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TESTICULAR CHANGES DUE TO GRADED DOSES OF NICOTINE IN ALBINO MICE

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Abstract : Administration of graded doses of nicotine (0.2 mg, 0.4 mg and 0.6 mg/100 g body weight) for 15 days to the adult mice reduced the weight of testis, number of spermatocytes and spermatids, but increased the number of spermatogonia which may be due to reduced conversion to subsequent stages. There is a high cholesterol content and Sudanophilic lipid accumulation in the treated testis. The weight of accessory sex organs which is dependent on androgens produced by the testis is also reduced. These changes are brought because of the non-availability of pituitary gonadotrophins essential for initiation and completion of spermatogenesis and steroidogenesis in the testis due to the administration of nicotine, which being CNS depressor might have caused inhibition in the neural stimulus essential for release of pituitary gonadotrophins

Key words : nicotine testis spermatogensis steroidogensis gonadotrophins

INTRODUCTION

Nicotine, an isolated product of tobacco plant acts on CNS, first as stimulant and later as depressant bringing many neurophysiological changes in an individual. Smaller doses of nicotine produce tremors and larger doses cause convulsions in man and laboratory animals (1). The hypothalamus which is a part of CNS regulates the release of pituitary gonadotrophins i.e FSH and LH through the neural stimulus to gonadotrophin releasing hormone - GnRH (2). According to several investigators nicotine inhibits the release of FSH and LH from the pituitary acting through hypothalamus blocking the neural stimulus to the gonadotrophin releasing hormone (3–6). As the gonadotrophins are essential for the testicular function and both FSH and LH are required for the completion of spermatogenesis (7), it is of interest to study the effects of nicotine on spermatogenesis and also on the biochemical parameters of testis in mice.

METHODS

Male albino mice (Swiss strain) of 80 to 90 days old, weighing 30 to 35 gms were used for experimentation. The animals were maintained under laboratory conditions

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with lighting schedule of 12 hrs light and 12 hrs darkness. They were fed with balanced diet as prescribed by CFTRI, Mysore and water *ad libitum*. The mice divided into 4 groups of eight animals and treated as follows.

Group 1– Received 0.2 ml of Physiological saline/100 g body weight.

Group 2– Received 0.4 mg of Nicotine/100 g body weight in 0.2 ml of saline.

Group 3– Received 0.4 mg of Nicotine/100 g body weight in 0.2 ml of Saline.

Group 4– Received 0.4 mg of Nicotine/100 g body weight in 0.2 ml of Saline.

All the injections were given intraperitoneally every day between 10.30 to 11.30 AM for 15 days. The experimental animals were autopsied by cervical dislocation on the next day of the last injection. The testis, epididymis, seminal vesicle and vas deferens were dissected out and weighed upto the nearest mg on electronic balance. The organ weights were calculated per 100 gm body weight using the following formula: Organ weight (mg)/body weight (gm) × 100 = mg/100 gm. The left testis was processed for the estimation of cholesterol (8) and protein (9). The portion of right testis was fixed in Bouin's fluid and calciumformal for histological and histochemical studies respectively, were processed and embedded in paraffin wax, sectioned at 5 μ thickness. The sections were stained with haematoxyline- eosin for histological observations and with Sudan black B for histochemical localization of lipids.

The micrometric measurements such as diameter of testis & seminiferous tubules were measured by the help of ocular and stage micrometer from randomly selected twenty round sections from each group. Statistical analysis was done by using students `t` test and the values were judged significant if $P < 0.05$.

RESULTS

Gravimetric and micrometric changes of the testis (Table I) : Administration of nicotine to the adult albino mice at the dose level of 0.2 mg, 0.4 mg and 0.6 mg/100 g body weight has caused significant ($P < 0.001$) decrease in the weight of the testis with respective inhibition of 24.41%, 26.92% and 28.55% in the growth. Though there is a reduction in the diameter of testis with administration of all the three doses, a

TABLE I : Gravimetric, micrometric and biochemical changes of the testis due to nicotine administration.

Duration 15 days

Treatment mg/100 g body wt.	*Wt. of testis mg/100 g body wt.*	*Diameter of testis (mm)*	*Diameter of tubules (μm)*	*Cholestrol (μg/100 mg testis)*	*Protein (μg/100 mg testis)*
Saline	752.66±31.58	3.51±0.12	168.8±7.1	150.70±5.20	220.90±4.60
0.2 mg Nicotine	568.54±32.69**	2.90±0.24	158.0±7.0	210.49±1.80**	200.00±4.30*
0.4 mg Nicotine	550.00±14.14**	2.56±0.18	127.7±6.0*	270.58±1.60**	150.76±13.10**
0.6 mg Nicotine	537.77±13.42**	2.30±0.14*	118.4±5.2	290.29±0.90**	102.80±16.40**

*P < 0.01 and **P<0.001 when compared to saline treated controls.
M + S.E. : Arithmatic mean + standard error of eight animals.

TABLE II : Effect of nicotine on the total count of spermatogenic elements per seminiferous tuble in albino mice.

Duration 15 days

Treatment mg/100 g body wt.	*Spermatogonia*	*Spermatocytes*		*Spermatids*	
		Preleptotene	*Zygotene*	*Elongated*	*Round*
Saline	13.50±2.36	38.43±3.21	41.20±2.34	80.41±2.98	43.24±3.80
0.2 mg Nicotine	13.75±1.20	38.29±2.68	28.48±1.68*	48.23±1.28**	29.62±2.18**
0.4 mg Nicotine	15.67±1.42	36.49±2.12	25.92±2.68**	46.72±2.73**	26.21±3.12
0.6 mg Nicotine	19.23±2.10	38.21±4.01	30.91±3.26*	40.31±3.46*	42.62±2.08**

*P<0.01 and** P<0.001 when compared to saline treated controls.
M + S.E : Arithmatic mean+standard error of eight animals.

significant ($P < 0.001$) reduction is seen only with 0.6 mg. The diameter of seminiferous tubule is significantly ($P < 0.001$) decreased with 0.4 mg and 0.6 mg nicotine administration.

Histological changes (Table II) : Though there is a gradual increase in the number of spermatogonia, it is isgnificant ($P < 0.01$) only with 0.6 mg nicotine treatment. But the number of spermatocytes and spermatids is decreased gradually with the increase in the dose of nicotine. This indicates the slow conversion of spermatogonia to spermatocytes and spermatids, which is dependent on pituitary FSH.

Therefore though spermatogenesis is not completely arrested there is a significant ($P < 0.01$) inhibition in the spermatogenic process. The high accumulation of Sudanophilic lipids in the Leydig cell and seminiferous tubules of treated mice also indicates the lack of pituitary LH (Figs. 1 and 2).

Biochemical changes of Testis (Table I): Cholesterol level is increased in all the nicotine treated groups in comparison with

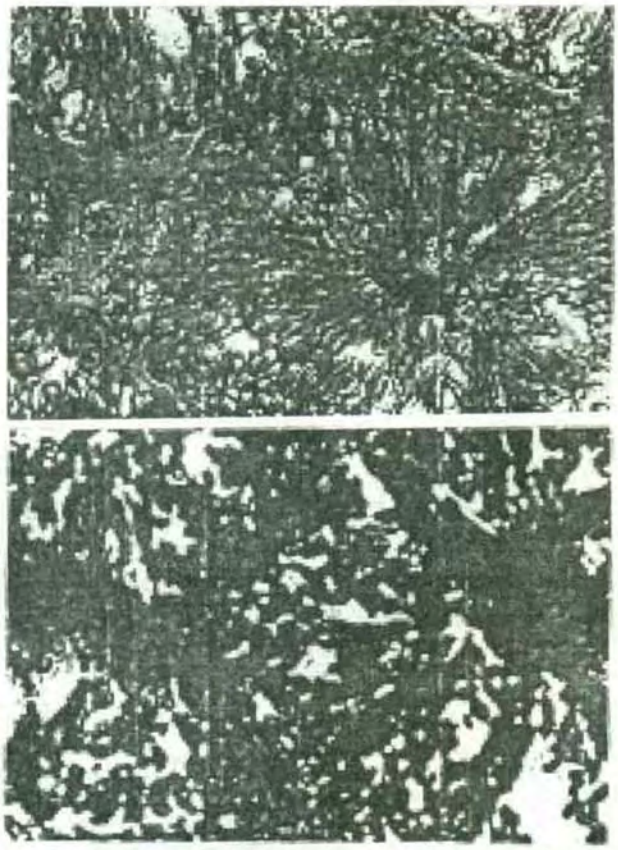

Fig. 1: Section of the testis showing moderate accumulation of Sudan Black B+ve lipid in the interstitum and seminiferous tubules.

Fig: 2: treated with 0.6 mg nicotine exhibiting increase in the accumulation of Sudan Black B +ve lipid in the interstitium and seminiferous tubule. Note the accumulation within the lumen of seminiferous tubules.

saline treated controls, which is highly significant. (P <0.001). The protein content of the testis is decreased significantly (P < 0.01) in all the drug treated animals.

Gravimetric changes in the accessory reproductive organs (Table III): Low dose of nicotine (0.2 mg) has reduced the weight of seminal vesicle and prostate gland significantly (P <0.01). While medium dose of nicotine (0.4 mg) has significantly (P<0.01) decreased not only the weights of seminal vesicle and prostate gland but also that of epididymis. The high dose of nicotine (0.6 mg) has significantly (P < 0.001) reduced the weight of all the accesory organs indicating higher dose is more effective. This significant decrease in the weights of accessory sex organs is due to acute scarcity of androgens.

TABLE III : Effect of Nicotine on accessary sex organs in male mice.

Duration 15 days

Treatment mg/100 g body wt.	*Organ weights mg/100 g body weight*			
	Epididymis	*Seminal vesicle*	*Prostate gland*	*Vas deferens*
Saline	306.11 ± 15.2	384.0 ± 21.4	124.28±3.48	75.94 ± 4.80
0.2 mg Nicotine	298.4 ± 20.8	214.01 ± 18.2***	100.21±9.81*	68.44 ± 9.20
0.4 mg Nicotine	280.4 ± 16.8*	138.6 ± 13.8***	82.6±3.78***	69.41 ± 4.2
0.6 mg Nicotine	230.6 ± 18.4**	130.4 ± 8.6***	68.21±4.2***	60.01 ± 4.32**

*P<0.05, **P<0.01 and ***P<0.001 when compared to saline treated controls.
M + S.E. : Arithmatic mean+standard error of eight animals.

There was no significant change in the body weight of nicotine treated mice is observed.

DISCUSSION

Nicotine being CNS influencing drug alters the secretion and release of pituitary gonadotrophins via hypothalamo-hypophyseal axis (3). Further studies indicate that administration of this drug decreases the serum levels of LH, FSH and prolactin in castrated rat (6) and also in human beings (10). Therefore, the observed reduction in the weight of testis after prolonged nicotine treatment may be due to the continuous availability of low levels of pituitary gonadotrophins (6).

In the present study it is observed that there is a significant reduction in the total number of spermatocytes and spermatids but not the spermatogonia in nicotine treated mice. This effect of nicotine can be attributed to the inhibition of pituitary FSH release, as FSH is essential for initiation and maintenance of spermatogenesis (11).

The reduction in the weight of the accessory reproductive organs and increase in the total cholesterol content of testis supports the inhibition of androgen production due to nicotine treatment. The significant decrease in the weights of accessory sex organs is due to acute scarcity of androgens. This is because of inadequate availability of LH in nicotine treated mice (12), as LH is essential for steroidogenesis in the Leydig cells (13).

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