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Effect of Vitamin A on Weight-Loss and Haematotoxicity Associated with Gasoline Vapours Exposure in Wistar Rats

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Abstract: The effect of vitamin A on weight-loss, growth-depression and haematotoxicity associated with gasoline vapours exposure was assessed in male and female Wistar albino rats. The rats were exposed to ungraded concentrations of gasoline vapours (6 h daily) for 20 weeks. Vitamin A (retinol) at prophylactic dosage (400 IU kg^{-1} day^{-1}) was orally administered to the rats in the last two weeks of exposure. The levels of haemoglobin (Hb), haematocrit or Packed Cell Volume (PCV), Red Blood Cells (RBC), weight gain and growth rate in the male and female rats exposed to the vapours were significantly lower ($p<0.05$) compared respectively to the levels obtained for male and female control rats. On the other hand, the levels of White Blood Cells (WBC) in the male and female test rats were significantly higher ($p<0.05$) compared respectively with the level obtained for male and female control rats. These observations indicated that exposure to gasoline vapours produced haematotoxicity, weight loss and growth depression in rats. However, administration of vitamin A was observed to produce a significant regain ($p<0.05$) in weight-loss, growth-depression and haematotoxicity observed to be associated with exposure to gasoline vapours, although the females were noted to respond more favourably than the males. This suggests that vitamin A may be used to reverse or prevent weight-loss, growth-depression and haematotoxicity in subjects exposed to gasoline vapours.

Key words: Gasoline vapours, haematotoxicity, weight-loss, growth-depression, vitamin A

INTRODUCTION

Gasoline vapours may be derived from direct evaporation or combustion of liquid gasoline. These vapours, being ubiquitous in the environment, constitute some components of petroleum pollutants in the air. The commonest sites of exposure to these pollutants from gasoline vapours include refineries, oil fields, refueling stations, petrochemical industries, motor mechanical workshops and traffic-congested areas. Hence, the population at greater risk of frequent exposure includes those occupationally exposed. As well as those residing in traffic-congested areas. Reports indicate that chronically exposed individuals are the oil drillers, refinery workers, petrochemical workers, refuel station attendants and motor mechanics (EHC 20, 1982; Carballo *et al*., 1994; Rabble and Wong, 1996).

Literature reports indicate that more saturated hydrocarbons than unsaturated aromatic hydrocarbons are found in human and animal blood after inhalation exposure to equal concentrations (Zahlsen *et al*., 1993). 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, our recent studies showed that inhalation exposure to composite constituents of kerosene and petrol vapours exhibit haematotoxic effect in Wistar albino rats (Uboh *et al*., 2005). According to Rothman *et al*. (1996) the levels of total white blood cells, absolute lymphocyte counts, platelets, red blood cells and haematocrit were observed to be reduced among workers heavily exposed to benzene.

Most of the literature reports available on the effect of gasoline vapours inhalation on total body weight are on mice. And these reports suggest that the adverse effect of gasoline vapours inhalation on total body weight is species-and sex-dependent, with male mice being more vulnerable to the effect. There is paucity of information on the ways of ameliorating the weight loss and haematotoxic effects reported to be associated with 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

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ameliorate the toxicity effect. Among the antioxidants that has attracted the attention of researchers in the recent times is vitamin A.

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. Interconversions between 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; Marcus and Coulston, 1996). 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. Hence, this study aimed at assessing the effect (s) of vitamin A on haematotoxic effect on weight loss associated with inhalation exposure to gasoline vapours in male and female rats.

MATERIALS AND METHODS

Experimental animals: Forty-eight adult Wistar albino rats (twenty-four males and twenty-four females) weighing 181.5±19.3 g were obtained from the animal house of the College of Medical Sciences, University of Calabar, Calabar-Nigeria and used for this study (February-June 2007). The rats were divided according to sex into eight groups with six rats each, as follows:

- Male control group, without exposure to gasoline vapours inhalation
- Male test group, i.e the group exposed to gasoline vapours by inhalation only
- Male test group, concomitantly treated with vitamin A daily for the last two weeks of the inhalation exposure
- Female control group, without exposure to gasoline vapours inhalation
- Female test group, exposed to gasoline vapours by inhalation only
- Female test group, concomitantly treated with vitamin A daily for the last two weeks of the inhalation exposure

The rats were acclimatized in the experimental animal house for five days before 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 Institutional Animals 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). The Premium Motor Spirit (PMS) blend of liquid 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 vitamin A: After eighteen weeks of pre-inhalation exposure to the gasoline vapours, the rats in groups V and VI were treated once daily with 400 IU kg^{-1} of vitamin A (retinol), i.e., normal prophylactic dose, concomitantly with gasoline vapours inhalation for the remaining two weeks. Treatment was done orally by intragastric syringe after solubilizing the vitamin 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 analyses. 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 (1993a, b). 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).

Determination of weight increase and growth rate: Total body weight of each rat was measured using a chemical balance, before and after the experimental period (and recorded as initial and final body weight, i.e., IBW and FBW, respectively). The mean body weight for each group was determined from the measured total body weights. Weight changes were expressed as percentage weight increase and percentage growth rate, where:

- Percentage weight increase was calculated from the formula:

$$\frac{\text{FBW-IBW}}{\text{IBW}} \times 100$$

- Percentage growth rate was calculated from the formula:

$$\frac{\text{FBW-IBW}}{y} \times 100$$

Where:
y = Number of days exposed

Statistical analyses: 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 groups and the control groups. A significant change was accepted at $p<0.05$.

RESULTS

The results of this study show that the levels of Hb, PCV and RBC in the male rats exposed to gasoline vapour, i.e., male test (or group II) rats and female test (or group V) rats were significantly lower $p<0.05$) compared, respectively with the levels obtained for male control (or group I) rats and female control (or group IV) rats (Table 1). The results also showed that the levels of WBC obtained for male test rats and female test rats were significantly higher ($p<0.05$) compared, respectively with the level obtained for male control rats and female control rats (Table 1). However, there was no significant difference in the levels of these parameters 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 male test groups treated with vitamin A, were 118.0±4.6 g L^{-1}, 46.7±2.1%, $6.99\times10^{6}\pm2.00\times10^{2}$ cells mm^{-3} and $4.66\times10^{4}\pm5.33\times10^{2}$ cells mm^{-3}, respectively, while 120.1±6.7 g L^{-1}, 44.8±3.3%, $6.44\times10^{6}\pm3.06\times10^{2}$ cells mm^{-3} and $2.95\times10^{4}\pm8.65\times10^{2}$ cells mm^{-3}, respectively, were the values obtained for rats in group VI, i.e, the female test group treated with vitamin A (Table 1). Also, there was no significant difference among the rats in the treated groups. However, these results showed that the levels of Hb and PCV obtained for rats in group VI were significantly higher ($p<0.05$) compared to the levels obtained for the rats in group V and insignificantly lower ($p>0.05$) compared to the levels obtained for the rats in group IV. However, the RBC level in group VI was significantly higher ($p<0.05$) compared to the level in group V and significantly lower ($p<0.05$) compared to the level in group IV. Also, the levels of Hb, PCV and RBC obtained for group III were significantly higher ($p<0.05$) compared to the level obtained for group II, though significantly lower ($p<0.05$) compared to the levels obtained for group I. Moreover, the level of WBC obtained for rats in group III was significantly lower ($p<0.05$) compared to the level obtained for rats in group II, but significantly higher $p<0.05$) compared to the level obtained for rats in group I. Similarly, the level of WBC obtained for rats in group VI was significantly lower ($p<0.05$) compared to the level obtained for rats in group V, but significantly higher ($p<0.05$) compared to the level in group IV (Table 1). The results obtained from this study indicated that vitamin A may enhance recovery from haematotoxicity associated with gasoline vapours exposure in rats, with the females being the more favoured sex.

The results of the effect of vitamin A on weight-loss and growth-depression associated with gasoline vapours exposure in male and female rats are shown in Table 2. The results showed that the initial total body weights recorded for male rats in experimental control group, experimental test group and vitamin A-treated group, compared with the final total body weight gave percentage weight increase and growth rate of 20.9±5.3 and 32.3±5.6, 11.7±3.9 and 8.3±4.1 and 18.1±4.2 and 28.3±4.6%, respectively. Also, the initial total body weights recorded for female rats in experimental control group, experimental test group and vitamin A-treated group, compared with the final total body weights gave percentage weight increase and growth rates of 20.0±5.1

Table 1: Effect of vitamin A on gasoline-vapours-induced alterations in haematological indices in male and female rats

Group	Hb (g L^{-1})	PCV (%)	RBC (cell mm^{-3})	WBC (Cell mm^{-3})
I (Mc)	123.2±2.9	48.0±1.3	$7.89\times10^{6}\pm2.61\times10^{2}$	$4.39\times10^{4}\pm5.51\times10^{2}$
II (Mt)	94.1±6.4*	44.0±0.6*	$6.05\times10^{6}\pm3.63\times10^{2*}$	$5.06\times10^{4}\pm7.41\times10^{2*}$
III (Mvita)	118.0±4.6+*	46.7±2.1+*	$6.99\times10^{6}\pm2.00\times10^{2+*}$	$4.66\times10^{4}\pm5.33\times10^{2+*}$
IV (Fc)	121.4±8.1	45.2±1.1	$6.90\times10^{6}\pm1.93\times10^{2}$	$2.48\times10^{4}\pm8.79\times10^{2}$
V (Ft)	93.1±6.7**	36.8±0.9**	$4.35\times10^{6}\pm4.56\times10^{2**}$	$4.86\times10^{4}\pm6.11\times10^{2**}$
VI (FvitA)	120.1±6.7x©	44.8±3.3x©	$6.44\times10^{6}\pm3.06\times10^{2x**}$	$2.95\times10^{4}\pm8.65\times10^{2x**}$

Values are presented as mean±SEM n = 8, *$p<0.05$ compared with group I (control) +$p<0.05$ compared with group II; **$p<0.05$ compared with group IV (control), x$p<0.05$ compared with group V, ©$p>0.05$ compared with group IV. Mc = male control; Mt = male test, exposed to gasoline vapours only; MvitA = male test treated with vitamin A. Fc = female control; Ft = female test, exposed to gasoline vapours only; FvitA = female test treated with vitamin A

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

Group	IBW (g)	FBW (g)	PWI (%)	PGR (%)
I (Mc)	185.1±13.0	223.8±17.5	20.9±5.3	32.3±5.6
II (Mt)	187.8±11.8$^{\odot}$	209.8±19.5*	11.7±3.9*	18.3±4.1*
III (MvitA)	187.7±19.4$^{\odot}$	221.6±20.7$^{+\odot}$	18.1±4.2$^{+\odot}$	28.3±4.6$^{+\odot}$
IV (Fc)	164.8±10.7	197.7±12.0	20.0±5.1	27.4±4.5
V (Ft)	185.5±11.4**	197.1±10.7$^{\odot}$	6.3±1.5**	9.7±1.8**
VI (FvitA)	166.5±9.7$^{x\odot}$	199.3±6.6$^{\odot}$	19.7±3.7$^{x\odot}$	19.0±3.1^{x**}

Values are presented as mean±SEM; n = 8; *p<0.05 compared with group I; $^{\odot}$p>0.05 compared with group I; $^{+}$p<0.05 compared with group II; **p<0.05 compared with group IV; ^{x}p<0.05 compared with group V; $^{\odot}$ p>0.05 compared with group VI; IBW = Initial Body Weight; FBW = Final body weight; PWI = Percentage weight increase; PGR = Percentage Growth Rate

and 27.4±4.5, 6.3±1.5 and 9.7±1.8 and 19.7±3.7 and 19.0±3.1%, respectively. These result showed that inhalation exposure of male and female rats to gasoline vapours produced a significant decrease ($p<0.05$) in percentage weight increase and growth rate, indicating a condition of weight-loss and growth-depression.

However, the weight-loss and growth-depression effects tend to be more severe in female than male rats. It was also observed that administration of vitamin A to both male and female rats in the experimental test group produced a significant regain ($p<0.05$) in weight loss and growth depression. From these results, it was also noted that the weight-loss and growth-depression regain was more rapid in females than males. These observations indicated that vitamin A may also be useful in ameliorating the weight-loss and growth-depression associated with inhalation exposure to gasoline vapours.

DISCUSSION

Weight-loss, growth-depression and haematotoxicity effects reported in this study to be associated with long-term exposure of male and female rats to gasoline vapours agree with our earlier report (Uboh *et al*., 2005). Moreover, the observations made from the results of this study confirmed our recent laboratory findings that weight-loss and growth-depression associated with exposure of rats to gasoline vapours are sex-dependent and that the female rats are more vulnerable. However, the effect of the gasoline vapours on the total body weights of female rats reported in this study disagrees with the reports of Standeven and Goldsworthy (1994) and Tilbury *et al*. (1993) for female mice. The specific molecular basis and hence, the mechanism of sex-dependent influence of gasoline vapours on the weight increased and growth rate in rat is not clear; but the interplay of endocrine system may be suspected.

The haematotoxic effect reported in this study to be associated with exposure of rats to gasoline vapours has also been reported in 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 gasoline vapours exposure may be diverse. The 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 harzardous effects. According to Synder and Hedli (1996), haematotoxic effect associated with benzene toxicity involves both bone marrow depression and luekaemogenesis, 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-hexanedione) 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).

We also observed in our recent study that inhalation exposure to ungraded concentrations of kerosene and petrol vapours caused a significant decrease in percentage total body weight increase and growth rate in rats (Uboh *et al*., 2005). 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 methyltertiary butylether (MTBE), American Petroleum Institute (API) 91-01 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 sex-dependent influence of gasoline vapours on the weight increase and growth rate pattern reported in this study may be suggested to be due to sex differences in the actions and functions of these hormonal signaling systems. Our previous studies showed that the adverse effect of exposure to gasoline fumes on sex hormones profile in rats is sex-dependent, with the females being more vulnerable (Uboh *et al*., 2007). It is possible that the constituents of the vapours interact with the signaling systems in a sex-dependent pattern 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 vitamin A enhanced the reversal of haematotoxic effect and the regain of weight-loss, caused by gasoline vapours exposure, in male and female rats. However, the vitamin was observed to be more potent in ameliorating the weight-loss and haematotoxic effects in female than male rats. The actual mechanism whereby the vitamin enhances weight-loss regain and reversal of haematotoxic effect in rats; and why the female rats tend to respond better than males are not very clear. However, the antioxidative and protective roles of vitamin A reported in the literature (Lotan, 1999; Knert *et al*., 1999; Marcus and Coulston, 1996), may be suggested to be implicated. It may be suspected that the vitamin interact with the haematopoietic growth factors/committed 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 in sex-dependent pattern. 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; Chatterjea 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). While some of these growth factors act on a variety of cell types, others have relatively specific targets and this specificity could be sex-dependent. From the observations made in this study and that of our previous report (Uboh *et al*., 2007), it may be assumed that due to hormonal differences, the vitamin interacted with the growth factors and other metabolic processes more in female than male rats 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 vitamin A in female than male rats exposed to gasoline vapours are reported in this study. In conclusion, the result of this study showed that vitamin A may be used to enhance recovery from or prevent weight loss, growth depression and haematotoxicity associated with exposure to gasoline vapours.

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