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Nitric oxide synthase inhibition with L-NAME ameliorates nicotine-induced reproductive organ decrease in male rat

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ABSTRACT

Testicular integrity is compromised during illness or infection leading to temporary or permanent infertility and studies have associated elevated nitric oxide (NO) with infertility. This study was designed to investigate the effect of inhibiting nitric oxide synthase on nicotine-induced reproductive organ decrease in male rats. Forty-eight male Wistar rats (160-180g) were randomly assigned to six groups and treated orally for 30 days with saline (control), nicotine (0.5mg/kg, 1.0mg/kg) with or without N^G Nitro-L-Arginine Methyl Ester (L- NAME, 50mg/Kg). At the end of the experiment, the animals were sacrificed and their productive organs were removed and weighed immediately. There was no significant difference in the mean body weight of the experimental group. Testicular and epididymal weight was significantly decreased in the nicotine treated groups. Serum testosterone level was also significantly decreased in a dose-related manner in nicotine-treated groups. However, co-treatment with L-NAME effectively reversed the nicotine-mediated alterations in weight of reproductive organs and testosterone when compared to nicotine only. Taken together, the present data indicate the abilities of L-NAME to ameliorate nicotine-induced testicular and epididymal alteration in male rats.

Keywords: Nicotine, N^G Nitro-L-Arginine Methyl Ester (L-NAME), testis, testosterone, rats

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INTRODUCTION

There is a considerable body of clinical evidence suggesting that testicular function is compromised during illness or infection, resulting in a temporary or permanent impairment of fertility [1–3]. Recently, generation of Reactive Oxygen Species (ROS) in male reproductive tract has become a major cause for concern because of their potential toxic effects at high levels on reproductive function. Nitric oxide (NO) is one of the ROS implicated in variety of physiologic cell signaling mechanisms in many tissues. NO has been documented as an important molecule regulating the biology and physiology of reproductive function. [4]

NO Production occurs through the action of one of three nitric oxide synthase (NOS) enzymes. Immunohistochemical studies have shown that the endothelial (type III) isoform is present in both Sertoli cells and human Leydig [5], and that the neuronal isoform (type I) is present in human and rat testes [6-9]. Viggiano et al showed that inhibition of NO synthase by L-NAME inhibits the acrosome reaction in mouse spermatozoa [10].

Studies on the administration of the broad-spectrum NO inhibitor, L-nitro-L-arginine methyl ester (L-NAME) to adult male rats resulted in an elevation in serum testosterone levels 2 h post-injection, indicating the involvement of

NO in the regulation of normal testosterone production [9, 11]. Previous studies on the effect of nicotine on male reproductive function concluded that nicotine associated decrease in weight and size of the reproductive organs is associated with decrease serum testosterone level [12]. It has therefore been hypothesized that NO is important in the male reproductive system, and may play a role in male reproductive function.

The use of nicotine seems to remain a broad public health concern since several million of humans use nicotine worldwide through smoking for a prolonged period of time. Infertility among couples of child bearing age is also on the rise. In spite of the growing knowledge of effects of NO on reproduction and the association between nicotine and male reproductive dysfunction, little is known about the effect of inhibiting NOS and its adverse effect on male reproductive organ. Given the pre-existing studies linking NO and testosterone production and altered organ integrity with decreased testosterone, the aim of the present study was to investigate the potential role of L-NAME in ameliorating the nicotine associated decrease in reproductive organ.

MATERIALS AND METHODS

Drug preparation

Nicotine*:* Nicotine hydrogen tartrate (95% Nicotine) (BDH Chemicals Ltd., Poole, England) was used in the study. The nicotine dosage freshly prepared in normal saline for each group of animals was delivered at 0.5 *mg*/*kg* and 1.0 *mg/kg* per body weight. The working solutions were stored in foil-wrapped glass bottle at 4°*C* for no longer than ten days.

Nitric oxide (NO) synthesis inhibition: N^G-nitro-L-arginine methylester (L-NAME; Sigma Chemicals St Louis, MO, USA), a nitric oxide synthase (NOS) inhibitor was administered in the drinking water at a dose calculated to provide 50mg/kg/day to rats. This was administered in light-proof bottles for a period of 4 weeks. It was used to determine the role of NO synthesis in nicotine induced infertility.

Animals and treatments:

Experiments were performed on forty male Wistar rats, 2.5 month old and whose average weight ranged between 190 *g* and 210 *g* obtained from the Animal House, College of Medicine, University of Ibadan, Oyo State, Nigeria. Animals were divided into six equal groups with *ad libitum* access to rat chow and drinking water. Animals were also maintained in a well-ventilated room with a 12/12-hour light/ dark condition at room temperature. The experiment was conducted in accordance with the Guidelines of the U.S. National Institute of Health (NIH) on the care and use of laboratory animals. The male animals in the six groups were treated for 30 days and they included the control group that received 0.2 *ml/kg* normal saline, 0.5 *mg/kg* nicotine-treated group, 1.0 *mg/kg* nicotine-treated group, 50mg/ kg L-NAME, 0.5 mg/kg nicotine and 50mg/ kg L-NAME and 1.0 mg/kg nicotine and 50mg/ kg L-NAME. The two latter groups served as the intervention groups.

Blood Sample Collection

Blood (2ml) was collected from each animal via the retro-orbital sinus with 70µl heparinized capillary tube under anaesthesia and put into plain sample bottle for testosterone analysis. The sample was centrifuged at 3000 rpm for five minutes. The serum was used to analyze the level of testosterone

Organ Collection

The animals were dissected and the reproductive organs (testes, epididymis, prostate gland and seminal vesicle) were removed, cleared of adherent tissues and weighed immediately with an electronic weighing balance, model DT 1000 England with a capacity of 0.1 to 1000g.

Testosterone assay procedure.

An enzyme –based immunoassay (EIA) system was used to measure testosterone level in serum samples collected. The EIA kit was obtained from immunometrics (London, UK) and contained a testosterone EIA enzyme label, testosterone EIA substrate reagent and EIA quality control sample. A quality control was carried out at the beginning and at the end of the assay to ascertain the acceptability with respect to bias and within batch variation. The EIA kit used had a sensitivity of approximately 0.3nmol/M (0.1g/mL) of testosterone. The intra and inter assay variations were 10.02% and 10.12% respectively.

Statistical analysis*:* The results are presented as means±SEM for each group. Differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by the Duncan's multiple range Post hoc test for pairwise comparisons. All statistical comparisons and tests were performed using SPSS (SPSS Inc., Chicago, IL., USA) for Windows. $P<0.05$ was accepted as significant.

RESULTS

Effect of nicotine and L-NAME on body weight

There were no significant difference ($p > 0.05$) in the mean body weight of nicotine and L-NAME treated rats during the experimental period when compared with the control group during the experimental period as shown in table 1.

Effect of nicotine and L-NAME on organ weight

Effect of nicotine and L-NAME on mean testicular weight

Table 1: Body weight changes of experimental rats treated with nicotine and L-NAME

DOSE	Before treatment (g)	Week 1(g)	Week 2 (g)	Week 3 (g)	Week 4 (g)
Control	200.45±4.42	202.25±4.30	208.25±4.10	218.23±5.21	222.60±4.80
L-NAME (50mg/kg BW)	198.86±5.06	204.64±5.32	211.34±4.78	221.68±5.68	234.78±5.21
0.5mg/kg BW	202.00±5.73	201.61±6.66	203.43±4.98	208.43±5.60	212.55±6.23
1.0mg/kg BW	199.85±6.12	199.37±6.62	200.58±5.45	205.13±4.50	209.55±5.48
0.5 mg/kg + L-NAME	201.45±5.84	203.43±5.93	207.31±5.63	212.01±5.32	223.63±5.97
1.0 mg/kg+ L-NAME	204.77±5.63	207.64±5.93	212.45±5.82	218.22±5.72	227.43±5.58

Values are expressed as Means ±SEM of 8 rats per group. Means in columns with different superscript letters are significantly different; p<0.05

Table 2: Reproductive organ weight changes of experimental rats treated with nicotine and L-NAME

DOSE	Testes (g)	Epididymis (g)	Prostate (g)	Seminal vesicle (g)
Control	1.48 ± 0.04^a	0.38 ± 0.02^a	0.44 ± 0.04^a	1.18 ± 0.38^a
L-NAME (50mg/kg BW)	1.32±0.04^a	0.31 ± 0.02^a	0.45 ± 0.04^a	1.21 ± 0.30^a
0.5mg/kg BW	1.28 ± 0.03^b	0.28 ± 0.03^b	0.45 ± 0.05^a	1.00 ± 0.24^a
1.0mg/kg BW	1.12 ± 0.02^b	0.21 ± 0.03^b	0.48 ± 0.06^a	1.14 ± 0.32^a
0.5 mg/kg + L-NAME	1.47 ± 0.04^a	0.37 ± 0.04^a	0.43 ± 0.04^a	1.08 ± 0.32^a
1.0 mg/kg + L-NAME	1.42 ± 0.02^a	0.37 ± 0.03^a	0.47 ± 0.04^a	1.12 ± 0.28^a

Values are expressed as Means ±SEM of 8 rats per group. Means in columns with different superscript letters are significantly different; p<0.05

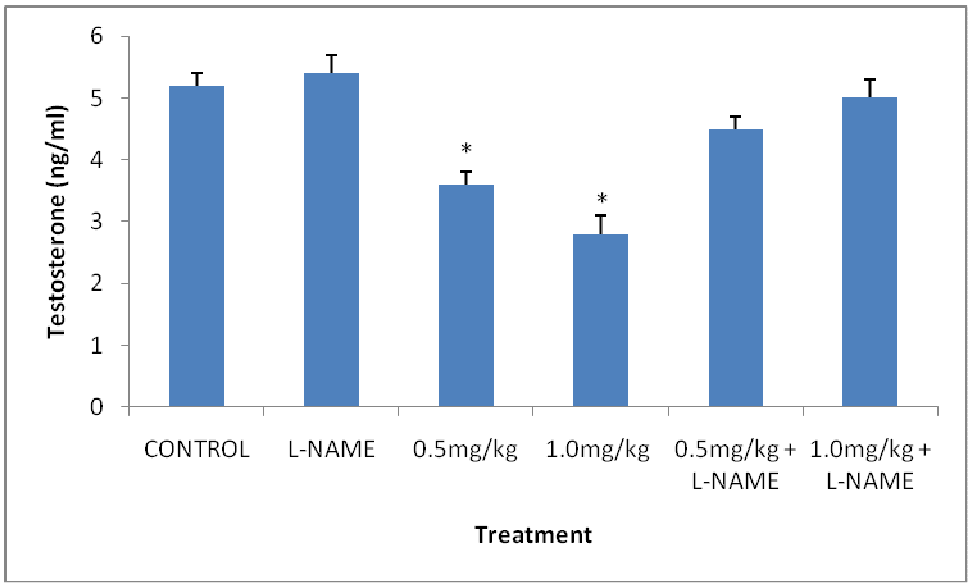

Figure 1: Serum testosterone level in male rats treated with nicotine
*Values are expressed as mean ±SEM of 8 rats. *= p<0.05 vs control*

The result showed that there was a dose-related significant decrease ($p < 0.05$) in the mean testicular weight of rats administered with 0.5mg/kg B.W and 1.0mg/kg B.W nicotine when compared with their control. However, L-NAME treated group and the intervention groups had comparable values when compared with the control group as shown in table 2.

Effect of nicotine and L-NAME on mean epididymal weight

The mean epididymal weight of rats that received the two doses of nicotine was significantly decreased ($p < 0.05$) when compared with the control. The observed decrease is dose-related. However, L-NAME treated group and the intervention groups had comparable values when compared with the control group as shown in table 2.

Effect of nicotine and L-NAME on mean prostate weight
The treated groups had comparable values for the mean prostate weight when compared with their control counterpart as shown in table 2.

Effect of nicotine and L-NAME on mean seminal vesicle weight
There were no significant difference ($p > 0.05$) mean seminal vesicle weight of nicotine and L-NAME treated rats during the experimental period when compared with the control group as shown in table 2.

Effect of nicotine and L-NAME on serum level of testosterone
The mean serum testosterone level of rats administered with 0.5 mg/kg B.W and 1.0 mg/kg of nicotine was significantly decreased ($p<0.05$) when compared with the control group. This decrease is dose related. However, L-NAME 0.5mg/kg BW + L-NAME and 1.0 mg/kg BW + L-NAME groups showed an insignificant decrease ($p>0.05$) as shown in figure 1.

DISCUSSION

The data presented herein clearly indicate profound restoration of the adverse reproductive effects of nicotine on male reproductive organ and testosterone with co- administration of NOS inhibitor; L-NAME in rats. Previous study on nicotine treatment has been associated with decrease reproductive organ weight and testosterone subsequently leading to infertility in earlier studies [12, 13].

To date, this is the first investigation for a relationship between the effects of nitric oxide synthase inhibitor (L-NAME) and nicotine on male reproductive organ weight in rats.

In this study, the weight of the organs of the treated rats showed variable response to the two doses of nicotine. Of great interest is the mean weight of the testis and epididymis of animals administered with 0.5mg/kg and 1.0mg/kg B.W that showed a significant decrease. The decrease in the mean body weight of these organ correlates with the decrease in the serum testosterone level obtained from this study.

The reversibility of the decrease in organ weight, coupled with the increase in testosterone in the co-treated groups showed that L-NAME was acting through changes in androgen levels. Thus, in the present experiments, L-NAME was probably acting by promoting testosterone synthesis and secretion probably through inhibiting the formation of NO in the reproductive organs. The increase in testosterone levels agrees with previous studies that observed increased testosterone secretion with L-NAME administration [14, 15]. The results of this study also indicate that the synthesis of NO through NOS may play a role in the regulation of the serum levels of testosterone.

It may therefore be inferred that low concentrations of NO may protect against the adverse effect of nicotine on testosterone thereby preventing the decrease in testicular and epididymal weight since testosterone is vital for development, growth and normal functioning of the testis and the male accessory reproductive glands [16]. In contrast, excessive generation of NO under pathological conditions such as cigarette smoking may reduce testosterone level. Several studies have confirmed the role of NO in modulation of sexual and reproductive function and, it has also been suggested that NO might be involved in different testicular abnormalities [17, 18]. Previous studies that evaluated the testicular NO level of nicotine treated rats concluded that nicotine caused significant increase in testicular NO level [19].

CONCLUSION

The present data suggests that NO is an important mediator in the pathogenesis of infertility with nicotine treatment. It also indicates the abilities of L-NAME to ameliorate nicotine-induced testicular and epididymal alteration in male rats. However, further investigations are still needed to confirm the exert mechanism of NO inhibition since L-NAME is a broad-spectrum NO inhibitor.

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