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# **Ruinous Effect on Male Reproductive Hormones of** Wistar Albino Rats Exposed to Air Freshener

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### Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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# **ABSTRACT**

Background: Air fresheners have become indispensible in many living homes and offices, marketed with the promise of creating a clean, healthy, and sweet-smelling indoor atmosphere. Many of these products contain harmful chemicals, hence are of public health significance. This study evaluated the effects of acute exposure to gel air freshener on male reproductive hormones of wistar albino rats.

Aim: This study was designed to examine the effects of exposure to air-freshener on male reproductive hormones of Wistar Albino rats.

Study Design: Eighteen (18) Male wistar albino rats were divided into 6 groups (A-F) and three in each group. Rats in groups B-F were exposed to Air Freshener 194q via inhalation in their cages for 28 days. Rats in group A (control) were not exposed to Air Freshener. At the end of the experiment, rats were sacrificed. Blood samples were collected via cardiac puncture, male reproductive hormones; Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Testosterone (TET) and Prolactin (PRL) were determined using standard methods.

**Results:** The results obtained revealed that exposure to air freshener led to an adverse alteration in the levels of the hormones. The serum concentration of FSH and PRL increased significantly at p<0.05, while LH and TET serum concentration were significantly decreased at p<0.05.

**Conclusion:** This result has revealed that exposure of male wistar albino rats to gel air freshener adversely affected male reproductive hormones, and suggests that it may cause infertility in men. However, the effect is dependent on the duration of exposure, therefore, the reduction in duration of exposure to air freshener is suggested.

Keywords: Air freshener; testosterone; luteinizing hormone; follicle stimulating hormone; prolactin.

# 1. INTRODUCTION

Air fresheners are fragrance- containing products commonly used in homes, offices and cars to mask unpleasant odors [1,2]. There are various types, such as incense sticks, scented candles. aerosols, liquids, solids, gels, and electric diffusers [3]. The increasing application of these products in both private and public places is based on the belief that they improve sensory appeal through elimination of odors in the environment [1,2]. Air Fresheners eliminate offensive odors by releasing chemical substances into the air, strong enough to mask unpleasant odors [4]. However, studies have shown that these fragrances contain toxic chemicals that may pose health challenges to the regular users, hence are of major public health concern [1,3,4]. The major chemical constituents found in air fresheners include volatile organic compounds (VOCs), such as benzene, toluene, ethylene and limonene [5], which are known toadd to environmental pollution [1]. Studies have shown that VOCs cause a wide range of adverse effects such as drowsiness, dizziness, headache, tremor and coma [1,4]. Phthalates, another VOCs in air fresheners have been shown to cause hormonal imbalance, birth defects. reproductive dysfunctions, cardiovascular and respiratory diseases, immune dysfunctions and neurotoxicity via increased generation of reactive oxygen species (ROS) and depletion of antioxidant enzymes [4-7]. Oxidative products including ROS have been implicated in the degeneration of several neuronal pathways especially cholinergic system [5,6]. Studies revealed that exposure to air-freshner altered adversely the antioxidant status in wistar albino rats [8].

Infertility is the inability to achieve pregnancy following twelve months of unprotected sex [7]. About 50% of male infertility is found in infertile couples [8,9]. Some of the etiologies of declining male infertility can be related to falling androgen

levels, decreased sexual activity, and DNA integrity [9]. Gonadotropin releasing hormone (GnRH) secreted by the hypothalamus stimulates the release of gonadotropins i.e follicle stimulating hormone (FSH) and luteinizing hormone (LH)from the pituitary gland [10]. LH regulates testosterone (TET) synthesis by the extratubularleydig cells, FSH spermiocytogenesis and spermiogenesis by affecting both the germinal epithelium and sertoli cells [11]. The levels of these hormones are under negative feedback control by the gonads. Testosterone is responsible for the normal growth, development of male sex organs, and maintenance of secondary sex characteristics. Failure of pituitary gland to secrete FSH and LH will result in disruption of testicular function leading to infertility [12]. Prolactin is a hormone synthesized the adenohypophyse in allactotrophs, expression of prolactin receptors choroid plexuses and hypothalamus presupposes its role in the regulation of male fertility [13,14]. Testosterone regulates prolactin secretion through a long feedback mechanism [15,16]. Thus, this study was designed to evaluate the effects of Air Fresheners on male reproductive Hormones in Wistar Albino Rats.

### 2. MATERIALS AND METHODS

### 2.1 Laboratory Animals

Eighteen (18) male Wistar albino rats (80-100 kg) used in this study were procured from the University of Port Harcourt Biochemistry Department Animal farm, and housed in a well ventilated wire mesh at standard conditions, with unrestricted access to rodent foods and water.

### 2.2 Experimental Design

Eighteen wistar albino rats of 80kg-100kg were used. They were divided into six groups of three rats each and allowed to acclimatize for 7 days. Group A served as a control (was not exposed to

air freshener). Gel air fresheners of 194g weight were kept in a specially built cage to sublime and saturate the cage with its content. The experimental animals excluding the control were expose to the air fresheners at varying length of time. Animals in group B were exposed for 4 hours, group C exposed for 6 hours, group D exposed for 8 hours, group E exposed for 10 hours and group F exposed for 12hours daily for twenty-eight days. The animals were placed in the cage saturated with the gel air freshener to inhale and absorb the content of the air freshener. At the end of the experimental exposure, the rats were sacrificed and blood samples were collected by direct cardiac puncture into a sterile plain bottle for analysis of follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Testosterone (TET), Prolactin (PRL).

# 2.3 Measurement of Male Reproductive Hormones

Serum levels of testosterone, Follicle Stimulating Hormone, Luteinizing Hormone, and Prolactin were measured using Enzyme-Linked Immunosorbent Assay (ELISA) according to the methods described byManata et al. [17] and Beltran et al. [18]

# 2.4 Statistical Analysis

Data were subjected to analysis using Analysis of Variance (ANOVA) with the aid of Graph Pad Prism. Data from each parameter was expressed as mean ± Standard Deviation.Data were considered to be significantly different at 95% confidence level (P < .05).

### 3. RESULTS

The results of the effect of Acute exposure to air freshener on male reproductive Hormones of Wistar Albino rats are presented in Table 1.

Values are expressed as Mean ± Standard Deviation.

- Values with different superscript show significant difference at p<.05 level.</p>
- Values with the same superscripts shows no significant difference at p<.05 level.</p>
- ❖ FSH Follicle Stimulating Hormone
- LH Luteinizing Hormone
- ❖ TET Testosterone
- PRL Prolactin

### 4. DISCUSSION

This study evaluated the effects of air freshener on male reproductive hormone profile of Wistar albino rats.

The serum levels of FSH were significantly increased in the rats exposed to air freshener when compared with the levels in the control rats at p<0.05 (Table 1). This corresponds to the findings of Airaodion et al., [19], they reported significant increase in serum FSH concentrations when animals were exposed to insecticides. This could be as a result of feedback inhibition of anterior pituitary suppression [20]. suppression of feedback inhibition secondarily increase the secretion of FSH. The mechanism through which air freshener affects male reproductive hormones is not clear, but may be similar to that of insecticides, because they are both air pollutants [19]. The increase in serum FSH levels may reflect impaired testicular activity, resulting in altered feedback mechanism between the testes and the hypothalamic pituitary axis, through damaged sertoli cells and decreased secretion of inhibin [21]. Under normal concentration, FSH affects independently and in concert with testosterone, the proliferation, maturation and function of the supporting Sertoli cells that produce regulatory signals and nutrients for the maintenance of developing germ cells [22].

Exposure of animals to gel air freshener in this study was observed to have significantly reduced the levels of serum LH when compared with levels in the control group at p<.05 (Table 1).

Table 1. Results of the male reproductive hormones when exposed to air freshener

	MALE REPRODUCTIVE HORMONES			
GROUPS	FSH (mIU/mL)	LH (mIU/mL)	TET (mIU/mL)	PRL (mIU/mL)
A (Control)	1.00 <sup>a</sup> ±0.00	8.57 <sup>a</sup> ±0.15	2.60 <sup>a</sup> ±0.35	20.33° ± 0.58
B (4 hours' exposure)	O.45 <sup>a</sup> ±0.13	$6.78^{b} \pm 0.03$	$2.05^{b} \pm 0.08$	22.23 <sup>b</sup> ±0.21
C (6 hours' exposure)	1.20 <sup>b</sup> ±0.10	$3.30^{\circ} \pm 0.10$	1.74 <sup>b</sup> ±0.08	26.43° ±0.51
D (8 hours' exposure)	1.50 <sup>b</sup> ±0.61	$2.97^{c} \pm 0.06$	1.56 <sup>b</sup> ±0.07	26.87° ±1.50
E(10 hours' exposure)	2.57 <sup>b</sup> ±0.06	$2.50^{c} \pm 0.00$	1.57 <sup>b</sup> ±0.43	22.50 <sup>b</sup> ±0.50
F (12 hours exposure)	$3.83^{c} \pm 0.06$	$0.86^{d} \pm 0.71$	1.44 <sup>b</sup> ±0.36	24.67° ±0.56

This result is in accordance with the research result of Augustine et al., [23], they reported the effect of air freshener on sperm quality and male sex hormones, which showed that exposure of laboratory animals to air freshener reduced reduced LH recorded LH.The in study may be due to direct toxic effects of the air freshener on cells and tissue, also may be due to imbalanced hormonal levels The air freshener used in this research may have affected the testis tissue or migrate into the pituitary gland, which is capable of causing significant in the LH. The role of LH in male is stimulation of Testosterone production by Leydig cells [23].

The reduction of serum TET levels as seen in the result of this study (Table 1) agrees with the findings of Darbe, [25], who reported that air pollution can have serious hormonal effects, as many environmental pollutants (chemicals) have been reported to interfere in the functioning of endocrine system. TET in male is responsible for normal growth and development of sex organs, as well as secondary sex characteristics maintenance. A high level of intratesticular TET is an absolute prerequisite for sperm production and function, TET is responsible for the improvement of sperm motility and epididymis function [26] The mechanism on how these hormones are affected by the chemical pollutants is similar to that of the ubiquitous chemical, Bisphenol A, which acts like sex hormones, thereby binding to the receptors of the sex hormones, hence, leading to disruption in the functioning of the endocrine system and causing alteration in the concentration of sex hormones [27].

The increased serum Prolactin levels in the rats exposed to air freshener when compared withlevel in the control group at p<.05 (Table 1) may be as a result of inhibition of Hypothalamic regulatory factors, adenohyphyseallactotrophs express a testosterone-responsive inhibitory mechanism for prolactin synthesis, hence, the reduced TET levels may have induced the increased prolactin levels [16]. Hyperprolactinemia is known to suppress TET synthesis and male fertility through prolactininduced hypersecretion of adrenal corticoids or by inhibiting the secretion of gonadotropinreleasing hormone (GnRH) through prolactin hypothalamic dopaminergic receptors on neurons [28-30].

### 5. CONCLUSION

This study has revealed that exposure of male Wistar albino rats to gel air freshener adversely affects male reproductive hormones, and may cause infertility in men.

The effect of air freshener is dependent on the duration of exposure, therefore, the reduction in duration of exposure to air freshener is suggested.

### CONSENT

It is not applicable.

# **ETHICAL CONSIDERATIONS**

The ethical guidelines governing the use of laboratory animals for research as set by the Rivers State University Animal Care and Use of Research Ethics Committee were followed. The internationally accepted National Institute of Health (NIH) Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) were also observed. All efforts were in place to totally eliminate animal sufferings.

### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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