

## The Effect of Varied Doses of Nicotine on Some Morphometric Parameters of the Testis in Albino Wistar Rats

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### Abstract

**Background:** This study is aimed at determining the effect of nicotine on male fertility by evaluating some morphometric parameters of male Wistar rat such as testicular weight, seminiferous tubule diameter, height of epithelium, sertoli-germ cell ratio and Johnsen's score in order to assess the spermatogenic index.

**Methods:** 20 adult male rats were randomly divided into four groups, the test groups were administered with 0.2mg/100g, 0.4/100g and 0.6/100g body weight of nicotine base daily for 30 days using a polythene catheter orally, while the control were administered with 2mls 0.9% physiological saline. Histological slides of the testis were made and data obtained from the slides.

**Results:** nicotine caused a significant reduction ( $P < 0.05$ ) and ( $P < 0.01$ ) in the mean values of the morphometric parameters of the test group compared with control. The Johnsen's Score results were  $5.4 \pm 0.51^*$ ,  $5.2 \pm 0.37^*$  for groups 2 and 3 respectively. For seminiferous tubule diameter the significant values were  $208.0 \pm 4.22^{**}$ ,  $184.5 \pm 11.31^{**}$  in groups 2 and 3, whilst the height of epithelium showed a significant reduction of  $153.9 \pm 13.58^*$ ,  $101.6 \pm 1.35^{**}$ ,  $76.6 \pm 6.65^{**}$  for groups 1, 2 and 3 respectively. Sertoli-germ cell ratio showed a significant reduction in groups 2 and 3 with values of  $3.6 \pm 0.77^{**}$ ,  $1.5 \pm 0.16^{**}$  for groups 2, and 3 respectively, testicular weight showed significant reduction of  $1.2 \pm 0.05^{**}$ ,  $1.1 \pm 0.05^{**}$  for group 2 and 3. The results stated are only those that showed a significant reduction at 95% confidence level when compared with the control.

**Conclusion:** It was concluded that nicotine exerted an adverse effects on the spermatogenic index with concomitant reduction in reproductive potentials of the male rat. Nicotine and nicotine- based products should therefore be taken with caution in cases of infertility in man and animal.

**Key words:** *Morphometric parameters, Johnsen's Score, Spermatogenic index, fertility*

Infertility can be defined as the failure to conceive despite having regular unprotected sexual intercourse for a period of twelve months. It is said that approximately one in ten people suffer from this disability with 50% of the causes being attributed to the male factor (De Kretser and Baker 1999) though many factors associated with female infertility have been mentioned with subsequent advances in the management of infertility amongst them, not much has been done in respect to managing male infertility. Some factors have however been listed as possible causes of male infertility prominent among them is a previous history of sexually transmitted diseases. Other factors like tobacco smoking have been mentioned though more with female infertility but have also been implicated in male infertility.

Nicotine is an alkaloid found in the nightshade family of plants (Solanaceae); biosynthesis takes place in the roots and accumulation occurs in the leaves. It constitutes approximately 0.6–3.0% of the dry weight of tobacco (NIH) and is present in the range of 2–7  $\mu\text{g}/\text{kg}$  of various edible plants. It functions as an antiherbivore chemical; therefore, nicotine was widely used as an insecticide in the past (Rodgman et al, 2009) and nicotine analogue such as imidacloprid are currently widely used. It being a central nervous system influencing drug interferes in many endocrine activities related to reproduction. It inhibits the release of FSH and LH from the pituitary (Blake et al., 1972; Blake, 1974, 1978). Nicotine administration or exposure to cigarette smoke inhalation to rats and hamsters results in testicular degeneration (Vicizian, 1968; Tsilikov, 1969). A study done by Gambo et al (2013) showed dose dependent testicular degeneration following the administration of varied doses of an aqueous extract of tobacco.

Though there are studies such as this, the incidence of tobacco smoking is said to be on the rise as of 2002, about twenty percent of young teens (13-15) smoke worldwide. 80,000-100,000 children begin smoking every day. Half of those who begin smoking in adolescent years are projected to go on to smoke for 15 to 20 years (WHO, 2002). In the developing world tobacco consumption is rising by 3.4% annually (WHO, 2002).

The rising incidence in tobacco smoking particularly in the developing world as well as the little knowledge of the effect of tobacco on male fertility in comparison to the females necessitates studies such as these to determine the effect nicotine on spermatogenesis, whilst making an attempt to count and score the germ cells using the male albino wistar rat as a model.

## **MATERIALS AND METHODS**

### **Animals**

20 male Wistar rats weighing 220 - 280 g were used in the study. All animals were kept in the animal house of the University of Jos. They were maintained at room temperature and 12 hours light/dark cycle. All the experimental procedures were done following the experimental guidelines of Institutional Animal Ethics Committee (IAEC).

### **Chemicals**

Testosterone, Oestrogen, FSH, LH, Progesterone, EIA(Enzyme-linked Immunosorbent Assay) kit was purchased from Monobind Inc. Lake forest U.S.A. Nicotine hydrogen tartrate salt ( $C_{10}H_{14}N_2C_4H_6O_6$ ) purchased from sigma Aldrich catalog number **N5260-25G**.

### **Experimental protocol**

Four groups with 5 rats selected randomly in each group were formed. Groups 1-3 were the test groups, whilst group 4 was used as the control, each rat in group 1 was treated with 0.2 mg/100g body weight /p.o of nicotine daily for 30 days, groups 2 and 3 were given 0.4mg/ 100g and 0.6mg/ 100g body weight of nicotine per os daily for 30 days respectively. While group 4 (control group) was given 2ml of 0.9% physiological saline solution for the same period of exposure.

### **Sample collection**

The rats were anaesthetized using ether and afterward sacrificed by cervical dislocation and blood sample collected by cardiac puncture. Orchidectomy was performed by open castration method. A midline or pre-scrotal incision was made and the testicles were milked out of the incision site and weighed with the aid of OHAUS electric weighing balance.

The testicles were exposed by incising the tunica vaginalis.

### **Histological procedures**

After the extraction of the testis from the animal's body, the organ was promptly and adequately treated with 10% formal saline (fixation) in order to preserve its structure and molecular composition. After fixation, the piece of organ was dehydrated by bathing it successfully in graded mixture of ethanol and water (70 - 100%). The ethanol was then replaced with a solvent miscible with the embedding medium (xylene). As the tissues were infiltrated with xylene, it became transparent (clearing). Once the tissue has been impregnated by xylene it was placed in melted paraffin in an oven maintained at 58 - 60°C (embedding). The heat caused the solvent to evaporate and the spaces within the tissues become filled with paraffin. The tissue together with its impregnating paraffin hardens after been taken out of the oven this was done with the aid of the Leica TP120 automatic tissue processor. The hard block containing the tissue was then taken to the microtome and sectioned by the microtome steel. The sections were then floated on water and transferred to a glass slide and stained with hematoxylin and eosin stains with the aid of the leica auto stainer XL. The slides were then viewed under light microscope with varying magnification. Spermatogenesis was assessed by a method which depended upon scoring 'cross sectional' profiles of seminiferous tubules according to Johnsen's criteria. (Johnsen S.G1970) The criteria use scores from 1 to 10 and are as follows: 10. Complete spermatogenesis with many spermatozoa. 9. Many spermatozoa present but germinal epithelium disorganized with marked sloughing or obliteration of lumen. 8. Only a few (<5) spermatozoa. 7. No spermatozoa but many spermatids. 6. No spermatozoa and only a few spermatids (<5). 5. No spermatozoa or spermatids but many spermatocytes. 4. No spermatozoa or spermatids and only a few spermatocytes(<5). 3. Spermatogonia are the only germ cells present. 2. No germ cells but sertoli cells are present. 1. No cells in tubular section.

Diameter of seminiferous tubules was measured using Leica 1000 DM microscope and X10 objective lens with ocular micrometer whilst the measurement for the height of epithelium was done at X40 magnification. The sertoli/germ cell ratio was also determined, using stage 7 spermatids as the chosen line of germ cells on photomicrographs of the histological slides. The pathologist involved in the count was blind to the grouping and dosage of nicotine given to the rats.

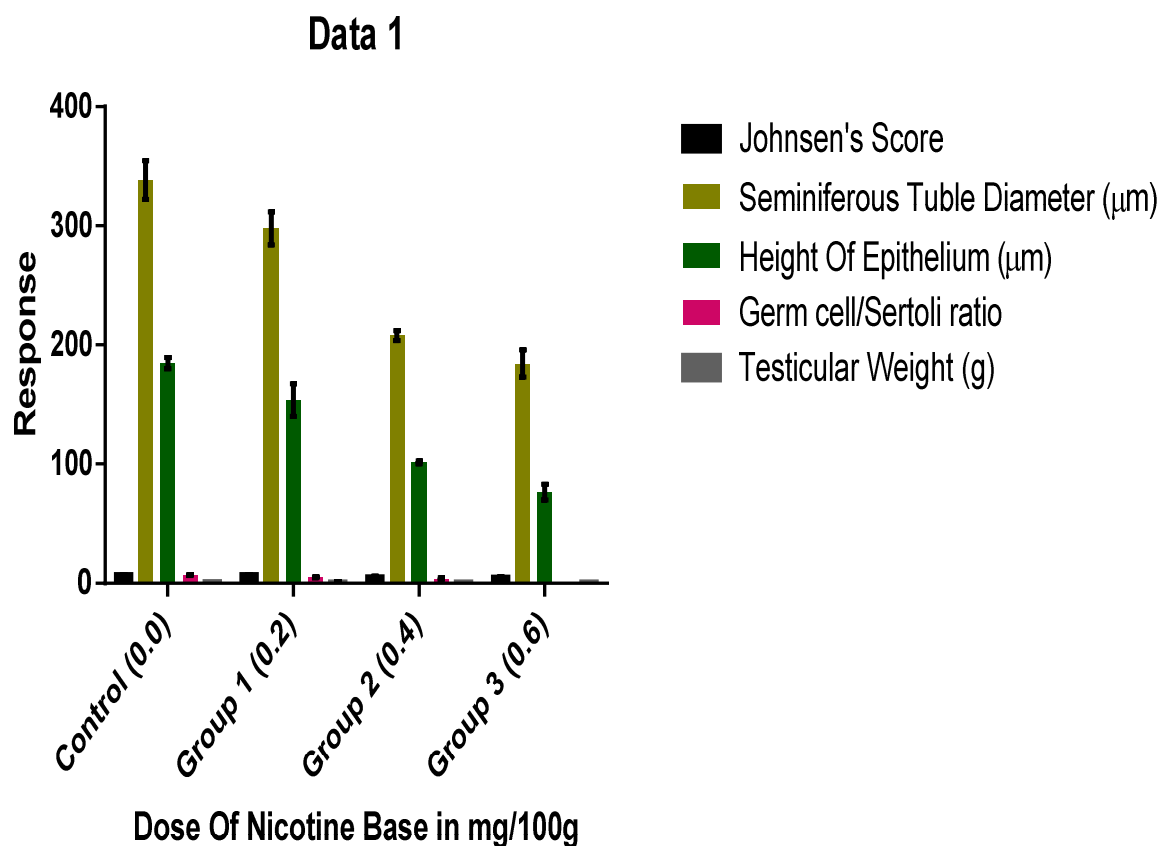
### Statistical analysis

Statistical analysis was done using graphpad instat3 tool to conduct one way analysis of variance (ANOVA). The graphpad prism 6 was used to generate a bar chart of the grouped data.

### Results

Are presented as the mean  $\pm$  standard error of mean. The results showed a significant dose dependent decrease at all dose levels for the height of the epithelium, whilst showing a significant decrease in seminiferous tubule diameter, Johnsen's score and sertoli – germ cell ratio at the doses of 0.4mg/100g and 0.6mg/100g of nicotine base only.

Figure 1: bar chart showing morphometric parameters measured.



**Discussion :** The sertoli- germ cell ratio, Johnsen's score, testicular weight, height of the germinal epithelium and seminiferous tubule diameter were used in this study to evaluate the effect of administration of varied doses of nicotine over a period of thirty days on spermatogenesis, using the Wistar rat as animal model. The sertoli to elongated germ cell ratio as well as the Johnsen's score was used to evaluate the spermatogenic index, a decrease in the sertoli to elongated spermatid ratio or Johnsen score indicates a fall in spermatogenesis (Aruna *et al* 2009). The results showed a significant dose dependent decrease at all dose levels for the height of the epithelium, whilst showing a significant decrease in seminiferous tubule diameter, Johnsen's score and sertoli – germ cell ratio at the doses of 0.4mg/100g and 0.6mg/100g of nicotine base only, corroborating the findings of Egesie *et al* (2013) at the preliminary stage of this study, which reported the histological slides as having a dose dependent decrease on spermatogenic cells and distortion of the cyto-architecture of the seminiferous tubule of the testis. The decrease spermatogenic index is also in tandem with the findings of decrease sperm count in the study.

The finding of decreased sertoli to elongated germ cell ratio corroborates studies done by Rajpurkar (2000) where they observed a decrease in the ratio as well as a decrease in seminiferous tubule diameter in rats exposed to cigarettes smoke, which was also observed in this study, they however did not observe any significant difference in the height of epithelium of the rats exposed to cigarettes smokes as opposed to this study which

observed a significant difference in the height of the epithelium in the seminiferous tubules of tubules of the rats given high doses of nicotine base. The observed difference in the findings may be as a result of the difference in the modes of administration as well as the probable higher dose given as direct nicotine base in this study. The observed decrease in the morphometric parameters measured may be as a result of the inhibition of the release of gonadotrophins, follicle stimulating hormone and luteinizing from the anterior pituitary (Blake et al., 1972; Blake, 1974, 1978). These hormones are necessary for proper development of the gonads as well as the process of spermatogenesis.

### Conclusion

Thus it can be stated from this study that the intake of nicotine especially at doses greater than 2mg/100g for a period of 30 days in male albino wistar rats adversely altered the morphometric parameters measured with a concomitant decrease in spermatogenic index, thus nicotine and nicotine – based products should be taken with caution particularly in cases of infertility.

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