

**EFFECT OF INHALATION EXPOSURE TO GASOLINE
ON SEX HORMONES PROFILE IN
WISTAR ALBINO RATS**

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Abstract

12 adult male and 12 female *Wistar* albino rats were used to measure the levels of the serum follicle stimulating hormone (FSH), the luteinizing hormone (LH), the estradiol (E₂) and the progesterone in female rats, as well as the FSH, the LH and the testosterone in male rats, following 8-hour daily inhalation exposure to gasoline vapours for 6 weeks, to assess the effect of the vapours on the reproductive integrity of experimental animals. The results showed that the levels of serum FSH and LH obtained for female rats in the test group (3.48±0.03 and 19.97±1.20mIU/ml, respectively) were insignificantly lower (p>0.05), compared respectively to the levels obtained for female rats in the control group (3.62±0.12 and 20.06±0.23mIU/ml, respectively), whereas the levels of serum estradiol and progesterone obtained for female rats in the test group (25.02±2.32pg/ml and 0.18±0.02mg/ml, respectively) were significantly lower (p<0.05), compared respectively to the levels obtained for female rats in the control group (39.84±3.64pg/ml and 0.33±0.02mg/ml, respectively). On the other hand, the levels of serum FSH and LH obtained for male rats in the test group (2.87±0.21 and 3.09±0.32mIU/ml, respectively) were insignificantly higher (p>0.05), compared to the levels obtained for male rats in the control group (2.75±0.14 and 2.93±0.12mIU/ml, respectively). At the same time, the level of serum testosterone in the male test rats (7.82±2.17ng/dl) was significantly higher (p<0.05), compared to the level in the male control rats 4.66±1.85ng/gl. The results of this study showed that the adverse effect of inhalation exposure to gasoline fumes on the reproductive integrity in rats is sex-dependent, with the females being more vulnerable.

Key words: Gasoline vapours, environment, petroleum derivatives, testosterone, estradiol, sex hormones, reproductive integrity.

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INTRODUCTION

Gasoline fumes are ubiquitous in the environment, and a great percentage of the urban population is known to be frequently exposed to the fumes directly or indirectly. However, it has been reported that chronically exposed population includes petrochemical workers, refuel station attendants, refinery workers, oil drillers and motor mechanics (1, 2). Those residing in traffic-congested areas may as well be at risk of exposure to low levels of fumes' pollutants over a long period of time.

Hazardous effects of the inhalation exposure to gasoline fumes have been reported both in experimental animals and humans (1, 3-6). According to Suhanova and Melnikova (7), disturbances in the menstrual function were common in female workers in petroleum refineries, especially in those who have been subject to long term exposures. Similarly, disturbances in the menstrual cycle have been reported by Beskrovnaja *et al.* (8) in workers with more than five years service as female operators of plants producing rubber materials and having petroleum solvent vapour concentration of 250-350mg/m³ in the air. There are also some reports on the adverse effect of gasoline fumes inhalation on the reproductive function of female mice (9, 10). Furthermore, there are some reports on the effect of aging on the sex hormones concentrations in experimental animals (11-15). Bromoethane, chloroethane and ethylene oxides have been reported to induce uterine neoplasms in B6C3F1 mice from 2 year NTP inhalation bioassays (16). Hormonal disruption in agricultural workers and experimental animal studies suggest that exposure to organophosphorus pesticides disrupted the hypothalamic pituitary endocrine function and also indicated that FSH and LH are the most affected (17).

The reproductive integrity in males has been assessed from the level of follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone in circulation, while the level of estradiol and progesterone, in addition to LH and FSH in circulation, is used in the assessment of the reproductive integrity in females (18, 19). The FSH and LH (gonadotropins) are produced in the pituitary gland under the influence of estradiol and progesterone in females, and testosterone in males, through a feedback regulation mechanism. Estradiol and progesterone are produced mainly in the ovaries in non-pregnant females, while testosterone are produced primarily in the testes in males. Testosterone is required for normal spermatogenic activities, and has been used to assess the effect of certain chemicals on the male reproductive function (20, 21). According to Megevan *et al.* (22), decreased libido, and hence impotency may be caused by a low testosterone level. This implies that an increased testosterone level may increase the libido and reproductive functions.

The female reproductive functions, integrity and characteristics are regulated by the female sex hormones, mainly estradiol and progesterone, which are in turn regulated by FSH and LH. The polycystic ovarian syndrome (PCOS) is one of the contributing factors to infertility and women with PCOS are reported to have a low estrogen level in circulation (23). Hence, a low estrogen level may be used as a

diagnostic index for infertility in females. An investigation into the effect of inhalation exposure to gasoline fumes on serum sex hormones profile in male and female rats is therefore of clinical significance in the management of infertility in workers in petroleum industry.

MATERIALS AND METHODS

Experimental animals

Twenty-four adult albino *Wistar* rats (twelve males and twelve females) weighing 160.7 ± 5.3 g were obtained from the animal house of Biochemistry Department, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria. The rats were divided into four groups, according to sex (i.e., male control, male test, female control and female test groups) consisting of six rats each. The rats were transferred to the experimental animal house, and were kept under normal room temperature and humidity during the period of the experiment. The animals were kept in stainless steel cages (60cm \times 30cm \times 45cm) and were fed on normal rat pellets. Both control and test animals 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 method of exposure employed in this study was by inhalation as previously described (6). In this exposure method, the animals in the test groups were exposed to gasoline vapours in the exposure chambers. The animal cages housing the test groups were placed in an exposure chamber measuring 150cm \times 90cm \times 210cm. Two highly perforated 1000ml cans containing 500ml of gasoline were placed in the exposure chamber and the animals were allowed to inhale the vapours generated from the direct evaporation of liquid gasoline from the cans at ambient humidity and temperature. The exposure period of 8 hours (8.00 am till 4.00 pm) daily was adopted for 6 weeks. After each day of exposure the animals were transferred to the gasoline vapour-free section of the experimental animal house. The liquid gasoline (PMS blend) was obtained from Mobil refueling station, Marian Road, Calabar, Nigeria. At the end of the experimental period, the animals were anaesthetized with chloroform and dissected for the collection of blood samples. The blood samples were collected 20 hours after the last day of exposure.

Collection and analysis of blood

Blood samples were collected by cardiac puncture into a set of plain sample bottles, and allowed to clot. The clotted blood samples were spun in a bench top centrifuge (MSE, England) to obtain sera. The serum samples were separated into another set of plain sample tubes. The separated serum samples were stored in the refrigerator until required for the hormonal assay. All assays were done within 24 hours of the sample collection. The serum samples were assayed for FSH, LH, estradiol, progesterone and testosterone using enzymes immunoassay methods. The

respective immunoassay reagent kits were obtained from Diagnostic Automation Inc., 23961 Craftman Road, Suite E/E, Calabasas, CA 91302. Microplate reader (Dialab Instruments Ltd.) was used in taking the absorbance. Calculations of the concentrations of hormones were made according to the method given in the kits handbook.

Statistical analysis

Students' t-test was used 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 effects of gasoline fumes inhalation on serum sex hormones of male and female rats are summarized in Table 1.

Table 1. Effect of gasoline vapores inhalation on serum sex hormones profile of male and female Wistar albino rats

Group	FSH (mIU/ml)	LH (mIU/ml)	Testosterone (ng/dl)	Estradiol (pg/ml)	Progesterone (mg/ml)
Male					
Control	2.78 ± 0.14	2.98 ± 0.12	4.66 ± 1.18	-	-
Male	2.80 ± 0.21	3.01 ± 0.32	$7.82 \pm 2.17^*$	-	-
Female					
Control	3.62 ± 0.12	20.06 ± 0.23	39.84 ± 3.64	39.84 ± 3.64	0.33 ± 0.02
Female	3.48 ± 0.03	19.97 ± 1.20	-	25.02 ± 2.32	0.18 ± 0.02

The results showed that both FSH and LH levels obtained for male rats in the test group (2.80 ± 0.21 and 3.01 ± 0.32 mIU/ml, respectively) were insignificantly higher ($p > 0.05$) compared with the levels obtained for male rats in the control group (2.78 ± 0.14 and 2.98 ± 0.12 mIU/ml, respectively) while the level of testosterone obtained for male rats in the test group (7.82 ± 2.17 ng/dl) was significantly higher ($p < 0.05$) compared with the level obtained for the male rats in the control group (4.66 ± 1.85 ng/dl). This indicates that while gasoline fumes inhalation has no significant effect on the serum FSH and LH levels, it increases the level of serum testosterone in male rats. From the results of this study it was also observed that inhalation exposure of female rats to gasoline fumes resulted in a decrease in the levels of serum FSH (3.48 ± 0.03 mIU/ml) and LH (19.97 ± 1.20 mIU/ml), compared with the levels in the female control rats (3.62 ± 0.12 and 20.06 ± 0.23 mIU/ml respectively). However, the decreases in FSH and LH in this experiment were not significant ($p > 0.05$). Interestingly, the levels of serum estradiol and progesterone in the female test rats (25.02 ± 2.32 pg/ml and 0.18 ± 0.02 mg/ml, respectively) were observed to be significantly reduced ($p < 0.05$).

compared respectively to the levels in the female control rats (39.84 ± 3.64 pg/ml and 0.33 ± 0.02 mg/ml) respectively. These observations indicated that gasoline fumes inhalation resulted in a significant reduction in the concentrations of estradiol and progesterone released into circulation, without any significant effect on the level of serum FSH and LH in female rats.

DISCUSSION

Sex hormones are known to regulate the reproductive functions and characteristics in both male and female organisms (24, 25). Measurement of serum sex hormones profile is therefore very useful in assessing the reproductive integrity in both animals and humans. According to Jenner *et al.* (26), serum estradiol measurement is a valuable index in evaluating a variety of menstrual dysfunctions in females. This indicates that suppression of reproductive functions may be evidenced in reduced serum sex hormone profile. Several factors implicated in these include genetic defects, surgery, and exposure to certain chemical agents (20, 21, 23).

Sex hormones (estradiol and progesterone in females, and testosterone in males), are produced primarily in the gonads under the influence of FSH and LH. The increases in the concentrations of sex hormones are known to exert positive feedback influence at the level of the pituitary gland, where they regulate the secretion of gonadotropins (27, 28). In non-pregnant females with a normal menstrual cycle, the progesterone level remains relatively constant throughout the follicular phase of the menstrual cycle and then increases rapidly following ovulation, while the estradiol secretion follows a cyclic biphasic pattern, with the highest concentration found immediately prior to ovulation (18, 20, 29).

In this study, we observed that exposure to gasoline fumes results in a significant decrease in serum estradiol and progesterone levels in female rats, without any significant effect on the serum gonadotropins level. This result agrees with the earlier report of Histalera *et al.* (30), that women who have been in contact with petroleum solvents have reduced estrogen levels in the blood. Suhanova and Melninkova (7) also reported that disturbances in the menstrual functions are common in female workers in petroleum refineries, especially in those who have been subject to long term exposure to hydrocarbons, hydrogen sulphide and other related products. The low serum estradiol and progesterone levels observed in this study indicate that the constituents of gasoline fumes, if allowed into the body, can disturb the normal activity/metabolism of mammalian ovaries, and hence, the reproductive functions in female rats. Although the specific mechanism(s) by which gasoline fumes cause a decrease in the levels of serum estradiol and progesterone is not clearly understood, it may be assumed that the hydrocarbons present in the fumes may interact at the ovarian level with some specific receptors to reduce the sensitivity of the ovaries to the stimulating effect of gonadotropins.

From the result of this study, we also observed that inhalation exposure to gasoline fumes resulted in an increased testosterone level in male rats, without affecting the levels of serum gonadotropins. This observation showed that the effect of gasoline fumes on the male testicular function is the reverse of that of the ovarian functions, with respect to sex hormones. The mechanism(s) through which serum testosterone level is increased in male rats on exposure to gasoline fumes by inhalation is a subject for further studies. It is well known that high testosterone level increases libido, and hence, reproductive functions in males. In conclusion, the effect of inhalation exposure to gasoline vapours on reproductive functions in rats is sex-dependent, with the female rats being more adversely affected. Also clinical application of this finding may be of importance to the health management of workers in the petroleum industry.

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