

## Effects of Nicotine on Sperm Characteristics and Fertility Profile in Adult Male Rats: A Possible Role of Cessation

Ibukun Peter Oyeyipo<sup>1\*</sup>, Yinusa Raji<sup>2</sup>, Benjamin Obukowho Emikpe<sup>3</sup>, Adeyombo Folashade Bolarinwa<sup>2</sup>

1- Department of Physiology, College of Health Sciences, Osun State University, Osun State, Nigeria

2- Department of Physiology, College of Medicine, University of Ibadan, Oyo State, Nigeria

3- Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ibadan, Oyo State, Nigeria

### Abstract

**Introduction:** Infertility is common among couples of child-bearing age and approximately half of known causes of primary infertility are attributable to male factor. It is still unclear whether the injurious effects of cigarette smoking on sperm characteristics and infertility are due to nicotine. Therefore, the present study investigated the effects of orally administered of nicotine on sperm characteristics and libido in adult male albino rats. The study also sought nicotine effects on fertility rate, litter size and weight in female animals cohabited with nicotine treated male rats.

**Methods:** Forty male and twenty-five female rats were used for the study. The male rats were divided into five groups and were treated for a period of 30 days with nicotine 0.5 mg/kg (low dose) and 1.0 mg/kg (high dose) per body weight while the control rats received 0.2 ml/kg normal saline. The fourth and fifth groups were gavaged with 0.5 mg/kg and 1.0 mg/kg body weight of nicotine but were left untreated for another 30 days. These groups served as the recovery groups. At the end of each experimental period, sperm analysis, fertility study, litter weight and size were determined.

**Results:** Sperm motility and count significantly decreased ( $P < 0.05$ ) while the percentage of abnormality significantly increased ( $P < 0.05$ ) in both treatment groups. However, there was an insignificant decrease ( $P > 0.05$ ) in the viability and semen volume of the treated groups. Fertility studies revealed that nicotine reduced libido in male rats, litter weight and number delivered by the untreated female during the experiments.

**Conclusion:** The present study showed that nicotine has a dose-dependent deleterious effect on the sperm characteristics and that fertility is ameliorated by nicotine cessation in male rats.

**Keywords:** Fertility, Litter weight, Nicotine, Rat, Smoking, Sperm.

**To cite this article:** Oyeyipo IP, Raji Y, Emikpe BO, Bolarinwa AF. Effects of Nicotine on Sperm Characteristics and Fertility Profile in Adult Male Rats: A Possible Role of Cessation. *J Reprod Infertil.* 2011;12(3):201-207.

\* Corresponding Author:  
Ibukun Peter Oyeyipo,  
Department of Physiology,  
College of Health  
Sciences, Osun State  
University, Osun State,  
Nigeria  
E-mail:  
greatibuks@yahoo.com

Received: Jan. 15, 2011  
Accepted: May 22, 2011

### Introduction

Infertility is common among couples of child-bearing age. Approximately half of known causes of primary infertility are attributable to male factor (1, 2). However the etiology of male factors infertility is poorly understood. While

some individuals may be genetically predisposed to be sub-fertile (3), there are major epigenetic factors which are implicated as potential causes of male infertility. The male reproductive system is known to be highly sensitive to many chemicals

and drugs which have been found to pose adverse effects on male reproductive capacity under certain conditions (4).

The health consequences of cigarette smoking and the use of tobacco products are well known. These are an important cause of increased mortality and morbidity in the developed countries and the prevalence is increasing in the developing world as well (5). Despite the increasing knowledge on the adverse reproductive effects of smoking, it is relatively unclear whether or not, nicotine has the same effects on male reproductive activities especially in apparently reproductively active subjects.

Nicotine can be consumed in different forms ranging from smokeless tobacco products such as snuff and chewing tobacco to the more often consumed form: smoked tobacco. Cigarette tobacco contains several substances (6). Carbohydrates and proteins are the most representative components but alkaloids are significantly present as well. Nicotine, in particular, represents 90% - 95% of the total alkaloids. Nicotine is a highly toxic substance and it is absorbed quickly through the respiratory tract, oral mucosa and skin. Approximately 80%-90% of nicotine is metabolized by the liver, but the kidney and lungs are involved as well (7).

Cigarette smoking is not only a potent cause of lung cancer but also has been associated with low birth weight, preterm delivery and abortion in women who are addicted to it. It also causes menstrual irregularities, pregnancy complications, and decreased fertility in women (8). Moreover, cigarette smoking inhibits spermatogenesis and causes decreased steroidogenesis in men (9, 10). Cigarette smoking has also been shown to have anti-estrogenic effects in women (11). In males, the effects of smoking on androgen is important, given the recent interest in the association between low androgen levels, the metabolic syndrome and coronary heart disease (12). Other adverse effects of smoking include premature ejaculation and reduced penile erection; however, these depend on individual sensitivity or susceptibility (12).

It has been previously demonstrated that exposure to reference cigarette smoke resulted in reduced birth weight in rats under experimental condition (13, 14) while oral administration of nico-

tine have been associated with testicular degeneration, disorganization of the cytoarchitecture and decreased serum testosterone levels (15). In addition, nicotine has been shown to have adverse effects on fertility potentials of female albino rats by reducing the weight and disorganizing the histology of some vital visceral and reproductive organs (16).

In spite of the growing knowledge on the adverse reproductive effects of smoking, it is relatively unclear whether or not, nicotine has the same effects on male reproductive activities as it relates to its effects on sperm cells, male fertility indices and subsequent effects of cessation on these reproductive parameters. The present study was, therefore, designed to investigate the effects of nicotine on reproductive functions during treatment and recovery periods.

### Methods

**Nicotine preparation:** Nicotine hydrogen tartrate (95% Nicotine) (BDH Chemicals Ltd., Poole, England) was used in the study. The nicotine dosage freshly prepared in normal saline for each group of animals was delivered at 0.5 mg/kg and 1.0 mg/kg per body weight. The working solutions were stored in foil-wrapped glass bottle at 4°C for no longer than ten days.

**Animals and treatments:** Experiments were performed on forty male and twenty-five female Sprague-Dawley rats, 2 - 2.5 month old and whose average weight ranged between 150 g and 180 g obtained from the Animal House, College of Medicine, University of Ibadan, Oyo State, Nigeria. Animals were divided into five equal groups with *ad libitum* access to rat chow and drinking water. Animals were also maintained in a well-ventilated room with a 12/12-hour light/ dark condition at room temperature. The experiment was conducted in accordance with the Guidelines of the U.S. National Institute of Health (NIH) on the care and use of laboratory animals. The male animals in the five groups were treated for 30 days and they included the control group that received 0.2 ml/kg normal saline, 0.5 mg/kg nicotine-treated group, 1.0 mg/kg nicotine-treated group, 0.5 mg/kg nicotine-treated group but left untreated for another 30 days and 1.0 mg/kg nicotine-treated group but left untreated for another 30 days.

**Libido test:** To observe the libido-oriented mounting behaviour, non-estrous untreated female rats were paired on the 30<sup>th</sup> day at 6.00 pm. The male rats assuming the copulatory position over the female rats, but failing to achieve intromission was considered as a mount (17). Male rats from each group were chosen and suitably marked. The rats were placed in a clear aquarium and were allowed to acclimatize for 15 minutes. Afterwards a non-estrous female rat was introduced into the arena. The number of mounts were recorded for 15 minutes. This process was also done for the recovery groups.

**Fertility studies:** A total of 25 untreated fertile, prestrous female rats were used for the fertility test. Five untreated female rats were cohabited with a male rat from one of the five male groups on the 31<sup>st</sup> day of treatment except the recovery groups whose cohabitation commenced on the 31<sup>st</sup> day of the recovery period. All animals were cohabited for 5 days according to earlier studies (17). The presence of a vaginal plug was accepted as the index for a positive mating and it was taken as day one of pregnancy (18). A fertility test was calculated using the following formula:

$$\% \text{ Fertility Success} = \frac{\text{Pregnancy Females} \times 100}{\text{Mated females}} \quad (19)$$

The number of litters delivered and their body weights were determined.

**Semen collection:** The left testis was removed along with its epididymis. The caudal epididymis was separated from the testis and lacerated to collect the semen with a microscope slide for semen characteristics evaluation as previously described (20).

**Sperm characteristics analysis:** Progressive motility was tested immediately. Semen was squeezed on a pre-warmed slide, two drops of warm 2.9% sodium citrate was added to it. This was then covered with a cover slip, examined and scored under the microscope using x40 objective with reduced light (21).

A viability study (percentage of live spermatozoa) was done using eosin/nigrosin stain. Semen was squeezed onto a microscope slide and two drops of the stain were added. The motile (live) sperm cells were unstained while the non-motile (dead) sperms absorbed the stain. The stained and the unstained sperm cells were counted using x40

microscope objectives and an average value for each was recorded from which percentage viability was calculated.

Sperm morphology was evaluated by staining the sperm smears on microscope slides with two drops of Walls and Ewas stain after they were air-dried. The slides were examined under the microscope under oil immersion with x100 objective. The abnormal sperm cells were counted and the percentage calculated according to the method described by Wyrobek and Bruce (22). The epididymis was immersed in 5 ml normal saline in a measuring cylinder and the volume displaced was taken as the volume of the epididymis. Sperm count was done under a microscope with the aid of the improved Neubauer hemocytometer. Counting was done in five Thoma chambers (23).

**Statistical analysis:** The results are presented as mean  $\pm$  SEM for each group. The differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range Post hoc test for pairwise comparisons. For sperm abnormalities, the data was analyzed using  $\chi^2$  test. All statistical comparisons and tests were performed using SPSS (SPSS Inc., Chicago, IL., USA) for Windows.

## Results

### Effect of nicotine on semen characteristics

**Motility:** Daily oral nicotine administration of 0.5 mg/kg and 1.0 mg/kg per body weight for a period of four weeks significantly decreased ( $P < 0.05$ ) the progressive motility of the sperm when compared with the control group. Although, this observation was dose-dependent. Rats in the recovery groups of these treatments also showed a decrease in their mean progressive motility when compared with the controls as shown in Table 1.

**Epididymal sperm count:** The mean sperm counts per epididymal volume of rats administered with 0.5 mg/kg and 1.0 mg/kg body weight significantly decreased ( $P < 0.05$ ) when compared with the controls. This decrease was dose-dependent. There was also a decrease in the mean epididymal sperm counts of the recovery groups for 0.5 mg/kg and 1.0 mg/kg B.W. as shown in Table 1.

**Viability (live/dead ratio):** An insignificant decrease ( $P > 0.05$ ) was recorded for the mean percentage of live sperms in rats treated with both 0.5 mg/kg and 1.0 mg/kg body weight treated

**Table 1.** Semen parameters of experimental rats treated with nicotine

Dose	Motility (%)	Live/Dead ratio (%)	Volume ( $\mu$ l)	Count ( $\times 10^6/ml$ )
Control	91.00 $\pm$ 2.00 <sup>a</sup>	96.80 $\pm$ 1.47 <sup>a</sup>	5.20 $\pm$ 0.00 <sup>a</sup>	103.60 $\pm$ 8.8 <sup>a</sup>
0.5 mg/kg B.W.	60.00 $\pm$ 6.32 <sup>b</sup>	92.60 $\pm$ 3.32 <sup>a</sup>	5.18 $\pm$ 0.04 <sup>a</sup>	71.80 $\pm$ 5.20 <sup>b</sup>
1.0 mg/kg B.W.	44.00 $\pm$ 4.90 <sup>b</sup>	91.20 $\pm$ 6.65 <sup>a</sup>	5.16 $\pm$ 0.05 <sup>a</sup>	51.40 $\pm$ 5.20 <sup>b</sup>
0.5 mg/kg B.W. recovery	82.00 $\pm$ 4.00 <sup>a</sup>	94.60 $\pm$ 2.58 <sup>a</sup>	5.18 $\pm$ 0.04 <sup>a</sup>	93.40 $\pm$ 6.50 <sup>a</sup>
1.0 mg/kg B.W. recovery	68.00 $\pm$ 7.48 <sup>b</sup>	91.00 $\pm$ 3.74 <sup>a</sup>	5.18 $\pm$ 0.04 <sup>a</sup>	70.80 $\pm$ 3.60 <sup>b</sup>

Values are expressed as Means  $\pm$  SEM of 8 rats per group. Means in columns with different superscript letters are significantly different;  $p < 0.05$

groups when compared with the controls. However, the same trend was recorded for the recovery groups of both treatment regimens when compared with the control group as shown in Table 1.

**Epididymal volume:** The results showed that nicotine caused an insignificant decrease ( $P > 0.05$ ) in the epididymal volume for both 0.5 mg/kg and 10.0 mg/kg B.W. nicotine treated groups when compared with the control group. This trend was also recorded for the recovery groups of these treatments when compared with the control group as shown in Table 1.

**Morphology:** The most common abnormality encountered during the morphological examination of the sperms in the rats that received the two daily doses of nicotine was the “curve tail” which accounted for 60% of the observed abnormalities. Though, there seemed to be a dose-dependent morphological abnormality. The observed “curve tail” abnormality was statistically significant ( $P < 0.05$ ) when compared with the controls. The recovery groups showed fewer occurrences of the morphological aberration as recorded in the Table 2 and Figure 1.

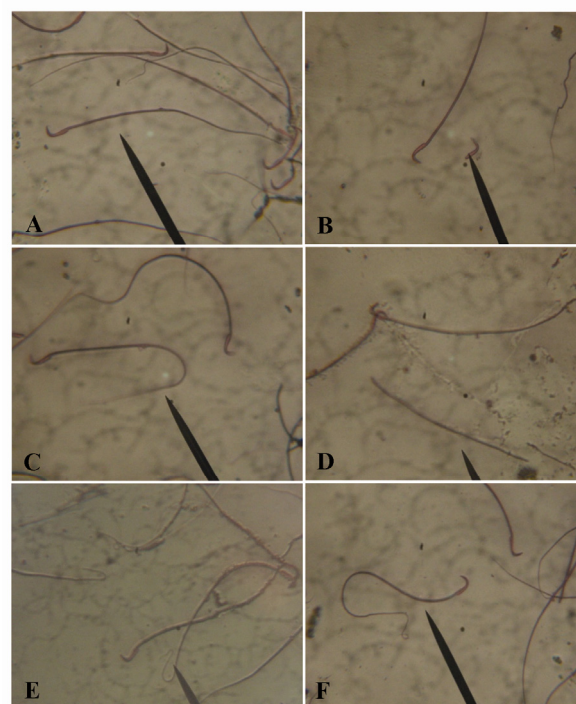
**Effect of nicotine in male fertility**

**Libido score:** Rats treated with 0.5 mg/kg and 1.0 mg/kg B.W. nicotine for four weeks had a significant decrease ( $P < 0.01$ ) in their libido when compared with their control counterparts. The observed decrease was dose-dependent (Table 3). The recovery groups for these treatments also had a significant decrease ( $P < 0.01$ ) in their libido when compared with the control group (Table 3).

**Fertility percentage:** The female rats used for mating in the control group had 100% fertility rate while 0.5 mg/kg and 1.0 mg/kg B.W. nicotine-treated rats had 40% and 0% fertility rates, respectively. The recovery group for 0.5 mg/kg B.W. had a fertility rate of 80% while the recovery

group for 1.0 mg/kg B.W. had a fertility rate of 60% (Table 3). Female rats cohabited with male rats from the high-dose group did not conceive throughout the study period. However, these effects were reversible after nicotine was withdrawn from the rats.

**Litter weight:** Female rats used for mating in the control group produced an average litter size of 6.24  $\pm$  0.1. An average of 5.3  $\pm$  0.1 litter weight was produced by female rats used to mate male rats that received 0.5 mg/kg B.W. (low dose) nicotine. The recovery group of 0.5 mg/kg B.W.



**Figure 1.** Photomicrograph showing sperm from the cauda epididymis of male albino rats; A: normal sperm from the control rats; B-F: sperm showing defects in morphology after nicotine treatment; B: tail less sperm; C: Curve tail; D: Headless sperm; E: looped tail; F: coiled tail (arrow) (eosin/ nigrosin Mag. X100)



**Table 2.** Sperm abnormalities of experimental rats treated with nicotine

Sperm parameters	Control	0.5 mg/kg B.W.	1.0 mg/kg B.W.	0.5 mg/kg B.W. recovery	1.0 mg/kg B.W. recovery
Tailless head	2	4	5	4	6
Headless sperm	2	3	5	4	5
Rudimentary tail	2	6	10	1	2
Curved tail	10	38	60	21	20
Curved midpiece	3	6	7	4	6
Bend midpiece	5	5	8	4	3
Coiled-tail	1	3	2	2	2
Swapped-tail	1	3	2	1	1
Total number of abnormal sperm	26	68	99	41	53
Total number of normal sperm	379	342	301	359	347
% of Abnormal cells	6.87 <sup>a</sup>	19.88 <sup>b</sup>	32.89 <sup>c</sup>	11.42 <sup>a,d</sup>	15.27 <sup>d</sup>

Numbers in rows with different superscript letters are significantly different;  $p < 0.01$

and 1.0 mg/kg B.W. nicotine-treated rats had  $5.47 \pm 0.33$  and  $5.15 \pm 0.05$  litter weights, respectively (Table 3).

**Litter size:** Female rats used for mating in the control group had an average litter size of  $7.80 \pm 0.4$ . An average litter size of  $4.50 \pm 0.5$  was produced by female rats used in mating the 0.5 mg/kg B.W. treated animals. The recovery groups of 0.5 mg/kg B.W. and 1.0 mg/kg B.W. treated rats had an average litter size of  $6.33 \pm 0.5$  and  $3.50 \pm 0.5$ , respectively (Table 3).

### Discussion

The results of this investigation demonstrated that nicotine has deleterious effects on the reproductive functions of male rats, sufficient to cause reversible infertility. Rats were used in this study because rats have been shown to have a well-defined reproductive system and all the compounds, that have been used to cause anti-fertility effects in males have been found to be active in

rats (24). The present study indicates that nicotine-treatment in male rats could affect the weight and number of the litter delivered by untreated female rats.

Male Sprague-Dawley rats treated with nicotine for 30 days had a decreased sperm motility.

Viability (percentage of live sperms) was also reduced in rats with the two different doses of nicotine used in the study in a dose-dependent manner. The reduction in sperm viability agreed with reduction in the progressive sperm motility because immobile sperms were considered dead as they took up the Eosin/Nigrosin stain when the smear was examined. This result may also be due to the effect of nicotine on the epididymis by acting as a spermatotoxic agent on maturing or matured spermatozoa (25).

The epididymal sperm count of the rats treated with nicotine, 0.5 mg/kg and 1.0 mg/kg body weight, was significantly reduced when compared with their control counterparts. This decrease in

**Table 3.** Fertility potentials of experimental rats treated with nicotine

Dose	Libido score	Litter size	Litter weight (g)	Percentage fertility (%)
Control	$11.40 \pm 1.01$ <sup>a</sup>	$7.80 \pm 0.40$ <sup>a</sup>	$6.24 \pm 0.10$ <sup>a</sup>	100 <sup>a</sup>
0.5 mg/kg B.W.	$6.80 \pm 1.94$ <sup>b</sup>	$4.50 \pm 0.50$ <sup>b</sup>	$5.30 \pm 0.10$ <sup>a</sup>	40 <sup>b</sup>
1.0 mg/kg B.W.	$3.80 \pm 0.75$ <sup>b</sup>	$0.00 \pm 0.00$ <sup>b</sup>	$0.00 \pm 0.00$ <sup>b</sup>	0 <sup>b</sup>
0.5 mg/kg B.W. recovery	$8.20 \pm 2.32$ <sup>a</sup>	$6.33 \pm 0.47$ <sup>a</sup>	$5.47 \pm 0.33$ <sup>a</sup>	80 <sup>a</sup>
1.0 mg/kg B.W. recovery	$7.00 \pm 2.09$ <sup>b</sup>	$3.50 \pm 0.50$ <sup>b</sup>	$5.15 \pm 0.05$ <sup>a</sup>	60 <sup>a</sup>

Values are expressed as Means $\pm$ SEM of 8 rats per group. Means in columns with different superscript letters are significantly different;  $p < 0.05$

epididymal sperm count could be connected to the decrease in the serum testosterone level reported in our previous study (15). Testosterone was reported to act on the seminiferous tubules to initiate and maintain spermatogenesis (26). The arrest of spermatogenesis may probably occur as a consequence of reduction in serum testosterone which had been shown to be essential for the completion of meiotic division during spermatogenesis. It was shown that nicotine could adversely affect cell division. There was an appreciable increase in the epididymal sperm count of rats in the recovery group showing that the effect of nicotine on sperm count may be ameliorated by nicotine cessation.

The most prominent morphological abnormalities observed in the nicotine-treated rats were curve tail, rudimentary tail, curve mid-piece and bent mid-piece forms. These secondary abnormalities usually occur during epididymal transit, maturation and storage of sperm during which period the spermatozoa develop motility (27). The recovery groups showed a decrease in the occurrence of these abnormalities which further showed that the rats had recovered from the deleterious effect of nicotine.

Fertility studies show a significant decrease in the libido score of rats treated with nicotine. This is probably associated with the decrease in the serum testosterone level as observed in our previous study (15) because testosterone has been associated with increased sexuality, physical and mental energy, stamina and vitality (28). High testosterone has also been shown to account for increased sperm count, fertility, as well as sexual drive (29).

The decrease in the average litter size produced by the untreated female rats used in mating the treated male rats might be due to the effects of nicotine on the progressive epididymal sperm motility. This study showed, for the first time, the recovery of male fertility after cessation of nicotine in adult male albino rats as the libido score, fertility rate, litter weight and size significantly increased showing an improved fertility index.

### Conclusion

The present investigation shows that nicotine reduces fertility in adult male rats and adversely affect litters' weight and size delivered by

untreated female rats. The data also indicate that nicotine withdrawal for a particular period of time could ameliorate the observed effects. However, further investigation is encouraged to confirm the role and mechanisms of action that nicotine leaves on male infertility.

### References

1. Yales CA, Thomas C, Kovacs GT, De Krester DM. Androgen, male factor, infertility and IVF. In: Wood C, Trouson A, editors. Clinical in vitro fertilization. USA: Springer-Verlag; 1989. pp. 95-111.
2. Yeşilli C, Mungan G, Seçkiner I, Akduman B, Açık-göz S, Altan K, et al. Effect of varicocele on sperm creatine kinase, HspA2 chaperone protein (creatine kinase-M type), LDH, LDH-X, and lipid peroxidation product levels in infertile men with varicocele. *Urology*. 2005;66(3):610-5.
3. Reijo R, Alagappan RK, Patrizio P, Page DC. Severe oligozoospermia resulting from deletions of azoospermia factor gene on Y chromosome. *Lancet*. 1996;347(9011):1290-3.
4. Bonde JP. Male fertility. In: Comhaire FM, editor. Chapman and Hall Medicals. New York: Chapman and Hall; 1996. p. 266-84.
5. Kapoor D, Jones TH. Smoking and hormones in health and endocrine disorders. *Eur J Endocrinol*. 2005;152(4):491-9.
6. Russell MA, Jarvis MJ, Devitt G, Feyerabend C. Nicotine intake by snuff users. *Br Med J (Clin Res Ed)*. 1981;283(6295):814-7.
7. Armitage AK, Dollery CT, George CF, Houseman TH, Lewis PJ, Turner DM. Absorption and metabolism of nicotine from cigarettes. *Br Med J*. 1975;4(5992):313-6.
8. Weisberg E. Smoking and reproductive health. *Clin Reprod Fertil*. 1985;3(3):175-86.
9. Aydos K, Güven MC, Can B, Ergün A. Nicotine toxicity to the ultrastructure of the testis in rats. *BJU Int*. 2001;88(6):622-6.
10. Mlynarcikova A, Fickova M, Scsukova S. Ovarian intrafollicular processes as a target for cigarette smoke components and selected environmental reproductive disruptors. *Endocr Regul*. 2005;39(1):21-32.
11. Tankó LB, Christiansen C. An update on the anti-estrogenic effect of smoking: a literature review with implications for researchers and practitioners. *Menopause*. 2004;11(1):104-9.

12. Jones TH, Jones RD, Channer KS. Testosterone and its beneficial actions on cardiovascular disorders. In: Plotsky PM, Hunzicker-Dunne M, Rose JC, Brenner RM, editors. Recent research developments in endocrinology & metabolism, Volume 1. USA: Transworld research network; 2003. p. 143-68.
13. Carmines EL, Gaworski CL, Faqi AS, Rajendran N. In utero exposure to 1R4F reference cigarette smoke: evaluation of developmental toxicity. *Toxicol Sci.* 2003;75(1):134-47.
14. Gaworski CL, Carmines EL, Faqi AS, Rajendran N. In utero and lactation exposure of rats to 1R4F reference cigarette mainstream smoke: effect on prenatal and postnatal development. *Toxicol Sci.* 2004;79(1):157-69.
15. Oyeyipo IP, Raji Y, Emikpe BO, Bolarinwa AF. Effects of oral administration of nicotine on organ weight, serum testosterone level and testicular pathology in adult male rats. *Niger J Psychol Sci.* 2010;25(1):81-86.
16. Iranloye BO, Bolarinwa AF. Effect of nicotine administration on weight and histology of some vital visceral organs in female albino rats. *Niger J Physiol Sci.* 2009;24(1):7-12.
17. Dhawan K, Sharma A. Prevention of chronic alcohol and nicotine-induced azospermia, sterility and decreased libido, by a novel tri-substituted benzoflavone moiety from *Passiflora incarnata* Linneaus in healthy male rats. *Life Sci.* 2002;71(26):3059-69.
18. Bolarinwa Y. Gastric acid secretion in pregnant and lactating rat. *Trop Vet.* 1993;11:35-38.
19. Raji Y, Oloyo AK, Morakinyo AO. Effect of methanol extract of *Ricinus communis* seed on reproduction of male rats. *Asian J Androl.* 2006;8(1):115-21.
20. Raji Y, Udoh US, Mewoyeka OO, Ononye FC, Bolarinwa AF. Implication of reproductive endocrine malfunction in male antifertility efficacy of *Azadirachta indica* extract in rats. *Afr J Med Med Sci.* 2003;32(2):159-65.
21. Morrissey RE, Schwetz BA, Lamb JC 4th, Ross MD, Teague JL, Morris RW. Evaluation of rodent sperm, vaginal cytology, and reproductive organ weight data from National Toxicology Program 13-week studies. *Fundam Appl Toxicol.* 1988;11(2):343-58.
22. Wyrobek AJ, Bruce WR. The induction of sperm shape abnormalities in mice and humans. In: Hollaender A, De Serres FJ. *Chemical mutagens* Vol. 5. New York: Plenum Press; 1980. p. 257-85.
23. Freund M, Carol B. Factors affecting haemocytometer count of sperm concentration in human semen. *J Reprod Fertil.* 1964;8:149-55.
24. Andrzej B, Hahn DW, Foldsey RG, Megure JL. Experimental studies in the development of male contraceptives. In: Zatučni G, Goldsmith A, Spieller JM, Saara JJ, editors. *Male contraception, advances and future prospects.* New York: Harper and Row; 1985. p. 158-82.
25. Pacifici R, Altieri I, Gandini L, Lenzi A, Passa AR, Pichini S, et al. Environmental tobacco smoke: nicotine and cotinine concentration in semen. *Environ Res.* 1995;68(1):69-72.
26. Sharpe RM, Maddocks S, Millar M, Kerr JB, Saunders PT, McKinnell C. Testosterone and spermatogenesis. Identification of stage-specific, androgen-regulated proteins secreted by adult rat seminiferous tubules. *J Androl.* 1992;13(2):172-84.
27. Tulsiani DR, Orgebin-Crist MC, Skudlarek MD. Role of luminal fluid glycosyltransferases and glycosidases in the modification of rat sperm plasma membrane glycoproteins during epididymal maturation. *J Reprod Fertil Suppl.* 1998;53:85-97.
28. Mooradian AD, Morley JE, Korenman SG. Biological actions of androgens. *Endocr Rev.* 1987;8(1):1-28.
29. Yamamoto Y, Isoyama E, Sofikitis N, Miyagawa I. Effects of smoking on testicular function and fertilizing potential in rats. *Urol Res.* 1998;26(1):45-8.