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Cigarette smoke alters testicular and epididymal histology in adult Wistar rats

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Abstract:

BACKGROUND: Cigarette smoking is a major public health problem. Different disease conditions have been linked to smoking as a risk factor, including infertility, with most studies focusing on semen quality.

AIM: The current study aimed at determining the effects of cigarette smoking on the histology of both the testes and epididymis in animal models.

MATERIALS AND METHODS: Sixteen adult male Wistar rats were grouped into four groups: Control Group A and 3 treated Groups B, C, and D, exposed to 1, 2, and 3 sticks of cigarette, respectively. Smoking chambers, made of cylindrical containers, were constructed indigenously and used for the exposure procedure. Each stick was suspended within the chamber and allowed to completely burn for about 10 min. Cigarette exposure was carried out for 28 consecutive days. Both the testes and caudal epididymis were examined histologically.

RESULTS: There was distortion of the testicular and epididymal architecture and varying degrees of structural degeneration, especially in the group exposed to the highest concentration of cigarette smoke; spermatogenic cells appeared to decrease in population and there was a reduction in the density of mature spermatozoa in the lumen of the epididymis and seminiferous tubules.

CONCLUSION: The architectural changes in the testes and epididymis explain the reasons for low or poor semen quality in subjects exposed to cigarette smoke, and possible impaired reproduction.

Keywords:

Cigarette smoke, epididymis, histology, testis

Introduction

Cigarette smoking is a leading cause of preventable death and disease worldwide (Zavos and Zarmakoupis-Zavos, 1999). The major constituents of cigarette smoke that affect human health are nicotine, tar, and carbon monoxide (Hammond *et al.*, 2006), while others are however not insignificant. Nicotine and tar are responsible for the acute and chronic effects of tobacco, respectively. Both active and passive smoking have the potential to harm almost every organ in the body.

Nicotine is one of the most widely used licit drugs, as well as one of the most insidiously addicting substances, both because tolerance develops rapidly and most users have extremely long-lasting craving for it when trying to quit (Katzung, 2001). Of the twenty million smokers who attempt quitting every year, only less than 10% succeed (Michael-Titus *et al.*, 2007). Addiction to nicotine, the main psychoactive component of tobacco, constitutes one of the most serious health problems worldwide (Michael-Titus *et al.*, 2007).

Carbon monoxide (CO) makes up 2%–6% of cigarette smoke, and this percentage rises in smokers (Mirza *et al.*, 2005). Carbon monoxide reduces the oxygen-carrying capacity of the blood, as it competes with

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oxygen for hemoglobin, leading to tissue hypoxia. This acts in synergy with the vasoconstrictive effects of nicotine, to increase oxidative stress and tissue ischemia, thereby contributing significantly to the adverse effects of cigarette smoking on the process of spermatogenesis (Ahmadnia *et al*., 2007).

Oxidative stress, inflammation, atherosclerosis, and ischemic processes are potential mechanisms for the untoward effects of cigarette smoke (Swan and Lessov-Schlaggar, 2007). Smoking affects both males and females from conception to adulthood. Some reproductive studies showed that smoking reduces spermatogenesis, and the mature sperm cells that are eventually produced have compromised quality and low fertilizing capacity (Soares, 2009; Mostafa, 2010). Severe chromosomal damage occurs at different phases of spermatogenesis in male smokers, which could prevent oocyte fertilization and embryogenesis (Lahdetie, 1986). Childhood neurological conditions such as hyperactivity, learning disabilities, and other behavioral disorders have been associated with prenatal exposure to cigarette smoke (Durazzo *et al*., 2007). Paternal cigarette smoke exposure affects the embryonic ability for implantation (Kapawa *et al*., 2004).

The aim of the current study was to determine the effects of cigarette smoke exposure on the histology of the testis and epididymis in experimental animals.

Materials and Methods

Laboratory animals

Sixteen Wistar rats with average weight of 125 g were used. They were bred under ideal condition in the Experimental Animal House of the Department of Anatomy, University of Ilorin. Growers mash and clean water were provided, and animals were allowed to acclimatize for 2 weeks before commencement of the experiment.

Exposure to cigarette smoke

The rats were divided into four groups: Group A, B, C, and D. Group A served as the Control, and the rats in this group were not exposed to cigarette smoke, while Groups B, C, and D were exposed to smoke from 1, 2, and 3 sticks of cigarette (Pall Mall®), respectively, daily for a period of 28 days. Four cylindrical buckets were used to construct indigenous smoking chambers for the exposure procedure (Omotoso *et al*., 2013). A circular hole of about 1 cm in diameter was made on the lid of each bucket. The cigarettes were suspended in the buckets using threads that were tied around them. Lighters were used to light up candles and the cigarettes were lit from the candles. The lids were opened periodically to prevent suffocation.

Specimen collection

The rats were sacrificed 24 h after the last administration by cervical dislocation. An abdominal incision was made to deliver the testes and caudal epididymis, which were thereafter placed in 10% formal saline, and processed for histological studies using hematoxylin and eosin staining techniques.

Results

The rats that were exposed to cigarette smoke had marked reduction in body weight and growth rate when compared to the Control rats [Table 1]. Those exposed to three sticks of cigarette had the least growth rate as indicated by the lowest record of weight difference in this group.

The general architecture of the testes and caudal epididymis was distorted mostly in the group exposed to the highest concentration of cigarette smoke, with signs of disintegration and degeneration of cells [Figures 1b-d and 2b-d]. These signs included a gradual decrease in the population of spermatogenic cells, irregular outline and disordered arrangement of the cells, breaking off of the flagella from the mature sperms, mild reduction in the number of Leydig cells, and reduced quantity of mature sperm cells within the lumen of the seminiferous tubules and caudal epididymis in exposed Wistar rats [Figure 1b-d], when compared with the Control [Figures 1a and 2a].

Discussion

The development of sperm cells begins with the differentiation of spermatogonia in the seminiferous tubules, which after series of events transform into the mature spermatozoa. For viable spermatozoa with reproductive capability to be produced, spermatogenesis must go unhindered and undisturbed. An insult to the body or specifically directed at the reproductive organs, like the testes, could distort spermatogenesis and impair reproductive functions in males, due to low or poor quality and characteristics of sperm cells. A significant correlation exists between cigarette smoking and altered reproductive physiology (Ahmadnia *et al*., 2007).

Various components of tobacco smoke are capable of crossing the blood-testis barrier, thereby inducing significant alteration in sperm quality, such as

Table 1: Changes in weights of experimental rats

Group	Final weight (g)	Initial weight (g)	Weight difference (g)
Group A: Control	214.70±9.20	131.25±8.17	83.45
Group B: 1 stick	198.23±2.67	165.48±4.15	32.75
Group C: 2 sticks	196.82±11.01	154.80±1.23	42.02
Group D: 3 sticks	192.27±16.53	161.42±4.44	30.85

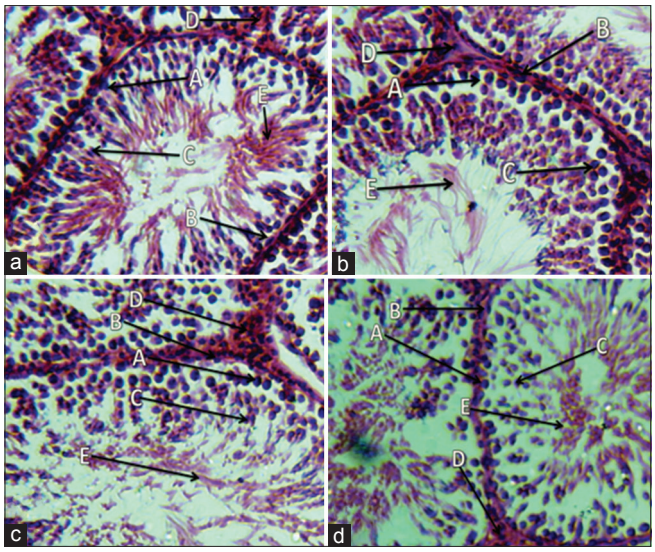

Figure 1: Photomicrographs of the seminiferous tubules of rats, showing A - Spermatogonia, B - Sertoli cells, C - Primary spermatocytes, D - Leydig cells and E - Spermatozoa. (a) Group A (Control) showed normal testicular structure. (b) Larger spermatogonia and low count of spermatozoa. (c) Mild reduction in spermatogonia, more centrally located primary spermatocytes, and irregularly arranged sperm cells. (d) Reduced sperm cells and distortions of sperm cells within lumen; slightly reduced number of Leydig cells (H and E, ×200)

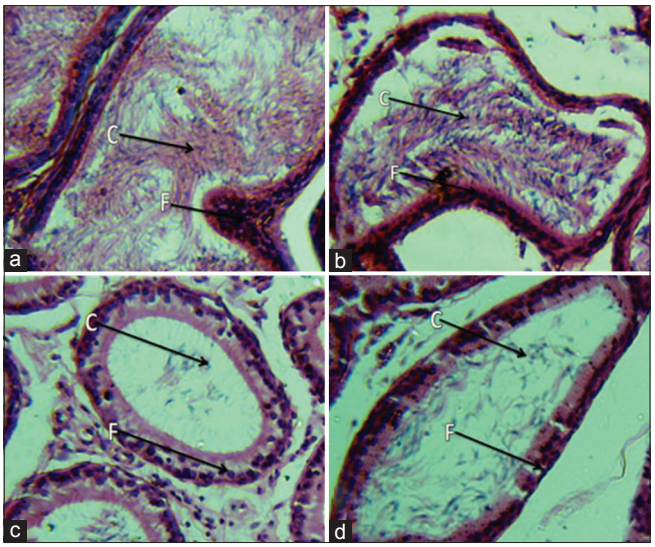

Figure 2: Photomicrographs of the caudal epididymis: (a) Group A (Control) showed a normal epididymal architecture; (b) Group B: slightly reduced number of spermatozoa (c) and basal cells (f) compared with Control. (c) Group C: Tubules appeared reduced in diameter, with significant reduction in the population of spermatozoa and basal cells; (d) Group D showed significant reduction in spermatozoa and basal cells of the caudal epididymis (H and E, ×200)

decrease in concentration, morphology, and motility of spermatozoa (Sepaniak *et al*., 2005). Although not very clear, the mechanisms proposed for the effects of cigarette smoke on reproductive function include increased oxidative stress and DNA damage (Sepaniak *et al*., 2005). According to Kothari and colleagues (Kothari *et al*., 2010), smoking is a major exogenous source of reactive oxygen species, which are capable of inducing lipid peroxidation, DNA damage, and apoptosis of sperm cells.

The various histological changes observed in the current studies, such as decrease in the number of spermatogenic cells, irregular outline and disordered arrangement of the cells, breaking off of the flagella from the mature sperms, mild reduction in the number of Leydig cells, and also a reduction in the density of spermatozoa in the lumen of both the seminiferous tubules and epididymis of treated rats, were as a result of the exposure to cigarette smoke.

With increasing quantity of cigarettes smoked per day, the adverse effects on body tissues and organs become more evident and deleterious in smokers (Nadeem *et al*., 2012). The detrimental effects of cigarette smoke reported on semen quality were a possible reflection of what happens at the cellular and tissue level. Histological observation from the current work on the seminiferous tubules and epididymis of exposed rats showed that with increasing dosage or concentration of cigarette smoke, there was a gradual depletion and disintegration of the mature sperm cells in the lumens of the seminiferous tubules when compared with the Control animals. Decrease in the population of mature sperm cells could be due to a reduced population of early spermatogenic cells series, disruption, or destruction or death of growing cells, or some other developmental abnormalities occasioned by oxidative stress, tissue ischemia, apoptosis, or any of the proposed mechanisms.

Abnormal sperm cells are demonstrable by seminalysis, in terms of their morphological appearance, life and death ratio, concentration or count, motility, and progression. An earlier study reported a significant reduction in percentage of morphologically normal spermatozoa in animals exposed to cigarette smoke (Omotoso *et al*., 2011). Other studies also observed induced apoptosis of germinal cells, a reduction in the number of germ cells and Leydig cells, the diameter of the seminiferous tubules, and some other morphometric changes in the testes of exposed subjects (Ahmadnia *et al*., 2007; Rajpurkar *et al*., 2010).

A deficiency in the secretory functions of the Leydig and Sertoli cells could occur in cigarette smoking, resulting in an impaired epididymal sperm maturation process and a diminished capacity of the spermatozoa to penetrate oocytes (Kapawa *et al*., 2004); and the dysfunction of Leydig cells could impair the secretion of sex hormones (Yamamoto *et al*., 1988).

Aside the histological changes reported, animals exposed to cigarette smoke in the current study also showed less appetite for feeds, with consequent reduction in weight

and growth rate. Nicotine is believed to suppress food intake by modifying the levels of neuroregulatory substances that initiate or suppress feeding (Wack and Rodin, 1982); metabolic rate is also increased, thereby increasing energy utilization (Audi *et al*., 2006).

Conclusion

Exposure to cigarette smoke is a threat to male reproductive structures and functions and could contribute significantly to male infertility.

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Conflicts of interest
There are no conflicts of interest.

References

1. Ahmadnia H., Ghambari M., Moradi M.R., Khaje-Dalouee M. (2007). Effect of cigarette smoke on spermatogenesis in rats. Urol J 4:159-63.
2. Audi S.S., Abraham M.E., Borker A.S. (2006). Effect of cigarette smoke on body weight, food intake and reproductive organs in adult albino rats. Indian J Exp Biol 44:562-5.
3. Durazzo T.C., Gazdzinski S., Meyerhoff D.J. (2007). The neurobiological and neurocognitive consequences of chronic cigarette smoking in alcohol use disorders. Alcohol Alcohol 42:174-85.
4. Hammond D., Fong G.T., Cummings K.M., O'Connor R.J., Giovino G.A., McNeill A. (2006). Cigarette yields and human exposure: A comparison of alternative testing regimens. Cancer Epidemiol Biomarkers Prev 15:1495-501.
5. Kapawa A., Giannakis D., Tsoukanelis K., Kanakas N., Baltogiannis D., Agapitos E., *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.
6. Katzung B.G. (2001). Drugs of abuse. In: Basic and Clinical Pharmacology. 8th ed. Lange Medical Books/McGraw-Hill, New York.
7. Kothari S., Thompson A., Agarwal A., Plessis S.S. (2010). Free radicals: Their beneficial and detrimental effects on sperm function. Indian J Exp Biol 48:425-35.
8. Lahdetie J. (1986). Micronucleated spermatids in the seminal fluid of smokers and non-smokers. Mutat Res 172:255-63.
9. Michael-Titus A., Revest P., Shortland P. (2007). Addiction. In: The Nervous System: Basic Science and Clinical Conditions. 1st ed. Elsevier, Churchill Livingstone, 329-31.
10. Mirza A., Eder V., Rochefort G.Y., Hyvelin J., Machet M.C., Fauchier L., Bonnet P. (2005). CO inhalation at dose corresponding to tobacco smoke worsens cardiac remodeling after experimental myocardial infarction in rats. Toxicol Sci 85:976-82.
11. Mostafa T. (2010). Cigarette smoking and male infertility. J Adv Res 1:179-86.
12. Nadeem F., Fahim A., Bugti S. (2012). Effects of cigarette smoking on male fertility. Turk J Med Sci 42 Suppl 2:1400-5.
13. Omotoso G.O., Enaibe B.U., Caxton-Martins E.A. (2013). Microarchitectural changes in the superior colliculli of male Wistar rats after cigarette and cotton wool smoke exposure. Trop J Health Sci 20:37-40.
14. Omotoso G.O., Olagunju A.A., Enaibe B.U., Oyabambi A.O., Olawuyi T.S. (2011). Alteration in semen characteristics and testicular histology of male Wistar rats following exposure to cigarette smoke. West Afr J Assist Reprod 2:45-9.
15. Rajpurkar A., Li H., Dhabuwala C.B. (2010). Morphometric analysis of rat testis following chronic exposure to cigarette smoke. J Environ Pathol Toxicol Oncol 19:363-8.
16. Sepaniak S., Forges T., Monnier-Barbarino P. (2005). Consequences of cigarette smoking on male fertility. J Gynecol Obstet Biol Reprod 34:3S102-11.
17. Soares S. (2009). Cigarette smoking and fertility. Reprod Biol Insights 2:39-46.
18. Swan G.E., Lessov-Schlaggar C.N. (2007). The effects of tobacco smoke and nicotine on cognition and the brain. Neuropsychol Rev 17:259-73.
19. Wack J.T., Rodin J. (1982). Smoking and its effects on body weight and systems of caloric regulation. Am J Clin Nutr 35:36.
20. Yamamoto Y., Isoyama E., Sofikitis N., Miyagawa I. (1988). Effects of smoking on testicular function and fertilizing potential in rats. Urol Res 26:45-8.
21. Zavos P.M., Zarmakoupis-Zavos P.N. (1999). Impact of cigarette smoking on human reproduction: Its effects on male and female fecundity. Technology 6:9-16.