

Alterations in the levels of female sex hormones following administration of nicotine to Wistar rats

Gabriel Olaiya Omotoso^{1*}, R Eniola Kadir¹, Ade Stephen Alabi¹, Luqman Aribidesi Olayaki², Omowumi Femi-Akinlosotu³

^{1*}*Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria.*

²*Department of Physiology, College of Health Sciences, University of Ilorin;*

³*Department of Anatomy, College of Medicine, University of Ibadan, Nigeria*

Corresponding Email: gabrielolaiya@yahoo.com, omotoso.go@unilorin.edu.ng
Tel: +2347030505707

Abstract

The indiscriminate use of nicotine is a serious public health challenge worldwide. In this work, the effect of nicotine was studied on female sex hormones using adult female Wistar rats. Fifteen (15) animals were grouped into three equally as, Group A (Control, given 0.1 ml of normal saline), Group B (given 0.03 mg/kg nicotine), and Group C (received 0.06 mg/kg nicotine). All administration was via the intraperitoneal route, once daily for five (5) consecutive days. All the animals were sacrificed by cervical dislocation about 24 hours after the last treatment, and intracardial blood samples obtained. Using appropriate biochemical kits, the serum levels of follicle-stimulating hormone (FSH), luteinising hormone (LH), prolactin, oestrogen and progesterone were determined. The results showed a dose-dependent significant increase in serum levels of the gonadotropins and progesterone, and a marked increase in serum levels of oestrogen and prolactin in animals treated with low dose nicotine, but significantly low levels of these hormones were recorded in animals treated with higher dose of nicotine. Administration of nicotine affects the female sex hormones adversely, and this by extension could affect female reproductive activities.

Keywords: female rats, nicotine, sex hormones, gonadotropin, progesteron

Introduction

A very common source of exposure to nicotine is through cigarette smoking, which is a major risk factor for the development of many clinical conditions, including cardiovascular diseases, metabolic disorders, and the induction of a variety of

neoplasias (1-3). With increasing percentage of women smokers, the deleterious effects of tobacco smoking on female reproductive functions have been reported by various authors (2, 4, 5). Women smokers have a risk of impaired fertility (4-6), apart from other effects smoking has on other body organs and structures.

Tobacco contains thousands of constituents, out of which nicotine is a major component, and is responsible for many of the toxic effects of tobacco products. The possible mechanisms of the effect of cigarette smoking on fertility include its effects on angiogenic processes (7) and apoptosis (2). A direct inhibition of aromatase by nicotine and its alkaloids could also result in decreased oestrogen levels as seen in women who smoke (8).

Administration of nicotine induces oxidative stress, significantly promotes lipid peroxidation in ovarian tissue and significantly reduces ovarian antioxidant enzymes activities (9, 10).

Liu *et al.*, (11) studied *in vitro* maturation of bovine oocytes and observed that nicotine affects bovine oocyte cumulus cell expansion, maturation rate, and chromosomal complement in a dose-dependent and an oocyte-stage-dependent manner. Similarly, according to Li *et al.*, (12) nicotine affects oocyte maturation, and subsequently results in poor blastocyst development.

Follicular growth is a crucial step in normal ovulation and fertilization (2); nicotine induces granulosa cell apoptosis, which probably underlies some of the fertility disorders associated with cigarette smoking (2). Furthermore, ovarian steroidogenesis is altered by administration of nicotine, resulting in increased serum progesterone and decreased oestrogen levels (5, 13). In addition, nicotine also causes inhibition of androgen production by the *theca interna* (13). Studies by Patil *et al.*, (14) recorded reduction in number of follicles, uterine weight and diameter, thickness of myometrium and endometrium, and height of epithelium in albino rats treated with nicotine.

Aside its direct effect on adult women, the effect following prenatal exposures has also been studied in the offspring. Prenatal and postnatal exposure to nicotine has been observed by Holloway *et al.*, (5) to result in delayed ovarian dysfunction in female offspring. Studies in male offspring showed that if nicotine is given during pregnancy or lactation, it induces transient structural changes in the testis and epididymis of male offspring (15).

The current study looked at the effects of intraperitoneal nicotine administration on female sex hormones.

Materials and Methods

Experimental Animals

Fifteen (15) adult female Wistar rats of mean weight 188.00 ± 5.77 g were divided into three groups. The animals were housed in different cages and fed on standard rat feeds and water. Group A animals were given 0.1 ml of normal saline, serving as Control; Group B had 0.03 mg/kg nicotine in 0.05 ml vehicle; while Group C received 0.06 mg/kg nicotine in 0.1 ml vehicle. All administration was intraperitoneal, once daily for 5 consecutive days.

Specimen Collection

The animals were sacrificed by cervical dislocation about 24 hours after the last intraperitoneal administration, and blood samples were collected intra-cardially into plain specimen bottles (16) for onward hormonal assay, using sex hormonal assay kit from Monobind Inc[®], USA. A spectrophotometric method was adopted in determining the levels of follicle-stimulating hormone, luteinising hormone, prolactin, oestrogen and progesterone in the serum of experimental rats used.

Statistical Analysis of Data

Using SPSS software version 15.0, the data obtained were expressed as means $\pm$ SEM, and subjected to Student's t-test. The level of significance was taken at p values < 0.05 .

Results

The level of luteinising hormone (LH) significantly increased in the treatment groups compared with the Control ($p < 0.05$), with Group C which received a higher dose of intraperitoneal nicotine (0.06 mg/kg) having the higher level than Group B (0.03 mg/kg), and the difference between these two groups were statistically significant ($p < 0.05$) (Table 1).

The serum level of follicle-stimulating hormone (FSH) also increased in the treatment groups, but not as marked as serum LH; the increase was also statistically significant ($p < 0.05$) in the high dose group compared with the Control. However, only a marginal difference existed between the Control and Group B that received a lower dose of nicotine, and this was not statistically significant ($p > 0.05$). FSH level was higher in the high dose Group C than in the low dose Group B, with statistically significant difference ($p < 0.05$) (Table 1).

Serum prolactin decreased significantly ($p < 0.05$) in the higher dose Group C, but increased significantly ($p < 0.05$) in the low dose treatment Group B, compared with

the Control Group A. The difference in prolactin levels between Treatment Groups B and C was statistically significant ($p < 0.05$) (Table 1).

There was also increase in the serum level of progesterone in the treatment groups, which was significant when compared with the progesterone level in the Control animals ($p < 0.05$). Although the higher dose group had a higher level of the Progesterone hormone when compared with the low dose group, the difference was not statistically significant ($p > 0.05$) (Table 1).

Serum level of oestrogen significantly reduced in Group C, which received a higher dose of nicotine compared with Control Group A, while Group B treated with a lower dose had a very high oestrogen level compared with the Control ($p < 0.05$). The difference between the treatment groups was also significant ($p < 0.05$) (Table 1).

Discussion

A very common source of nicotine is tobacco, and its consumption has increased in some population, in form of cigarette smoking. Of concern is the increase in the percentage of women who smoke. The adverse effects of nicotine and other components of tobacco have been demonstrated in various organs and systems of the body. The current study however examined the effects on female sex hormones in relation to the reproductive functions of women. This work further confirmed earlier studies that showed a decrease in the level of oestrogen in women smokers (5, 8, 13). The higher dose of nicotine used in this study led to a significantly lower serum level of oestrogen compared with the Control, and surprisingly, the animals that received a lower dose had a higher level of oestrogen compared to the Control ($p < 0.05$).

Cigarette smoking adversely affects ovarian function. It is anti-oestrogenic, causing a direct inhibitory effect on granulosa cell aromatase activity, and also, less specific cytotoxic effects (8, 17). In the current study, the serum levels of progesterone were significantly raised in animals treated with nicotine in a dose-dependent fashion, contrary to a previous report where administration of nicotine caused a dose-dependent inhibition of progesterone synthesis (18). Even though the reason for the differences might not be very clear, studies by Holloway *et al* (5) on postnatal ovarian function following foetal exposure to nicotine, reported increased serum progesterone levels in the female offspring.

The gonadotropins and prolactin are peptide hormones, whose serum levels were altered in animals treated with nicotine. A dose-dependent increase in serum levels of LH was noted in this study, with a very marked increase in the high dose group, signifying the function of the hormone in stimulating the synthesis of oestrogen to increase the level of the latter. Usually, the control of hormone secretion is via a

negative feedback mechanism that ensures a proper level of hormone activity at the target tissue. However, in a few instances, positive feedback occurs when the biological action of the hormone causes additional secretion of the hormone. The stimulatory effect of oestrogen on the anterior pituitary before ovulation results in a surge of LH, and the latter further stimulates the ovaries to secrete more oestrogen, which in turn causes more secretion of LH. Eventually, LH reaches an appropriate concentration, and typical negative feedback control of hormone secretion is then exerted (19).

Given the various forms of hormonal alterations observed in female animals given intraperitoneal nicotine administration, there is the possibility of a resultant adverse implication on female reproductive function.

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Result

Table 1: Results of Serum Hormonal Assay (n=5 per group)

Groups		LH (mIU/ml)	FSH (mIU/ml)	Prolactin (muU/ml)	Progesterone (ng/ml)	Oestrogen (ng/ml)
A: Control		0.26±0.02	0.29±0.01	0.42±0.01	20.41±0.7	333.72±30.7
B: 0.03 mg/kg Nicotine	0.03	0.36±0.02*‡	0.29±0.01‡	0.63±0.02*‡	31.98±0.8*	720.56±20.9*‡
C: 0.06 mg/kg Nicotine	0.06	0.89±0.03*‡	0.37±0.01*‡	0.23±0.02*‡	33.09±0.5*	237.80±3.7*‡

*Statistically significant difference compared with the Control Group;

‡ Statistically significant difference between treatment groups (p< 0.05).

LH- luteinising hormone, FSH- follicle-stimulating hormone