

Effect of Nicotine on Reproductive Hormones in Adult Male Mice (*Mus musculus*)

S. Sharif*, N. Fatima, T. Farasat and A. A Latif

Department of Zoology, Lahore College for Women University, Lahore, Pakistan

ABSTRACT

The study was conducted to determine the direct effect of nicotine administration on reproductive hormones in adult male mice (*Mus musculus*). Adult mice (n=60) were randomly divided into two groups viz. experimental (n=40) and control (n=20) groups. Experimental group was injected with nicotine subcutaneously (1mg/ kg body weight); whereas, the control group was administered with normal saline solution subcutaneously for 6 weeks. At the end of six weeks, blood was collected to determine the concentrations of luteinizing hormone and testosterone using enzyme linked immunosorbent assay (ELISA). Results showed that nicotine significantly ($p < 0.05$) decreased the levels of luteinizing hormone and testosterone in the experimental group compared to the control group. It was concluded that intake of nicotine may cause some deleterious effects on reproductive hormones in mice.

Key words: Nicotine, luteinizing hormone, testosterone, mice

INTRODUCTION

Nicotine is mainly obtained from the tobacco plant which belongs to the family *Nicotiana tabacum*. This plant has been used for centuries and can be chewed, sniffed or smoked (Jain et al., 2003). Animal studies on the male and female rodents showed that these animals exhibited diverse sensitivities to the effects of nicotine (Schechter and Rosecrans, 1971). The major effects of nicotine on animals and humans include injury of lungs, increased plasma free fatty acids, elevated blood pressure and increased pulse rate (Kavitharaj and Vijayammal, 1999; Liu et al., 2001; Benowitz et al., 2002; Pausova et al., 2003; Valenca et al., 2004). Inhalation of nicotine by smoking largely affects the functioning of thyroid, pituitary, adrenal, ovarian and testicular glands (Kapoor and Jones, 2005). In males, nicotine has been found to decrease steroidogenesis and inhibits spermatogenesis (Mlynarcikova et al., 2005).

Previous reports demonstrated that nicotine is largely responsible for the inhibition of leutinizing hormone (LH), follicular stimulating hormone (FSH) and prolactin from pituitary (Anderson et al., 1982). The maturation and growth of testis is controlled by gonadotropins which in turn regulate the release of androgen (Odell and Swerdloff, 1976; Ketelslegers et al., 1978; Huhtaniemi et al., 1982). Therefore, both gonadotropins and testosterone are responsible for the initiation of spermatogenesis (Ojeda and Urbanski, 1994). The LH is a major tropic regulator of Leydig cells without which the production of androgens is not possible (Huhtaniemi and Toppari, 1995). Leydig cells secrete testosterone when they are stimulated by LH and the concentration of testosterone rises almost in direct proportion to the quantity of LH available.

The results of various studies to demonstrate the effect of nicotine on serum testosterone levels are largely contradictory because of the complications in the hormonal assays. Therefore, the present study was designed

*Corresponding author:
ssharif1978@yahoo.com

aiming at evaluating the effects of nicotine on serum testosterone (T) and LH levels in adult male mice.

MATERIALS AND METHODS

Experimental Design

A total of 65 one month-old male albino mice (*Mus musculus*) were procured from the Veterinary Research Institute, Lahore, Pakistan with the body weight ranging from 19-27g. The mice were kept in the Animal House, Lahore College for Women University, Lahore, Pakistan. These animals were kept in wire cages in groups of 20 animals per cage and fed a standard pellet diet. Feed and water was provided ad libitum. The animals were kept under standard management and feeding conditions throughout the experiment and maintained on a 12-h light/12-h dark cycle with ambient temperature of 20-22°C. The relative humidity was 60%.

The mice were acclimatized for a month prior to experiment till the animals were weighed about 30-39g. To decrease the effects of circadian rhythm, treatments were carried out between 9 to 10 AM. During acclimatizing period, 5 mice died. After acclimatization period, the mice were distributed in two groups viz control (n=20) and experimental (n=40) groups. The experimental group was administered with nicotine hydrogen tartarate subcutaneously at the dose rate of 1.0 mg/ kg of body weight/ daily for a period of six weeks. The control group was treated with subcutaneous injection of normal saline (0.1 ml/kg body weight). No mortality was recorded during the experimental period of six weeks. In each group animal were weighed individually. Feed intake (g/cage/day) was observed between the experimental and control groups. Daily weighed feed was provided early morning and then on next day the remaining feed was weighed again per cage to determine the amount of feed consumed.

Estimation of Reproductive Hormones

For the assessment of reproductive hormones, the blood samples were collected in EDTA coated tubes by cardiac puncture directly from the ventricle of the heart after anesthetizing the animal. The collected blood was centrifuged at 5,000 rpm for 10 minutes to separate of plasma that was stored at -20°C till analyzed.

At the time of analysis, the plasma samples were thawed and plasma levels of LH and testosterone were determined using standard kits. The kit (Model # E90441Mu 96 Tests, USCN, Life Science Inc., USA) was used for the assessment of LH and the kit (Model # E90458Mu 96 Tests, USCN, Life Science Inc., USA) was used for the determination of testosterone level in the plasma samples. The experiment was conducted after the approval of the Animals Ethical Committee, Lahore College for Women University, Lahore.

Statistical Analysis

Data, presented as Mean \pm SE, were statistically analyzed using Independent Sample Student's "t" Test and $p < 0.05$ was considered to be significant.

RESULTS

The mean body weight before and after acclimatization were 19.95 ± 0.04 g and 26.51 ± 0.06 g, respectively. During the acclimatizing period, the body weight gain was approximately 6.56 g (Figure 1). The food intake as well as body weight of experimental mice decreased as compare to the control group during the experimental period (Figures 2 and 3).

The plasma concentration of LH was higher ($p < 0.05$) in the control group, when compared with the experimental group (Table 1). Similar outcome was observed for the plasma concentration of testosterone that was significantly ($p < 0.05$) decreased in

experimental group compared with the control group.

Table 1 Mean ± SE of reproductive hormones in adult male mice with and without administration of nicotine

Hormones	Group	
	Control	Experimental
Luteinizing hormone (ng/mL)	31.26± 0.75	29.14* ± 0.24
Testosterone (ng/mL)	1.9 ± 0.35	1.4* ± 0.01

*Significant difference at $p < 0.05$

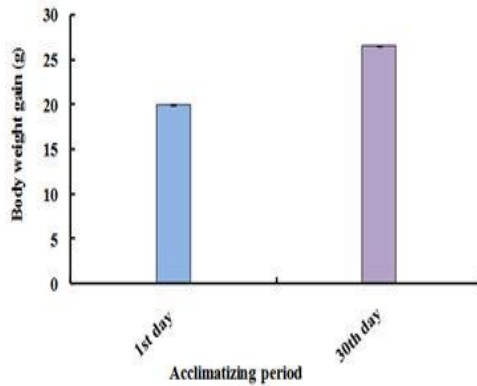


Figure 1 Body weight gain in adult male mice during acclimatizing period of one month

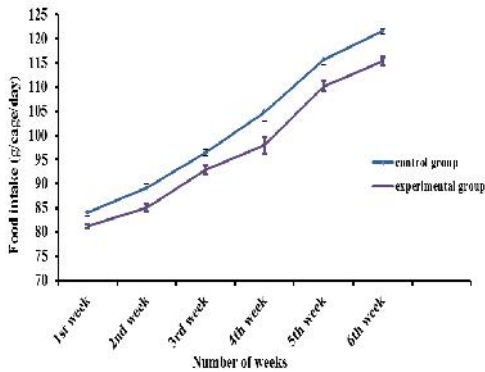


Figure 2 Graph showing decreasing trend of food intake (g/cage/day) in experimental group compared to control group during 6 weeks of nicotine administration

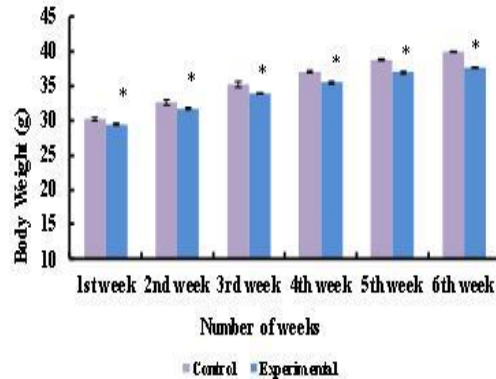


Figure 3 Body weight (mean±SE) in control and experimental group during 6 weeks of nicotine administration. * $p < 0.05$ significant difference

DISCUSSION

The present study was investigated the effects of nicotine on changes occurring in reproductive hormone levels in adult male mice. Results showed a significant ($p < 0.05$) decrease in body weight gain and food intake in nicotine-treated adult male mice compared with the saline-treated mice. Many other studies conducted on changes in body weight and food intake due to nicotine confirmed our present results. Audi et al. (2006) showed that administration of nicotine in rats caused a significant decrease in body weight gain and food intake. Similarly, Wack and Rodin (1982) concluded that decrease in food intake and body weight was because nicotine exerted its effect on the neuroregulatory substances which have

major role in suppression or boosting of food intake mechanism.

Nicotine affects the levels of reproductive hormones and it is well demonstrated in our study that nicotine administration to mice significantly ($p < 0.05$) reduced the LH level. Our results are similar to the results of Yoshinaga et al. (1979) who demonstrated that nicotine administration to rats lowered LH levels and LH/FSH ratio. Blake (1972) reported that subcutaneous administration of nicotine resulted in lower LH secretion. The site of this nicotine effect was supposed to be the medial basal hypothalamus because nicotine caused inhibition of LH secretion.

Nicotine has also been reported to have a direct effect on the testicular tissues and ultimately affects spermatogenesis (Zovas et al., 1999). Nicotine administration to male mice resulted in damage to the tissues of testis due to the cytotoxic effects of nicotine on spermatogenic cells or through the inhibition of synthesis of prostaglandins which played a vital role in the maintenance of reproductive system of mice (Polyzos et al. 2009). It has been reported by Olayaki et al. (2008) that high nicotine smoking might initiate rapid release of prolactin by causing higher concentration of endogenous opioids, which in turn might result in the inhibition of dopamine release. The release of hypothalamic LHRH, which controls the release of LH from pituitary, was inhibited by endogenous opioid peptides. The increase in endogenous opioid peptides due to cigarette smoking might show lower levels of LH.

In present study, nicotine caused a significant ($p < 0.05$) decrease in testosterone level in adult male mice. Kavitharaj and Vijayammal (1999) reported that nicotine was found to have adverse effects on the functions of gonads in males, in addition, to its part in the lowering of serum testosterone levels. It has also been demonstrated that nicotine also reduced the secretion of serum

testosterone, LH and follicle stimulating hormone (Wang et al., 2005). In another study, Mittler et al. (1983) suggested that the low level of testosterone in smokers was due to increased activity of liver hydroxylase, an enzyme known to increase the metabolism of testosterone. Riesenfeld and Oliva (1988) found that nicotine exerted adverse effects on gonadal functions as it induces some biochemical changes in testis. A number of previous studies showed that nicotine caused cytotoxic effects resulting in the inhibition of the prostaglandins production and reduced testosterone levels which are essential for male reproduction (Favaro and Cagnon, 2006; Ahmadnia et al., 2007; and Yamamoto et al., 1998)

In conclusion, reproductive hormonal levels were affected by the administration of nicotine that may affect the fertility.

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