ORIGINAL ARTICLE

The Ameliorating Role of Oral Administration of Basella Alba Methanol Extract in Nicotine-Induced Testicular Toxicity

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Abstract

Background: Nicotine is a potential cause of male infertility due to its ability to induce testicular damage. This study investigated the influence of *Basella Alba* (BA) on possible nicotine-induced testicular damage.
Methods: Twenty male Wistar rats were divided into four random groups namely; healthy control (HC) in which rats were administered only normal saline, Nicotine control (NC) which received high dose nicotine only (1.0mg/kg), Low dose nicotine, *Basella Alba* treatment group (LDN + BA) given nicotine (0.5mg/kg) plus methanol extract of *Basella Alba* (200mg/kg) and High dose nicotine, *Basella Alba* treatment group (HDN + BA) in which rats received nicotine (1.0mg/kg) plus *Basella Alba* extract (200mg/kg). Rats were euthanized via exsanguination after five weeks of treatment and testes extracted for histological analysis. Blood samples were also analyzed for gonadal hormones.
Results: There was a significant decline ($p<0.05$) in body weight, testicular weight, and serum testosterone but elevated follicle stimulating hormone (FSH) and luteinizing hormone (LH) in NC rats when compared with HC. Rats in LDN + BA and HDN + BA showed a significant increase ($p<0.05$) in serum testosterone and a corresponding decrease ($p<0.05$) in FSH and LH levels when compared to rats in NC. Additionally, testicular photomicrographs revealed significant histoarchitectural distortions in NC rats when compared to HC. These anomalies were found to be either milder or absent in LDN + BA and HDN + BA groups.
Conclusions: Nicotine induces both structural and functional testicular damage that is ameliorated by *Basella Alba* possibly due to the high antioxidant composition.

Keywords: Oral, Methanol, Nicotine, Testis, Toxicity

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INTRODUCTION

Since ancient times, medicinal plants have been a major therapeutic agent with one or more of their organs used for curative purposes or as precursors for the synthesis of various drugs. *Basella Alba* remains a rather under-explored medicinal plant despite its cosmopolitan status. It is a rapidly growing succulent plant that contains several biologically active compounds such as carbohydrates, alkaloids, steroids, and many more substances with vital health benefits [1]. *Basella Alba* serves several functions including being an antioxidant, hepatoprotective, androgenic, antiulcer, central nervous system depressant, cytotoxic, and antibacterial [2, 3, 4, 5, 6, 7].

Nicotine has been named among a wider variants of chemicals and drugs that have been found to adversely affect the male reproductive system and consequently with a potential to cause male infertility [8]. The consumption of cigarette has been the major source of nicotine intake in humans with each stick of cigarette estimated to contain about 10 milligram of nicotine [9]. Nicotine was originally isolated from the tobacco plant in 1828 by chemists Wilhelm Heinrich Posselt and Karl Ludwig Riemann and named after the French ambassador in Portugal, Jean Nicot de Villemain, who mainly promoted the health advantages [10]. However, studies conducted afterwards revealed that nicotine is actually very toxic and can be rapidly absorbed into the blood stream through the respiratory system, oral mucosa, and the skin, thereby exerting widespread deleterious effects [11].

Nicotine has also been revealed to inhibit the release of follicular stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland. Further studies

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demonstrated that nicotine decreases testosterone and androstenedione concentrations in rats by competitive inhibition of multiple stages in testosterone biosynthesis [12]. Cotinine, a metabolite of nicotine has also been reported to have similar effect and recent studies have shown that nicotine could affect spermatogenesis and cause a depreciation in the quality of semen [13]. Additionally, there is significant evidence from human studies that nicotine contains harmful mutagens and carcinogens capable of stimulating nuclear deoxyribonucleic acid (DNA) damage in spermatozoa, which may trigger defective semen quality [14].

Androgens are essential for male reproductive function and androgen resistance has been implicated in certain forms of male infertility [15]. Estradiol is also essential in the development and maintenance of male reproductive function as it has been found to play vital roles in spermatogenesis as well as in the modulation of erection and libido [16]. *Basella Alba* is capable of modulating testicular androgen-estrogen homeostasis, which contributes to the improvement of male reproductive function [17]. It also has a positive effect on Leydig cell viability and enhances the level of testicular aromatase mRNA whose activities has significant benefit for the male reproductive function [17, 18]. Additionally, the rich presence of terpenoid compounds in *Basella Alba* is believed to produce testosterone-enhancing effects [4]. Owing to these beneficial effects of *Basella Alba* on male reproductive functions and many more pro-fertility potentials that have been attributed to the plant, this study was designed to explore the possibility of beneficial roles for it on nicotine-induced testicular toxicity in male Wistar rats. The study is aimed specifically at investigating the possible effects of *Basella Alba* on the main endocrine function of the testes (production of testosterone), which has been reportedly impacted negatively by nicotine toxicity.

METHODS

Study Design

Twenty male Wistar rats were used for this study, each weighing between 180-220g. The rats were procured from the Animal House of the Physiology Department at Bowen University, Iwo. Osun State, Nigeria. The animals were divided into four groups each containing five Wistar rats and housed in four separate industrial cages at standard conditions as follows;

Healthy control group (HC)-Rats were given only normal saline at 0.2ml/ 100g body weight daily throughout the period of the experiment.

Nicotine control group (NC)-Rats were given only nicotine at high dose (1.0mg/kg daily) throughout the period of the experiment.

Low dose nicotine + methanol extract of *Basella Alba* treatment group (LDN + Ba) – Rats were given low dose nicotine (0.5mg/kg) daily and methanol extract of *Basella Alba* at the recommended daily dose of 200mg/kg [19].

High dose nicotine + methanol extract of *Basella Alba* treatment group (HDN + Ba) – Rats were given high dose nicotine (1.0mg/kg) and methanol extract of *Basella Alba* (200mg/kg) daily.

All treatments were administered orally by gavage via oropharyngeal cannula and animals were fed with commercially available rat pellet and water ad libitum and left for two weeks to acclimatize to the study environment before the commencement of treatments. All procedures involving the use of animals in this study complied with the guiding principles for research involving animals as recommended by the declaration of Helsinki and the Guiding principles in the care and use of animals [20].

Plant Materials

Fresh *Basella Alba* leaves were obtained from Ogbomoso, Oyo State, Nigeria. The plant was identified and authenticated in the Department of Botany, University of Ibadan with voucher number, (UIH-22391). The leaves were detached from the stems, washed in clean water, and allowed to air-dry at room temperature. The dried leaves were powderized using an electric blender and then weighed. 336 grams of the powdered leaves was soaked in 3 litres of methanol (70% V/V) and then warmed in a water bath for an hour at a low temperature of 40°C to avoid denaturing the protein components while consistently stirring the mixture [21]. It was then left at room temperature for seventy-two (72) hours on a shaker to ensure it is constantly stirred. Cheesecloth was used for filtration after the 72 hours of cooling and the residue discarded. The filtrate was evaporated to dryness using a rotatory evaporator. The weight of the extract obtained was 75.42g giving a percentage yield of 22.45%. The extract was then dissolved in normal saline and preserved in a refrigerator at 2.8°C for the purpose of this study.

Preparation and Dosage of Nicotine Solution

Liquid nicotine (Alchem Inc., Minnesota USA) was used for this study. 10 microliters of Nicotine was dissolved in 20 milliliters of normal saline to get a stock solution with concentration of 0.2g/0.4ml. The dosage of nicotine administered to rats in each group was 0.5mg/kg for low dose and 1.0mg/kg for high dose [8]. Nicotine dilution was prepared daily to ensure freshness and the required potency.

Weighing of Animals and Testes

Rats in all four groups were weighed before commencement of experiment to record baseline weights and then weights were subsequently recorded weekly using a digital weighing balance. Following euthanization, each animal was dissected, where testes excised, freed of all fat tissues, and weighed with the aid of a sensitive organ weighing balance.

Collection of Blood Samples for Analysis

After the five weeks of administration, all animals were anaesthetized with dimethyl ether and euthanized via exsanguination. Blood samples were collected via cardiac puncture and they were preserved in lithium heparinized EDTA bottles to prevent coagulation and then centrifuged for ten minutes at a speed of 5000rpm. The supernatant was preserved in plain bottles for hormonal assays. The samples were analyzed to determine the concentration of testosterone, luteinizing hormone (LH) and follicle stimulating hormone

(FSH). The analysis was carried out via the tube-based enzyme immunoassay (EIA) method. The protocol adopted for the hormonal assays was in accordance to the method described for the kit (Immunometric Limited, UK).

Histological Analysis

Following excision, the right testicular sample from each rat was fixed in Bouin's fluid and preserved for histological studies. The samples were washed, trimmed, embedded in paraffin and then sectioned to 5um thickness and stained with Haematoxylin and Eosin stains for histological analysis. Slides were examined under microscope according to Luna's method and with the guidance of a histopathologist who was blinded to the study.

Statistical Analysis

All quantitative data were expressed as Mean ± SEM. Results were subjected to statistical analyses using GraphPad Prism (version 8.0). One way ANOVA was used to analyze the difference between groups and Newman-Keuls Multiple Comparison Test was conducted. Differences were considered significant at $p < 0.05$.

RESULTS

Effect of Methanol Extract of *Basella Alba* on Nicotine Induced Changes in Body Weight

The effect of methanol extract of *Basella Alba* (MeBA) on nicotine induced changes in body weight is represented in Table 1.The body weight of rats in nicotine control (NC), low dose nicotine + treatment (LDN+BA) and high dose nicotine + treatment (HDN+BA) significantly declined ($p < 0.05$).when compared to healthy control (HC). Body weight of rats in LDN+BA and HDN+BA groups were not significantly different ($p>0.05$), even though slightly increased in both groups when compared to NC rats.

Effect of Methanol Extract of *Basella Alba* on Nicotine Induced Changes in Testicular Weight

The effect of methanol extract *Basella Alba* on nicotine induced changes in weight of testes is represented in Table 1. It was observed that the weight of the testes of NC, LDN + BA and HDN + BA rats in comparison with healthy control rats, showed a decline that was statistically significant ($p < 0.05$).

While the decline in weight of testes seen in LDN + BA and HDN + BA groups were not significantly different from that in NC group statistically ($p > 0.05$), both treatment groups showed less decline in testicular weight.

Effect of Methanol Extract of *Basella Alba* on Nicotine Induced Changes in Serum Gonadal Hormones

The levels of gonadal hormones in rats treated with nicotine and *Basella Alba* is represented in Figure 1. Testosterone level in nicotine control rats was compared to that in healthy control group and a statistically significant decrease ($p<0.05$) was observed in the NC rats. Likewise, the level of testosterone in both nicotine treatment groups (LDN + BA and HDN +BA) were significantly higher when compared to that in nicotine control rats ($p<0.05$). The testosterone level in HDN + BA rats was not significantly different ($p>0.05$) from that in HC, while levels in LDN + BA was significantly higher.

The level of LH in NC rats was significantly increased when compared to that in healthy control group ($p<0.05$). However, the LH level of LDN + BA and HDN + BA showed a statistically significant decrease compared to healthy control ($p<0.05$). Furthermore, when the level of LH in nicotine low dose and high dose treatments were compared to nicotine control, they were both significant decreased ($p<0.05$).

The level of FSH in nicotine control rats was equally observed to be significantly increased when compared to healthy control group ($p<0.05$). There was however no statistically significant difference in FSH levels when both LDN + BA and HDN + BA were compared to healthy control ($p>0.05$). Furthermore, both treatment groups (LDN + BA and HDN + BA) recorded significant decreases in FSH levels when compared with the untreated nicotine control rats ($p<0.05$).

Histological Analyses of the Effect of Methanol Extract of *Basella Alba* on Nicotine Induced Changes on Cytoarchitecture of Testes

The testicular histopathological slides from the two control groups are presented in Figure 2. As seen in slide A, healthy control rat showed evidence of well-preserved testicular histoarchitecture that is characterized by apparently normal seminiferous tubule (ST), with well-rounded boundaries and organized epithelium. Additionally, evidence of active cell division and maturation of germ cells seen as terminally differentiated spermatozoa (yellow arrow) is observed at a higher magnification in slide C of Figure 2.

On the contrary, slides from nicotine control rats revealed significant histoarchitectural distortion of the testis with evidence of ballooned and elongated seminiferous tubules (black arrows, figure 2 B). There is also evidence of inactive or inhibited cell division with presence of terminally

Table 1. Effect of methanol extract of *Basella Alba* on nicotine induced changes in body weight and testicular weight after five weeks of treatment

GROUPS	BASELINE BODY WEIGHT (g)	BODY WEIGHT AFTER TREATMENT (g)	WEIGHT OF TESTES (g)
HC	195.10 ± 3.25	254.20 ± 13.18	2.98 ± 0.26
NC	198.60 ± 4.51	182.60 ± 6.65 $^{\alpha}$	1.35 ± 0.04 $^{\alpha}$
LDN + BA	200.20 ± 3.05	209.80 ± 11.75$^{\alpha}$	1.49 ± 0.07 $^{\alpha}$
HDN + BA	185.40 ± 2.24	184.60 ± 4.91 $^{\alpha}$	1.59 ± 0.25 $^{\alpha}$

α = Significant difference when compared to HC at $p < 0.05$, $n = 5$

undifferentiated spermatozoa (red arrows, Figure 2 D).

Figure 3 represent photomicrographs of histological slides of rat testes from the two *Basella Alba* treatment groups. LDN + BA treatment group showed significant improvement in seminiferous tubule histoarchitecture with evidence of few shrunken seminiferous tubules (green arrows, slide A) and active cell division and maturation of germ cells evidenced by some terminally differentiated spermatozoa (yellow arrow, slide C).

Slides from HDN + BA rats (Figure 3, B and D) also revealed seminiferous tubules with evidence of similar but milder abnormalities in shape (black arrows, slide B) when compared to slides from NC rats. The observed population of terminally differentiated spermatozoa (yellow arrows, slide D) are however, relatively less when compared to that in slides from LDN + BA rats.

DISCUSSION

The findings in the present study confirms among other major findings that nicotine significantly retards the expected gain in body weight and may even cause an outright weight loss in high dose consumption as observed in nicotine control rats. This is in consonance with previous studies where it has been documented that chronic nicotine administration results in decreased food intake thereby causing weight loss [22, 23]. Although treatment with *Basella Alba* was found to exert a beneficial effect in this regard by ameliorating the weight loss, this effect appears to be largely dependent on the dose of nicotine administered with the effect been relatively minimal in rats placed on high dose nicotine. Additionally, nicotine has been reported to cause an increase in lipolysis and also promote fat utilization for energy, [24] which suggests that the nicotine administered may have induced an increased body fat metabolism thereby contributing to the loss in body weight. *Basella Alba* is known to have a level of pro-insulinic effects and has been reported to induce regeneration of islet cells of Langerhans [25]. This may help in boosting the blood level of insulin and thereby countering the lipolytic effect of nicotine. In terms of composition, *Basella Alba* leaves are said to be high in proteins, fat, vitamins as well as minerals such as calcium and iron [26, 27]. These are vital nutrients that can also contribute significantly to tissue building and hence, weight gain.

As it is with the generalized body weight, nicotine also induced an immense deleterious effect on the weight of testis in this study which confirms earlier report by Jana and co-workers [28]. This can be attributed (in addition to the reasons given above for weight loss) to the nicotine-induced elimination of germ cells in the testes and the associated inhibition of germ cell maturation [28]. Furthermore, Nicotine induces significant damage to the tissues of the male reproductive organs including the testes via the oxidative stress pathway and this is commonly evident as a reduction in the weight of these organs [29, 30]. *Basella Alba* is reputed for its high composition of numerous antioxidant phytochemicals with a relative affinity for gonadal tissue effect [31], these component chemicals are expected to counter and neutralize to a significant proportion the potential nicotine-induced oxidative damages to testicular tissue. This

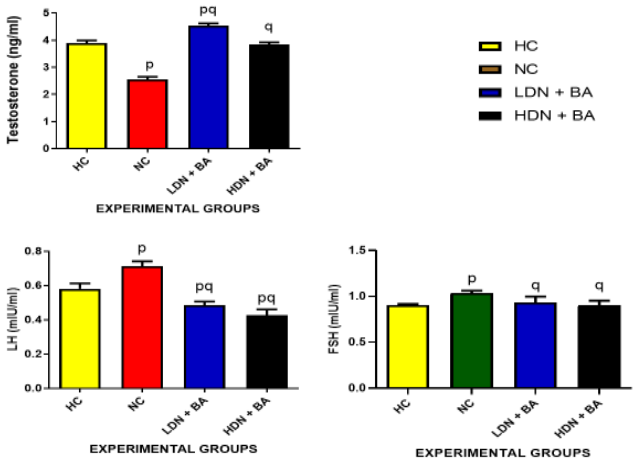

Figure 1. Effect of methanol extract of Basella Alba on nicotine induced changes in serum levels of gonadal hormone in male Wistar rats.
Values are expressed as Mean ± SEM; p indicates significant difference when compared to the HC at p<0.05; q indicates significant difference when compared to NC at p<0.05
ng/dl: nanograms per deciliter; mIU/ml: milli-International Units per millilitre

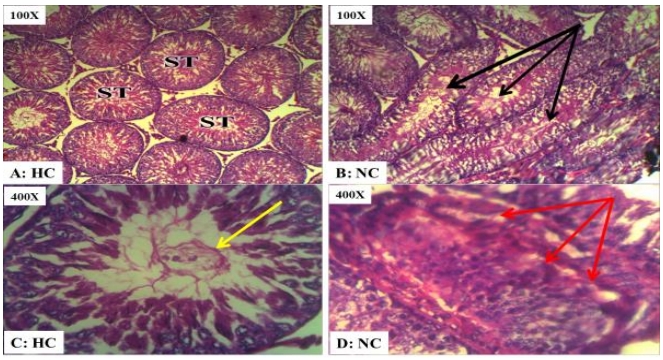

Figure 2. Photomicrograph of the cytoarchitecture of the testes showing normal histological features in healthy control rats versus features of nicotine induced testicular toxicity in nicotine control rats [X 100 and X400 Magnifications]
HC: Healthy control group, NC: Nicotine control group

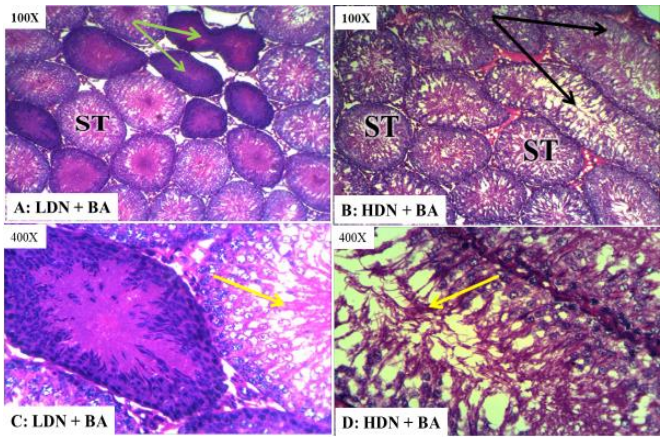

Figure 3. Photomicrograph of the cytoarchitecture of the testes showing effect of methanol extract of *Basella Alba* on nicotine induced testicular toxicity [X 100 and X400 Magnifications]
LDN + BA: Low dose nicotine Basella Alba treatment group, HDN + BA: High dose nicotine Basella Alba treatment group

way, the administration of *Basella Alba* helped to preserve testicular tissues as well as the weight and by extension, the functions, out of which only the endocrine component was explored in this study.

The decline in serum testosterone level accompanied by elevated FSH and LH observed in nicotine control group depicts a typical picture of primary hypogonadism. This pattern has been reported in earlier studies following nicotine toxicity and suggests local damage to the testosterone secreting Leydig cells of the testes [28, 32]. This is in contrast to the rather more systemic variant in which the pituitary production of FSH/LH is impeded thereby leading to low levels of testosterone (secondary hypogonadism). In this scenario, the elevated blood levels of FSH and LH is expected in the face of nicotine induced decline in testosterone level, since the negative feedback suppression of FSH/LH is now limited. In the *Basella Alba* treated groups, there was an appreciable increase in the testosterone level which inadvertently reversed the FSH and LH levels towards normal. The testosterone enhancement effect has been documented previously with the report that *Basella Alba* stimulated testosterone production by Leydig cells [4]. This beneficial role of *Basella Alba* could be attributed to the high presence of terpenoid compounds believed to produce testosterone-enhancing effect by increasing blood cholesterol level which is a substrate for steroid synthesis. Additionally, as can be deduced from the histopathological findings of this study, *Basella Alba* treatment appeared to induce a level a healing and regeneration of some of the nicotine damaged testicular tissues including the seminiferous tubular epithelium where the Leydig cells are located. The potential for *Basella Alba* to stimulate epithelial tissue recovery from insults was first described by Arokoyo et al., (2018) following a study in which treatment with aqueous extract of the plant was observed to result in re-proliferation of streptozotocin-damaged beta islet cells in diabetic Wistar rats [25]. Although the actual mechanism underlying this epithelial cell-protective role of *Basella Alba* cannot be completely substantiated from this study, it can be linked to the potent anti-inflammatory and antioxidant capacities of the plant [25, 31]. These attributes help to neutralize and limit the possible nicotine-induced oxidative and inflammatory processes, thereby preventing the progress of damage to the seminiferous epithelium and other testicular tissues.

CONCLUSION

Based on the results, it can be concluded that nicotine induces a dose dependent testicular toxicity, which results in both anatomical and functional distortions of the testes. This is predominantly characterized by primary hypogonadism that is significantly amenable to *Basella Alba* treatment largely due to the antioxidant and anti-inflammatory potentials of the plant.

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