

Effects of Oral Administration of Nicotine on Organ Weight, Serum Testosterone Level and Testicular Histology in Adult Male Rats

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Summary: This study investigated the effects of oral administration of nicotine on body and reproductive organ weight, serum testosterone level and testicular histology in adult male rats. Forty male rats divided into five groups and treated for a period of 30 days with 0.5mg/kg (low dose) and 1.0mg/kg (high dose) body weight of nicotine while the control rats received 0.2 ml/kg normal saline. The fourth and fifth groups were gavaged with 0.5mg/kg and 1.0mg/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, the animals were scarified and their reproductive organs were removed and weighed immediately. There was no significant change in the body weight. There was a significant decrease ($p < 0.05$) in the testicular and epididymal weight of rats for both treatments while the decrease in the seminal vesicle weight for both treatment groups was not significant. The prostate weight was not significantly increased in both groups. The recovery groups showed appreciable recovery in their organ weight. Serum level of testosterone of both groups was significantly decreased in a dose dependent manner when compared with those of the control rats. The histological section showed testicular degeneration and disorganization in the cytoarchitecture, as the observed changes were pronounced in the high dose group than the low dose group. However, there were both regeneration of the germinal epithelium and restructuring of the interstitium towards normal in the recovery groups. No lesion was observed in the epididymis of the rats. The results suggest that nicotine has deleterious effect on the male reproductive organ of albino rats ameliorated by nicotine cessation.
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INTRODUCTION

Despite worldwide anti-smoking campaigns, cigarette smoking is very common. The highest prevalence of smoking is observed in young adult males during their reproductive period (46% smokers between 20 and 39 years) (Langgassner, 1999). Recent estimates indicate that there are 46.2 million smokers in the United States alone, with smoking-related deaths numbering >440,000 each year (Centers of Disease control and prevention, 2006). Tobacco smoke contains numerous compounds, the important substances of medical significance being the carcinogens, irritant substances, nicotine, carbon monoxide and other gases (Stillman, *et al*, 1986). Nicotine is considered the primary chemical in tobacco that is responsible for engendering tobacco

use and dependence (Di Chiara, 2000, Harvey *et al* 2004).

Some effects of nicotine on reproduction have been reported (Patterson *et al*, 1990; Lambers and Clark 1996). Several epidemiological studies have revealed a consistent and highly significant incidence of infertility (Rodriguez-Rigau, *et al* 1982, Tuormaa, 1995 Yamamoto, *et al* 1998) as well as an increased risk of spontaneous abortion among smokers (Becker *et al*, 1968) but studies that showed the effects of nicotine on fertility were only seen for smoking more than 20 cigarette per day, though there was a trend for all levels of smoking (Laurent *et al*, 1992). Effect of nicotine administration on weight and histology of some male visceral organ in female albino rats has also been studied (Iranloye and Bolarinwa, 2005) however, there are a lot of controversies on the effect of nicotine on changes in testosterone level, but most

studies have associated nicotine with decreased serum testosterone (Patterson *et al*, 1990; James, 2001). Nevertheless, studies that comprehensively associated nicotine with altered body weight in rats did not account for its effect on recovery phase and male reproductive organs. In spite of the growing knowledge of adverse reproductive effects of smoking on reproduction, it is relatively unclear whether or not, nicotine has the same effects on male reproductive activities. However, no study has documented the effect of nicotine on organ weight, serum testosterone, testicular histology and subsequent effects of cessation on male reproductive parameters. The present study was therefore designed to investigate the effect of nicotine on reproductive functions during treatment and recovery.

MATERIALS AND METHODS

Nicotine Preparation

Nicotine hydrogen tartrate with product number 26140 (95% nicotine) was purchased from BDH chemical Ltd Poole England. The nicotine dosage freshly prepared in normal saline for each group of animals was delivered orally at 0.5mg/kg and 1.0mg/kg 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 40 male Sprague-Dawley rats whose average weight ranged between 150g and 180g (2-2.5 month old) 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 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 animals were divided into five groups; Control group that received 0.2 ml/kg normal saline, 0.5mg/kg nicotine treated, 1.0mg/kg nicotine treated, 0.5mg/kg nicotine treated but left untreated for another 30 days and 1.0mg/kg nicotine treated but left untreated for another 30 days. Control group received 0.2 ml of normal saline (vehicle) which was used to dissolve the nicotine hydrogen tartrate but nicotine-treated group received nicotine hydrogen tartrate by daily oral gavage through the use of oral cannula. The dosage used in this study i.e. 0.5mg/kg and 1.0mg/kg was chosen in relation to human studies to be the amount of nicotine

absorbed by taking 10 and 20 cigarettes respectively. This dosage was administered to mimic what obtains in tobacco smoking (Beckers and King, 1966). Body weight, food and water intake were monitored before and at the end of the 8-week experimental period.

Blood Sample Collection

Blood (2ml) was collected from each animal via the retro-orbital sinus with 70µl heparinized capillary tube (Ezzai, 1995) and put into plain sample bottle for testosterone analysis. The sample was centrifuged at 3000 rpm for five minutes. The serum was used to analyze the level of testosterone

Organ Collection

The animals were dissected and the reproductive organs (testes, epididymis, prostate gland and seminal vesicle) were removed, cleared of adherent tissues and weighed immediately with an electronic weighing balance, model DT 1000 England with a capacity of 0.1 to 1000g.

Histology

Testes and epididymis of the control and treated rats were fixed in Bouin's fluid for 6 hours before they were transferred into 10% formalin for histological evaluation. The tissues were routinely processed and examined under the light microscope. Photomicrograph of the slide was then taken.

Testosterone assay procedure.

An enzyme –based immunoassay (EIA) system was used to measure testosterone level in serum samples collected. The EIA kit was obtained from immunometrics (London, UK) and contained a testosterone EIA enzyme label, testosterone EIA substrate reagent and EIA quality control sample. A quality control was carried out at the beginning and at the end of the assay to ascertain the acceptability with respect to bias and within batch variation. The EIA kit used had a sensitivity of approximately 0.3nmol/M (0.1g/mL) of testosterone. The intra and inter assay variations were 10.02% and 10.12% respectively.

Statistical analysis: The results are presented as means±SEM for each group. Differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by the Duncan's multiple range Post hoc test for pairwise comparisons. All statistical comparisons and tests were performed using SPSS (SPSS Inc., Chicago, IL., USA) for Windows. P<0.05 was accepted as significant.

RESULTS

Effect of nicotine on body weight

There were no significant differences in the mean body weight of nicotine treated rats during the treatment period compared with the control group as shown in table 1(a). However, there was a significant weight gain of 17.46% for the control, 11.32% for 0.5mg/kg, and 7.84% for 1.0mg/kg treated group for the four weeks when the final weight of each group after treatment was compared with the initial weight before treatment. 0.5mg/kg recovery group and 1.0 mg/kg recovery group had a total weight gain of 25.37% and 24.27% weight gain respectively for the eight weeks experimental period.

Table 1:
Effect of nicotine on Percentage change in Body weight of male rats

DOSE	Body weight gain (%)
Control	17.46 ^a
0.5mg/kg BW	11.32 ^a
1.0mg/kg BW	7.84 ^b
0.5mg/kg BW recovery	25.37 ^c
1.0mg/kg BW recovery	24.27 ^c

Values are expressed as means±S.E.M of 8 rats per group. Means in rows showing different superscript letters ^{a,b,c} are significantly different; p<0.05.

Effect of nicotine on reproductive organ weight

The result showed that there is a significant decrease in the mean testicular weight of rats that received 0.5mg/kg B.W and 1.0mg/kg B.W in a dose-dependent manner, however, the recovery groups of this treatment showed no significant decrease in their mean testicular weight when compared with the control group. The mean epididymal weight of rats that received the two doses of nicotine was significantly decreased (p<0.05) when compared with the control. Their recovery groups showed no significant decrease in the mean epididymal weight when compared with their control counterparts as shown in table 2.

Table 2 shows that rats treated with 0.5mg/kg and 1.0mg/kg BW of nicotine for four weeks showed no

Table 2: Effect of nicotine on mean organ weight of male rats

DOSE	Testes (g)	Epididymis (g)	Prostate (g)	Seminal vesicle (g)
Control	1.45 ± 0.03 ^a	0.38 ± 0.04 ^a	0.44 ± 0.04 ^a	1.14 ± 0.32 ^a
0.5mg/kg BW	1.30 ± 0.02 ^b	0.30 ± 0.00 ^b	0.46 ± 0.05 ^a	1.04 ± 0.20 ^a
1.0mg/kg BW	1.17 ± 0.01 ^b	0.22 ± 0.07 ^b	0.48 ± 0.06 ^a	0.99 ± 0.48 ^a
0.5mg/kg BW recovery	1.42 ± 0.04 ^a	0.36 ± 0.04 ^a	0.40 ± 0.04 ^a	1.04 ± 0.36 ^a
1.0mg/kg BW recovery	1.37 ± 0.02 ^a	0.29 ± 0.05 ^b	0.48 ± 0.04 ^a	1.18 ± 0.24 ^a

Values are expressed as means±S.E.M of 8 rats per group. Means in rows showing different superscript letters ^{a,b} are significantly different; p<0.05.

significant increase in the mean prostate weight when compared with the control while an insignificant decrease in the mean prostate weight of 0.5mg/kg recovery group. An insignificant increase was observed for the mean prostate weight for 1.0mg/kg recovery group.

Rats treated with 0.5mg/kg and 1.0mg/kg B.W nicotine had an insignificant decrease in the mean seminal vesicle weight. The recovery groups for 0.5mg/kg B.W had an insignificant decrease in the mean seminal vesicle weight when compared with the control while groups treated with 1.0mg/kg B.W had an insignificant increase compared with the control group as shown in table 2.

Effect of nicotine on the histology of the testis of male albino rats

There was a reduction in the germ cells and sertoli cell population of nicotine treated groups. The severity of testicular degeneration and disruption of the interstitium was dose-dependent, as observed changes were more pronounced in the high dose group than the low dose group. However, there were both regeneration of the germinal epithelium and restructuring of the interstitium towards normal in the recovery group.

Effect of nicotine on the histology of the epididymis of male albino rats

The histological study shows that nicotine had no effect on the epididymis of the animals as shown in figure 2

Effect of nicotine on serum level of testosterone

Figure 3 shows that the mean serum testosterone level in rats that received 0.5 mg/kg B.W (low dose) and those that received 1.0 mg/kg B.W (high dose) of nicotine for four weeks was significantly decreased (p<0.05) in dose-dependent manner when compared with the control group. This decrease is dose dependent. The recovery groups of these treatment also showed a significant decrease (p<0.05) in serum level of testosterone when compared with the control group.

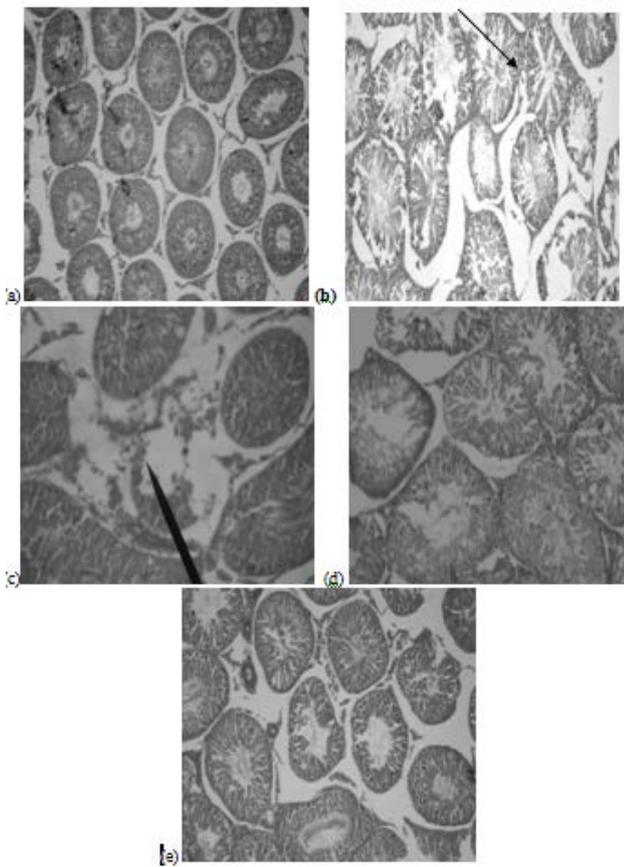


Figure1. Photomicrograph of testis of (a) control rat with normal seminiferous tubules epithelium, (Mag X 250 Hand E) (b) 0.5mg/kg treated rat showing moderate testicular degeneration, (Mag X 250 Hand E) (c) 1.0mg/kg treated rats showing focal testicular degeneration. (Mag X 250 Hand E) (d) 0.5mg/kg recovery group showing regeneration, (Mag X 250 Hand E) (e) high dose recovery group showing regeneration (Mag X 250 Hand E)

DISCUSSION

Several adverse effects of nicotine leading to various diseases and pathological conditions in man have been described (Hammer and Mitchell, 1979, Christensen *et al*, 1984, Wilkins *et al*, 1982, Pullan *et al*1994).

Some isolated effects of nicotine on reproductive system have also been studied both in male and female (Patterson *et al*, 1990, Hammer and Mitchell, 1979 and Kamaldeep, 2002) but none has been done to comprehensively evaluate the effects of nicotine on male reproductive organs, its association with histology, testosterone and recovery phase of treatment.

Nicotine treatment on rats had no significant effect on their mean body weight during the treatment period compared with the control group. However,

there was a significant decrease in weight gain of the animals that received the two doses and this effect was dose – dependent when the final weight of each group was compared with the initial weight. The observed decrease in body weight gain could be associated with the effect of nicotine in the increase of satiety and decrease in food intake as described earlier by Ramos *et al*, 2004.

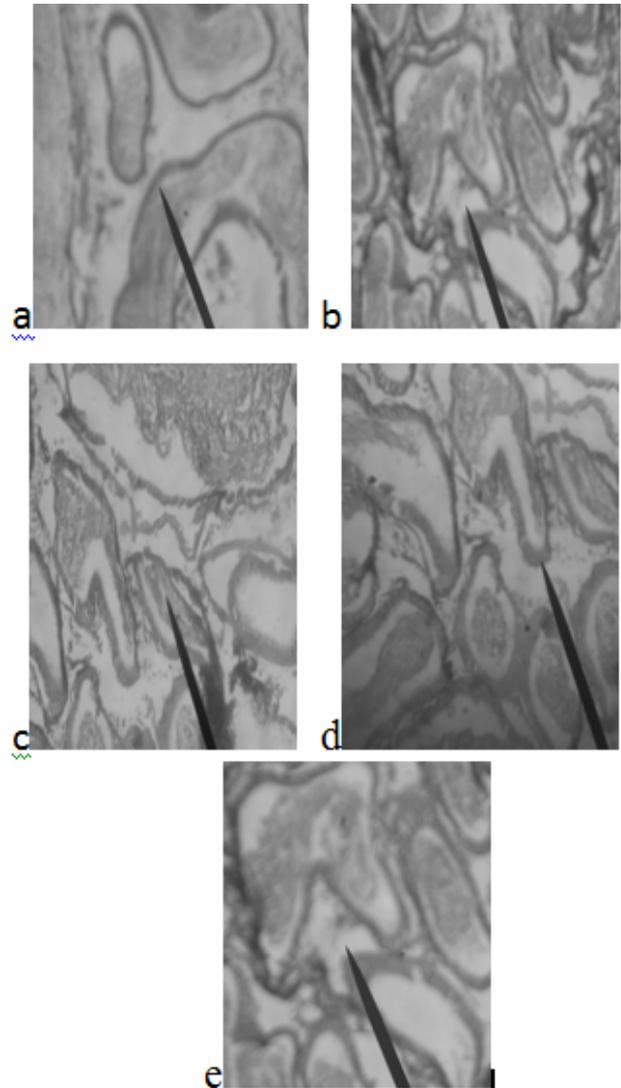


Figure 2 Photomicrograph of epididymis of (a) control rat with normal epididymis, (Mag X 250 Hand E) (b) 0.5mg/kg treated rat showing normal epididymis (Mag X 250 Hand E) (c) 1.0mg/kg treated rats normal epididymis (Mag X 250 Hand E) (d) 0.5mg/kg recovery group showing normal epididymis (Mag X 250 Hand E) (e) high dose recovery group showing normal epididymis (Mag X 250 Hand E)

The weight of the organs of the treated rats showed variable response to the two doses of nicotine. Of great interest in the mean weight of the

testis and epididymis of animals administered with 1.0mg/kg B.W that showed a significant decrease. There was also a decrease in the weight of other organs except for the prostate, though it was not significant. The decrease in the mean body weight of these organ correlates with the decrease in the serum testosterone level obtained from this study.

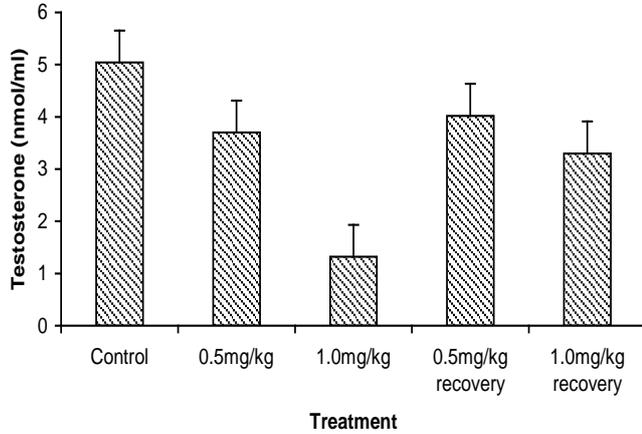


Figure 3: Effect of nicotine on serum level of testosterone in male rats. Values are expressed as means±S.E.M of 8 rats per group.

Testosterone is necessary for the development, growth and normal functioning of the testis and the male accessory reproductive gland. Low serum testosterone levels have been reported to adversely affect the structure, weight and functioning of the testis and epididymis. Hence, the reduction in weight of the testis, epididymis and seminal vesicle could be associated with the decrease in the serum level of testosterone in the nicotine treated rats (George and Welson 1986, Mooradian *et al*, 1997). The increase in mean weight of the prostate and liver could be due to the effect of nicotine in causing hypertrophy (Halliwell, 1993).

The decrease in serum testosterone level of rats treated with the two doses of nicotine must have been caused by the disruption of testicular cytoarchitecture by nicotine. Consequently this might have adversely affected Leydig cell number and functioning leading to decrease serum testosterone level since Leydig cells secrete testosterone (Preslock, 1989, Saez 1994).

The decrease in the serum testosterone level caused by nicotine could be associated with disruption of testicular cytoarchitecture which adversely affected leydig cell number and function, as well as the reproductive organs and fertility rate of the animals. It should be noted that the antifertility effects of nicotine observed in this study were ameliorated by nicotine cessation.

In conclusion, the results suggest that nicotine decrease serum testosterone level and have deleterious effect on the male reproductive organ and histology of albino rats.

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