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Original Article

Evaluation of some reproductive hormonal profile following the administration of varied doses of nicotine

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Abstract

Background: This study is aimed at determining the effect of nicotine on male fertility by evaluating some reproductive hormone parameters of male Wistar rat such as serum testosterone, luteinizing hormone (LH), prolactin and follicle stimulating hormone (FSH).

Methodology: A total of 20 adult male rats were randomly divided into four groups, the test groups were administered with 0.2 mg/100 g, 0.4/100 g and 0.6/100 g body weight of nicotine base daily for 30 days using a polythene catheter orally while the control were administered with 2 ml 0.9% physiological saline.

Results: Nicotine caused a significant reduction ($P < 0.05$) and ($P < 0.01$) in the mean values of the hormones of the test group compared with control. Serum testosterone concentration of 4.8 ± 1.30 ($P < 0.05$), 4.0 ± 2.25 ($P < 0.01$), 0.6 ± 0.64** were recorded for groups 1, 2 and 3 respectively. For FSH the significant values were 0.26 ± 0.11 ($P < 0.05$), 0.20 ± 0.12 ($P < 0.01$) in groups 2 and 3, whilst prolactin showed a significant reduction of 0.08 ± 0.08 ($P < 0.05$) only in group 3. LH showed a significant reduction in all the test groups with values of 0.48 ± 0.08 ($P < 0.01$), 0.24 ± 0.11 ($P < 0.01$) 0.08 ± 0.08 ($P < 0.01$) for groups 1, 2 and 3 respectively. 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 adverse effects on gonadotrophins secreted by the anterior pituitary 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.

Key words: Fertility, gonadotrophins, prolactin, testosterone

Introduction

The effect of nicotine on fertility is being widely studied, though more studies have been done on the effect on female fertility with results indicating that it adversely affects it. Studies are now being centered on its effect on male fertility particularly its effect on spermatogenesis, which is a remarkably complex but precise process yielding highly differentiated haploid germ cells from diploid stem cells as well as its effect on testicular histology.

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Nicotine which is an alkaloid is found in the nightshade family of plants (Solanaceae); its 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[1] and is present in the range of 2-7 μg/kg of various edible plants.[2] Though there are several routes of exposure to nicotine (oral, dermal and inhalational), the most frequently used route is inhalational while smoking tobacco.

In low concentrations (an average cigarette yields about 1 mg of absorbed nicotine), the substance acts as a stimulant in mammals while high concentrations (30-60 mg) can be fatal.[3]

The stimulant effect is the main factor responsible for the dependence-forming properties of tobacco smoking.[4] According to the American Heart Association, nicotine

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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.[5] As of 2002, about 20% of young teens (13-15) smoke world-wide, 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 15-20 years.[6] In the developing world tobacco consumption is rising by 3.4% annually.[6]

A study done by Egesie *et al.* in 2013 showed that the administration of varied doses of nicotine caused a dose dependent reduction in mean values of andrological parameters.[7] This is as a result of a decrease in germ cell development shown by scanty germ cells seen in the histological slides of the semineferous tubules with increased dose of nicotine base, whilst showing no significant change in sperm motility. Another study showed high content of testicular cholesterol, reduction in the weight of accessory reproductive organs along with their biochemical changes and absence of epididymal sperms after chronic nicotine treatment, indicating a delay caused by nicotine in the attainment of puberty.[8] The synthesis and release of androgens are dependent on the pituitary gonadotropins like follicle stimulating hormone (FSH) and luteinizing hormone (LH)/interstitial-cell stimulating hormone. Both FSH and LH are essential for testicular function and spermatogenesis.[9-11] Whereas luteinizing hormone is the main tropic hormone of the interstitial cells of leydig, without which testosterone secretion will be impaired, it is believed that FSH regulate spermatogenesis via its action on the sertoli cells.

Though several of these studies exist, not much has been done to evaluate the effect of nicotine on paracrine and endocrine mediators or their interactions on spermatogenesis. The increasing prevalence of smoking amongst teenagers in the developing world as well as rising concerns over the effect of nicotine on fertility necessitates studies such as these. This study was designed to evaluate the effect of varied doses of nicotine on the concentration of some reproductive hormones: FSH, LH, testosterone and prolactin.

Methodology

Animals

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

Chemicals

Testosterone, Oestrogen, FSH, LH, progesterone, enzyme-linked immunosorbent assay (ELISA) enzyme immuno assay (EIA) kit ways purchased from Monobind Inc. Lake forest USA. Nicotine hydrogen tartrate salt ($C_{10}H_{14}N_2 2C_4H_6O_6$) purchased from sigma Aldrich catalog number N5260-25G.

Experimental Protocol

Four groups with five 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/100 g body weight/p.o of nicotine daily for 30 days, groups 2 and 3 were given 0.4 mg/100 g and 0.6 mg/100 g body weight of nicotine per os daily for 30 days respectively. While group 4 (control group) was given 2 ml of 0.9% physiological saline solution for the same period of exposure.

Sample Collection

The rats were anesthetized using ether and afterward sacrificed by cervical dislocation and blood sample collected by cardiac puncture then centrifuged to obtain the serum.

Hormonal Assays

This was carried out with the use of the respective EIA Kit, for testosterone, prolactin, FSH, LH with product codes: Testosterone 3725-300, prolactin 725-090, FSH 425-090, LH 625-090 and ELISA microwells and microplate immunoassay using Statfax-2100 microplate reader.

Description of acetylcholinesterase (AChE) competitive EIAs

The serum gotten from centrifuged blood is used for the assay of testosterone, the assay is based on the competition between testosterone and a testosterone AChE conjugate (testosterone tracer) for a limited amount of testosterone antiserum. The concentration of the testosterone tracer is held constant while the concentration of testosterone varies; the amount of testosterone that is able to bind to the testosterone antiserum will be inversely proportional to the concentration of testosterone in the well. This antiserum-testosterone complex binds to mono-clonal anti-rabbit immunoglobulin G that has been previously attached to the well. The plate is washed to remove any unbound reagent and then Ellman's reagent (which contains the substrate to AChE) is added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 412 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of the testosterone tracer bound to the well, which is inversely proportional to the amount of free testosterone present in the well during the incubation; or absorbance α (bound testosterone tracer) α 1/(testosterone). The statfax-2100 utilizes the standards made available with the kits to generate a curve from which the value of the hormone concentration is extrapolated.

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Same procedure was carried out for FSH, LH and prolactin based on the same EIA principle using a prolactin anticholinesterase tracer for prolactin, an FSH tracer for FSH and a LH anticholinesterase tracer for LH.

Statistical Analysis

Statistical analysis was performed using the Graphpad Instat 3.1 tool (California USA) to conduct one-way analysis of variance and $P < 0.05$ were considered as significant.

Results

Results were presented as the mean ± standard deviation. Nicotine caused a significant reduction ($P < 0.05$) and ($P < 0.01$) in the mean values of serum testosterone concentration, FSH, prolactin and LH in the test when compared with the control. These changes occurred at dose levels of 0.2 mg/100 g for testosterone and from 0.4 mg/100 g for LH and FSH whilst only the highest dose of 0.6 mg/100 g had a significant effect for serum prolactin as shown in Table 1. The result showed a dose dependent decrease in the measured hormones.

Table 1: Serum concentration of prolactin, FSH, LH and testosterone

Parameters	Control (Group 4)	Group 1	Group 2	Group 3
Serum prolactin (ng/ml)	0.28±0.19	0.12±0.08	0.10±0.07	0.08±0.08*
Serum FSH (mU/ml)	0.50±0.15	0.40±0.12	0.26±0.11*	0.20±0.12**
Serum LH (mU/ml)	0.78±0.16	0.48±0.08**	0.24±0.11**	0.08±0.08**
Serum testosterone (nmol/l)	7.4±1.54	4.8±1.30*	4.0±2.25**	0.6±0.64**

*P<0.05, **P<0.01. FSH - Follicle stimulating hormone, LH - Luteinizing hormone

Discussion

The data generated from this study clearly shows a significant decrease in testosterone and LH at all dose levels whilst showing a significant decrease at 0.4 mg/100 g and 0.6 mg/100 mg for serum FSH, whereas for prolactin a significant decrease at $P < 0.5$ was obtained only for the highest dose. The decrease in gonadotrophins is in tandem with studies done by Blake,[12] and may adequately explain findings of Viczián[13] and Egesie *et al*.[7] that cigarette smoking or nicotine treatment results in testicular degeneration, deficiency of male sex hormone and reduction in sperm count, thus the low sperm count would probably be a result of decrease or absent androgens and FSH to adequately steer the process of spermatogenesis. The dose dependent decrease in these gonadotrophins after administration of nicotine suggest that nicotine being a central nervous system stimulant also interferes with endocrine secretion such as the release of gonadotrophins (FSH, LH) and prolactin from the anterior pituitary. The decrease in gonadotrophins may also explain the decrease in weight of the testes as observed by Egiese *et al*.[7] following administration of nicotine. Prolactin considered to be a hormone primarily meant for lactation in mammals, also inhibits FSH and gonadotrophin releasing hormone, the hormones that triggers ovulation and allows eggs to develop and mature, thus its common role is usually in females and high levels may be associated with infertility. Though the role of prolactin in males is not well-known, it has come into scrutiny of late with studies showing an increase in prolactin levels in expectant fathers as well as new fathers while compared to non-expectant fathers. This increase was associated with increased emotional attachment as well as paternal care and fathers with more prolactin showed more emotional attachment and paternal care to their children.[14-16] It is in view of these findings that prolactin is being known in some quarters as the hormone of paternity. In the present study, prolactin is the least affected by nicotine, though the slight decrease might suggest that expectant and new fathers be advised to either stop smoking or reduce their nicotine intake in the interest of the child.

The limitations of this study includes the difficulty and cost of procuring the EIA kits, the short shelf life of the reagent s in the kits thus prompting us to hasten the study as well as our inability to conduct a time scale experiment to determine the exact time that the observed changes begin to occur for all the hormones. Further studies should be carried out to include the time scale experiment, histological examination of the pituitary gland and the hypothalamus using an animal model to determine if there is an associated distortion of the said glands while keeping in view the possibility of modifying nicotine and using it as an anti-fertility drug in males.

Conclusion

The outcome of this study showed that the administration of varied doses of *nicotine* caused a reduction in mean values of reproductive hormonal parameters (FSH, LH, prolactin and testosterone). This may be as a result of the inhibitory effect of nicotine on the anterior pituitary. This indicates that prolonged intake/administration of nicotine may induce infertility. Nicotine (cigarettes) should therefore be taken with caution in both man and animal particularly in cases of infertility.

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