of spent lubricating oil was 6.7 showing that it was slightly acidic.

Figure 1

Table 1: Hepatotoxic effects of WSF of spent lubricating oil on serum AST, ALT, ALP and LDH in wistar albino rats.

Group	AST	ALT	ALP	LDH
Control	78.6±1.29^a	32.8±0.97^a	41.6±0.68^a	4.9±0.38^a
10 % WSF of SLO	88.0±2.53^b	36.8±1.43^b	44.4±0.81^b	5.8±0.12^b
50% WSF of SLO	117.2±8.59^c	44.8±2.60^c	50.4±2.36^c	7.4±0.26^c
100% WSF of SLO	169.4±1.29^d	63.4±1.81^d	65.2±2.73^c	11.9±0.38^d

Values are means ±SEM. n=6 in each group. Means with different superscript letters (a, b, c, d) in the same column are significantly different at the 0.05 level.

Figure 2

Fig. 1a: a section of the rat liver showing normal architecture of control rats

Figure 3

Fig.1b: a section of the rat liver treated with 10% WSF of spent lubricating oil showing disorganized cords of hepatocytes

Figure 4

Fig.1c: a section of the rat liver treated with 50% WSF of spent lubricating oil showing fatty change and obliteration

Figure 5

Fig. 1d: a section of the rat liver treated with 100% WSF of spent lubricating oil showing of sinusoids macrovesicular fatty change and enlargement of hepatocytes

DISCUSSION

Limited information regarding the impact of chronic exposure of terrestrial animals to aqueous hydrocarbons such as WSF of spent lubricating oil was the motive for the present study. Treatment of rats with different concentrations of WSF of spent lubricating oil resulted to a significant hepatic damage in a concentration dependent manner as elicited by the elevated levels of serum marker enzymes (AST, ALT, ALP) and LDH. These marker enzymes are cytoplasmic in origin and are released into the circulation after cellular damage [19, 20]. The rise in the enzyme AST with a corresponding increase in the levels of ALT as observed in this study corroborates the findings of Sallie et al., [21] who observed that the rise in the enzyme AST is usually accompanied by an elevation in the levels of ALT, which plays a vital role in the conversion of amino acids to keto acids. The role of ALT in the conversion of -amino acids to keto acids is also well documented [22]. In the assessment of liver damage, the determination of enzyme levels such as AST, ALT is largely used [23]. Necrosis or membrane damage releases the enzyme into circulation and hence it can be measured in the serum. High levels of AST indicates liver damage, such as that caused by viral hepatitis as well as cardiac infarction and muscle injury, AST catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore ALT is more specific for to the liver, and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative

of cellular leakage and loss of functional integrity of cell membrane in liver [24]. Both AST and ALT levels increase due to toxic compounds affecting the integrity of liver cells [25] , this is in agreement with the findings in this study. It is suggestive that the WSF of spent lubricating oil is hepatotoxic and has adversely affected the integrity of the liver of rats administered different concentrations (10, 50, and 100%) of WSF of spent lubricating oil in this study. Serum ALP on the other hand is a membrane bound glycoprotein enzyme with a high concentration in sinusoids and endothelium. This enzyme reaches the liver mainly from the bone. It is excreted into the bile; therefore its elevation in serum occurs in hepatobiliary diseases [26]. Increase in serum level of ALP has been reported to be due to increased synthesis, in the presence of increasing biliary pressure [27].

The slight acidity of the WSF (pH 6.7) observed in this study is similar to the findings of Ogali et al., [17]. Who reported that the slight acidity of the WSF could probably be due to the high acidity associated with used hydrocarbon-based oils. However, in the course of using lubricating oils for engine operations, the oil ages and deteriorates by oxidation, decomposition, and polymerization. The hydrocarbon matrix of the oil may undergo free-radical autoxidation, forming carboxylic acids, aldehydes, and alcohols [28]. These processes result in increased acidity and formation of deposits that thicken the oil. Several other factors such as high engine temperature, which is characteristic of tropical conditions, are also known to enhance the ageing and acidic characteristics of oils. Such high levels of acidity might pass on to the aqueous phase by the solubilized hydrocarbons. Thus, the more time the used engine oil is likely to reside on the water body, the more acidic its WSF becomes [17]. Therefore, the hepatotoxic effects of WSF of spent lubricating oil as observed in this study could be attributed to the generation of free radicals as a result of autoxidation, decomposition and polymerization.

Histopathological examinations of the liver tissues of the experimental animals (Figs. 1a-d) indicate that exposure to WSF of spent lubricating oil affected the structural integrity of the liver cells. This is characterized by the presence of fatty change, enlargement of hepatocytes, macrovesicular fatty change and obliteration of sinusoids of the liver and disorganized cords of hepatocytes. This implies that the liver is one of the major target organs of aqueous hydrocarbons such as the WSF of spent lubricating oil – induced injury. The cumulative oxidative damage is therefore likely to be one of the underlying mechanisms responsible for the hepatotoxic effects of WSF of spent lubricating oil as observed in this study.

In conclusion, the results of this study suggest that repeated exposure to WSF of spent lubricating oil may elicit hepatotoxicity, thereby impairing the normal function of the liver.

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