



EVALUATION OF ORAL ADMINISTRATION OF NICOTINE ON LIBIDO AND SPERM QUALITIES IN MALE WISTAR RATS.

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ABSTRACT

The burden of implication of Nicotine-laden cigarette on human reproductive cells and sexual performance is legendary and whether or not nicotine is directly involved remains to be unraveled hence, this study was designed to investigate effects of oral administration of nicotine on libido and sperm qualities of male wistar rats. Twenty (20) male were used for the study with each rat weighing between 80-160g. The rats were obtained from the Experimental Animal Unit of the Department of Human Physiology, University of Port Harcourt, Rivers State and were housed in conventional wire mesh cages under normal day/light cycle. The animals were allowed free access to water and feed throughout the period of the experiment. The male rats were divided into four (4) groups, with each group consisting of five (5) rats each as follows; Group 1(the control group); receive 0.2 ml/kg normal saline for four weeks. Group 2: received 0.5mg/kg of Nicotine for four weeks. Group 3(Test group 2); received 1mg/kg of Nicotine for four weeks. Group 4(Test group 3); received 1.5mg/kg of Nicotine for four weeks. Libido test was carried out and semen collection was done using standard procedures, sperm characteristics (i.e count, motility, viability, morphology and volume) were studied with a standard apparatus, procedures and method. Evaluation of administration of nicotine on libido and sperm qualities in male wistar rats was investigated and the results of this investigation demonstrated that nicotine has deleterious effects on the reproductive functions of male rats, sufficient to cause reversible infertility. Viability (percentage of live sperms) was also reduced in rats with the three different doses of nicotine used in the study in a dose-dependent manner. Fertility studies show a significant decrease in the libido score of rats treated with nicotine. The reduction in sperm viability is a function of reduction in the progressive sperm motility. The present study revealed that nicotine-treatment in male rats could affect the significantly, sperm qualities, fertility potential and libido by untreated female rats. Rats treated with nicotine for 30 days had a decreased sperm motility.

KEYWORDS: Nicotine, sperm qualities, Libido, Fertility, sperm motility.

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. (Anderson et al, 1984, Andrzej et al, 1985). 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 anti- herbivore chemical; therefore, nicotine was widely used as an insecticide in the past (Aydes et al, 2001, Bancroft, 2000, Basson & Brotto, 2003, bassoon, 2001) 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. (Berk et al, 1986, Bruin et al, 2007). 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, Carmines et al, 2003).

MATERIALS AND METHODS

A good laboratory practice protocol was observed when all experiments were conducted. The recommendations for quality standards of biomedical research were noted and implemented.

Collection of experimental animals

Twenty (20) male and twenty five (25) female rats were used for the study with each rat weighing between 80-

160g. The rats were obtained from the Experimental Animal Unit of the Department of Human Physiology, University of Port Harcourt, Rivers State and were housed in conventional wire mesh cages under normal day/light cycle.

The animals were allowed free access to water and feed throughout the period of the experiment. Animal feed was purchased from Rumuosi local market Port Harcourt consisting of Maize grains, Wheat brand, Groundnut, Palm kernel, Fish meal. It also contains minerals like sodium and magnesium.

Acclimatization of animals

Animals were weighted and kept in a wire gauze cage floored with saw dust to maintain dryness, under favourable condition for two weeks. The animals were fed and properly catered for.

Experimental Design

The male rats were divided into four (4) groups, with each group consisting of five (5) rats each as follows; Group 1(the control group); receive 0.2 ml/kg normal saline for four weeks, Group 2(Test group 1): received 0.5mg/kg of Nicotine for four weeks, Group 3(Test group 2); received 1mg/kg of Nicotine for four weeks and Group 4(Test group 3); received 1.5mg/kg of Nicotine for four weeks.

Nicotine Preparation

Nicotine hydrogen tartrate (95% Nicotine) (BDH Chemicals Ltd., Poole, England) was used in the study. The nicotine dosage was freshly prepared in normal saline and administered at a dose of 0.5/1.0/1.5ml/100g b.w. via the intra-peritoneal route.

Acute Toxicity Test

LD 50 of nicotine (was found to be 50mg/kg) was carried out using the modified method of Karber (Karber, 1931) to determine the safe concentrations to be administered to the animals in order to achieve the desired level of the chemical in the animals' body.

Libido Test

To observe the libido-oriented mounting behaviour, non-estrous untreated female rats were paired on the 30th day at 6.00 *pm*. The male rats assuming the copulatory position over the female rats, but failing to achieve intromission was considered as a mount. Male rats from each group was chosen and suitably marked.

The rats were placed in a clear aquarium and allowed to acclimatize for 15 minutes. After wards a non-estrous female rat was introduced into the arena. The number of mounts was recorded for 15 minutes. This process was also done for the recovery groups.

Fertility Studies

A total of 20 untreated fertile, pre-oestrous female rats were used for the fertility test. One untreated female rats

was made to co-habit with a male rat each from all the four male groups on the 31st day of treatment. All animals co-habited for 5 days according to earlier studies. The presence of a vaginal plug was accepted as the index for a positive mating and it was taken as day one of pregnancy.

Semen Collection

The left testis was removed along with its epididymis from each male rat. The caudal epididymis was separated from the testis and lacerated to collect the semen with a microscope slide for semen characteristics evaluation, as previously described.

Sperm characteristics analysis

1. Progressive motility was tested immediately.
2. A viability study (percentage of live spermatozoa) was done using eosin/nigrosin stain.
3. Sperm morphology was evaluated by staining the sperm smears on microscope slides with two drops of Walls and Ewas stain after they were air-dried.
4. Sperm count was done under a microscope with the aid of the improved Neubauer hemocytometer.

Procedures for Sperm Analysis

Estimation of Percentage Motile Spermatozoa

One drop of well-mixed liquefied semen was placed on a slide, and cover with a glass. 40X objective with condensers iris closed sufficiently to give good contrast was used, examination of several fields of the preparation for the motile spermatozoa was done and the approximate percentages that are actively motile was recorded.

Motility parameters (i.e Motile cells, Active cells, Sluggish Cells, Dead cells).

Morphology (Principle & Procedure)

Make a thin smear of the liquefied well mixed semen on a slide. While still wet, fix the smear with 95% ethanol for 5-10minute and allow to air -dry.

Wash the smear with sodium bicarbonate formal solution to remove any mucus which may be present.

Rinse the smear with several change of water. Cover the smear with dilute (1 in 20) carbon fuchsin and allow to stain for 3 times. Wash off the stain with water.

Counter stain, by covering the smear with dilute (1-20) methylene blue for 2 minutes wash off the stain with water drain and allow the smear to air-dry.

Staining and Microscopic Viewing (Staining Result) for Defects such as

Head Defect, Mild Pieces, Tail.

Sperm Count

Using a Pasteur pipette, fill an improved Neubauer chamber with the diluted semen. Wait for 3-5 minute for the spermatozoa to settle.

Using the number of spermatozoa in 1ml of fluid by multiplying the number counted by 100.000.

Normal Specimens Contain;

60-150 million spermatozoa 1 ml. The addition of a drop of eosin to the preparation helps differentiate viable from non-viable spermatozoa, non-viable spermatozoa stain red while viable spermatozoa remains unstained.

In normal specimens, Over 70% of the spermatozoa are motile most specimens contain over 80% motile forms. The spermatozoa remain motile for several hours.

1 in 20 dilution (i.e 19 drops of semen diluent and 1 drop of semen) into a test tube, mix gently and use a Pasteur pipette, fill an improved Neubauer counting chamber

with the diluted semen, wait for 3 minute for the spermatozoa to settle. Using the X10 microscope with a condenser, iris closes sufficiently to give good contrast, count the number spermatozoa. In an area of 4 mm², (i.e 4 large squares.) Calculate the number of spermatozoa in 1ml of fluid by multiplying the number counted by 100.000.

Statistical Analysis

The results are presented as mean sem for each group. The differences among groups were analysed using one-way analysis of variance (ANOVA) version 20.0 followed by Duncan's multiple range posthoc test for pairwise comparisons. For sperm abnormalities, the data was analysed using X2 test.

RESULTS

The outcomes of this study are presented in this section in tables, graphs and charts respectively. These were followed by the interpretations and the discussions of findings.

Table 1: Nicotine effect on libido in study animals.

S/No.	Groups	Mounting Frequency (%)	Intromission Frequency (%)	Ejaculate (ml)
1.	Control	10.60±0.51	2.60±0.51	0.80±0.20
2.	0.5ml/kg Nicotine	7.00±0.55*	1.80±0.58	0.60±0.25
3.	1ml/kg Nicotine	8.20±0.49*	1.00±0.45*	0.40±0.37*
4.	1.5ml.kg Nicotine	5.60±1.25*	0.80±0.37*	0.20±0.25*

Values are presented in mean ± SEM. n= 5. P ≤ 0.05 *means values are statistically significant when compared to the control.

Table 2: Nicotine effects on libido in study animals (Latency).

S/n	Groups	Mounting Latency	Intromission Latency
1	Control	30.30 ± 0.21	32.60 ± 0.25
2	0.5mg/kg nicotine	21.50 ± 0.25*	21.90 ± 0.51
3	1mg/kg nicotine	24.80 ± 0.40*	10.50 ± 0.55*
4	1.5mg/kg	15.50 ± 1.05*	2.50 ± 0.56*

Values represented in ± SEM n=5, P ≤ 0.05 * means values are statistically significant when compared to the control.

Table 3: Sperm Motility parameters of experimental rats treated with nicotine.

S/No.	Groups	Percentage Motile Cells(%)	Percentage Active Cells (%)	Percentage Sluggish Cells (%)	Percentage Dead Cells (%)
1.	Control	93.00±2.74	87.00±1.23	5.00±0.34	7.00±1.23
2.	0.5ml/kg Nicotine	58.00±34.93*	49.00±14.35*	9.00±2.45*	22.00±15.6*
3.	1ml.kg Nicotine	38.40±6.35*	28.00±3.39*	10.80±1.80*	31.60±2.84*
4.	1.5ml/kg Nicotine	22.00±15.25*	13.00±6.44*	29.80±1.46*	48.00±6.82*

Values are presented in mean ± SEM. n= 5. P ≤ 0.05 *means values are statistically significant when compared to the control.

Table 4: Nicotine effect on sperm count and morphology in study animals.

S/No	Groups	Total Sperm Count (%)	Head Defect (%)	Tail Defect (%)	Mid Piece Defect (%)
1.	Control	80.20±6.46	0.40±0.25	0.80±0.49	0.60±0.25
2.	0.5ml/kg nicotine	38.40±3.57*	2.20±0.49*	2.60±0.25*	0.40±0.25
3	1ml/kg nicotine	32.80±0.92*	3.20±0.92*	2.20±0.37*	0.40±0.25
4.	1.5 ml/kg nicotine	32.80±6.05*	2.00±0.84*	4.60±0.25*	0.60±0.25

Values are presented in mean ± sem. n= 5. P ≤ 0.05 *means values are statistically significant when compared to the control.

Table 5: Nicotine effect on sperm cell viability in study animals.

S/No.	Groups	Viable cells (%)	Non-viable cells (%)
1.	Control	95.00±0.84	5.00±0.83
2.	0.5ml/kg nicotine	53.00±0.55*	7.00±0.55*
3.	1ml/kg nicotine	22.40±0.60*	7.60±0.60*
4	1.5ml.kg nicotine	13.80±0.49*	10.20±0.49*

Values are presented in mean ± sem. n= 5. P ≤ 0.05 *means values are statistically significant when compared to the control.

DISCUSSION

Evaluation of administration of nicotine orally on libido and sperm qualities in male wistar rats was investigated and the results of this investigation demonstrated that nicotine has harmful impact on the reproductive functions of male rats, sufficient to cause reversible infertility. The present study indicates that nicotine-treatment in male rats could affect the significantly, fertility potential and libido by untreated female rats. Rats induced with nicotine for four weeks had reduced motility of sperm. Increased latencies of mount and intromission are suggestive arising from increase in libido due to rise in anterior pituitary hormones which cause the stimulation dopamine receptor (cartel et al, 1988).

There was an appreciable increase in the epididymal sperm count of rats in the group with the lowest concentration showing that the effect of nicotine on sperm count may be ameliorated by minimal use of nicotine or complete cessation of it. It was also discovered that sperm viability was decreased with induction of nicotine. The sperm viability reduction is in agreement with progressive sperm motility reduction because immobile sperms are assumed dead during examination of the smear. This could be attributed to impact of nicotine on epididymis when working as spermatotoxic agent on already matured and maturing spermatozoa (Chu et al., 2013).

The well-known morphological problem discovered in nicotine induced specimen are curved tail, curved mid piece, rudimentary tail, bent mid-piece. These problems usually happen at sperm transit, sperm maturation and

storage at these periods, spermatozoa motility is developed (Clair et al., 1994).

Not minding that classical erection frequency measurement was not carried out in this research, both intromission and mount frequencies that are parameters of potency, libido and vigor reduced during and after treatment with nicotine (Agmo, 1997). Thus, while decreased mount frequency is a sign for weakened sexual motivation, decreased intromission frequency is sign for deficiency of erection, penile orientation, and decreased ejaculatory activation by nicotine (Crenshaw & Goldberg 1996). Increased latencies of mount and intromission are suggestive arising from increase in libido due to rise in anterior pituitary hormones, which cause the stimulation dopamine receptor (Crowley et al., 2003).

CONCLUSION

The outcome of this research indicated that regular consumption of nicotine (main element of cigarettes) can have negative impact sexual motivation in male, fertility potential and performance during administration for four weeks at doses of 1 and 1.5 mg/kg. The impact of the cigarette smoking, which are connected to its nicotinic component, appears during prolong period of usage and can justify the use of endogenous nicotine as a sexual suppressant mainly when used uncontrollably, abusively and over a long period of time.

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