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NICOTINE INDUCED OVARIAN AND UTERINE CHANGES IN ALBINO MICE

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Abstract : Nicotine at the dose level of 0.3 mg/100 g body weight was administered to normal cycling mice for 15 days through oral and intraperitoneal routes. At autopsy on 16th day significant reduction in the ovarian and uterine weight was observed. Histological observations showed decrease in the number and size of Graafian follicles, corpora lutea and increase in the atretic follicles in the ovary. The uterus showed absence of endometrial glands, decrease in the height of myometrium, endometrium and its epithelial cells. The total cholesterol content of the ovary and uterus is increased whereas the protein content is decreased. This antagonistic action of nicotine to gonadotrophins is discussed.

Key words : nicotine atretic follicle ovarian steroids

INTRODUCTION

Nicotine is one of the few liquid alkaloids which is widely consumed by cigarette smoking and tobacco chewing. Carcinogenic potential of nicotine and its effect on central nervous system is well documented. Investigations made to know the effect of nicotine on endocrine system indicated that it causes the discharge of epinephrine from adrenal medulla, reduces the production of corticosteroids and increases the level of prolactin, ACTH, vasopressin and growth hormone (1–4). It is also reported that nicotine in high doses increases the release of catecholamines and inhibits the aldosterone synthesis in the rat adrenal cortex (5–6). Reports of nicotine on reproduction are scanty. However, Mattison has revealed the adverse effects of cigarette smoking on gametogenesis upto implantation in human (7). Weisburg reviewed that, smoking causes menstrual irregularities, pregnancy complications and decreases fertility in women (8). Therefore, the present study is undertaken to understand the direct involvement of nicotine on reproduction.

METHODS

Normal cycling, healthy albino female mice of 60 days were used for the experiment. The animals were maintained in the standard laboratory conditions and fed with balanced diet as prescribed by

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Central Food and Technological Research Institute (CFTRI), Mysore, India and water *ad libitum* at room temperature of 28 ± 2°C.

The animals were divided into four groups, each consisting of 6 animals. Based on the earlier studies in our laboratory the effective dose 0.3 mg/100g body weight was selected. The treatment was started from estrous phase of the cycle only as the ovarian and uterine activities change markedly from one phase to another phase. One group was treated with 0.3 mg nicotine/ 100g body weight, in saline orally and another group was treated with the same dose intraperitoneally (i.p.). Suitable saline treated controls were maintained. The treatment was given once a day between 10.00 AM and 11.00 AM for 15 days. All the experimental mice were sacrificed by decapitation on 16th day, 24 hours after the final dose.

The body weight was recorded. Ovary and uterus were dissected out, freed from adherent tissue and weighed on Anamed electronic balance. Organs from left side of each animal were processed for histological studies. The number of Graafian follicle, atretic follicle and corpora lutea was made from randomly choosen 20 sections from each group. Micrometric measurements such as diameter of follicles, copora lutea, and diameter of uterus, thickness of myometrium, endometrium and epithelial cell height were also made from randomly selected 20 sections which appeared round in cross section from each group. Micrometric measurements were made by using stage and ocular micrometer.

Cholesterol content from the right side ovary and uterus was estimated by Libermann and Burchard's reaction as described by Peter and Vanslyke (9). Protein content of ovary and uterus was estimated by Lowry's method (10). Stastical analysis was carried out by using student "t" test.

RESULTS

Body weight

There is no significant change in the body weight of the mice due to treatment of nicotine for 15 days, either orally or intraperitoneally compared to their respective control groups.

TABLE I : Effect of Nicotine on gravimetric and biochemical changes of ovary and uterus in albino mice.

	Weight (mg/100 g body wt.)		*Cholesterol (µg/mg)*		*Protein (µg/mg)*	
	Ovary	*Uterus*	*Ovary*	*Uterus*	*Ovary*	*Uterus*
Saline (oral)	103.31±1.74	436.16±4.40	22.57±0.21	8.60±0.06	66.15±0.06	13.8±0.04
Nicotine (oral)	66.11±1.10*	395.26±4.31*	28.83±0.19**	9.17±0.04**	65.54±0.19	12.00±0.01
Saline (IP)	99.20±1.56	424.18±4.10	23.42±0.02	9.01±0.02	67.98±1.42	14.2±0.09
Nicotine (IP)	33.41±1.57**	170.69±2.67**	37.33±0.32**	16.6±0.01**	55.03±0.06**	7.00±0.01**

M±S = Arithemetic Mean ± Standard Error.
*P<0.01; **P<0.001 compared to respective control.

TABLE II : Effect of Nicotine on gravimetric and biochemical changes of ovary and uterus in albino mice.

	Graafin Follicle		*Atretic follicle*		*Corpora lutea*	
	No.	*Diameter (μm)*	*No.*	*Diameter (μm)*	*No.*	*Diameter (μm)*
Saline (oral)	1.0±0.0	23.99±1.62	–	–	13.0±1.82	28.04±1.8
Nicotine (oral)	0.08±0.01	18.76±0.15*	2.20±0.12	22.18±0.63	9.5±0.1*	12.68±0.52*
Saline (IP)	1.1±0.38	23.64±1.25	–	–	12.92±1.84	28.4±2.1
Nicotine (IP)	1.0±6.21	16.5±0.20*	3.16±0.14	22.41±1.24	5.58±1.32**	19.2±0.30**

M±S = Arithemetic Mean ± Standard Error.
*P<0.01; **P<0.001 compared to respective control.

Gravimetric changes (Table I)

The ovarian and uterine weights of the oral nicotine treated mice showed significant decrease (P<0.01) compared to that of controls and highly significant (P<0.001) reduction of the same was seen in the group that received nicotine intraperitoneally.

Biochemical changes (Table I)

Highly significant (P<0.001) increase in the cholesterol content of ovary and uterus was seen in both oral and i.p. nicotine treated groups. Whereas, the protein content of ovary and uterus was decreased significantly (P<0.001) only in i.p. nicotine administered group compared to the control group of mice.

Histological and histometric changes in the ovary (Table II)

Histological observations of the ovary of nicotine treated groups showed decrease in the number of Graffian follicles and corpora lutea (P<0.01 and P<0.001) compared to the saline treated control groups. There was induction of atresia in the developing and antral follicles as granulosa cells were seen to infiltrate the antrum. The size of Graffian follicles and corpora lutea was decreased as evident from significant (P<0.01 and P<0.001) reduction in their diameters in the nicotine treated groups compared to the respective control groups.

TABLE III : Effect of Nicotine on histometric changes of uterus in albino mice.

	Diameter of uterus (μm)	*Thickness of myometrium (μm)*	*Thickness of endometrium (μm)*	*Height of epithelium (μm)*
Saline (Oral)	148.55±0.90	6.60±0.35	34.44±2.04	1.02±0.08
Nicotine (Oral)	58.71±0.47**	2.74±0.27**	11.74±1.33**	0.64±0.11**
Saline (IP)	139.49±0.98	6.40±0.29	33.42±2.08	1.06±0.02
Nicotine (IP)	37.36±0.27**	3.10±0.07**	7.59±0.41**	0.24±0.01**

M ± S = Arithemetic Mean ± Standard Error
*P<0.01, **P<0.001 compared to respective control.

Histological and histometric changes in uterus (Table III)

There was significant reduction in the diameter of uterus, thickness of endometrium and myometrium and epithelial cell height ($P<0.01$) in nicotine treated groups compared to their respective control groups. A reduction in the secretion of endometrial gland was also observed.

DISCUSSION

It is well known that hypothalamus regulates the rhythmic release of pituitary gonadotrophins, i.e., FSH, LH and prolactin through neural stimulus to GnRH (11). The orderly event of follicular growth and ovulation depends upon the pituitary FSH, LH and prolactin. Investigations on nicotine indicate that nicotine being a central nervous system influencing drug inhibits the release of gonadotrophins from pituitary (12–14). The studies also indicate that nicotine blocks ovulation by inhibiting the LH surge from pituitary in rats (15). In the present study, as the drug was administered between 10.00 and 11.00 AM every day, it covers the "critical period" of LH surge, thus postponing the ovulation for one day by interfering with 24 hours periodicity for gonadotrophin release (16–17).

FSH stimulates the differentiation of granulosa cells and promotes the follicular development (18–20). In the present investigation, the reduction in the number of Graafian follicle in the ovary of nicotine treated mice indicates the inhibition of follicular growth which is gonadotrophin dependent.Decrease in the number of corpora lutea in the nicotine treated mice indicates the reduction in the rate of ovulation leading to follicular atresia.

Uterine growth depends upon the ovarian estrogen secretion. Estrogen primarily acts upon the surface epithelium and the glands within endometrium (21). Progesterone acts on estrogen primed uterus and prepares the uterine epithelium from proliferative to secretory state (21). In the present investigation, reduction in the uterine diameter, reduced thickness of its myometrium and endometrium and reduced secretions from endometrial glands indicate the inhibition of ovarian steroid biosynthesis necessary for growth of the uterus and reproductive cyclicity.

High accumulation of cholesterol content in ovary and uterus of experimental mice may be attributed to the lowered steriodogenesis, which is dependent on availability of pituitary gonadotrophins (22). This observation is supported by the studies of Kasson and Hsuch (23) and Meyer and Carr (24), which reveal that nicotine alters the optimum steriod synthesis. The low protein content of the ovary indicates the retarded ovarian growth as FSH is essential for protein synthesis in gonads (25). The blockade of pituitary FSH release in nicotine treated mice might have resulted in the low protein content. The low level of protein observed in the uterus may be due to reduced availability of ovarian estrogen.

Intraperitoneal route of administration of nicotine is found more effective than that of the oral, which may be due to the fact that intraperitoneal route facilitate the rapid absorption of the drug. The oral administration of nicotine is less effective

which may due to its *first-pass effect* in the liver, where the drug is subjected to biotransformation through hepatic microsomal enzymes-drug metabolizing systems and become less potent (26-27).

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