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***Original Paper***

# ABNORMAL PRESENTATION OF SERUM MICRO-NUTRIENTS IN WISTAR RATS: CONSEQUENCE OF VIRGIN ENGINE OIL EXPOSURE

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Very many harmful practices are found in Africa. The use of engine oil for various therapeutic and cosmetics purposes, is one of them. Petroleum products are made up of toxic components; therefore the effects of virgin oil on micro-nutrient levels are being investigated. 30 rats divided equally into 5 groups were named GROUPS 1-5. GROUP 1, GROUP 2, GROUP 3, and GROUP 4 were treated with 0.5, 0.5, 1.0, and 1.0 mg/kg body weight of engine oil respectively. GROUP 1 and GROUP 3 were administered by oral-route while GROUP 2 and GROUP 4 were dosed through the dermal-route. GROUP 5 served as the control. On the 31st day, blood samples were obtained from the rats, centrifuged and the resultant serum stored at - 20ºC until the time they were utilized for analysis of vitamins (High Performance Liquid Chromatography); as well as Mg and trace elements (Atomic Absorption Spectrometry). $P \leq 0.05$ was considered significant. Significant differences were observed for folic acid, Mg, Mn, and Zn as well as vitamins A, C, E, and D irrespective of dose and route of administration. These results suggest that exposure to engine oil can alter micro-nutrient metabolism.

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## INTRODUCTION

Petroleum products constitute significant hazard to the environment and exposure to their hydrocarbon components equally constitute significant threats to human health.[1] Refined fractions of crude oil contain aliphatic, aromatic, and various branched saturated and unsaturated hydrocarbons of different levels.[2,3] The domestic and industrial importance of petroleum, especially the refined forms cannot be overemphasized. Because it is so widely employed for many purposes, most common routes of contact remain through inhalation, dermal contact, and ingestion of petroleum-contaminated food and water. While exposure for occupational reasons are diverse, contact for therapeutic reasons e.g. snake poison antidote, anti-sore throat agent, remain another veritable source of concern. The presence of aliphatic and aromatic hydrocarbons which are the major components of petroleum products and their consequent metabolism result in generation of free radical species in various tissues.[4]

In Nigeria, the need for a study of this nature cannot be divorced from the fact that human contact with refined fractions of crude oil is increasing as a result of incessant oil spills and proliferation of sales outlet. The study of Ita and Udofia[4] revealed the devastating effect of refined products such as gasoline, kerosene and diesel on hematological markers. From the result of that study, it was established that Red Blood Cell (RBC), Packed Cell Volume (PCV) and Hemoglobin (Hb) content were significantly lower in petrol, kerosene and diesel than the control group. Other hematological indices like lymphocytes, neutrophils and eosinophil were also grossly abnormal in refined product exposed rats.

While results from many studies have suggested that exposure to petroleum hydrocarbons can result in cardiotoxicity[5,6] ; hepatotoxicity[7,8] ; nephrotoxicity[3,9] and hematoxicity[10], there is dearth of data on impact of virgin engine oil on micro-nutrient metabolism. The objective of this study is to establish whether incessant exposure to virgin engine oil may be a cause of serum micro-nutrient alterations in a mammalian species.

## MATERIAL AND METHODS

**Material:** The AP engine oil used for the study was purchased in Osogbo, Osun State, Nigeria in December, 2011.

**Animals and Animal Care:** This study was carried out in conformity with National and International Laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research; as promulgated and adopted by United States Institutes of Health (1985). The animal experiment study was carried out at the Animal House of the Experimental Animal Unit of the Faculty of Veterinary Medicine, University of Ibadan, from where the animals were obtained. Thirty female Wistar rats which had weight range of 200-240 g were left to acclimatize for two weeks prior to commencement of the experiment. Animals were housed in cages at ambient temperature of 23±3°C and a 12 h light, 12 h dark cycle. All the animals were fed standard laboratory diet and given water *ad libitum*.

**Experimental Design:** The experimental animals were divided into 5 groups consisting of 6 rats per group, and named GROUP 1, GROUP 2, GROUP 3, GROUP 4, and GROUP 5. GROUP 1, GROUP 2, GROUP 3, and GROUP 4 were administered with 0.5, 0.5, 1.0, and 1.0 mg/kg body weight of engine oil respectively. The route of administration for GROUP 1 and GROUP 3 was by oral route while GROUP 2 and GROUP 4 were dosed through the dermal route. GROUP 5 served as the control. Those in oral route received the test agent as contaminant of feed, in which engine oil was freshly and thoroughly mixed with feed every

morning before being supplied to the rats. On the other hand, dermal exposure occurs each day through application of engine oil to the neck region to prevent oral contact. Treatment lasted for a period of 30 days.

Blood samples were obtained from the rats between 11: 00 and 13:00, on the 31st day, bleeding was through the retro-orbital plexus. The samples obtained were dispensed into anti-coagulant free bottle, and subjected to centrifugation at 3000 g for ten minutes. The serum obtained was stored at - 20ºC until required for analysis.

**Estimation of serum micro-nutrients:** Estimation of the serum levels of vitamins, i.e. folic acid, thiamine, niacin, riboflavin, and vitamins A, B12, C, D and E were carried out by High Performance Liquid Chromatographic (HPLC) technique. The HPLC equipment supplied by Waters® Corporation (Milford, MA, USA) was used for this purpose. In addition, the columns used for the analysis were also supplied by Waters® Corporation. On the other hand, serum levels of Zn, Cu, Se, Mn, Mg, Co, Cr, Fe and Mo were determined by the Atomic Absorption Spectrometric method. Buck Scientific 205 Atomic Absorption used for mineral estimation was obtained from Buck Scientific, East Norwalk, Connecticut, USA.

**Statistical analysis:** The mean values of the serum levels of the vitamins and minerals for control and each of the treatment groups were compared using the analysis of variance (ANOVA). Value of $P \leq 0.05$ was considered significant.

## RESULTS

The results of the study are presented in **Tables 1-5**. Administration of engine oil to rats caused significant decreases ($p<0.05$) in the serum levels of Zn, Cu, Mn, and Se at 0.5 and 1.0 mL/kg body weight (BW) for oral route. For dermal route significant decreases ($p<0.05$) were recorded for these elements also at 1.0 mL/kg BW, but only Zn and Mn were significantly decreases ($p<0.05$) at 0.5 mL/kg BW, while Cu and Se were not significantly different (**Table 1**). Of all the elements presented in **Table 2** only serum levels of Fe was not significantly different ($p>0.05$) at both dosage levels for oral exposed rats, Mg, Cr, Mo, and Co were significantly decreased ($p<0.05$). On the other hand, for dermal route, while Mg was significantly reduced ($p<0.05$) for 0.5 and 1.0 mL/kg BW, Cr and Mo were not significantly different ($p>0.05$) at both dosage levels. Serum concentrations of vitamins A, C and E were significantly reduced ($p<0.05$) at 0.5 and 1.0 ml/kg BW for both dermal and orally administered rats (**Table 3**). In **Tables 4 and 5** folic acid, niacin, riboflavin, pyridoxine, thiamine and vitamin D were significantly different ($p<0.05$) for oral route at 0.5 mL/kg BW, only folic, thiamine and vitamin D were significantly different ($p<0.05$) for dermal route. Niacin, riboflavin, pyridoxine were not significantly different. Serum levels of folic acid, niacin, riboflavin, pyridoxine, thiamine and vitamin D were significantly different ($p<0.05$) for both oral and dermal routes irrespective of the dosage levels except thiamine and vitamin D that were not significantly different ($p>0.05$) for dermal route. Result of ANOVA showed that intergroup comparison between control, oral and dermal routes revealed significance for all except Cu at 0.05 mL/kg (**Table 1**); and Fe at both dosage levels (**Table 2**)

**Table 1**: Serum levels of zinc, copper, selenium and manganese in rats administered with engine oil at two dosage levels.

| | 0.5 mL/kg body weight | | | | 1.0 mL/kg body weight | | | |
|---|---|---|---|---|---|---|---|---|
| | Zn (µmol/L) ǂ | Cu (µmol/L) | Se (µmol/L) ǂ | Mn (nmol/L) ǂ | Zn (µmol/L) ǂ | Cu (µmol/L) ǂ | Se (µmol/L) ǂ | Mn (nmol/L) ǂ |
| Control | 22.80±2.06 | 19.00±2.38 | 1.82±0.21 | 14.78±1.27 | 22.80±2.06 | 19.00±3.38 | 1.82±0.21 | 14.78±1.27 |
| Oral Route | 16.50±1.73* | 18.14±1.25 | 1.61±0.24* | 12.00±0.74* | 12.71±1.83* | 14.02±1.27* | 1.05±0.22* | 9.32±1.44* |
| Dermal Route | 19.18±1.90* | 20.43±0.89 | 1.70±0.30 | 12.05±0.66* | 16.54±1.92* | 17.30±1.91* | 1.54±0.17* | 9.04±0.82* |

*Results are expressed as mean ± standard deviation. *p <0.05 is significant when compared with control using Student's t test. ǂP < 0.05 when control, dermal and oral groups were compared using ANOVA, n=6.*

**Table 2:** Serum iron, magnesium, chromium, molybdenum and cobalt in rats administered with trace quantity of engine oil at two dosage levels.

| | 0.5 mL/kg body weight | | | | | 1.0 mL/kg body weight | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Fe (µg/dl) | Mg (mmol/L) ǂ | Cr (nmol/L) ǂ | Mo nmol/L) ǂ | Co (nmol/L) ǂ | Fe (µg/dl) | Mg (mmol/L) ǂ | Cr (nmol/L) ǂ | Mo (nmol/L) | Co (nmol/L) ǂ |
| Control | 108.84±8.53 | 1.06±0.11 | 214.02±9.47 | 9.58±1.33 | 5.91±0.63 | 108.84±8.53 | 1.06±0.11 | 214.02±9.47 | 9.58±1.33 | 5.91±0.63 |
| Oral | 109.66±7.24 | 0.80±0.11* | 190±7.33* | 8.35±1.11* | 4.24±0.55* | 102.73±7.08 | 0.88±0.14* | 181.76±6.75* | 9.00±1.05* | 4.18±0.75* |
| Dermal | 110.19±10.57 | 0.91±0.09* | 215.47±6.87 | 10.05±0.93 | 5.88±0.38 | 106.74±5.32 | 0.94±0.08* | 218.90±8.81 | 8.93±1.20 | 4.99±0.27* |

*Results are expressed as mean ± standard deviation. *p <0.05 is significant when compared with control using Student's t test. ǂP < 0.05 when control, dermal and oral groups were compared using ANOVA, n=6.*

**Table 3:** Serum levels of antioxidant vitamins in engine oil administered rats at two dosage levels.

| | 0.5 mL/kg body weight | | | 1.0 mL/kg body weight | | |
|---|---|---|---|---|---|---|
| | Vitamin A (µmol/L) ǂ | Vitamin C (mmol/L) ǂ | Vitamin E (µmol/L) ǂ | Vitamin A (µmol/L) ǂ | Vitamin C (mmol/L) ǂ | Vitamin E (µmol/L) ǂ |
| Control | 2.21±0.21 | 66.01±3.98 | 26.16±1.62 | 2.21±0.21 | 66.01±3.98 | 26.16±1.62 |
| Oral | 1.71±0.18* | 52.13±3.42* | 20.35±2.13* | 1.69±0.38* | 40.48±2.14* | 16.11±1.19* |
| Dermal | 1.94±0.15* | 57.08±5.56* | 19.66±1.73* | 1.88±0.22* | 50.14±2.72* | 18.49±1.96* |

*Results are expressed as mean ± standard deviation. *p <0.05 is significant when compared with control using Student's t test. ǂP < 0.05 when control, dermal and oral groups were compared using ANOVA, n=6.*

**Table 4:** Serum vitamin levels in rats administered with trace quantity of engine oil at 0.5 mL/kg body weight.

| | Riboflavin (nmol/L) ǂ | Folic (nmol/L) ǂ | Niacin (nmol/L) ǂ | Thiamine (nmol/L) ǂ | Pyridoxine (nmol/L) ǂ | Vitamin D (nmol/L) ǂ |
|---|---|---|---|---|---|---|
| control | 739.71±20.56 | 14.20±2.59 | 64.44±5.87 | 130.91±18.93 | 90.01±11.80 | 136.38±14.75 |
| Oral | 715.62±16.90* | 11.27±0.53* | 53.99±3.73* | 119.81±4.63* | 79.33±1.25* | 129.50±3.71* |
| Dermal | 722. 08±11.47 | 13.20±1.20* | 63.24±4.25 | 120.18±2.99* | 90.85±1.21 | 126±10.49* |

*Results are expressed as mean ± standard deviation. *p <0.05 is significant when compared with control using Student's t test. ǂP < 0.05 when control, dermal and oral groups were compared using ANOVA, n=6.*

**Table 5:** Serum vitamin levels in rats administered with trace quantity of engine oil at 1.0 mL/kg body weight.

| | Riboflavin (nmol/L) ǂ | Folic (nmol/L) ǂ | Niacin (nmol/L) ǂ | Thiamine (nmol/L) ǂ | Pyridoxine (nmol/L) ǂ | Vitamin D (nmol/L) ǂ |
|---|---|---|---|---|---|---|
| control | 739.71±20.56 | 14.20±2.59 | 64.44±5.87 | 130.91±18.93 | 90.01±11.80 | 136.38±14.75 |
| Oral | 662.13±17.16 * | 12.49±0.99 * | 55.19±3.76 * | 115.58±10.58 * | 77.62±5.13* | 122.48±8.21* |
| Dermal | 671. 83±15.92* | 11.51±1.38 * | 53.00±5.43 * | 131.44±10.66 | 83.06±12.01* | 130.39±7.35 |

*Results are expressed as mean ± deviation. *p <0.05 is significant when compared with control using Student's t test. ǂP < 0.05 when control, dermal and oral groups were compared using ANOVA, n=6.*

## DISCUSSION

Petroleum products used for a variety of purposes have been established to induce toxicity in innumerable ways if ingested by man or experimental animals. For example the hepato-nephrotoxic effects of agents like kerosene and petrol are well documented.[4,11,12] Serum micro-nutrient alterations as a result of exposure to xenobiotics in many mammalian species have also been noted. Earlier studies such as that of Iyanda[13] have identified that kerosene; another petroleum product caused decreases in the levels of Fe, Mo, Co, Mn and Mg as well as significant increase in the level of Cr irrespective of the route of administration. Although the results of the same study[13] showed that Mo and Mn were not significantly different in rats dosed through the dermal route.

The result of the present study revealed significant differences in a wider array of micro-nutrients. The slight difference in the results obtained for the kerosene study[13] and the present one on engine oil may not be unconnected with differences in their constituents which have the likely effect of modulating the rate of absorption. Since it is known that the rate of absorption varies with concentration of the chemical at the absorbing surface, which is related with degree of exposure and the dissolution of the chemical.

The slight difference in the micro-nutrient presentations of oral route compared with dermal route may be ascribed to differences in morphology of cells at sites of petroleum application in both sets of experimental animals. Lehman-McKeeman[14] identified that the rate of toxicant absorption is determined by the area/site of application, as it relates to the peculiarity of the epithelial layer through which absorption takes place (e.g., the thickness of the stratum corneum in the skin), the type of the subepithelial microcirculation, and the physicochemical properties of the toxicant. All these therefore may help in modulating micro-nutrient metabolism.

In addition, it should also not be surprising that the results of rats in dermal-exposed groups are different from orally administered ones because in the course of transfer from the site of

exposure to the systemic circulation, toxicants may be eliminated. This may be the basis of the differences in micro-nutrient presentation of dermal and oral route. Pre-systemic elimination is not uncommon with chemicals administered orally and absorbed from the gastrointestinal (GI) tract because they must first be transported through the GI mucosal cells, liver, and lung before being distributed to the rest of the body (target tissue) by the systemic circulation. Agents like ethanol, cyclosporine, and morphine have been identified to be so affected, in which the GI mucosa and the liver remove a significant portion of a toxicant during its passage through these tissues, thereby reducing its systemic availability.

According to Lim *et al.*[15], ethanol is known to be oxidized by alcohol dehydrogenase in the gastric mucosa, whereas Lin *et al.*[16] have identified that cyclosporine is re-transferred from the enterocyte into the intestinal lumen by P-glycoprotein (an ATP-dependent xenobiotic transporter) and is also hydroxylated by cytochrome P450 (CYP3A4) in these cells. Morphine on the other hand is glucuronidated in intestinal mucosa and liver, and manganese is taken up from the portal blood into liver and excreted into bile. The type of processing may hinder a considerable quantity of chemicals from reaching the systemic blood. This seems not to have occurred with orally administered engine oil as the level of micro-nutrients were generally lower in engine oil orally administered rats compared with control. Signifying that high level of this petroleum product was made available to the systemic that induced free radical generation.

What then is the implication of these significant alterations? Micro-nutrients such as vitamins A, C and E are well known antioxidants whereas zinc, copper, manganese and selenium are co-factors for anti-oxidant enzymes (superoxide dismutase and glutathione peroxidase). These in association with other members of antioxidant defence system maintain a balance between the rate of free radical generation and elimination. Since inadequate elimination process can results in oxidation of cellular biomolecule leading to structural and cellular alterations of vital organs. Deficiency of components of antioxidant defence system has been linked with various pathological conditions. The irony of this is that for those humans who are exposed to petroleum products for therapeutic reasons due to harsh financial conditions, they may be prone to oxidative stress-induced diseases as this study reveals that engine oil irrespective of exposure route can lead to micro-nutrient deficiency.

While a possible link between virgin engine oil exposure and micro-nutrient alteration has been established through this study; and a possible virgin engine oil exposure and increase in free radical generation that may cause pathological conditions is speculative, data are available that suggest that exposure to petroleum product can result in abnormality in hematological indices.[17] According to Ita and Idofia[4] Red Blood Cell (RBC), Packed Cell Volume (PCV) and Haemoglobin (Hb) content were significantly lower with petrol, kerosene and diesel exposure than the control group. To give credence to the role of free radical in micro-nutrient depletion observed in this study is the fact that all nutrients with antioxidant properties such as Zn, Cu, Mn, Se, vitamins A, C, and E were significantly decreased irrespective of route of administration. While iron is known for its vital role in hemoglobin formation, it was not significantly different, but cobalamin and folic acid were significantly decreased. These two are vital ingredients in the process of erythropoesis.

## CONCLUSION

These results of significant differences in level of many of the vitamins that possess anti-oxidant properties and minerals that are vital components of antioxidant defence system, means that exposure to engine even at low level may predispose to various disorders associated with depleted vitamin and mineral levels. This should be of great concern because many of those that use engine oil for therapeutic or cosmetic purpose in the impoverished parts of the developing world do so for lack of adequate financial resources. Poverty is also a major cause of malnutrition worldwide; this means that when both (engine oil exposure and malnutrition) co-exist in an individual, the micro-nutrient depletion that is common to either of them will be markedly aggravated.

**Conflict of interest: nil**

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