Formaldehyde and anti-fertility: correlation between testicular cytoarchitecture and semen analysis

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SUMMARY

The anti-fertility effect of formaldehyde was investigated on the testicular cytoarchitecture and semen of Sprague-Dawley rats exposed to formaldehyde by inhalation. Thirty adult male rats were randomly selected into three (3) groups of ten (10) rats each. The treatment group 2 and group 3 were exposed to 40% stock concentration of formaldehyde for 1 min/m^2 and 2 min/m^2 respectively daily for four (4) weeks of experimental period. The control group (group 1) was allowed free access to natural air throughout the experimental period. Testis was dissected out following cervical dislocation and fixed in Bouins fluid for histological processing. Caudal Epididymis was also dissected out and suspended in 1ml of Hams F- 10 solution for estimation of sperm concentration and motility.

Semen analysis revealed a significant reduction in sperm concentration and sperm motility in the treated groups. Histological alterations in the seminiferous tubule and degeneration of spermatogenic cells were observed in the treated groups compared with the control. Reduction in sperm concentration and motility and histological alteration of the testes in the treated rats showed that formaldehyde might have toxicity on the reproductive organ.

Key words: Formaldehyde – Testis – Semen – Anti-fertility – Cyto-architecture, - Spermatogenesis

INTRODUCTION

Infertility is one of the major health problems among married couples (Agarwal and Prabakaran, 2005). Approximately 20-30% of couples with infertility can be traced to combined female and male factors (Oyewopo et al., 2018). Several conditions and factors can interfere with sperm development and reduce sperm quality and quantity. Such factors may include: drug treatment, toxin, infectious organisms, air pollution and malnutrition (Fujii et al., 2003, Olaniyan et al., 2021a, Olaniyan et al., 2021c).

In each testis are 200 to 300 lobules, and within each lobule are 1 to 4 convoluted lobules

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composed of germinal epithelia cell, called seminiferous tubules, and between the tubules are groups of interstitial cells (of Leydig) that secrete the hormone testosterone after puberty (Sheweita et al., 2005; Olaniyan et al., 2021d). Successful spermatogenesis take place at a temperature of about 3^{o}C below normal body temperature and require hormonal regulation such as Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Testosterone (Sheweita et al., 2005).

Formaldehyde is a chemical primarily used for preservation of tissues in research and in manufacturing industry where resin, particle board, plywood, leather, paper and pharmaceutical products are produced (Wong et al., 2006). It is also a flammable, choking, colorless, and reactive gas. Exposure to formaldehyde can be from both direct environmental source and from metabolism of xenobiotics (Wong et al., 2006). However, it has been shown that the level of exposure to formaldehyde in occupational environment is relatively high as compared to other sources (Wong et al, 2006). There are various sources of formaldehyde; the major anthropogenic sources that grossly affect human and others animals are the indoor, environment or long-term outdoor pollution, which poses a significant threat to public health (Yu et al., 2005; Tang et al., 2009). Formaldehyde is a volatile, highly soluble compound in water which can rapidly diffuse into many tissues and cause distortion of cells (Metz et al., 2004).

Emerging evidence supports an association between formaldehyde exposure and multiple adverse health effects (Tang et al., 2009).

It was reported that long-term exposure to formaldehyde negatively affects the hematological parameters and respiratory system; it also causes irritation of the eyes and nasal pathway in animals (Yu et al., 2005).

The reproductive and developmental toxicity associated with formaldehyde exposure remains inconclusive (Duong et al., 2011), and this prompted us to investigate the formaldehyde action on the testicular cytoarchitecture and semen analysis of adult male Sprague Dawley rats.

MATERIALS AND METHODS

Experimental Animal

A total of 30 male adult Sprague-Dawley rats weighing between 160-200 g were obtained from the animal house Department of Physiology, Ladoke Akintola University, Ogbomosho, Oyo State. The rats were allowed to acclimatize for 2 weeks at photo periodic condition of 12hrs light and 12 hrs darkness natural cycles, and were fed with a standard Pfizer diet obtained from Bendel Feeds Ilorin and water *ad libitum.* T experiment was carried out in the Department of Anatomy University of Ilorin, in accordance with the Animal Experimentation Committee Regulation.

Experiment design

The animals were randomly selected into three (3) groups, each comprising ten (10) Wistar rats. 40% stock concentrations of formaldehyde were soaked in Cotton wool and the animals were exposed to the 40% stock concentration by inhalation at varying time per meter square area. Group 1 (control) were exposed to natural fresh air throughout the experimental period. Group 2 and Group 3 were exposed to 40% concentration of formaldehyde by inhalation for a period of 1 minutes/m^2 and 2 minutes/m^2 area of exposure respectively daily for the 4 weeks of experimental period between 0900 and 1000 hours. Animals were sacrificed by cervical dislocation at the end of 4th week.

Sample collection and processing

The animals were euthanized by cervical dislocation twenty-four hours after last administration and the testes tissue were excised. The testis from each rat was fixed in Bouin's fluid for routine histological haematoxylin & eosin (H/E), while the caudal epididymis used in sperm analysis.

Histological Analysis

Testes were carefully excised, cut in slabs of 0.5 cm thick, fixed in Bouins fluid and processed routinely for paraffin embedding. 5µ sections were obtained with rotatory microtome and processed for Heamatoxylin and Eosin Stain (H&E). Sections

were observed with light microscope and photomicrographs were taken for further analysis (Bustos-Obregon et al., 2003).

Sperm analysis

The caudal epididymis was dissected out, several incisions (1 mm) were made in the caudal epididymis, which was suspended in 1ml of Ham's f-10 solution after 3-5 minutes of incubation at 37°C. The sperm swim up and the concentration and motility were determined by using new improved Neubauer Haemocytometer (Tang et al., 2003; Olaniyan et al., 2021b).

RESULTS

Semen analysis revealed a significant reduction ($p<0.05$) in sperm concentration and motility in the treated group as compared with the control group table 1. Spermatocyte concentration significantly decrease in relation to the time of exposure to 40% stock solution of formaldehydes. Animals exposed for 2 minutes per meter square area demonstrated marked reduction in the sperm concentration associated with loss of motility integrity when compared with the control animals without restriction to the normal air exhalation and inhalation. Formaldehyde exposure for the period for 1 minute per area square demonstrated mild impact on the sperm characteristics and quality.

There was significant histological alteration in the testes of group 2 and group 3 rats that were exposed to the formaldehyde as compared with the control. There was disruption of the seminiferous tubule and degeneration of spermatogenic cells. There was also edema of the interstitial connective tissue in the treated groups compared to the control (Fig. 1). Animals in the control group expressed the histological architecture of the testes, presenting the three types of the cells: the spermatogonia population, the Sertoli and the interstitial Leydig cells in the interstitial spaces. All stages of spermatogonia cells were expressed across the seminiferous tubules and the mature Spermatocytes in the ad-luminal compartment. Abnormal widening of the interstitial spaces, de-arrangement in the lining basal membranes and seminiferous tubules shrinkage were observed in the animals exposed for a period of 1 minute per square area. Complete loss of spermatogonia cells across the tubules was observed in the group with the long period of exposure per unit area, loss of the basal membrane integrity and complete breakdown of the interstitial spaces as evident in the loss of quality in the sperm characteristics.

DISCUSSION

The anti-fertility effect of formaldehyde was investigated on the testicular cytoarchitecture and on semen analysis in Sprague-Dawley rats treated with formaldehyde. The usual quantity of semen ejaculated at each coitus averages 3.5 million in man, and varies according to species (Fujii et al., 2003). When the number of sperm per each milliliter falls below normal, the animal is likely to be infertile. Even though only a single sperm is necessary for fertilization, the ejaculate usually must contain a tremendous number of sperm from which only one sperm will fertilize the ovum (Fujii et al., 2003). Whenever the majority of sperms are morphologically abnormal or are found to be non motile, the condition is likely to be infertile, even though the remaining sperms may appear to be normal (Fujii et al., 2003).

The present study revealed that there was decreased sperm concentration and reduction in motility of sperm of the treated rats as compared with the control, which is in tandem with the previous study conducted by Zhou et al. (2006) in male humans, and this is further supported by

Table 1. Sperm concentration and motility of the treated rats compared with controls.

	No. of rats	Sperm concentration× 10^6	Sperm Motility%
Group 1	10	60.14 ± 0.70	75.25 ± 6.26
Group 2	10	56.20 ± 0.52	62.15 ± 3.17
Group 3	10	22.13 ± 0.68*	40.15 ±3.12*

Values are mean ± SEM. (n): * indicates significant different when compared with the control group ($p<0.05$)

another study on adult male rats with large dose formaldehyde exposure (Zhou et al., 2011).

The decrease in sperm concentration and reduction in sperm motility caused by the action of formaldehyde showed that formaldehyde may cause infertility in animals depending on the length of exposure, but not with low-dose exposure (Zhou et al., 2011). Study by Collins et al. (2001) in both humans and animals contradicts this report, even though other routes apart from inhalation were disregarded.

Histological observation of the testicular cytoarchitecture showed disarrangement of seminiferous tubules and destruction of spermatogenic cells in the treated group, which was worst in the group 3 animals. This histological alteration of the testes may alter the initiation and maintenance of spermatogenesis in the rats according to Dare et al. (2021), who report a decrease in the number of Spermatogonia and differentiating cells in the testes of rats treated with cadmium chloride, a free-radical-generating agent similar to the effect of exposure to formaldehyde on the sperm characteristics and the histological architecture observed in this study.

CONCLUSION

This study has shown that formaldehyde affects the cytoarchitecture and sperm characteristics of adult male Sprague-Dawley rats, and can be reasonably concluded to have toxic effects on the reproductive organs of the male rats and, by extension, humans.

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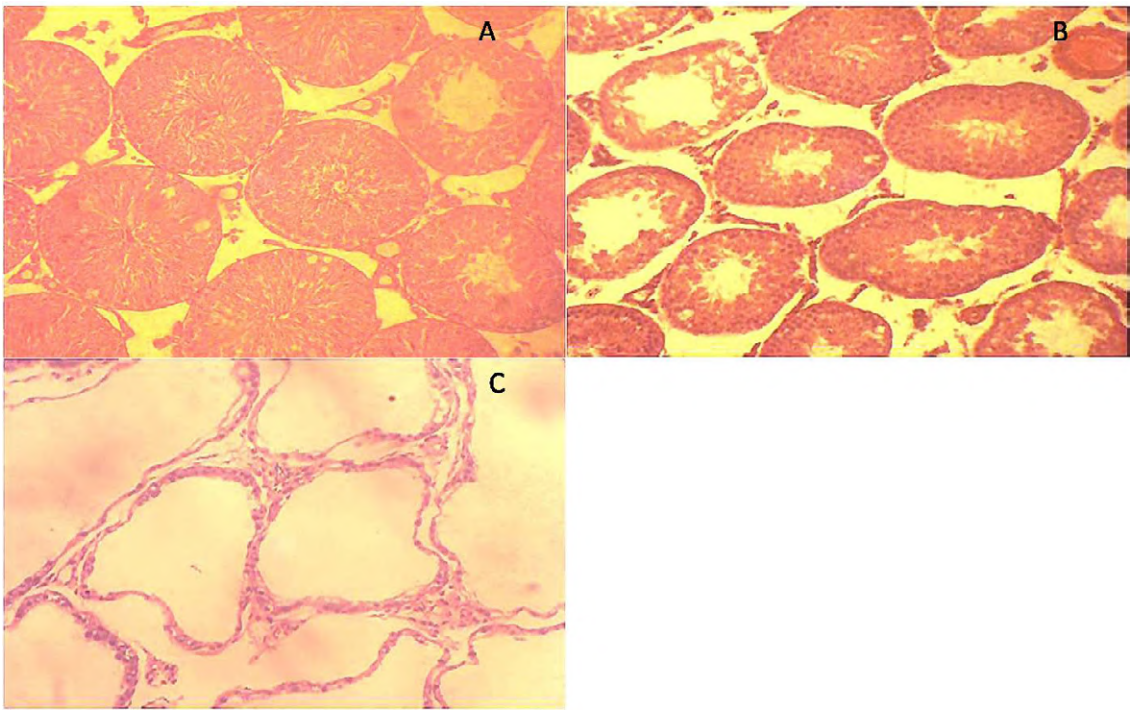

Fig. 1.- A: Cross section of the testes of control rats showing the normal architecture of the testis. **B:** Cross section of the seminiferous tubules of rat treated with 40% FA for 1 min/m^2 showing disruption in the spermatogenic cell. **C:** Cross section of seminiferous tubules of rat treated with 40% FA for 2 min/m^2 showing degeneration of spermatogenic cell. H&E staining, x400.

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