Comparative Effect of Vitamins A and E on Gasoline Vapours-Induced Haematotoxicity and Weight-Loss in Male Rats

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Abstract: Comparative effect of vitamins A and E on gasoline vapours haematotoxicity, growth-depression and weight-loss was assessed in male Wistar albino rats. The rats were exposed to gasoline vapours (17.8±2.6 cm³/h/m³/day), 6 h/day, 6 days/week for 20 weeks. Vitamins A (retinol) and E(α-tocopherol) at prophylactic dosage (400 and 200 IU/kg/day, respectively) were orally administered to the rats separately, in the last 2 weeks of exposure. The levels of haemoglobin (Hb), haematocrit or Packed Cell Volume (PCV), Red Blood Cells (RBC), growth-rate and weight-gain in the rats exposed to the vapours were significantly lower (p<0.05) compared, respectively to the levels obtained for control rats. On the other hand, the levels of White Blood Cells (WBC) in the test rats were significantly higher (p<0.05) compared, respectively with the levels obtained for male control rats. These observations indicate that exposure to gasoline vapours may cause haematotoxicity, growth-depression and weight-loss in male rats. However, administration of vitamins A and E was observed to produce a significant recovery (p<0.05) in haematotoxicity, growth-depression and weight-loss observed to be associated with exposure to gasoline vapours, although, the rats administered with vitamin E were noted to respond more favourably than those administered with vitamin A. This suggests that although retinol and α-tocopherol may be used to reverse or prevent haematotoxicity, growth-depression and weight-loss in subjects exposed to gasoline vapours, the reversal potency of α-tocopherol is higher than that of retinol.

Key words: Gasoline vapours, haematotoxicity, retinol, α-tocopherol

INTRODUCTION

Growth-depression, weight-loss and haematotoxicity have been reported to be associated with exposure to gasoline vapours (Uboh et al., 2008). Gasoline is one of the fractionated products of crude oil. It is widely used as fuels for automobiles and some electricity generating machines. Liquid gasoline is known to be very volatile, with several organic and inorganic constituents. Gasoline vapours may be derived from direct evaporation of liquid gasoline. These vapours are ubiquitous in the environment and constitute some components of petroleum pollutants in the air. Exposures to these pollutants are common in the refineries, oil fields, refueling stations, petrochemical industries, motor mechanical workshops and traffic-congested areas. However, the population at greater risk of frequent exposure includes those occupationally exposed, as well as those residing in traffic-congested areas. It has been reported that the oil drillers, refinery workers, petrochemical workers, refuel station attendants and motor mechanics suffer the greater risk of chronic exposures to petroleum pollutants (EHC, 1982, Carballo et al., 1994, Rabbie and Wong, 1996).

According to Zahlsen et al. (1993), more saturated hydrocarbons than unsaturated aromatic hydrocarbons are found in human and animal blood after inhalation exposure to equal concentrations. Some of the gasoline vapours' constituents (such as alkanes, benzenes, tetraethyl lead and xylene) have been reported to be haematotoxic in humans and experimental animals (D'Azevedo et al., 1996, Rothman et al., 1996; Synder and Hedli, 1996). Also, the recent studies showed that exposure to composite constituents of kerosene and gasoline (petrol) vapours (fumes) weight-loss and haematotoxicity in Wistar albino rats (Uboh et al., 2005, 2008). Also, the levels of total white blood cells, absolute lymphocyte counts, platelets, red blood cells and haematoctrit have been reported to be reduced among workers heavily exposed to benzene (Rothman et al., 1996).

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In the recent times, the major concern of the environmental and biochemical toxicologists has been how to devise measures that can abate or reverse the adverse effects associated with exposure to environmental pollutants. There is paucity of information on the ways of ameliorating the weight loss and haematotoxic effects reported to be associated with inhalation exposure to gasoline vapours. Since, the toxicity effect associated with exposure to gasoline vapours’ constituents may be an indication of tissue, or tissue components-reactive metabolite species interactions in the body, it is believed that the presence of antioxidants may ameliorate the toxicity effect. Some antioxidants are naturally present in the body, while others have to be provided as micronutrients. Among the antioxidants that has attracted the attention of researchers in the recent times include vitamins A and E. In the recent study, it was observed that vitamin A enhanced the reversal of haematotoxic effect as well as the regain of weight-loss and growth-depression associated with exposure to gasoline vapours in male and female rats (Uboh et al., 2008). Hence, among the various biochemical functions of vitamins A and E, their antioxidative and protective role has attracted more investigations in the recent times (Lotan, 1999; Knert et al., 1999; Marcus and Coulston, 1996). Retinol, alpha tocopherol and the related compounds have been reported to possess apparent ability to interfere with some chemical reactions that may potentiate carcinogenesis (Jialal and Grundy, 1993) Administration of retinol and other retinoids to animals is reported to delay arrest and even reverse progression of premalignant cells and malignant characteristics.

Vitamin A is obtained from β-carotene and it belongs to retinoid family. It exists in several chemical forms, such as retinol, retinoic acid and retinal. Intercoversion among these chemical forms readily occur in the body. Vitamin A is also present as a retinyl ester in the tissues of animals. Among the various biochemical functions of the vitamin A, its antioxidative and protective role has attracted more investigations in the recent times (Lotan, 1999; Knert et al., 1999). Retinol and the related compounds are reported to possess apparent ability to interfere with carcinogenesis. Administration of retinol and other retinoids to animals is reported to delay, arrest and even reverse progression of premalignant cells and malignant characteristics. Vitamin E is another important fat-soluble antioxidant vitamin. There are about eight naturally occurring tocopherols with vitamin E activity. Among these, α-tocopherol is considered to be the most important tocopherol, since it has been reported to constitute about 90% of the tocopherols in animal tissues and displays the greatest biological activity in bioassay systems (Marcus and Coulston, 1996). The α-tocopherol is observed to the major lipid soluble antioxidant vitamin protecting membranes and lipoproteins from injury by free radicals. Also, vitamin E have been shown to reduce the oxidative susceptibility of lipoproteins and may have antiatherosclerotic effects (Jialal and Grundy, 1993; Reaven et al., 1993; Verlangieri and Bush, 1992). In hypercholesterolemic animal models, vitamin E was reported to have increased resistance to lipoprotein oxidation and preserved normal endothelial function (Andersson et al., 1994). Moreover, the epidemiologic studies also indicate an inverse association between the intake of vitamin E and coronary heart disease (Kushi et al., 1996; Heitzer et al., 1999). Hence, this study aimed at assessing the effect(s) of vitamins A and E on haematotoxic effect, growth-depression and weight loss associated with exposure to gasoline vapours in male rats.

MATERIALS AND METHODS

Experimental animals: A study on the effect of vitamins A and E on gasoline vapours-induced haematotoxicity and weight-loss in male rats was carried out from July to November, 2008 in the Department of Biochemistry, University of Calabar, Calabar, Nigeria.

Thirty two matured male Wistar albino rats weighing 160.2±10.1 g were obtained from the animal house of the College of Medical Sciences, University of Calabar, Calabar-Nigeria and used for this study. The rats were divided into four groups with eight rats each, as follows:

- **Group I:** Normal control group, without exposure to gasoline vapours
- **Group II:** Experimental test control group, exposed to gasoline vapours only
- **Group III:** Test group 1, concomitantly administered with vitamin A daily for the last 2 weeks of the exposure
- **Group IV:** Test group 2, concomitantly treated with vitamin E daily for the last 2 weeks of the exposure

The rats were acclimatized in the experimental animal house for 1 week before the commencement of the experiment. The animals, housed in stainless steel cages, were fed with the normal rat pellets. All the rats in both test and control groups were allowed free access to food and water throughout the experimental period. All animal experiments were carried out in accordance with the guidelines of the Institutional Animal Ethics Committee.

Exposure to gasoline vapours: The animals in the test groups were exposed to gasoline vapours in the exposure chambers. A modified nose inhalation exposure method previously described, was used to expose the animals in test groups to upgraded concentration of the vapours.
generated from direct evaporation of liquid gasoline (Uboh et al., 2005, 2008). The Premium Motor Spirit (PMS) blend of liquid unleaded gasoline used in this study was obtained from Mobil refueling station, Marian Road, Calabar, Nigeria. The test animals were allowed to inhale the evaporating vapours in the chambers during the exposure period. An exposure period of 6 h (9.00 am to 3.00 pm) daily, 6 days per week, was adopted for 20 weeks. At the end of the experimental period, the animals were sedated with chloroform and dissected for collection of blood specimen.

**Treatment of the rats with vitamins A and E:** After 18 weeks of pre-inhalation exposure to the gasoline vapours, the rats in groups III and IV were, respectively administered, once daily, with 400 IU kg⁻¹ of vitamin A (retinol) and 200 IU kg⁻¹ of vitamin E (α-tocopherol), i.e., at normal prophylactic dose, concomitantly with exposure to gasoline vapours for the remaining 2 weeks. Administration of the vitamins was done by oral gavaging using intragastric syringe after solubilizing the vitamins with Goya olive oil, as the vehicle.

**Collection and analysis of blood:** Blood samples were collected by cardiac puncture into heparinised sample bottles for haematological analysis. The whole blood specimens were used for the determination of the levels of haemoglobin, haematocrit, red blood and white blood cells counts. Haemoglobin and haematocrit levels were determined by the methods described by Alexander and Griffiths (1993). All absorbance readings for haemoglobin determinations were taken using DREL 3000 HACH (England) model spectrophotometer. The total red and white blood cells were counted by the microscopic visual identification methods described by Dacie and Lewis (1975).

**Statistical analysis:** The results were analyzed by one-way Analysis of Variance (ANOVA) followed by student's t-test to evaluate the significance of the difference between the mean value of the measured parameters in the respective test and the control groups. A significant change was accepted at p<0.05.

![Graph](image)

**Fig. 1:** Effect of vitamin A and E on percentage comparative change in haematological indices in male rats following exposure to gasoline vapours. 
- MvitA: Male test treated with vitamin A, 
- MvitE: Male test treated with vitamin E

**Table 1:** Effect of vitamins A and E on gasoline vapours-induced alterations in haematological indices in male rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hb (mg dL⁻¹)</th>
<th>PCV (%)</th>
<th>RBC (cell mm⁻³)</th>
<th>WBC (cell mm⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Mc)</td>
<td>12.1±1.3</td>
<td>46.2±1.6</td>
<td>7.69±1.2.45×10⁷</td>
<td>4.392±10⁶±5.51×10³</td>
</tr>
<tr>
<td>II (Mt)</td>
<td>9.2±0.6*</td>
<td>24.7±2.1*</td>
<td>6.19±10⁶±2.00×10⁷</td>
<td>6.333±10⁶±6.60×10⁷</td>
</tr>
<tr>
<td>III (MvitA)</td>
<td>11.6±0.7*</td>
<td>44.8±1.9*</td>
<td>6.36±10⁶±1.60×10⁷</td>
<td>5.058±10⁶±7.41×10⁷</td>
</tr>
<tr>
<td>IV (MvitE)</td>
<td>11.9±1.4*</td>
<td>45.0±6.6*</td>
<td>6.55±10⁶±3.63×10⁷</td>
<td>4.958±10⁶±5.35×10⁷</td>
</tr>
</tbody>
</table>

Values are presented as Mean±SEM (n = 8), *p<0.05 compared with group I (control), **p<0.05 compared with group II. Mc: Male control, Mt: Male test exposed to gasoline vapours only; MvitA: Male test treated with vitamin A; MvitE: Male test treated with vitamin E

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### RESULTS

The results of this study are shown in Table 1 and Fig. 1. From these results, the levels of Hb, PCV and RBC in the experimental test control rats, exposed to gasoline vapour only (i.e., group II), were significantly lower (p<0.05) compared, respectively with the levels obtained for rats in the normal control (i.e., group I). The results also showed that the level of WBC obtained for rats in the experimental test group was significantly higher (p<0.05) compared respectively, with the level obtained for the normal control rats (Table 1). Moreover, a significant difference (p<0.05) in the levels of these parameters was observed within or among the various respective groups.

The mean values of Hb, PCV, RBC and WBC obtained for rats in group III, (i.e, the test rats treated...
with vitamin A) were 11.6±0.7 mg dl⁻¹, 44.8±1.9%, 6.36×10⁵±6.1×10⁵ and 5.05×10⁵±7.41×10⁵ cells mm⁻³, respectively, while 11.9±0.4 mg dl⁻¹, 45.0±0.6%, 6.55×10⁵±3.63×10⁵ and 4.58×10⁵±5.33×10⁵ cells mm⁻³, respectively were the values obtained for rats in group VI, i.e., the test rats treated with vitamin E (Table 1). Also, there was no significant difference (p>0.05) among the rats in the treated groups. Although, these results showed that the levels of Hb, PCV and RBC obtained for rats in groups III and VI were significantly higher (p<0.05) compared, respectively to the levels obtained for the experimental test rats, i.e., group II (9.2±0.6 mg dl⁻¹, 42.7±2.1% and 6.19×10⁵±2.00×10⁵ cells mm⁻³, respectively) there was no significant difference (p>0.05) when compared to the levels obtained for the rats in the normal control i.e., group I (12.1±1.3 mg dl⁻¹, 46.2±1.6% and 7.69×10⁵±2.45×10⁵ cells mm⁻³, respectively). Moreover, the level of WBC obtained for rats in groups III and IV was significantly lower (p<0.05) compared, respectively, to the level obtained for rats in group II (6.33×10⁵±6.46×10⁵ cells mm⁻³), but insignificantly higher (p>0.05) compared, respectively, to the level obtained for rats in group I (4.39×10⁵±5.1×10⁵ cells mm⁻³) (Table 1). The results obtained from this study indicated that vitamins A and E may enhance recovery from haematotoxicity associated with exposure to gasoline vapours in rats, with vitamin E being more potent than vitamin A.

The results showed that the initial total body weights recorded for rats in normal control group, experimental test group, vitamins A and E treated groups (164.5±12.4, 166.3±11.3, 166.2±13.4 and 165.6±14.8 g, respectively), compared with their final total body weights (213.6±10.3, 199.5±12.5, 211.4±11.4 and 213.5±12.5 g, respectively) gave percentage weight increase and growth rate of 29.6±4.6, 40.9±4.5, 20.0±4.7, 27.4±3.8, 27.2±3.8, 37.7±4.2, 28.8±3.4 and 39.8±4.6%, respectively. From these results, it is clear that exposure of male rats to gasoline vapours produces a significant decrease (p<0.05) in percentage weight increase and growth rate, indicating a condition of weight-loss and growth-depression, which are reversed by the administration of vitamins A and E. However, the results showed that vitamin E tends to be more effective in ameliorating weight-loss and growth-depression associated with exposure to gasoline vapours in male rats (Table 2).

**DISCUSSION**

Haematotoxicity, growth-depression and weight-loss reported in this study to be associated with exposure of male rats to gasoline vapours agree with the earlier reports (Uboh et al., 2005, 2008). The haematotoxic effect reported in this study to be associated with exposure of rats to gasoline vapours also correlates with the result of the study reported for rats and humans occupationally exposed to benzene and xylene (D’Azevedo et al., 1996; Rothman et al., 1996). Although, the mechanism of haematotoxicity causation reported in this study is not clear, it may be suggested that the chemical constituents of gasoline vapours might have interacted with the bone marrow to depress the rate at which the haematopoietic committed stem cells are synthesized, according to the report of Synder and Hedli (1996) for benzene, or interacted with the red blood cells membrane protein to increase the rate of red blood cells destruction as reported by Valentine et al. (1993) for carbon disulphide. Also, the increased white blood cell levels observed after the exposure of rats to gasoline vapours may be as a result of immunologic response to the foreign chemical agents introduced into the system by the vapours.

Generally, the mechanism of haematotoxicity expression associated with exposure to gasoline vapours may be diverse. The hydrocarbons and other chemical constituents of the gasoline vapours may be metabolized in the body to reactive species which interact with the tissues to exhibit their toxic or hazardous effects. According to Synder and Hedli (1996), haematotoxic effect associated with benzene toxicity involves both bone marrow depression and luskaemogenesis, caused by damage to multiple classes of haematopoietic cells and a variety of haematopoietic functions. Also, the toxic metabolite of hexane (i.e., 2, 5-hexanediene) and carbon disulphide have been reported to covalently cross-link red cells axonal membrane proteins (such as γ-diketones) and cause damages to these cells (Valentine et al., 1993; Amarnath et al., 1991). Since, gasoline contains different types of hydrocarbons, it may be assumed that the haematotoxicity reported in this study follows similar mechanisms reported for such hydrocarbons as benzene and hexane.

It is also observed in the recent studies that inhalation exposure to ungraded concentrations of kerosene and petrol vapours caused a significant decrease in percentage total body weight increase and

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**Table 2: Effect of vitamins A and E on gasoline vapours-induced alterations in weight increase and growth rate in male rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>IBW (g)</th>
<th>FBW (g)</th>
<th>PWI (%)</th>
<th>PGR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Mc)</td>
<td>164.5±2.4</td>
<td>213.6±10.3</td>
<td>29.6±4.6</td>
<td>40.9±4.5</td>
</tr>
<tr>
<td>II (M0)</td>
<td>166.3±11.3</td>
<td>199.5±12.5</td>
<td>20.0±4.7</td>
<td>27.4±3.8</td>
</tr>
<tr>
<td>III (M0V)</td>
<td>166.2±13.4</td>
<td>211.4±11.4</td>
<td>27.2±3.8</td>
<td>37.7±4.2</td>
</tr>
<tr>
<td>IV (V0E)</td>
<td>165.6±14.8</td>
<td>213.5±12.5</td>
<td>28.8±3.4</td>
<td>39.8±4.6</td>
</tr>
</tbody>
</table>

Values are presented as Mean±SEM; (n=8); *p<0.05 compared with group I; **p<0.05 compared with group II; IBW: Initial body weight, FBW: Final body weight, PWI: Percentage weight increase, PGR: Percentage growth rate.
growth rate in rats (Ubboh et al., 2005, 2008). Moreover, Tilbury et al. (1993) reported that insignificantly higher mean in-life body weights were observed in female mice, whereas a significantly higher mean in-life weights were observed in the male mice exposed to 67 ppm Unleaded Gasoline (UG) during week 6-9 of the experiment. With different UG formulations, it has been reported that methylietria butylether (MTBE), American Petroleum Institute (API) 9-0, and PS-6 blends of UG did not significantly alter body weights of mice after 3 to 21 days of inhalation exposure (Moser et al., 1996). Also, female mice exposed to 2039 ppm UG vapour for 13 weeks were reported to have gained weight over the exposure period (Standeven and Goldsworthy, 1994; Tilbury et al., 1993).

The molecular events in cell growth, hence total body weight increase and growth rates are complex and involve an increasing array of intercellular pathways and molecules. According to Cotran et al. (1999), aberrations in such pathways may underlie the uncontrolled growth (as in cancer) and abnormal cellular responses in a variety of diseases. Also, the molecular mechanisms of growth inhibition are similar to those of growth stimulation and intertwine along their intercellular routes. It has been established that growth stimulation and inhibition proceed through a variety of intercellular signaling systems (Murray, 2000a). These signaling systems, which are mainly hormonal, operate through the stimulation or inhibition of specific polypeptide synthesis (Massague, 1998). The influence of gasoline vapours on the weight increase and growth rate reported in this study may be suggested due to the interaction of the gasoline vapours’ constituents with these signaling systems, to inhibit the growth stimulation signals and stimulate the growth inhibitory signaling system in a pathway leading to growth depression and weight loss.

It was also observed in this study that vitamins A and E enhanced the reversal of haematotoxic effect and the regain of weight-loss, caused by gasoline vapours exposure, in male rats. However, vitamin E was observed to be more potent in ameliorating the weight-loss and haematotoxic effects than vitamin A. The effect of vitamin A recorded in this study supports the earlier report that vitamin A may be used to enhance recovery from weight-loss, growth-depression and haematotoxicity associated with exposure to gasoline vapours (Ubboh et al., 2008). Also, the observed higher potency of vitamin E reported here gives an indication that α-tocopherol is observed to be the major lipid soluble antioxidant vitamin protecting membranes and lipoproteins from injury by free radicals. Although, the actual mechanism(s) whereby the vitamins enhance weight-loss regain and reversal of haematotoxic effect in rats is (are) not very clear. However, the antioxidative and protective roles of vitamins A and E reported in the study (Lotan, 1999, Knert et al., 1999; Marcus and Coulston, 1996), may be suggested to be implicated. Prevention of reactive metabolites formation or rapid scavenging of the generated reactive species by the antioxidants may be useful in preventing the toxicity effects of different reactive metabolites. For example, Peshlow and Hesse (1991), Maellaro et al. (1994) and Sheweita et al. (2001) reported that antioxidants have proved to be effective in protecting the liver against carbon tetrachloride-induced hepatotoxicity. With the antioxidative and protective roles of these vitamins, the toxic effects of the various reactive metabolites responsible for the haematotoxicity, weight-loss and growth-depression observed to be associated with exposure to gasoline vapours, are assumed to be reversed or prevented. In another view, it may also be suspected that the vitamins interact with the haematopoietic growth factorscommitted stem cells, the growth stimulation signaling systems and the various growth factors to stimulate rapid synthesis of blood cells as well as growth and weight increase. Among the several growth factors-x (TGF-X), Platelet-Derived Growth Factor (PDGF), Fibroblast Growth Factor (FGF), Vascular Endothelial Growth Factor (VEGF) and cytokinins, among others (Murray, 2000a; Chatterjeea and Shinde, 2002; Sporn and Roberts, 1983). Also, stimulations of haematopoietic growth factors and erythropoietin systems have been reported to enhance rapid synthesis of blood cells (Murray, 2000b). From the observations made in this study, it may be assumed that the vitamins interacted with the growth factors and other metabolic processes to stimulate growth signaling as well as haematopoietic growth factors and erythropoietin systems (Murray, 2000b). Hence, rapid reversal of haematotoxic effect and enhancement of weight-loss and growth-depression regain by vitamins A and E in male rats exposed to gasoline vapours are reported in this study. In conclusion, the result of this study showed that vitamins A and E may be used to enhance recovery from or prevent weight loss, growth depression and haematotoxicity associated with exposure to gasoline vapours. Hence, these vitamins may be recommended at prophylactic dosages to those who are routinely occupationally exposed to gasoline vapours. However, the specific mechanism(s) through which vitamins A and E effect the recovery or reversal of weight loss, growth depression and haematotoxicity associated with exposure to gasoline vapours is a subject for further study.
REFERENCES


