Journal of

# Pharmacology and Toxicology

ISSN 1816-496X

Academic
Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 9 (2): 68-81, 2014
ISSN 1816-496X / DOI: 10.3923/jpt.2014.68.81
© 2014 Academic Journals Inc.

# Rhodinol-based Incense Testiculotoxicity in Albino Rats: Testicular Histology, Spermatogenic and Biochemical Evaluations

[1]A.M. Akingbade, [1]L.C. Saalu, [1]O.O. Oyebanji, [1]D.A. Oyeniran, [1]O.O. Akande and [2]G.G. Akunna
[1]Department of Anatomy, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria
[2]Department of Anatomy, Federal University Nudfu-Alike Ikwo, Ebony State, Nigeria

*Corresponding Author: Linus C. Saalu, Department of Anatomy, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria Tel: 08033200876*

## ABSTRACT

This study evaluates the testicular degeneration induced by Rhodinol-based incense using testicular histology, sperm characteristics, as well as testicular oxidative status bio-markers. Twenty four male adult albino rats (10-12 weeks old) weighing 200-230 g were divided into four groups (A, B, C and D) of six rats each. Group A served as the control group and the animals in this group were exposed to 1 g of natural solid air freshner. Groups B, C and D, were exposed to 1, 2 and 3 g of rhodinol-based incense smoke, respectively for 30-40 min every day for 62 days. All the exposures were via whole body inhalation. The animals were sacrificed 24 h after. The results obtained from this study showed a significant ($p<0.05$) decrease in the gross anatomical parameters of absolute and relative testicular weights in rats exposed to 2 g and 3 g rhodinol based incense when compared to the control group. The rhodinol-based incense exposed groups of rats also demonstrated a reduction of basal seminiferous epithelia, testicular atrophy, germinal aplasia and hypo-spermatozoa formation. Furthermore, there was a statistically significant ($p<0.05$) decrease in sperm count, sperm motility, normal sperm morphology and a significant ($p<0.05$) increase in total abnormal sperm morphology in group of animals exposed to 2 and 3 g rhodinol-based incense when compared to the control group. In addition, groups of animals exposed to 2 and 3 g rhodinol-based incense demonstrated a derangement in their oxidative status when compared to the control group as evidence by the significant ($p<0.05$) decreased in activities of superoxide peroxidase, catalase, glutathione, reduced glutathione and significant ($p<0.05$) increase malondialdehyde (a product of lipid perioxidation). Taken together, it was concluded that rhodinol-based incense produces testicular derangement in testicular histology, sperm parameters and oxidative status in albino rats. This derangement may be mediated at least in part through the oxidative pathway.

**Key words:** Rhodinol, oxidative stress, infertility, histology, rats

## INTRODUCTION

Male infertility is one of the major health problems in our society and the possibility that some chemicals may disrupt the endocrine systems in humans and animals have received considerable attention in the scientific and public community (Saalu *et al.*, 2009).

Infertility and exposure to household substances have been correlated in several studies and many environmental xenobiotic chemicals, such as polychromatic biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), have been discovered to have estrogenic effects (Colborn *et al*., 1993; Sikka and Gurbuz, 2006). The cyto-toxic effect of perfume on neonatal mice was shown by Katarina *et al*. (2006). Also, a study of allethrin-based mosquito coil was shown to be testiculotoxic by Akunna *et al*. (2013).

Incense (from Latin incendere “to burn”) is composed of chemicals and aromatic materials, which release fragrant smoke when burned (Herrera, 2011). A typical incense stick can measure around 23 cm in length and last up to 1 h during burning. Incense is widely used in Africa countries including Nigeria. The fragrance of incense is used as a gesture of hospitality during special occasions like religious ceremonies in churches and prayer houses for creating spiritual atmosphere and by the Muslims clerics during prayers (Neal, 2003). Incense is also used in aromatherapy medications and for creating a mood masking bad odours (Hyams and Cushner, 2003).

Rhodinol is an active ingredient of incense called Viji®. Individuals are most likely to be exposed to rhodinol-based incense dermally or by inhalation during the manufacture, formulation and burning of this incense.

Oxidative pathway has been ascribed to rhodinol-based incense induced toxicity in organs (Fowler *et al*., 2004). The gonad is also considered the main target for environmental toxins. This organ has membranous structures rich in polyunsaturated fatty acid. Membrane polyunsaturated fatty acids are highly sensitive to oxidative stress manifested through lipid peroxidation, which usually result in loss of membrane integrity (Sokol, 1997).

A number of studies have reported the pathological and pharmacological effect of incense smoke on numerous organs such aslungs, skin and liver (Alarifi, 2005; Alokail *et al*., 2011). Even though there are various studies on the cytotoxic effects of various types of incense on several other organs, there is however, a dearth of information in the literature on the testiculotoxic effects of a rhodinol-based incense.

Aim in the present study is to investigate the effects of rhodinol-based incense on the testicular histology, sperm parameters and testicular oxidative status.

## MATERIALS AND METHODS

### Materials

**Incense:** A commonly used brand of incense ‘viji’® (1 g and 23 cm) containing 0.81% w/w rhodinol, 0.1% w/w resinoids and inert ingredients 0.1% w/w was purchased from optimist supermarket, Mende, Maryland, Lagos, Nigeria in the month of July, 2013.

**Air freshner:** Natural solid air freshner containing 100% essential oil (20 g) was purchased from Payworth, Nigeria L.T.D. in Ketu Lagos in the month of July, 2013.

**Animals:** Twenty four male albino rats (10 to 12 weeks old) weighing 200-230 g were obtained from the Animal House of Lagos State University College of Medicine, Ikeja, Lagos. An approval was sought and obtained from the departmental ethical committee on animal use. The rats were allowed to acclimatize for 3 weeks and were fed freely on standard commercial mouse cubes from Akintola and Son’s Livestock Feed Limited, Agege, Lagos, Nigeria.

Relatively constant environmental condition were maintained with proper aeration and good source of light (12 h light-12 h dark and 24±30°C). Food and water were provided *ad libitum*. The weighings and observations were done before the rats were exposed to rhodinol-based incense, respectively.

The weights of the animals were estimated at procurement, during acclimatization, at commencement of the experiments and twice within a week throughout the duration of the experiment, using an electronic analytical and precision balance (BA210S, d = 0.0001 g) (SatoriusGA, Goettingen, Germany).

Experimental procedures involving the animals and their care were conducted in conformity with International, National and institutional guidelines for the care of laboratory animals in Biomedical Research and Use of Laboratory Animals in Biomedical Research as promulgated by the CCAC (1985). Further, the animal experimental models used conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (WMA/APS, 2002).

## Methods

**Animal groupings and rhodinol-based incense exposure:** Four groups of rats (A, B, C and D) consisting of 6 animals each were housed separately in four undisturbed cages of size 5 $m^3$ with cross ventilation to avoid the cross exposure to incense smoke (Ahmed *et al*., 2013). The rats in group A served as the control and were exposed to 1 g of natural solid air freshner every day for 8 weeks consecutively. The rats in group B were exposed to the smoke emanating from the burning of 1 g of each rhodinol-based incense material via whole body inhalation for 30-40 min every day, for a period of 8 weeks (Wang *et al*., 1999; Ahmed *et al*., 2013) The rats in group C and D were exposed to the smoke emanating from theburning of 2 and 3 g of rhodinol-based incense material via whole body inhalation for 30-40 min every day, for a period of 8 weeks, respectively.

**Animal sacrifice and sample collection:** The rats at the time of sacrifice were first weighed and then anaesthetized by placing them in a closed jar containing cotton wool soaked in chloroform. The abdominal cavity was opened up through a midline abdominal incision to expose the reproductive organs. Then the testes and epididymis were excised. The weight of the testes of each animal was evaluated. The testes were weighed with an electronic analytical and precision balance (BA 210S, d = 0.0001- Sartoriusen GA, Goettingen, Germany). The volume of each testis was measured by water displacement method. The two testes of each rat were measured and the average value obtained for each of the two parameters was regarded as one observation.

One of the testes of each animal was fixed in 10% formol-saline for histological examination. Serum and the remaining testes of each animal were store at -25°C for biochemical assays.

### Determination of epididymal sperm parameters

**Progressive sperm motility:** This was done immediately after the semen collection. Semen was squeezed from the caudal epididymis onto a pre-warmed microscope slide (27°C) and two drops of warm 2.9% sodium citrate was added, the slide was then covered with a warm cover slip and examined under the microscope using X400 magnification. Ten fields of the microscope were randomly selected and the sperm motility of 10 sperms was assessed on each field. Therefore, the motility of 100 sperms was assessed randomly. Sperms were labeled as motile, sluggish or immotile. The percentage of motile sperms was defined as the number of motile sperms divided by the total number of counted sperms (i.e., 100) (Mohammad-Reza *et al*., 2005).

**Epididymal sperm concentration:** Spermatozoa in the right epididymis were counted by a modified method of Yokoi and Mayi (2004). Briefly, the epididymis was minced with anatomic scissors in 5 mL physiologic saline, placed in a rocker for 10 min and allowed to incubate at room temperature for 2 min. After incubation, the supernatant fluid was diluted 1:100 with solution containing 5 g sodium bicarbonate and 1 mL formalin (35%). Total sperm number was determined by using the new improved Neuber's counting chamber (haemocytometer). Approximately 10 μL of the diluted sperm suspension was transferred to each counting chamber of the haemocytometer and was allowed to stand for 5 min. This chamber was then placed under a binocular light microscope using an adjustable light source. The ruled part of the chamber was then focused and the number of spermatozoa counted in five 16-celled squares. The sperm concentration was calculated and expressed as $[X]\times10^6$ $mL^{-1}$ where, [X] is the number of spermatozoa in a 16-celled square.

**Sperm morphology:** The sperm cells were evaluated with the aid of light microscope at X400 magnification. Caudal sperm were taken from the original dilution for motility and diluted 1:20 with 10% neutral buffered formalin (Sigma Aldrich, Oakville, ON, Canada). Five hundred sperm from the sample were scored for morphological abnormalities (Atessahin *et al*., 2006). Briefly, in wet preparations using phase contrast optics, spermatozoa were categorized. In this study a spermatozoon was considered abnormal morphologically if it had one or more of the following features: Rudimentary tail, round head and detached head and was expressed as a percentage of morphologically normal sperm.

**Determination of biochemical parameters**

**Assay of testicular enzymatic antioxidants**

**Assay of catalase (CAT) activity:** Catalase activity was measured by the method described by Aebi (1974). To 0.5 mL of sample 5 μL of absolute ethanol was added and incubated for 30 min in ice bath, 0.45 mL of this aliquot 50 μL of 10% triton x-100 (1% final concentration) was added (Cohen *et al*., 1970). Catalase forms an inactive complex with $H_2O_2$, which is called Complex II. Ethanol reverses the inactivation, which occurs during the time interval from the preparation of tissue homogenate to the subsequent assay of catalase activity. Triton x-100 increases observable catalase levels, as the activation is complete. The enzyme reaction was started by adding 0.1 mL of sample (0.4-0.5 mg protein) to 2.9 mL of 50 mM phosphate buffer, pH 7.0 containing 12 mM $H_2O_2$. The absorbance was recorded at 240 nm immediately at 15 sec interval for 2 min. Activity of enzyme was expressed as U $mg^{-1}$ protein.

**Assay of superoxide dismutase (SOD) activity:** SOD activity was determined according to the method of Das *et al*. (2000) Using a negative assay system for detection of SOD by the photo reduction of riboflavin. In this method, superoxide radicals generated are allowed to react with hydroxylamine hydrochloride to produce nitrite, which in turn reacts with sulphanilic acid to produce diazonium compound that forms red azo compound by reacting with napthaloamine. The azo compound has absorption maxima at 543 nm. Superoxide dismutase scavenges superoxide radicals produced by photo reduction of riboflavin. The activity of superoxide dismutase is inversely proportional to the formation of red azo compound. In brief, the 1.4 mL of cocktail was prepared by adding following reagents: (1) 1.11 mL of 50 mM phosphate buffer pH 7.4, (2) 0.075 mL of 2 mM L-methionine, (3) 0.04 mL of 1% (v/v) triton X-100, (4) 0.075 mL of 10 mM HAC, (5) 0.1 mL

of 78.125 μM EDTA. To 1.4 mL of this prepared cocktail 0.1 mL of sample (containing 50-100 μg protein) and 0.1 mL of 40 μM riboflavin were added. A blank prepared without riboflavin and a control without sample was run with each set. The tubes were exposed to two 20 W fluorescent lamps fitted parallel to each other in an aluminum foil coated wooden chamber for 10 min. After incubation period, 1 mL of Griess Reagent was added. The Griess Reagent was prepared by adding equal volumes of 0.1% N (1-Napthylethylene diamine) and 1% sulfanilamide in 5% orthophosphoric acid.

The absorbance of tubes was read at 543 nm against the blank. Enzyme activity was expressed as U $mg^{-1}$ protein.

**Assay of glutathione peroxidase (GPx) activity:** Glutathione peroxidase activity was measured by the method described by Rotruck *et al.* (1973). The reaction mixture contained 2.0 mL of 0.4 M Tris-HCl buffer, pH 7.0 and 0.01 mL of 10 mM sodium azide, 0.2 mL of enzyme, 0.2 mL of 10 mM glutathione and 0.5 mL of 0.2 mM $H_2O_2$. The contents were incubated at 37°C for 10 min followed by the termination of the reaction by the addition of 0.4 mL 10% (v/v) TCA, centrifuged at 5000 rpm for 5 min. The absorbance of the product was read at 430 nm. Activity of enzyme was expressed as U $mg^{-1}$ protein.

**Assay of testicular non-enzymatic antioxidants**

**Assay of testicular reduced glutathione (GSH) concentration:** The estimation of reduced glutathione level was estimated according to the method of Griffith (1980). The mitochondrial and post-mitochondrial fractions were precipitated in ice cold 5% trichloroacetic acid containing 0.01N HCl and then subjected to centrifugation at 1000 g for 15 min. Following reagents were used for the assay: (1) 50 mM phosphate buffer containing 7 mM EDTA, pH 7.4, (2) 0.3 mM NADPH prepared in 50 mM phosphate buffer pH 7.4 containing 7 mM EDTA, (3) 6 mM DTNB, (4) GR (50 U $mL^{-1}$), (5) 2-vinyl pyridine and (6) 8 μM GSH. The absorbance was recorded at 412 nm. Reduced glutathione concentration was expressed as nmol $mg^{-1}$ protein.

**Estimation of lipid peroxidation (malondialdehyde):** Lipid peroxidation in the testicular tissue was estimated colorimetrically by thiobarbituric acid reactive substances (TBARS) method of Buege and Aust (1978). A principle component of TBARS being malondialdehyde (MDA), a product of lipid peroxidation. In brief, 0.1 mL of tissue homogenate in Tris-HCl buffer, pH 7.5 was treated with 2 mL of (1:1:1 ratio) TBA-TCA-HCl reagent (thiobarbituric acid 0.37%, 0.25 N HCl and 15% TCA) and placed in water bath for 15 min, cooled. The absorbance of clear supernatant was measured against reference blank at 535 nm. Concentration was calculated using the molar absorptivity of malondialdehyde which is $1.56\times10^5$ $M^{-1}$ $cm^{-1}$ and expressed as nmol $mg^{-1}$ protein.

**Statistical analysis:** All data were expressed as Mean±SD for n = 6. The level of homogeneity among the groups was tested using Analysis of Variance (ANOVA) as done by Snedecor and Cochran (1980). Where heterogeneity occurred, the groups were separated using Duncan Multiple Range Test (DMRT). A value of $p<0.05$ was considered to indicate a significant difference between groups (Duncan, 1957). Analysis of data was done using both electronic calculator and Statistical Package for Social Sciences (SPSS)/PC computer program (version 16.0 SPSS, Cary, NC, USA).

## RESULTS

**Effect of rhodinol-based incense on gross anatomical parameters of albino rats:** There was non-significant ($p>0.05$) decrease in testis weight, testis weight/body weight ratio and testis

Table 1: Effect of Rhodinol based-incense on gross anatomical parameters of albino rats

| Treatment groups | Body weight (g) | | | Testis | | Testis weight/body weight ratio |
|---|---|---|---|---|---|---|
| | Initial | Final | Different | Weight (g) | Volume (mL) | |
| A | 206.5±2.00 | 215±1.0 | 9.0 | 1.30±3.1 | 1.28±0.3 | 0.006 |
| B | 210.57±4.5 | 195.4±1.4 | 15.0* | 1.20±0.6 | 1.17±0.2 | 0.006 |
| C | 220.5±2.90 | 190±1.8 | 30.0* | 1.05±0.7* | 1.07±0.4* | 0.005* |
| D | 230.5±5.00 | 181±4.0 | 49.2* | 0.59±0.5* | 0.53±0.3* | 0.003* |

*p<0.05 significantly different from control, values are expressed as Mean±SD for n = 6 in each group, Group A, B, C and D: 1 g natural air freshner (100% essential oil) control, 1 g incense, 2 g incense and 3 g incense exposure for 8 weeks, respectively

Table 2: Effect of rhodinol based incense on the sperm parameters of male rat

| Treatment groups | Sperm count ($\times10^6$ mL$^{-1}$) | Sperm motility (%) | Sperm normal (%) | Morphologyabnormal(%) |
|---|---|---|---|---|
| A | 130.62±2.9 | 92.06±6.2 | 83.3±9.20 | 17.2±5.80 |
| B | 115.22±2.4 | 62.34±4.8 | 68.41±7.6 | 20.4±6.10 |
| C | 92.72±1.9* | 51.30±3.5* | 56.20±2.2* | 25.4±1.70 |
| D | 78.33±1.4* | 49.22±2.0* | 42.1±1.80* | 41.51±3.8* |

*p<0.05 significantly different from control, value are expressed as Mean±SD for n = 6 in each group, Group A, B, C and D: 1 g of natural air freshner (100% essential oil) control, 1 g incense, 2 g incense and 3 g incense exposure for 8 weeks, respectively

volume in 1 g incense exposed group when compared to the control counterpart, whereas statistically significant (p<0.05) decrease was observed in 2 and 3 g incense exposed group compare to the control counterpart (Table 1).

**Effect of rhodinol-based incense on the sperm parameters of male rat**

**Sperm count:** The group of rats exposed to 1 g of incense showed non-significant (p>0.05) decrease in sperm concentration (115.22±2.4$\times10^{-6}$ mL$^{-1}$) compared to the control group (130.62±2.9$\times10^{-6}$ mL$^{-1}$), 2 g incense exposed group provoked significantly (p<0.05) decreased sperm concentration (92.72±1.9$\times10^{-6}$ mL$^{-1}$) and 3 g incense exposed group showed marked oligospermia (78.33±1.4$\times10^{-6}$ mL$^{-1}$) with their sperm concentration being significantly lower (p<0.05)compared to the control group (Table 2).

**Sperm motility:** Although, the sperm motility of 1 g incense exposed group showed a lower non-significantly (p>0.05) (62.34±4.8%) compared to the control group (92.06±6.2%). However, the 2 g and 3 g incense exposed groups still had significantly lower (p<0.05) (51.30±3.5%) and (49.22±2.0%) value compared to the control counterpart (Table 2).

**Sperm morphology:** The 1 g incense group showed evidence of non-significantly (p>0.05) decrease in normal sperm morphology (68.41±7.6%) and non-significantly (p>0.05) increased in abnormal sperm morphology (20.4±6.1%) compared to the control group (83.3±9.2, 17.2±5.8%), respectively. The 2 g incense group however, showed a significant (p<0.05) decrease in normal sperm morphology (56.22±2.2%) and a significant (p<0.05) increase in abnormal sperm morphology (25.4±1.7%) when compared to the control group. Moreover, the rats exposed to 3 g incense also had significant (p<0.05) (42.1±1.8%) decrease in normal sperm morphology and a significant increase in abnormal sperm morphology (p<0.05) (41.51±3.8%) when compared to the control group (Table 2).

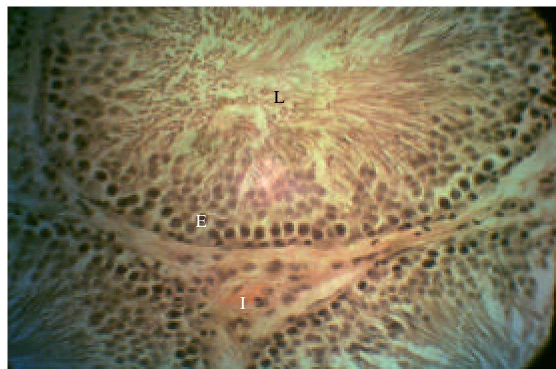


Fig. 1: Testis of group A rats (control), Treatment: Exposed to 1 g of air freshner containing 100% essential oil for 8 weeks, Stain: Haematoxylin and eosin. Magnification: X400, E: Seminiferous epithelium, L: Lumen of seminiferous tubule, I: Testicular interstitium

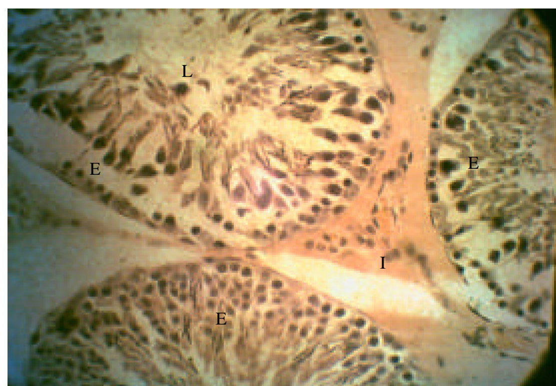


Fig. 2: Testis of group B rats, Treatment: Exposed to 1 g b.wt. of rhodinol based incense for 8 weeks, Stain: Haematoxylin and eosin, Magnification: X400, E: Seminiferous epithelium, L: Lumen of seminiferous tubule, I: Testicular interstitium

**Effect of rhodinol-based incense on the histological profiles of the testis:** As shown in Fig. 1, the sections of testes of control rats (Group A) showed normal testicular architecture with distinct seminiferous tubules, a normal cross sectional epithelial outline, numerous spermatozoa within their lumen, oval outlined with normal seminiferous epithelium and intact testicular interstitium.

In group B, the rats were exposed to 1 g of rhodinol-based incense and there was minimal damage in the testicular interstitium, the outlines of the seminiferous tubules were also moderately reduced and the spermatozoa within their lumen were altered compared to the control group (Fig. 2).

The seminiferous tubules of the rat in group C that were exposed to 2 g rhodinol-based incense showed degeneration in testicular architecture. The testicular interstitium were also reduced and were detached from the seminiferous tubules, isolating them from each other. The diameter of the lumen was reduced, characterized by slight vacuolization of the interstitium and reduced spermatozoa (Fig. 3).

Table 3: Effects of rhodinol based incense on testicular enzymatic antioxidants superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) of albino rat

| Groups | SOD (U $mg^{-1}$ protein) | CAT (U $mg^{-1}$ protein) | Gpx (nmol $mg^{-1}$ protein) |
|---|---|---|---|
| A | 3.65±2.10 | 12.83±1.8 | 1.10±2.1 |
| B | 2.71±1.41 | 12.00±1.7 | 0.92±1.3 |
| C | 2.36±1.60* | 9.31±0.1* | 0.71±1.8* |
| D | 1.72±1.60** | 6.50±2.1** | 0.56±0.8** |

*p<0.05 significantly different from control, values are expressed as Mean±SD for n = 6 in each group, Group A, B, C and D: 1 g of natural air freshner (100% essential oil) control 1 g incense, 2 g incense and 3 g incense exposure for 8 weeks, respectively

The rats in group D that were exposed to 3 g of rhodinol-based incense showed total degeneration of testicular interstitium, significant reduction in the diameter of seminiferous tubules and lumens devoid of spermatozoa were observed. There was also a significant reduction of the basal seminiferous epithelial cell (Fig. 4).

**Effects of rhodinol-based incense on activities of testicular enzymes superoxide peroxidase (SOD), catalase (CAT) and glutathione (GPx):** Incense exposed to group B rats caused a non-significant (p>0.05) decreased in SOD activity (2.71±1.41 U $mg^{-1}$ protein) when compared to the control values (3.65±2.1 U $mg^{-1}$ protein). Group C which was exposed to 2 g of incense had a significant (p<0.05) decrease in SOD activity (2.36±1.6 U $mg^{-1}$ protein) compared to the control counterparts. Rats exposed to 3 g of incense group D had an SOD activity level (p<0.05) (1.72±1.6 U $mg^{-1}$ proteins) which is significant compared to that of the control as shown on Table 3. The testicular activities of CAT group B rats exposed to 1 g of incense showed a non-significant (p>0.05) decrease in CAT values (12.00±1.7 U $mg^{-1}$ protein) when compared to that of the control counterpart (12.83±1.8 U $mg^{-1}$ protein). Group B (2 g incense group) rats, however, had a significant (p<0.05) reduction in testicular CAT activities (9.31±0.1 U $mg^{-1}$ protein) compared to the control counterpart. Incense exposure to 3 g incense caused highly significant (p<0.05) (6.50±2.1 U $mg^{-1}$ protein) decrease in testicular CAT activity compared to the control group (Table 3).

The testicular content of GPX group B rats exposed to 1 g of incense had non-significant (p>0.05) decrease in values (0.92±1.3 nmol $mg^{-1}$ protein) when compared to the control counterpart (1.10±2.1 nmol $mg^{-1}$ protein). Group C rats which had 2 g of incense showed significantly (p<0.05) decrease in values (0.71±1.8 nmol mg protein) when compared to the control value. Furthermore, group D (3 g incense group) rats also had significantly (p<0.05) decrease reduction in testicular GPx values (0.56±0.8 nmol $mg^{-1}$ protein) (Table 3).

**Effect of rhodinol-based incense on ticular non–enzymatic content of reduce glutathione (GSH) of albino rats:** A non-significant (p>0.05) decrease in GSH content (0.60±1.1 nmol $mg^{-1}$ protein) was observed in rats that were exposed to 1 g of incense when compared to that of the control animals (0.69±4.0 nmol $mg^{-1}$ protein). Moreover, 2 g of incense caused a significant (p<0.05) (0.47±1.9 nmol $mg^{-1}$ protein), decrease in GSH content when compared to the control counterpart. Furthermore, group D rats (3 g of incense) also showed a significant reduction in testicular GSH content (p<0.05) (0.37±1.8 nmol $mg^{-1}$ protein) compared to the values of the control groups (Table 4).

**Effects of rhodinol-based incense on malondiadehyde (MDA):** As shown in Table 4, the group B rats (1 g incense) had a non-significant (p>0.05) elevated testicular MDA value

Table 4: Effect of rhodinol-based incense on reduced glutathione (GSH) and malondialdehyde (MDA) of albino rats

| Groups | GSH (nmol $mg^{-1}$ protein) | MDA (nmol $mg^{-1}$ protein) |
|---|---|---|
| A | 0.69±4.0 | 0.30±2.0 |
| B | 0.60±1.1 | 0.35±1.1 |
| C | 0.47±1.9* | 0.49±3.1* |
| D | 0.37±1.8* | 0.60±2.1* |

*p<0.05 significantly different from control, Values are expressed as Mean±SD for n = 6 in each group, Group A, B, C and D: 1 g of natural air freshner (100% essential oil) control 1 g incense, 2 g incense and 3 g incense exposure for 8 weeks, respectively

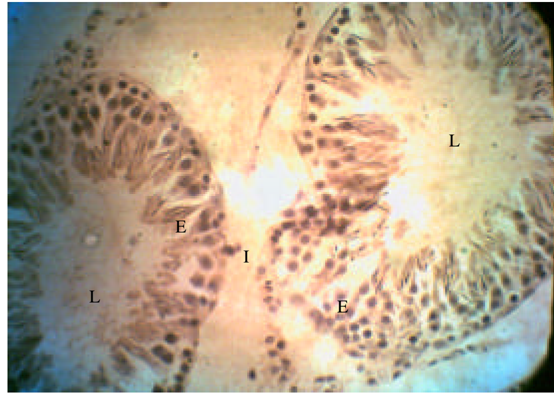


Fig. 3: Testis of group C rats, Treatment: Exposed to 2 g b.wt. of rhodinol based incense for 8 weeks, Stain: Haematoxylin and eosin. Magnification: X400, E: Seminiferous epithelium, L: Lumen of seminiferous tubule, I: Testicular interstitium

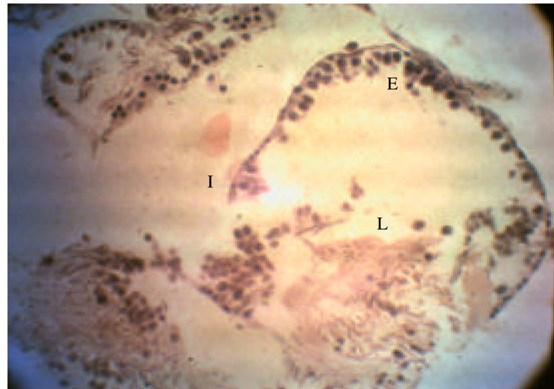


Fig. 4: Testis of group D rats, Treatment: Exposed to 3 g b.wt. of rhodinol based-incense for 8 weeks, Stain: Haematoxylin and eosin. Magnification: X400, E: Seminiferous epithelium, L: Lumen of seminiferous tubule, I: Testicular interstitium

(0.35±1.1 nmol $mg^{-1}$ protein) as compared to that of the control group (0.30±2.0 nmol $mg^{-1}$ protein). The group of rats that had 2 g of incense (0.49±3.1 nmol $mg^{-1}$ protein) showed appreciable elevated significant ($p<0.05$) testicular MDA value compared to that of the control group. The rats exposed to 3 g incense (group D) shows significant ($p<0.05$) elevated increase in the testicular MDA (0.60±2.1 nmol $mg^{-1}$ protein) compared to the control counterpart.

## DISCUSION

Numerous studies have shown that sperm membranes are rich in Polyunsaturated Fatty Acids (PUFA) and thus susceptible to attacks by Reactive Oxygen Species (ROS) or membrane lipid peroxide ions (Sanocka and Kurpisz, 2004; Sachdev and Davies, 2008). In biological system, vital macro molecules like proteins, lipid and Deoxyribonucleic Acid (DNA) may be oxidatively modified resulting in cell or tissue damage, hence the myriad of diseases linked to an excess production of free radicals (Willcox *et al.*, 2004). The present study was designed to evaluate the effect of rhodinol-based incense on the testes of adult albino rats using gross anatomical parameters, histological profiles, sperm parameters and oxidative status.

In this study, it was observed that the control group of animal models had a significant increase in gross anatomical parameters. The improved values of body weight of the control animals could mean that they were still in their active growth phase during the study (Saalu *et al.*, 2008). The findings from this study showed a significant decrease in the testes and body weights and testis volumes in rats exposed to incense smoke when compared to the controls groups. This is in concordance with the report of Yamamoto *et al.* (1998) and Ahmed *et al.* (2013) which also investigated incense exposure in animal models. The decrease in body and testicular weight of the animals that were exposed to rhodinol in this experiment are also in conformity with previous reports of considerable decrease in body and testicular weight as a result of seminiferous tubular derangements (Saalu *et al.*, 2008, 2009, 2010; Oyedeji *et al.*, 2013).

Furthermore, the observed body weights of rats in this study may also be due to a diminished food consumption caused by developed anorexia or decreased utilization of food (Gentry-Nielsen *et al.*, 2004) or may probably due to modification of protein metabolism, which was thought to be used for energy production rather than for growth. This may be as a result of rhodinol content in the incense smoke (Li *et al.*, 2003).

The testicular derangement demonstrated in this experimental model might have been as a result of active metabolites such as citronellol, geraniol and nerol generated by rhodinol which could have aided the production of lipid peroxides, resulting inhibition of mitochondrial action and eventually causing cell death (Aitken *et al.*, 1997; Agarwal *et al.*, 2006; Cocuzza *et al.*, 2007).

The histological evidences in this study showed degenerative changes characterized by vacuolization of the interstitium, reduced luminal spermatozoa and devoid spermatozoa in cross section of the seminiferous tubules of rats exposed to various concentration of incense (1, 2 and 3 g). This is in conformity with several other pervious reports on male infertility experiments in animal models involving cytotoxic chemicals (Saalu *et al.*, 2010; Akunna *et al.*, 2013). High doses exposure of bisphenol A led to concomitant leydig and germ cell apoptosis in pubertal mice (Li *et al.*, 2003). Lindone has also been shown to alter germinal epithelium and sertoli cells fragmentation in rat testes (Saradha *et al.*, 2008).

In this study, the incense exposed rats showed significant reductions in spermatozoa concentration, sperm motility and normal sperm morphology when compared to the control groups. These results are found to be consistence with several other reports on incense exposure (Kapawa *et al.*, 2004). Previous studies have also shown that incense exposure in animal models has led to decrease testicular sperm count, increase in percentage number of abnormal sperm and decrease in normal sperm morphology (Ahmed *et al.*, 2013). Rhodinol-based incense induced testicular toxicity is due mainly to oxidative stress (Fowler *et al.*, 2004). The increase oxidative stress damages the sperm membranes protein and DNA (Mallo *et al.*, 2001; Saleh *et al.*, 2002). This could explain the derangement in the sperm parameters in the animals that were exposed to the rhodinol-based incense.

The probable reasons for the degenerative evidence in this study could be as a result of increased oxidative stress, even though report has shown that ROS are products of normal cellular oxidative stress (Fujii *et al*., 2003). Another major source of ROS production has been implicated in the pathogenesis of poor sperm quality (Gate *et al*., 1999).

Reactive oxygen species are a product of normal cellular metabolism. Most of the body's energy is produced by enzymatically controlled reaction of oxygen with hydrogen in oxidative phosphorylation occurring within the mitochondria during oxidative metabolism (Ford, 2004; Maneesh and Jayalekshmi, 2006).

Rhodinol has been reported to generate reactive oxygen species which causes oxidative stress by production of potentially destructive reactive oxygen species that exceeds body's own natural antioxidant defense resulting in cellular damage (De Rosa *et al*., 2003; Willcox *et al*., 2004). This may explain the decrease in enzymatic and non-enzymatic antioxidants such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH) and reduced glutathione (GPX) obtained in this study. This is in conformity with the findings of Ahmed *et al*. (2013), which showed that there was decrease in both enzymatic and non-enzymatic antioxidants after exposure to incense smoke.

Furthermore, lipid peroxidation is a free-radical mediated propagation of oxidative insults to mainly PUFA involving several types of radicals and termination which may occur through enzymatic means or by free radical scavenging antioxidant (Cerolini *et al.,* 2000; Sanocka and Kurpisz, 2004).

However, the mammalian spermatozoon is particularly vulnerable to lipid peroxidation because of the molecular anatomy of its plasma membrane. Unlike somatic cells, mammalian sperm cells present highly specific lipidic composition with high content of PUFA, plasmolygens and sphingomylins. This unusual structure of sperm membrane is responsible for its flexibility and the functional ability of sperm cells. Moreover, spermatozoa's especially PUFA are the main substrates for peroxidation, which may provoke severe functional disorder of sperm (Sachdev and Davies, 2008; Hammadeh *et al*., 2009). This may explain why the testicular oxidative status of rats that received various grams of incense (2 and 3 g) were damaged as evidenced by the significant ($p<0.05$) increase in the activities of malondiadehyde (MDA), a product of lipid peroxidation. The Oxidative status of rats observed in this study was in consonance with that of Ahmed *et al*. (2013) which also showed an appreciable increase in MDA level after exposure to Arabian incense smoke.

## CONCLUSION

The results of this study showed that rhodinol-based incense causes the following effects on the albino rats testis:

- Testis histologic damage
- Deranged sperm parameters
- Reduced oxidative status

## RECOMMENDATIONS

Incense is used as a gesture of hospitality during special occasions like religious ceremonies in churches for creating spiritual atmosphere and by muslim clerics during prayers. The fact that

rhodinol based incense exerts a profound damage to the testis in laboratory animals warrants a further study for its testiculotoxicity in people who are more commonly exposed to this type of incense.

Despite these well-established toxic effects of rhodinol-based incense on the rat testis, there is a need for further investigations in humans to determine its lethal dose. This is even more pertinent when one consider reports that natural substances are selectively toxic to the mammalian tissue while sparing the lower animals such as rodents because they process more efficient xenobiotic biotransformation system than man (Laumann *et al*., 1995).

## REFRENCES


Aebi, H.C., 1974. Catalase. In: Methods of Enzymatic Analysis, Bergmeyer, H.U. (Ed.). Academic Press, New York, London, pp: 673-677.

Agarwal, A., S. Prabakaran and S.S. Allamaneni, 2006. Relationship between oxidative stress, varicocele and infertility: A meta-analysis. Reprod. Biomed. Online, 12: 630-633.

Ahmed, M., N. Al-Daghri, M.S. Alokail and T. Hussain, 2013. Potential changes in rat spermatogenesis and sperm parameters after inhalation of *Boswellia papyrifera* and *Boswellia carterii* incense. Int. J. Environ. Res. Public Health, 10: 830-844.

Aitken, R.J., H.M. Fisher, N. Fulton, E. Gomez, W. Knox, B. Lewis and S. Irvine, 1997. Reactive oxygen species generation by human spermatozoa is induced by exogenous NADPH and inhibited by the flavoprotein inhibitors diphenylene iodonium and quinacrine. Mol. Reprod. Dev., 47: 468-482.

Akunna, G.G., L.C. Saalu, B. Ogunlade, O.S. Ogunmodede and A.M. Akingbade, 2013. Anti-fertility role of allethrin based-mosquito coil on animal models. Int. J. Biol. Pharmacy Allied Sci., 2: 192-207.

Alarifi, S.A., 2005. Morphological evidence of apoptosis in hepatocytes of rats (*Rattus norvegicus*) exposed to Arabian Incense. J. Medical Sci., 5: 222-227.

Alokail, M.S., A.I. Mohammad and S.A. Al-Arafi, 2011. Antioxidant enzyme activity and lipid peroxidation in liver of wistar rats exposed to Arabian incense. Anim. Biol. J., 2: 1-9.

Atessahin, A., I. Karahan, G. Turk, S. Gur, S. Yilmaz and A.O. Ceribasi, 2006. Protective role of lycopene on cisplatin-induced changes in sperm characteristics, testicular damage and oxidative stress in rats. Reprod. Toxicol., 21: 42-47.

Buege, J.A. and S.D. Aust, 1978. Microsomal Lipid Peroxidation. In: Methods of Enzymology, Fleischer, S. and L. Packer (Eds.). Academic Press, New York, pp: 302-310.

CCAC, 1985. Guide to the Handling and Use of Experimental Animals. Vol. 23, NIH Publications, Ottawa, USA., pp: 45-47.

Cerolini, S., A. Maldjian, P. Surai and R. Noble, 2000. Viability, susceptibility to peroxidation and fatty acid composition of boar semen during liquid storage. Anim. Reprod. Sci., 58: 99-111.

Cocuzza, M., S.C. Sikka, K.S. Athayde and A. Agarwal, 2007. Clinical relevance of oxidative stress and sperm chromatin damage in male infertility: An evidence based analysis. Int. Braz. J. Urol., 33: 603-621.

Cohen, G., D. Dembiec and J. Marcus, 1970. Measurement of catalase activity in tissue extracts. Ann. Biochem., 34: 30-38.

Colborn, T., F.S. vom Saal and A.M. Soto, 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ. Health Perspect., 101: 378-384.

Das, K., L. Samanta and G.B.N. Chainy, 2000. A modified spectrophotometric assay of superoxide dismutase using nitrite formation by superoxide radicals. Ind. J. Biochem. Biophys., 37: 201-204.

De Rosa, M., S. Zarrilli, L. Paesano, U. Carbone and B. Boggia *et al*., 2003. Traffic pollutants affect fertility in men. Hum. Reprod., 18: 1055-1061.

Duncan, B.D., 1957. Multiple range tests for correlated and heteroscedastic means. Biometrics, 13: 164-176.

Ford, W.C.L., 2004. Regulation of sperm function by reactive oxygen species. Hum. Reprod. Update, 10: 387-399.

Fowler, B.A., M.H. Whittaker, M. Lipsky, G. Wang and X.Q. Chen, 2004. Oxidative stress induced by lead, cadmium and arsenic mixtures: 30-day, 90-day and 180-day drinking water studies in rats: An overview. Biometals, 17: 567-568.

Fujii, J., Y. Iuchi, S. Matsuki and T. Ishii, 2003. Cooperative function of antioxidant and redox systems against oxidative stress in male reproductive tissues Asian J. Androl., 5: 231-242.

Gate, L., J. Paul, G.N. Ba, K.D. Tew and H. Tapiero, 1999. Oxidative stress induced in pathologies: The role of antioxidants. Biomed. Pharmacother., 53: 169-180.

Gentry-Nielsen, M.J., E.V. Top, M.U. Snitily, C.A. Casey and L.C. Preheim, 2004. A rat model to determine the biomedical consequences of concurrent ethanol ingestion and cigarette smoke exposure. Alcoholism: Clin. Exp. Res., 28: 1120-1128.

Griffith, O.W., 1980. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal. Biochem., 106: 207-212.

Hammadeh, M.E., A.A. Filippos and M.F. Hamad, 2009. Reactive oxygen species and antioxidant in seminal plasma and their impact on male fertility. Int. J. Fertil. Sterility, 3: 87-110.

Herrera, M.D., 2011. Holy Smoke: The Use of Incense in the Catholic Church. Tixlini Scriptorium, San Luis Obispo, pp: 332-222.

Hyams, G. and S. Cushner, 2003. Incense: Rituals, Mystery, Lore. Chronicle Books, San Francisco, ISBN: 9780811839938, Pages: 96.

Kapawa, A., D. Giannakis, K. Tsoukanelis, N. Kanakas and D. Baltogiannis *et al*., 2004. Effects of paternal cigarette smoking on testicular function, sperm fertilizing capacity, embryonic development and blastocyst capacity for implantation in rats. Andrologia, 36: 57-68.

Katarina, K., B. Tomaz and M. Gregar, 2006. Reduced seminiferous tubule diameter in mice neonatally exposed to perfume. Slovenian Vet. Res., 43: 177-183.

Laumann, E., J. Gangnon, R. Michael and S. Michael, 1995. The Social Organization of Sexuality. Vol. 41, University of Chicago Press, Chicago, pp: 70-80.

Li, M.D., J.K. Kane and O. Konu, 2003. Nicotine, body weight and potential implications in the treatment of obesity. Curr. Top. Med. Chem., 3: 899-919.

Mallo, P.R., G.R. Pinto and C. Botelho, 2001. The influence of smoking on fertility, pregnancy and lactation. J. Pediatr., 77: 257-264.

Maneesh, M. and H. Jayalekshmi, 2006. Role of reactive oxygen species and antioxidants on pathophysiology of male reproduction. Indian J. Clin. Biochem., 21: 80-89.

Mohammad-Reza, P., D. Farzaneh, T.K. Taherch and P.P. Zoherb, 2005. The effects of hydroalcholic extract of *Actinidia chinensis* on sperm count and motility and blood levels of estradiol and testosterone in male rats. Ach. Iranina Med., 8: 211-216.

Neal, C.F., 2003. Incense: Crafting and Use of Magickal Scents. Llewellyn Publications, USA., ISBN: 9780738703367, Pages: 192.

Oyedeji, K.O., A.F. Bolarinwa and A.K. Adigun, 2013. Effect of aspirin on reproductive functions in male albino rats. IOSR J. Pharm. Biol. Sci., 4: 49-54.

Rotruck, J.T., A.L. Pope, H.E. Ganther, A.B. Swanson, D.G. Hafeman and W.G. Hoekstra, 1973. Selenium: Biochemical role as a component of glutathione peroxidase. Science, 179: 588-590.

Saalu, L.C., P.I. Jewo, I.O. Fadeyibi and S.O. Ikuerowo, 2008. The effect of unilateral varicocele on the contralateral testicular histo-morphology and function in *Rattus norvegicus*. J. Medical Sci., 8: 654-659.

Saalu, L.C., L.A. Enye and A.A. Osinubi, 2009. An assessment of the histomorphometric evidences of doxorubicin-induced testicular cytotoxicity in Wistar rats. Int. J. Med. Med. Sci., 1: 370-374.

Saalu, L.C., A.A. Osinubi and J.A. Olagunju, 2010. Early and delayed effects of doxorubicin on testicular oxidative status and spermatogenesis in rats. Int. J. Cancer Res., 6: 1-9.

Sachdev, S. and K.J.A. Davies, 2008. Production, detection and adaptive responses to free radicals in exercise. Free Radic. Biol. Med., 44: 215-223.

Saleh, R.A., A. Agarwa, R.K. Sharma, D.R. Nelson and A.J. Thomas, 2002. Effect of cigarette smoking on levels of seminal oxidative stress in infertile men: A prospective study. Fertil. Sterility, 78: 491-499.

Sanocka, D. and M. Kurpisz, 2004. Reactive oxygen species and sperm cells. Reprod. Biol. Endocrinol., Vol. 2. 10.1186/1477-7827-2-12

Saradha, B., S. Vaithinathan and P.P. Mathur, 2008. Single exposure to low dose of lindane causes transient decrease in testicular steroidogenesis in adult male Wistar rats. Toxicology, 244: 190-197.

Sikka, S.C. and N. Gurbuz, 2006. Reproductive Toxicity of Organophosphate and Carbamate Pesticides. In: Toxicology of Organophosphate and Carbamate Compounds, Gupta, R.C. (Eds.). Elsevir Inc., London, pp: 454-456.

Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods. 7th Edn., Iowa State University Press, Iowa, USA., ISBN-10: 0813815606, Pages: 507.

Sokol, R.Z., 1997. The Hypothalamic-Pituitary-Gonadal Axis as a Target for Toxicants. In: Comprehensive Toxicology, Sipes, I.G., C.A. McQueen, A.J. Gandolfi (Eds.). Vol. 10, Elsevier Science, Oxford, pp: 87-98.

WMA/APS, 2002. Guiding principles for research involving animals and human beings. Am. J. Physiol. Regul. Integr. Comp. Physiol., 283: R281-R283.

Wang, X.D., C. Liu, R.T. Bronson, D.E. Smith, N.I. Krinsky and R.M. Russell, 1999. Retinoid signaling and activator protein-1 expression in ferrets given β-carotene supplements and exposed to tobacco smoke. J. Natl. Cancer Inst., 91: 60-66.

Willcox, J.K., S.L. Ash and G.L. Catignani, 2004. Antioxidants and prevention of chronic disease. Crit. Rev. Food Sci. Nutr., 44: 275-295.

Yamamoto, Y., E. Isoyama, N. Sofikitis and I. Miyagawa, 1998. Effects of smoking on testicular function and fertilizing potential in rats. Urol. Res., 26: 45-48.

Yokoi, K. and Z.K. Mayi, 2004. Organ apoptosis with cytotoxic drugs. Toxicology, 290: 78-85.