Available online at http://www.ifgdg.org

Int. J. Biol. Chem. Sci. 12(2): 650-658, April 2018

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

Hepatotoxic and renal effects of the water soluble fractions of spent engine oil in Swiss albino mice

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ABSTRACT

Spent engine oil (SEO) contains toxic metals which may be leached into water supplies during precipitation. These metals may bioaccumulate during exposure, eliciting adverse effects on the liver and the kidneys, both target organs for several contaminants. This study determined the hepatotoxic and renal effects of the water soluble fractions (WSF) of SEO in mice. The levels of renal function biomarkers (creatinine and urea) and iron, zinc, copper, nickel, lead and cadmium levels in livers of twenty-four male albino mice administered daily oral gavages of 0%, 1%, 10% and 100% WSF of SEO for 30 days were evaluated. Creatinine (0.17±0.06 mg/dl) and urea (22.33±1.53 mg/dl) levels were lowest and highest respectively at 10%, although values were marginal but were not significantly different from control ($p>0.05$). Iron (61150.27±2300.25ug/l), Zn (25942.57±975.86), Cu (7412.20±278.81), and Ni (185.33±6.98ug/l) levels in the liver were significantly elevated ($p<0.05$) at 10%, while lead and cadmium showed dose-dependent but insignificant difference ($p>0.05$) compared with control and other treatments. Iron, zinc, copper and nickel levels at 10% were highest in the treatments. These results provide evidence for the adverse renal and hepatotoxic effects of water contaminated with SEO and highlight the need for its proper management.

Keywords: Hepatotoxicity, creatinine, urea, metal contaminants, waste oil.

INTRODUCTION

Spent Engine Oil (SEO) is lubricating oil that has given its service properties in a combustion engine. It is often disposed indiscriminately into drainages, open vacant plots and farmland by automobile and generator mechanics when the oil is changed during engine servicing and repair (Kayode et al., 2009). Toxic and carcinogenic substances found in the spent oil include polychlorinated biphenyls, chlorodibenzofurans, aliphatic and aromatic hydrocarbons, lubrication additives, decomposition products and heavy metal contaminants. The heavy metals include aluminium, chromium, tin, lead, manganese, cadmium, copper, zinc, iron, silicon and nickel which originate from the wearing down of the engine parts, with lead showing the highest concentration (Vazquez-Duhalt, 1989; Arise et al., 2012). However, the composition of the spent engine oil varies widely depending on factors such as the composition of the original crude oil, the process used during the refining, the efficiency and the type of engine it lubricates, the gasoline combustion products, oil and fuel additives and the length of time the oil remained in the engine (Ekeh et al., 2010).

DOI : https://dx.doi.org/10.4314/ijbcs.v12i2.3

Nigeria accounts for more than 87 million litres of spent engine oil waste annually (Patrick-Iwuanyanwu et al., 2009). Spilled oils in the environment are usually drawn into the soil due to gravity spreading laterally by capillary forces and soil heterogeneity until an impervious horizon such as bedrock, watertight clay or an aquifer is met (Chukwuma et al., 2012). This leads to the contamination of groundwater sources such as wells by the water soluble fraction (WSF) of the oil (Edema, 2012). When taken up by living cells and metabolized, it elicits adverse effects in living systems (Patrick-Iwuanyanwu et al., 2009). Groundwater has been observed as one of the various media by which human beings, plants and animals come into contact with petroleum hydrocarbon pollution (Chukwuma et al., 2012). The biological fate of the chemical components of SEO exposure in living organisms depends on the individual properties of the specific chemicals in the oil (Ujowundu et al., 2012). Heavy metals have been reported to accumulate in the liver and the kidney (Jihen et al., 2008). Some of these metals are extremely toxic to living systems especially when they are found to exceed regulatory limits. For example, cadmium has been reported as one of the most toxic metals in the environment because of its extremely long half-life (Jihen et al., 2008; Karimi et al., 2012). Its toxicity in the kidney and liver has also been reported by Nagano et al. (2000) and Deveci and Deveci (2011).

The occurrence of chronic kidney disease (CKD), a highly prevalent condition, has been escalating in recent years (Owiredu et al., 2012). Exposure to petroleum hydrocarbons and toxic heavy metals is also a risk factor for the impairment of renal function (Azeez et al., 2013). Urea (also known as Blood Urea Nitrogen) and Serum Creatinine are known biomarkers of renal function. Increases in urea in renal failure are caused by an impaired ability to excrete proteinaceous catabolites because of the marked reduction in glomerular filtration rate (GFR) (Robertson and Seguin, 2006). Increases in creatinine are also a result of decreased renal excretion. The aim of this study was therefore to evaluate the hepatotoxic and renal effects of the water soluble fractions of spent engine oil in Swiss albino mice. The specific objectives were to (1) determine the levels of heavy metals (Fe, Zn, Cu, Pb, Cr and Ni) in varying concentrations (0%, 1%, 10% and 100%) of the water soluble fractions of spent engine oil; (2) determine the levels of these metals in the liver of mice exposed to the same concentrations of the water soluble fractions of the waste oil and to (3) determine the level of the renal function biomarkers (Serum creatinine and blood urea nitrogen) in the kidney of mice exposed to the water soluble fractions of spent oil.

MATERIALS AND METHODS

Experimental animals

The experimental animals (twenty-four male albino mice) were procured from the Animal House of Ladoke Akintola University of Technology Teaching Hospital, Osogbo, Nigeria. They were acclimatized for two weeks prior to the commencement of the study (for details on acclimatization conditions, please see Babalola et al., 2016). We also obtained ethical approval from the Animal Use and Care Committee of the Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria before the commencement of the study.

Spent engine oil collection and preparation of the water-soluble fraction (WSF)

Spent Engine Oil was obtained from an automobile mechanic village at Ashi-Bodija, Ibadan. Two samples of two litres each were obtained and homogenized by mixing in equal

proportions of the ratio 1:1. The method described by Patrick-Iwuanyanwu et al., (2009) and adopted by Babalola et al. (2016) was used to prepare the water-soluble fraction (WSF) Babalola et al. (2016).

Preparation and analysis of different concentrations of WSF of spent engine oil

Various concentrations (1%, 10% and 100% of WSF of Spent Engine Oil) were prepared from the stock solution as stated in Babalola et al. (2016). Grab samples of each concentration were analyzed for heavy metals (Pb, Zn, Cd, Ni, Fe, and Cu) content.

Heavy metal analysis of the water soluble fraction

Heavy metal determination of the WSF of SEO was carried out according to the method adopted by Vickackaite et al. (1996). The water soluble fractions of SEO samples were preserved by the addition of 0.1mL concentrated nitric acid (HNO_3) to 1mL of the sample. Standard stock solutions of metals were prepared by dissolving the metals in the diluted nitric acid in a ratio of 1:1. Standard solutions of metals were prepared by dilution of the appropriate standard stock solution with redistilled water just before using. Thereafter, the heavy metals (Lead, Cadmium, Copper, Zinc and Iron) in the WSF of SEO were analyzed (three replicates) using the Atomic Adsorption Spectrophotometer (AAS).

Experimental design: treatment of animals

After the two week acclimatization period, four groups (A - D) of six animals each were constituted from the twenty-four experimental animals. The mice were subjected to treatments of the water soluble fraction of spent engine oil for 30 days and weighed at the commencement and termination of the experiment respectively. Group A mice served as the control and were provided daily with feed and distilled water only. Groups B – D mice were given daily oral gavages of 0.2 ml of 1%, 10% and 100% of WSF of Spent Engine Oil respectively and administered daily with feed and distilled water (Babalola et al., 2016).

Heavy metals analysis of the liver

At the end of the exposure period, the animals were anesthetized with chloroform and sacrificed by cervical dislocation after which the livers were surgically removed. Heavy metal determination in the liver was carried out according to the method adopted by Jihen et al. (2008). The liver samples for heavy metals analysis were oven-dried (60 ^{0}C) to constant weight. The dried samples (100 mg from each sample) were digested with 3 ml of pure nitric acid (HNO_3) at 90 ^{0}C for 24-48 hours. The volumes were then adjusted to 5 ml with deionized water. The heavy metals (lead, cadmium, copper, zinc and iron) in the liver samples were analyzed and estimated using the Atomic Adsorption Spectrophotometer (AAS).

Estimation of blood urea nitrogen and serum creatinine

Blood for renal function biomarkers analysis was collected using the orbital technique (Stone, 1954) into a plain sample bottle. The collected blood sample was allowed to coagulate, centrifuged at 3000 rpm for 10 minutes to obtain serum supernatant for biochemical analysis. Renal function biochemical markers (creatinine and urea) were measured as functional markers for nephrotoxicity. They were assessed using Randox Laboratory (UK) diagnostic kits. Blood urea was determined according to Weatherburn (1967), while serum creatinine was measured according to Henry et al. (1974).

Statistical analysis

Results were expressed as means ± standard deviation and all data were subjected to analysis of variance (ANOVA). Significant differences between the treatment means were expressed at 5% confidence level using the Duncan Multiple Range Tests.

RESULTS

Background analysis of heavy metals

Grab analysis of the treatment samples of the WSF of SEO for the six metals (Fe, Zn, Cu, Pb, Ni and Cd) showed a dose dependent increase in the concentration of the six metals from the control to 10% WSF of SEO followed by a marked decrease at 100% WSF (Table 1). The concentration of Iron (Fe) at control was 0.00µg/L. The range in the treatment was 886.10 µg/L at 100% WSF; 1092.20 µg/L at 1% WSF and 1330.20µg/L in 10% WSF. The concentration of Zinc (Zn) in the control was 3.00µg/L. The treatment ranged from 375 µg/L at 100% WSF; 463.40 µg/L at 1% WSF and 564.30µg/L in 10% WSF. The concentration of Copper (Cu) at control was 0.00µg/L. The treatment ranged from 107.40 µg/L at 100% WSF; 132.40 µg/L at 1% WSF to 161.20µg/L at 10% WSF. The concentration of Nickel (Ni) in the control was 1.20 µg/L. The treatment ranged from 2.70 µg/L at 100% WSF; 1.20 µg/L at 1% WSF and 4.00µg/L at 10% WSF. The concentration of Cadmium (Cd) in the control was 0.10µg/L. The treatment ranged from 0.30 µg/L at 1% WSF and 100% WSF to 0.40µg/L at 10% WSF. The concentration of Lead (Pb) in the control was 0.5 µg/L. The treatment ranged from 0.70 µg/L at 100% WSF; to 0.90 µg/L at 1% WSF and 1.10 µg/L at 10% WSF (Table 1).

Heavy metal accumulation in the liver

The results of the heavy metal accumulation in the liver of mice exposed to the three different concentrations of the WSF of spent engine oil and comparative control for 30 days is presented in Table 2. The results revealed the highest accumulation at 10% WSF of SEO for four metals; namely, Iron (Fe), Zinc (Zn), Copper (Cu) and Nickel (Ni) followed by an abrupt decrease at 100% WSF of SEO. Iron (Fe) was 41355.23±3816.17 µg/L in the control. The treatments ranged from 36766.67±1153.01 µg/L at 100% WSF; 44423.20±3399.69 µg/L at 1% WSF to 61150.27±2300.25 µg/L at 10% WSF. Zinc (Zn) was 17544.63±1619.17 µg/L in the control. The treatments ranged from 17170.00±865.33 µg/L at 100% WSF; 18846.23±1442.30 µg/L at 1% WSF to 25942.57±975.86 µg/L at 10% WSF. Copper (Cu) was 5012.77±462.62 µg/L in the control. The treatments ranged from 5352.33±571.34 µg/L at 100% WSF; 5384.63±412.07 µg/L at 1% WSF to 7412.20±278.81 µg/L at 10% WSF. Nickel (Ni) was 125.33±11.56 µg/L in the control. The treatments ranged from 108.57±12.52 µg/L at 100%; 134.60±10.28 µg/L at 1% WSF to 185.33±6.98 µg/L at 10% WSF. Statistical analysis however revealed that there were significant differences ($p<0.05$) from the control for all the four metals (Iron, Zinc, Copper and Nickel) at 10% WSF.

A critical look at the heavy metal levels in the liver of the exposed mice showed a dose dependent increase in the concentration of lead (Pb) and cadmium (Cd). Lead (Pb) was 34.83±3.20µg/L in the control. The range in the treatment was from 37.40±2.86 µg/L at 1% WSF; 51.50±1.95 µg/L at 10% WSF to 138.00±131.94 µg/L at 100% WSF. Cadmium was 12.07±1.10 µg/L in the control. The treatment ranged from 12.93±1.00 µg/L at 1% WSF; 17.83±0.68 µg/L at 10% WSF to 40.33±50.82 µg/L at 100% WSF. The increases, however, were not statistically significant ($p>0.05$) when compared with the control (Table 2).

Renal function biomakers

The results of the renal function biomarkers, serum creatinine and blood urea nitrogen (BUN) of mice exposed to three concentrations (1%, 10% and 100%) of 0.2 ml of WSF of Spent engine oil for 30 days are presented in Table 3.

Serum creatinine was 0.20±0.00 mg/dl in the control. The treatments ranged from 0.17±0.06 mg/dl at 10% WSF; 0.23±0.12 mg/dl at 1% WSF to 0.27±0.12 mg/dl at 100% WSF (Table 3). There was no significant difference ($p>0.05$) from the control in the treatment groups.

Urea was 21.67±0.57 mg/dl in the control group. The treatment values ranged from 21.33±1.53 mg/dl at 100% WSF; 21.67±1.53 mg/dl at 1% WSF to 22.33±1.53 mg/dl at 10% WSF (Table 3). There was no significant difference ($p>0.05$) from the control in the treatment groups.

Table 1: Heavy metals (iron, zinc, copper, lead, nickel and cadmium) accumulation in grab water samples of the WSF of SEO.

SAMPLE	Fe (µg/L)	Zn (µg/L)	Cu (µg/L)	Pb (µg/L)	Ni (µg/L)	Cd (µg/L)
CONTROL	0.00	3.00	0.00	0.50	1.20	0.10
1% WSF	1092.20	463.40	132.40	0.90	1.20	0.30
10% WSF	1330.20	564.30	161.20	1.10	4.00	0.40
100% WSF	886.10	375.00	107.40	0.70	2.70	0.30

Table 2: Heavy metals (iron, zinc, copper, lead, nickel and cadmium) accumulation in liver samples of mice exposed to WSF of SEO.

SAMPLE	Fe (µg/L)	Zn (µg/L)	Cu (µg/L)	Pb (µg/L)	Ni (µg/L)	Cd (µg/L)
CONTROL	41355.23 ± 3816.67^{bc}	17544.63 ± 1619.17^{b}	5012.77 ± 462.62^{b}	34.83 ± 3.20^{ab}	125.33 ± 11.56^{dc}	12.07 ± 1.10^{a}
1% WSF	44423.20 ± 3399.69^{b}	18846.23 ± 1442.30^{b}	5384.63 ± 412.07^{b}	37.40 ± 2.86^{ab}	134.60 ± 10.28^{c}	12.93 ± 1.00^{a}
10% WSF	61150.27 ± 2300.25^{a}	25942.57 ± 975.86^{a}	7412.20 ± 278.81^{a}	51.50 ± 1.95^{ab}	185.33 ± 6.98^{ab}	17.83 ± 0.68^{a}
100% WSF	36766.67 ± 1153.01^{c}	17170.00 ± 865.33^{b}	5352.33 ± 571.34^{b}	138.00 ± 131.94^{a}	108.57 ± 12.52^{d}	40.33 ± 50.82^{a}

(Means with the same letter are not significantly different ($p>0.05$).

Table 3: Levels of renal function biomarkers in mice exposed to WSF of SEO.

SAMPLE	Creatinine (mg/dl)	Bun (mg/dl)
CONTROL	0.20 ± 0.00^{a}	21.67 ± 0.57^{b}
1% WSF	0.23 ± 0.12^{a}	21.67 ± 1.53^{b}
10% WSF	0.17 ± 0.06^{a}	22.33 ± 1.53^{b}
100% WSF	0.27 ± 0.12^{a}	21.33 ± 1.53^{b}

(Means with the same letter are not significantly different ($p>0.05$).

DISCUSSION

The elemental analysis of the water soluble fraction of spent engine oil in this study confirmed the presence of toxic heavy metals with the highest levels of the metals at 10% SEO. This was also reflected in the pattern of accumulation of these metals in the liver of the exposed mice with the highest levels observed at 10% SEO and corroborated with reduced levels of serum creatinine and highest levels of urea also at 10% SEO. The reason for the increased levels of metal contaminants at 10% SEO is unclear. However, it may be reflective of the diverse, complex and often unpredictable nature of the effects of metal contaminants (Taylor and Maher, 2010). Heavy metals are known to primarily accumulate in the liver (Jihen et al., 2008). Accumulation occurs when internal dose exceeds detoxification and excretion capacity such that the accumulated metal is thus bioavailable to play a toxic role (Taylor and Maher, 2010). This perhaps explains the reduction in serum creatinine and elevated urea levels observed at 10% SEO. Although it was not statistically significant, this could be due to the short exposure period of 30 days which may have been insufficient for the toxic effects observed to be pronounced.

Metals have various levels of toxicity when present above acceptable levels in biological systems, although some may serve as cofactor or activators of enzymes (Goyer and Clarkson, 2001). Lead and Cadmium have been implicated in carcinogenesis (De Zwart and Slooff, 1987), suggesting that the water soluble fraction of spent engine oil may possess certain degree of deleterious effects. Zinc is one of the most important trace elements in the body and participates in the biological function of several proteins and enzymes (Maity et al., 2008). However, it is toxic to most organisms at higher doses (Gathwan et al., 2012). Iron has been said to be of little toxicological significance (Arise et al., 2012) because it is part of the essential elements that play important roles as prosthetic groups in enzymes and key metabolic activities in living organisms (Osuala et al., 2014). Signs of hepato and renal toxicities have been observed in animals as a result of exposure to Nickel including symptoms such as lethargy, ataxia, hypothermia, salivation, diarrhea, headache, vertigo, nausea, vomiting, insomnia and irritability (Das et al., 2008). Heavy metals are also known to be toxic to the kidney (Das et al., 2008; Jihen et al., 2008). This may lead to the impairment of the proper functioning of the kidney reflected in elevated levels of renal function biomarkers (Kumar et al., 2005). The kidney has also been reported as the primary organ of cadmium toxicity especially following chronic exposure (European Commission, 2002; Hu, 2002).

This study shows that serum Creatinine was highest at 100% WSF but lowest at 10% WSF compared with the control (Table 3). Urea had the highest value at 10% WSF compared with the control (Table 3). This was probably due to Nickel toxicity in the WSF of SEO as inferred from Das et al. (2008) who observed a decrease in serum urea due to Nickel. The increases observed, though not significant ($p>0.05$) may be due to the short exposure period and suggest that long-term exposure to the water soluble fraction of the spent oil may affect renal function over time. Furthermore, the lowest level of serum creatinine and the highest level of urea observed at 10% WSF may be due to the consistent high level of all metals at 10% WSF in the background analysis. Thus, it may be inferred that high level of heavy metals may suppress creatinine levels and increase urea levels in the blood.

Creatinine is a more reliable indicator of renal dysfunction compared with the blood urea nitrogen (BUN) because many extra-renal factors may influence urea concentration (Robertson and Seguin, 2006). Elevated levels of creatinine in animals exposed to petroleum hydrocarbons have been reported by several authors (Egwurugwu et al., 2013; Okoye et al., 2014). Orisakwe et al. (2004) also reported an increase in serum concentrations of

creatinine and potassium with degeneration and necrosis of glomeruli in rats treated with crude oil. Serum creatinine and urea concentrations are biomarkers of renal injury (Alimba et al., 2012) and the elevation of these biomarkers is usually associated with impairment of renal function (Kumar et al., 2005).

Increase in creatinine in WSF of SEO treated mice compared to the control may indicate kidney injury due to the depression of glomerular filtration rate and renal tubular cell injury by Cd (Hu, 2002) and other heavy metals analyzed in the WSF of SEO. Heavy metals also readily bioaccumulate in the kidney and are responsible for a high number of nephrotoxicity observed in mammals (Matos et al., 2010). It may be deduced from these findings that the possible nephrotoxicity observed in mice exposed to WSF of SEO may be associated with free radical formation. This may be as a result of the activities of heavy metals analyzed in this study including unanalyzed contaminants which may exhibit their damaging effects through the formation of reactive oxygen species (Alimba et al., 2012).

Conclusion

This study has provided evidence on the hepatotoxic and renal effects of exposure to the water soluble fraction of spent engine oil. Iron, copper, zinc and nickel showed elevated levels in mice liver owing to exposure to the water soluble fraction of the spent oil. It has also shown that spent engine oil contamination may elicit renal damage as shown from the levels of biomarkers of renal dysfunction: creatinine and urea. These portend great health risk to both human and animal populations who are constantly exposed to waste oil in the environment. Therefore, adequate measures should be taken to ensure proper disposal of waste engine oil.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

AAO conceptualized and supervised the study as well as managed the corrections of the manuscript while SOB carried out the experimental aspect and prepared the first draft of the manuscript.

ACKNOWLEDGEMENTS

The authors hereby acknowledge Mr. Akinloye Oyewole who participated in the field and experimental work. Also, Mr. Biodun Opaleye of IITA who assisted us in getting some of the laboratory facilities used for this research and Dr. Henry Osaiyuwu of the Department of Animal Science, University of Ibadan who gave professional assistance in the statistical analysis.

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