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Spermatotoxicity in Animal Models Exposed to Fragrance Components

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Various commonly-used products have been reported to contain chemicals that could disrupt estrogen and testosterone hormone. As trade secrets, these chemicals are generally listed as fragrance to mask individual identity. In this study, the reproductive implications of two commonly used perfumes (designated as F1 and F2) in Nigeria were carried out. Sixty adult male wistar rats (12-13 weeks old) were randomly divided into four groups (A-F) of ten rats each. Group A and B rats were exposed to 5 mL kg⁻¹ b.wt. of normal saline for 56 days and 112 days, respectively via whole body inhalation. Group C and D rats were exposed to 5 mL kg⁻¹ b.wt. of a fragrance product designated as F1 for a period of 56 days and 112 days, respectively while Group E and Group F rats were exposed to 5 mL kg⁻¹ b.wt. of a designated fragrance product F2 for a period of 56 days and 112 days, respectively. The results obtained from this study showed a significant ($p < 0.005$) decrease in body weight and absolute testicular weight of the rat models exposed to fragrance when compared to the control groups. It was also observed that the concentration, mobility, livability and morphology of spermatozoa from groups C, D, E and F were significantly lower ($p > 0.005$) when compared to values of the control group A and B. Based on the spermiographic evaluation from this study, fragrance materials could have an adverse effect on spermatozoa of the intact male wistar rats.

Key words: Fragrance, lipid peroxidation, spermatozoa, infertility

INTRODUCTION

Although researchers have severally reported the toxicity of fragrance exposure, most of the documentation spins around their dermatological implications. This is fairly acceptable as most fragrance-containing care products are applied directly on the skin (Vader and Christy, 2009). The exposure routes for industrial workers are mostly via the dermal and inhalation route while that of the consumers are via inhalation, dermal and oral routes (Caress and Steinemann, 2004; Vader and Christy, 2009).

It has been reported that through inhalation, ingestion and absorption, fragrance infiltrates the body and moves directly to the blood stream. Studies have shown that individual sensitivity to the effects of fragrance is relative (Elberling *et al.*, 2005, 2007) and the fact that different fragrances cause different symptoms may be an indication of its varying toxicity (Environmental Health Network, 2002). Symptoms ranging from severe mucosal discharge, sinus problems, tremor, asthmatic attack, sneezing, migraine headache, convulsions, hyperactivity, nausea, sore throat, cough, chest tightness to shortness of breath after fragrance exposure have been vastly documented (Guin and Berry, 1980; De Groot, 1987; Schleuter *et al.*, 1978).

Unswerving connection between memory and smell has been established (Rachel and Engen, 1996). This knowledge has resulted in placement of fragrance in the category of psychoactive drugs and highlighted the ability of fragrance to cross the brain barrier thereby resulting in potential damage to brain tissue (Andrea, 1997). Linalool, the most abundant fragrance substance has been indicated to cause lethargy, depression and severe respiratory difficulties after exposure (Dahom, 2011).

Research shows the ability of fragrance from citrus to efficiently boost metabolism, memory and alleviate food cravings and depression than were prescription anti-depressants, confirming the previous reports on its ability to permeate the brain barrier (Rachel and Engen, 1996).

Dose-dependent reduction in fecundity, median survival time and an increase in early life-stage mortality post administration of to musk xylene and musk ketone has been reported (Carlsson *et al.*, 2000). Diethyl phthalate, a common fragrance substance in perfume and cologne has been shown to damage sperm DNA in adult, cause premature breast formation in developing baby girls and abnormal sexual development in male fetuses including cryptorchidism (Duty *et al.*, 2003).

Synthetic musk fragrance ingredients which are widely highly distributed in many consumer products

have been examined in human blood, milk and fatty tissue. They represent a new group of human contaminants which are comparable with that of certain pesticides. Despite several reports on the toxic effect of fragrance, there is a dearth of literature ascertaining its effects on male fertility and testicular development (Thompson and Wansker, 1981).

Therefore, the solemn aim here in is to evaluate the possible effect of fragrance exposure on male infertility in animal models.

MATERIALS AND METHODS

Materials: Two commonly used perfumes in Nigeria designated as F1 and F2 were obtained from Bayous Cosmetics in Lagos on 23rd of April, 2012 and were kept under standard temperature.

Experimental procedure: Sixty adult male wistar rat (12-13 weeks old) weighing 190-220 g were used for the study. The rats were randomly divided into four groups (A-F) of ten rats and the average weight difference between and within groups did not exceed $\pm 20\%$ of the average weight of the sample population. Group A rats served as the first control and were exposed to 5 mL kg⁻¹ b.wt. of normal saline for 56 days. Group B rats served as the chronic control group and were exposed to 5 mL kg⁻¹ b.wt. of normal saline for a period of 112 days. Group C and Group D rats were exposed to 5 mL kg⁻¹ b.wt. of F1 for a period of 56 days and 112 days respectively. Group E and Group F animals were exposed to 5 mL kg⁻¹ b.wt. of F2 for a period of 56 days and 112 days, respectively. The study is consistent with the standard of the use of laboratory animals (WMA/APS, 2002; Akunna *et al.*, 2011).

Method of exposure: The exposure was done via whole body inhalation. Small balls of cotton wool were soaked with the fragrance (Experimental Groups) and normal saline (Control Groups), respectively. The wools were rightly placed in a Petri dish inside the cages and covered with perforated plastic container to prevent direct contact for an exposed duration of at least six hours per day throughout the period of study.

Animal sacrifice and sample collection: The rats were first weighed and then subjected to cervical dislocation. The abdominal cavity was opened up through a midline abdominal incision to expose the reproductive organs. The testes were excised and trimmed of all fat. The rats were then anaesthetized by placing them into a glass chamber containing cotton wool soaked in chloroform till

they lost consciousness. The testicles were then removed through a lower abdominal incision. The testes were then separated from the epididymis with the scalped blade. Sperm cells were sucked into a pre-warmed (37°C) Pasteur pipette from the caudal epididymis. It was flushed with 2-3 drops of 2.9% sodium citrate self kept at 37°C. Smears were prepared from these samples and strained with wells and Awa stain for morphological studies and eosin and nigrosin stain for live/dead ratio. Half of the spermatozoa samples collected were mixed with 0.5 mL of 2.9% sodium citrate solution for head forward unidirectional progressive motility. These were studied at $\times 40$ magnifications of the microscope (Zemjanis, 1970).

The mean percentages and standard error of mean were calculated for motility, live/dead ratio, Spermatozoa concentration and morphological studies. ANOVA (Analysis of Variance) was used to establish any significant difference in all these parameters (Snedecor, 1946).

RESULTS AND DISCUSSION

Changes in body weight, testis weight and volume of testes mean: As indicated in Fig. 1 and 2, the findings in our study showed a significant reduction in body weight, testicular weights and testicular volumes of the group of rats exposed to both F1 and F2. However, the group of rat exposed to F1 and F2 for a period of 112 days had a more significant ($p < 0.005$) weight loss when compared to the group that were exposed to F1 and F2 for a period of 56 days, suggesting a time dependent nature of toxicity.

Our results are in conformity with previous reports of considerable decrease in body and testicular weight due to seminiferous tubular derangements (Sofikitis and Miyagawa, 1992; Saalu *et al.*, 2010, 2009). Akunna *et al.* (2011) reported a significant loss in body weight of rats exposed to two Nigerian made perfumes. Although there was no significant difference between rats treated for the period of 154 days and rats treated for the period of 77 days. The testicular derangement indicated in the experimental model might have been as a result of active metabolites produced by the fragrance material (F1 and F2) which could have aided the production of lipid peroxides, resulting in oxidative degenerative changes in the cell and inhibition of mitochondrial action and eventually causing cell death (Akunna *et al.*, 2013). This could be the rationale behind the significant reduction in testicular weight and volume of treated animals.

In a study done by Carlsson *et al.* (2000), to evaluate the impact of musk ketone (a major fragrance component in perfume) on reproduction, exposed models had a significant reduction in body weight and gonad somatic

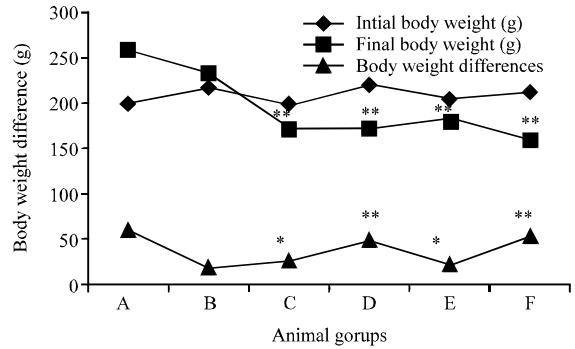


Fig. 1: Effect of Fragrance exposure (5 mL kg⁻¹ b.wt. for 56 days and 112 days) on the initial body weight, final body weight and body weight differences of experimental rats. * $p < 0.05$, ** $p < 0.005$ significantly different from control. Values are expressed as Mean \pm SD for $n = 10$ in each group, A: 5 mL kg⁻¹ b.wt. of normal saline for 56 days (Control), B: 5 mL kg⁻¹ b.wt. of normal saline for 112 days (Control), C: 5 mL kg⁻¹ b.wt. of Fragrance 1 (F1) for 56 days, D: 5 mL kg⁻¹ b.wt. of Fragrance 1 (F1) for 112 days, E: 5 mL kg⁻¹ b.wt. of Fragrance 2 (F2) for 56 days, F: 5 mL kg⁻¹ b.wt. of Fragrance 2 (F2) for 112 days

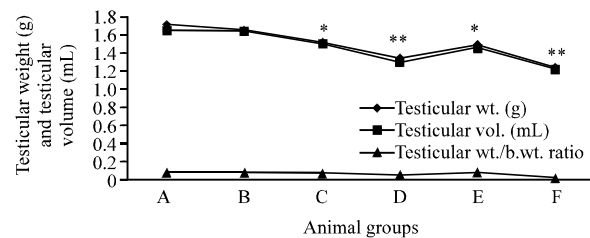


Fig. 2: Effect of Fragrance exposure (5 mL kg⁻¹ b.wt. for 56 days and 112 days) on testicular weight, testicular volume and testicular weight/body weight ratio of experimental rat, * $p < 0.05$, ** $p < 0.005$ significantly different from control. Values are expressed as Mean \pm SD for $n = 10$ in each group, A: 5 mL kg⁻¹ b.wt. of normal saline for 56 days (Control), B: 5 mL kg⁻¹ b.wt. of normal saline for 112 days (Control), C: 5 mL kg⁻¹ b.wt. of Fragrance 1 (F1) for 56 days, D: 5 mL kg⁻¹ b.wt. of Fragrance 1 (F1) for 112 days, E: 5 mL kg⁻¹ b.wt. of Fragrance 2 (F2) for 56 days, F: 5 mL kg⁻¹ b.wt. of Fragrance 2 (F2) for 112 days

index which is an indication of the toxic effect of fragrance. It should be noted however, that the control

Table 1: Effect of Fragrance exposure (5 mL kg⁻¹ b.wt. for 56 days and 112 days) on sperm count, sperm motility and livability of experimental rats

Treatment groups	Sperm Count (×10 ⁶ mL)	Sperm motility (%)	Live/Dead ratio (%)
A	128.3±3.1	82.1±5.2	88.50±0.3
B	132.2±4.6	91.2±1.1	90.2±2.1
C	70.1±2.2	45.3±1.7	69.3±5.5*
D	51.6±1.5**	25±9.200**	65.6±2.7*
E	72.3±5.1*	41.3±3.1*	70.4±4.3*
F	59.8±3.9	28.2±9.8	57.0±0.0**

*p<0.05, **p<0.005 significantly different from control. Values are expressed as Mean±SD for n =10 in each group, A: 5 mL kg⁻¹ b.wt. of normal saline for 56 days (Control), B: 5 mL kg⁻¹ b.wt. of normal saline for 112 days (Control), C: 5 mL kg⁻¹ b.wt. of Fragrance 1 (F1) for 56 days, D: 5 mL kg⁻¹ b.wt. of Fragrance 1 (F1) for 112 days, E: 5 mL kg⁻¹ b.wt. of Fragrance 2 (F2) for 56 days, F: 5 mL kg⁻¹ b.wt. of Fragrance 2 (F2) for 112 days

Table 2: Effect of Fragrance exposure (5 mL kg⁻¹ b.wt. for 56 days and 112 days) on sperm morphology of experimental rats

Groups	Headless tail (%)	Rudimentary tail (%)	Curved Mid-piece (%)	Curved tail (%)	Total abnormal (%)	Total normal (%)	Total cell counted (%)
A	6.0 (0.6)	5.6 (9.1)	11.4 (4.1)	0	23 (13.8)	860 (45)	883 (58.8)
B	6.3 (4.2)	5.0 (1.1)	10.2 (1.0)	0	21.5 (6.3)	980 (53.2)	1001.5 (59.5)
C	16.2 (0.1)	20 (5.1)	24 (5.1)	3.1 (5.0)	63.3 (15.3)	1102 (40.5)	1165.3 (55.8)
D	21.1 (3.3)	24.1 (0.2)	24.1 (8.1)	0	69.3 (11.6)	980 (38.4)	1049.3 (50)
E	18.3 (4.1)	21.2 (0.3)	23.3 (1.2)	0	62.8 (5.6)	920 (40.6)	982.8 (46.2)
F	16.2 (0.1)	17.3 (4.2)	26.1 (0.1)	2.2 (0.3)	61.8 (4.7)	890 (35.3)	951.8 (40)

*p<0.05, **p<0.005 significantly different from control. Values are expressed as Mean±SD for n =10 in each group, A: 5 mL kg⁻¹ b.wt. of normal saline for 56 days (Control), B: 5 mL kg⁻¹ b.wt. of normal saline for 112 days (Control), C: 5 mL kg⁻¹ b.wt. of Fragrance 1 (F1) for 56 days, D: 5 mL kg⁻¹ b.wt. of Fragrance 1 (F1) for 112 days, E: 5 mL kg⁻¹ b.wt. of Fragrance 2 (F2) for 56 days, F: 5 mL kg⁻¹ b.wt. of Fragrance 2 (F2) for 112 days

group of animal had a significant (p<0.005) increase in gross anatomical parameters which could mean that the control group of rat were still in their active growth phase during the study.

Sperm characteristics: As shown in Table 1, the groups of rat exposed to the two perfume for 112 days had a marked oligospermia (p<0.005), reduced sperm motility (p<0.005) and livability (p<0.005) when compared to the group exposed for 56 days which also had a significant decrease in sperm count (p<0.05), motility (p<0.05) and percentage amount of normal spermatozoa. These indicates a time dependent manner of fragrance toxicity. The morphological characteristic of the sperm cells in all the six groups of rats are as shown in Table 2, the following abnormalities were noticed both in the control group and the treated groups C, D, E, F; head less tail, rudimentary tail, curved mid-piece, curved tail. As shown in Table 2, there was a significant increase in percentage number of abnormal sperm of the exposed rat which was duration dependent. The observed decrease in sperm parameters could be as a result of the deleterious effect of fragrance on androgen-secreting capacity (Akunna *et al.*, 2013). Diminution in spermatozoa concentration and mobility with an increase in the percentage of abnormal sperms is directly related to infertility (Mazzilli *et al.*, 1994; Seed *et al.*, 1996). Sperm membranes are very vulnerable to oxidative insult owed to a high concentration of polyunsaturated fatty acids and the need for Sertoli cell barrier protection (Winterboum *et al.*, 1975; Zorogniotti and Sealfon, 1988; Weese *et al.*, 1993). Endocrine disruptors are chemical agents that have been severally documented as a possible cause of male infertility.

Currently, the process for deciding if a chemical is an environmental endocrine disruptor is to determine the effects of that chemical on the endocrine systems of humans and other animals. Fragrance has been indicated as a possible endocrine disrupting agent (Duty *et al.*, 2003). The most likely effect of endocrine disruption in men may be a reduction in sperm concentration, motility and elevation in percentage abnormal sperm which was evident in our study (Carlsen *et al.*, 1992; Sharpe, 1992).

CONCLUSION AND RECOMMENDATION

The overall results obtained in this study suggest that fragrance materials are toxic to spermatozoa of rat with the effect of a time dependent nature.

Despite the well established documentation on the effect of fragrance materials on human health, there is a need for further investigations in humans to determine its safety margin as far as fertility is concerned.

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