

Biochemical Effects of Nicotine on the Testis of Adult Male Rats

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Abstract: This study is aimed at determining the biochemical effect of Nicotine on male testis ices by evaluating, serum concentration of testosterone, androgen binding protein (ABP), FSH and LH. Nicotine caused a significant malfunction ($P < 0.05$) in the mean values of serum sexual hormones, testosterone, ABP, FSH and LH concentration in the testis when compared with the control. It was concluded that nicotine caused decreasing of testosterone and increasing of ABP, FSH and LH concentrations in the serum. Also the toxic effect of nicotine on the germ cell layers in somniferous tubule with concomitant reduction in reproductive potentials of the male rat was exerted. Nicotine should therefore be taken with caution in cases of infertility. A total of adult 30 male rats weighing from (250-300 g); were divided into three groups: Group 1 (n=10), the control group; group 2 (n=10), nicotine - low dose "0.25mg/kg" body weight; group 3 (n=10), nicotine – high dose "0.5mg/kg" body weight. After sacrifice, blood samples were collected in tubes, and separated the serum for the determination of testosterone, androgen binding protein (ABP), FSH and LH. The results showed that serum testosterone levels were significantly decreased in both treated rats groups, low and high dose, compared with control animals ($P < 0.05$). On the other hand, serum androgen binding protein (ABP), follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels were significantly increased in both treated rats groups, low and high dose, compared with control animals ($P < 0.05$). The present investigation was designed to study the biochemical effects of nicotine on testis of adult male albino rats.

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1. Introduction

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 µg/kg of various edible plants. It functions as an ant herbivore chemical; therefore, nicotine was widely used as an insecticide in the past (Rodgman and Thomas, 2009) and nicotine analogs such as imidacloprid are currently widely used. In low concentrations (an average cigarette yields about 1mg of absorbed nicotine), the substance acts as a stimulant in mammals, while high concentrations (30–60 mg) can be fatal. This stimulant effect is the main factor responsible for the dependence-forming properties of tobacco smoking (genetic science learning center Utah). According to the American Heart Association, nicotine addiction has historically been one of the hardest addictions to break, while the pharmacological and behavioral characteristics that determine tobacco addiction are similar to those determining addiction to heroin and cocaine (Connolly *et al.*, 2007). Cigarette smoke is a complex mixture of toxic chemicals including nicotine, carbon monoxide, and several recognized carcinogens and mutagens (Stedman,

1968). These toxicants are absorbed through the pulmonary vasculature and transported via the bloodstream causing cytotoxicity, genotoxicity, and tumorigenicity throughout the body (Stillman *et al.*, 1986 and Clair *et al.*, 1994). Nicotine is metabolized primarily by the liver, and to a lesser extent, the lung and kidney, with the primary metabolite being cotinine (Kyerematen *et al.*, 1990).

As of 2002, about twenty percent of young teens (13-15 years) smoke worldwide. 80,000-100,000 children begin smoking every day. Half of those who begin smoking in young years are projected to go on to smoke for 15 to 20 years (World Health Organization "WHO", 2002). In the developing world tobacco consumption is rising by 3.4% annually (WHO, 2002). While the association between inhalation of mainstream smoke and cardiovascular disease, respiratory disease and cancer has been established for many years, the impact of smoking on reproduction is recognized, but less well characterized and less well known. Cigarette smoking has a negative impact on the ability to become pregnant and carry a pregnancy to term. Virtually all scientific studies support the conclusion that smoking has an adverse impact on fertility. The prevalence of infertility is higher, and the time it takes to conceive is longer, in smokers

compared to nonsmokers. 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 (Weisberg, 1985). Moreover, cigarette smoking inhibits spermatogenesis and causes decreased steroidogenesis in men (Aydos *et al.*, 2001 and Mlynarcikova *et al.*, 2005). Cigarette smoking has also been shown to have anti-estrogenic effects in women (Tankó and Christiansen, 2004). 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 (Jones *et al.*, 2003). Other adverse effects of smoking include premature ejaculation and reduced penile erection; however, these depend on individual sensitivity or susceptibility (Jones *et al.*, 2003). Smoking appears to accelerate the loss of eggs and reproductive function and may advance the time of menopause by several years. Components in cigarette smoke have been shown to interfere with the ability of cells in the ovary to make estrogen and to cause a woman's eggs (oocytes) to be more prone to genetic abnormalities.

Cigarette smoking has been shown to stimulate the release of several anterior and posterior pituitary hormones. It increases the plasma levels of prolactin, growth hormone (GH), adrenocorticotrophin (ACTH), and arginine vasopressin (AVP) without significant changes in thyroid stimulating hormone (TSH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) (Seyler *et al.*, 1986). (However, there are controversies with the effect of cigarette smoking and reproductive hormones in men (Vine, 1996; Kapoor and Jones, 2005). Tobacco consumption has recently been documented to act as an endocrine disruptor on the male hormone profile, specifically on LH, testosterone, and prolactin levels (Blanco *et al.*, 2012). However, these group recommended basic research studies to determine what physiological mechanisms are involved in the endocrine effects of smoking, as well as which of the more than 4000 toxicants contained in tobacco smoke are responsible for these effects. The present study was, therefore, designed to investigate if nicotine induced infertility in male rats by measuring different hormones correlated with fertility.

2. Material and Methods

Nicotine Preparation

Nicotine hydrogen tartrate with product number 36733-1G (99% nicotine); was purchased from Sigma Chemical Corporation (Sigma Aldrich, St. Louis, Mo, USA). The dose of nicotine was delivered to each rat

via the subcutaneous injection daily throughout the period of experimentation (8 weeks).

The Nicotine dosage freshly prepared in normal saline for each group of animals was injected via a subcutaneous route with 0.25 mg/kg (low dose) and 0.5 mg/kg (high dose) body weight/day. The working solutions were stored in foil wrapped glass bottle at 4°C for no longer than ten days. We prepare nicotine solution 1ml=1gm concentration of nicotine 99%.

Animals and Experimentations

This study will be performed on thirty five adult male albino rats with an average weight of (250-300) gm. They will be fed milk, vegetables & bread daily. The rats will be kept under 12 hrs natural light /dark cycles and will be given water *ad libitum*.

The animals will be divided into three groups:

Group I (Control): Composed of 10 rats and injected subcutaneously with 1ml of normal saline for 8 weeks.

Group II: Composed of 10 rats and injected subcutaneously with (0.25mg/kg/rat/day) nicotine for 8 weeks.

Group III: Composed of 10 rats and injected subcutaneously with (0.5mg/kg/rat/day) nicotine for 8 weeks.

At the end of the experimentation; the animals were sacrificed by ether anesthesia and the 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 2500 rpm for ten minutes. The serum was used to analyze the level of testosterone, FSH, LH and androgen binding protein (ABP).

Hormonal assay

Andersen *et al.* (1984) described that the blood samples were spun at 2,500 rpm for 10 minutes in a table top centrifuge and the serum samples obtained were analyzed to determine the concentration of testosterone LH, FSH, and androgen binding protein (ABP). The analysis was carried via the tube-based enzyme immunoassay (EIA) method. The protocol used for the hormone was according to the method described for the kit (Immunometrics Limited, UK) and meet the World Health Organization (WHO) standards in research programmes for human reproduction.

In order to minimize the effect of diurnal fluctuation, all samples were obtained in baseline conditions between 8:00 A.M. and 9:30 A.M. The analysis was carried *via* the spectra U.V-VIS double beam U.D-3500 lapomed.INC (U.S.A).

Statistical analysis

All values were expressed as means \pm SD. Statistical analysis of data was performed using a one-way analysis of variance (ANOVA) and Tukey's post-hoc test. Differences with a value of $P < 0.05$ were considered as statistically significant.

3. Results

Effect of nicotine on mean hormone levels

Testosterone:

The mean serum testosterone level of rats that received 0.25 mg/kg body weight (B.W.) (low dose)

and those that received 0.5 mg/kg B.W. (high dose) of nicotine for eight weeks was significantly decreased ($P < 0.05$) when compared with the control group. The observed decrease is dose dependent as shown in (Table 1 and Figure 1"A").

Table (1): Effect of nicotine on Testosterone, ABP, FSH and LH in adult rats.

Parameters Groups	Testosterone (ng/ml)	ABP (mIU/ml)	FSH (mIU/ml)	LH (mIU/ml)
Control	1.88 ± 0.47	26 ± 3.08	15.5 ± 1.75	18.25 ± 1.8
Low dose	1.46 ± 0.23*	35 ± 1.78*	19 ± 0.89 ^N	21.8 ± 0.96 ^N
High dose	1.04 ± 0.08*	39 ± 1.37*	20.6 ± 0.67*	22.6 ± 0.97 ^N

Data presented as mean ± standard deviation.

* At significantly different at $P < 0.05$ as compared with control values.

^N NOT significantly different at $P > 0.05$ as compared with control values.

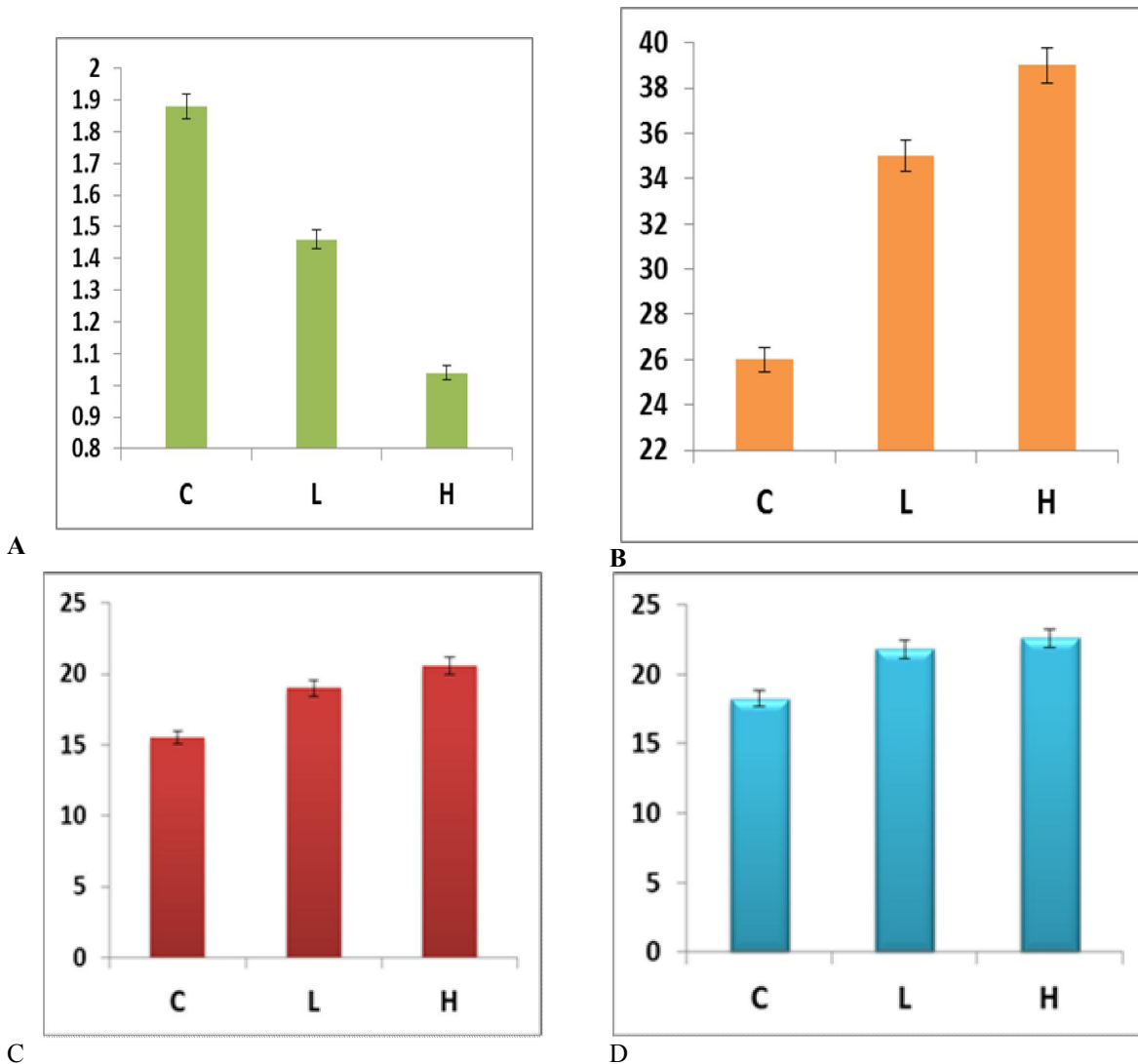


Figure (1): Effect of nicotine on Testosterone (A), ABP (B), FSH (C) and LH (D) in serum blood of adult rats.

Androgen binding protein (ABP):

The results showed that there was a significant increase ($P < 0.05$) in the mean serum androgen binding protein level in the 0.25mg/kg B.W. group when values compared with the control group. However, 0.5mg/kg B.W. group showed also a significant increase ($P < 0.05$) when compared with the control group. The observed increase is dose dependent as shown in (Table 1 and Figure 1"B").

Follicle stimulating hormone (FSH):

The serum FSH level of rats that received 0.5 mg/kg B.W. nicotine daily for eight weeks was significantly increased ($P < 0.05$) when compared with the control group. However, 0.25 mg/kg B.W. nicotine increased the serum FSH level in a non-significant manner ($P > 0.05$) when compared with their control counterpart as shown in (Table 1 and Figure 1"C").

Luteinizing hormone (LH):

The results showed that there was non-significant increase ($P > 0.05$) in the mean serum LH level of rats that received the two doses of nicotine 0.25mg/kg B.W. and 0.5mg/kg B.W. daily for 8 weeks when compared with their control. These results are presented in (Table 1 and Figure 1"D").

4. Discussion

Present results show that nicotine significantly decreases the serum level of testosterone in case of both doses and significantly increases the circulating levels of androgen binding protein (ABP) in both doses of nicotine also and FSH in the high dose of nicotine only (0.5mg/kg B.W.). Nicotine increases the serum level of luteinizing hormone in a non-significant manner as compared with the control.

Several studies have been performed on the harmful effects of smoking on the genital system of humans and rats (Stillman *et al.*, 1986).

Testosterone, being an important androgen plays a pivotal role in several aspects of sexual maturation, behavior, spermatogenesis, differentiation, and maintenance of accessory sex organs (Ojeda and Urbanski, 1994). The synthesis and release of androgens is dependent on the pituitary gonadotrophins, which are FSH and LH. Both FSH and LH are essential for testicular function and spermatogenesis. LH is the main tropic regulator of Leydig cell function without which androgen production is not possible (Huthaniemi and Toppari, 1995). They also reported that; both FSH and LH are essential for testicular function and spermatogenesis. LH is the main tropic regulator of Leydig cell function without which androgen production is not possible.

The results obtained from this study is in concomitance with (Ibukun *et al.*, 2013) who reported a significant decrease in serum testosterone level of rats treated with the two doses of nicotine.

Oyeyipo *et al.* (2010) reported that 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.

Cigarette smoking has been shown to stimulate the release of several anterior and posterior pituitary hormones. It increases the plasma levels of luteinizing hormone (LH), and follicle - stimulating hormone (FSH) without significant changes; except for the high dose of nicotine which induced a significant increase in the plasma level of (FSH). On the other hand, nicotine with the two doses prompted a significant increase in the plasma level of (ABP). This study also demonstrates that nicotine treatments cause a non-significant increase ($P > 0.05$) in the mean serum LH in male rats in the low and high treated groups. It is well-known that testosterone production by Leydig cell is primary under the control of LH and stimulation of LH is usually followed by stimulation of testosterone. Increased LH level in serum and decreased testosterone observed disagrees with this concept. These results are in concomitance with (Huthaniemi and Toppari, 1995). The increase in LH is also consistent with previous studies (Trummer *et al.*, 2002; Ramlau *et al.*, 2007).

The serum level of FSH of the rats treated with 0.5 mg/kg B.W. (high dose) of nicotine was significantly increased when compared with their control counterparts. While; the low dose (0.25 mg/kg B.W.) of nicotine induced insignificantly increased in the serum level of FSH of rats. These results are in agreement with (Heidary *et al.*, 2012) who found that an increased serum FSH level in cigarette smoking rats was also compatible to decreased serum testosterone level, since it is expected that FSH level to be enhanced following testosterone decline as a compensatory mechanism to elevate the testosterone level.

ABP is a protein found in the testicular cytosol or secreted by Sertoli cells in the rete testis fluid. It has a high affinity for androgens and binds specifically 5 alpha-DHT and testosterone. The results showed that there was a significant increase ($P < 0.05$) in the mean serum ABP levels in the two tested doses of nicotine when values were compared with the control.

Finally, we can say that our results are compatible with the hypothesis that nicotine through tobacco consumption may act as an endocrine disruptor on male hormone profile, specifically on LH, FSH, testosterone, and ABP levels. It is reasonable that the decreased testosterone level is

associated with testicular dysfunction rather than a pituitary disorder. However, the effects of nicotine on reproductive hormones were dose dependent.

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