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Research article

The Protective effects of chlorophytum borivilianum on Nicotine-induced reproductive toxicity, oxidative damage, histological changes and haematotoxicity in male rats

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Key words: Nicotine, Antioxidant, testosterone, oxidativestress, antioxidant, Chlorophytum borivilianum.

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Abstract

Objectives: Nicotine (NIC), a major constituent of cigarette, adversely affects male reproductive hormones, sperm indices and fertilizing potential. Chlorophytum borivilianum (Chlb), known as "white gold" in Indian Ayurveda, improves male reproductive performance. The present study was conducted to assess whether Chlb roots alcoholic extract could serve as a protective agent against NIC-induced reproductive toxicity in rats. **Material and method**: 24 adult Wister male rats were randomly divided into 4 groups : 1) Control 2) Nicotine 3) Root extract of Chlb and 4) NIC + Chlb extract. Sperm indices, Gonadotrophic hormones LH & FSH, and testosterone as well as anti oxidant capacity were measured. **Result and conclusion**: Intraperitoneal injection of NIC (3mg/kg body weight) for 21 days showed significant reduction in testicular weight, sperm count, motility, serum level of LH, FSH, testosterone and testicular endogenous antioxidant. The above- mentioned parameters were restored by Chlb co-administration. The findings indicate that Chlb may potentially be protective against NIC-induced testicular toxicity.

Introduction

Male reproductive system is affected by various chemicals and drugs which lead to infertility in them. It has been reported that 8-15% of couples become infertile in their reproductive age [1]. Male infertility is more prevalent compared to female infertility and smoking is implicated as potential cause of infertility in male. The major ingredients of cigarette, chewing tobacco products are nicotine (NIC) in particulate phase and carbon monoxide (CO) in gaseous phase. Nicotine is rapidly absorbed through respiratory system [2]. It has been reported that NIC inhibits FSH and LH, secreted from pituitary gland [3]. Nic as well as its metabolite cotinine decreases testosterone concentration by competitive inhibition of multiple stages of testosterone biosynthesis [4]. More recent studies have shown that, NIC obviously affects spermatogenesis [5-6], and hypoyhalomo-hypophyseal-gonadal axis [7]. Aydos *et al*, [8] reported that, NIC adversely affected spermatogenesis, epididymal sperm count, motility and fertilizing potential of sperms. Nicotine had been found to cause oxidative stress in testes and in brain by over producing reactive oxygen species and by decreasing antioxidant defence mechanism [9-10].
The use of plant or plant-based products to stimulate sexual desire and to enhance performance and enjoyment is almost as old as the human race itself. This is also reflected in the report from WHO which stated that traditional medicine is used as primary health care product by about 70-80% world population [11]. Chlophytum borivilianum or safed musli (family: Lilliacae) is a medicinal plant in India and is considered as white gold in Ayurveda [12]. This plant has been reported to have a number of biological properties including antimicrobial, anti-inflammatory, hepatoprotective and is used to cure impotency, sterility, enhance male potency [13]. It has been reported that aqueous extract of dried roots of Chlb enhances the sexual arousal, vigour, and libido in Wister rats [14]. The extract increases sperm count significantly. In case of streptozotocin and alloxan induced hyperglycemia, the aqueous extract from the plant resulted in amelioration of sexual dysfunction, resulted in improved sexual performance.
Based on these evidences, the present study was performed to uncover the new aspects from the possible mechanism(s), by which NIC is able to cause adverse impact on male reproductive system and the ameliorative effects of Chlb for these noxious effects.

Materials and methods

Experimental animals

The present was performed on twenty four adult Wister male rats of similar age with an average weight of 110±10 g. All animal experiments were performed

according to the ethical guidelines suggested by the Institutional Animal Ethics Committee (IAEC) of Serampore College, West Bengal, India. Approval number from animal ethical committee is 02/p/s/sc/IAEC/2017. The animals were maintained in an environmentally controlled animal house and in a 12 hour light/dark schedule with free access to water supply. All rats were acclimatized for about two weeks prior to the beginning of the study

Plant material

The Chlorophytum borivilianum (Safed musli) was harvested from natural habitat around the city of Serampore, Hooghly, West Bengal between (March-June). The identification of the collected plant was confirmed scientifically by the Botany department of the college, Calcutta University. The plant was deposited at herbarium with the number: SER/3074.

Preparation of the alcoholic extract

The alcoholic extract of dried roots of the plant was prepared by infusion of the finely dried material into 70% methanol at 20ºC for about 48 hours (REF). The infusion was filtered and concentrated with rotary machine. The concentrated and completely dried extract was diluted in distilled water immediately before use.

Nicotine

Nicotine (NIC) was procured from Sigma-Aldrich (USA) working nicotine prepared by highest body weight of rat, i.e. 3mg/kg body weight. The dose and administration route were selected according to previous studies [9].

Experimental protocol

The Twenty four rats were randomly divided into 4 groups (n=6) as follows Group 1: Control (untreated, receive saline) Group 2: Nicotine (administrated by intraperitoneal injection of nicotine 3mg/kg body weight Group 3: Rats were force feed C. borivilianum root aqueous extract at 250mg/kg body weight/day. Group 4: Plant extract with nicotine (treated with dose C. borivilianum root aqueous extract at 250mg/kg body weight/day rat. Treatments were carried for 21 days.

Body and reproductive organ weight

Body weights were recorded weekly during experimental period. At end of the experiment, the animals were sacrificed by decapitation under ether anaesthesia. Body weights were recorded before sacrifice and male reproductive organs quickly removed and weighted.

Plasma preparation and determination of LH, FSH, and testosterone

After the treatment periods were over (21 days), the animals of all groups were anaesthetized and sacrificed by cervical dislocation, which is one of the recommended physical methods of euthanasia by IAEC. Blood was drawn from heart and plasma was separated for assay of LH, FSH, and Testosterone. Plasma level of LH, FSH was examined by using the accubind ELISA kit obtained from Monobind, USA. All samples were assayed in duplicate. The sensitivity was 0.8 IU/ml. To avoid interassay variation, all samples were run at one time.

Plasma level of testosterone was estimated by the ELISA kit obtained from DRG Inc. Germany. All samples were assayed in duplicate. The sensitivity was 0.75pg/ml for testosterone. To avoid interassay variation, all samples were run at once.

Measurement of oxidative stress

For oxidative stress testes samples were homogenized in phosphate buffer (pH 7.4) using homogenizer and then it is centrifuged at 2000 r.p.m.

SOD superoxide dismutage (SOD)

The nitro blue tetrazolium (NBT) method of Beauchamp and Fridovich, which is based on the inhibition of NBT reduction by SOD, was used for the determination of SOD activities. The relative absorbance was then converted into of SOD activity per mL or per mg protein, where one unit of SOD activity was equivalent to the quality of SOD that caused a 50% reduction in background of NBT reduction [15].

Determination of catalase

Catalase activity was determined according to the method of Aebi 1952 [16] by following the decomposition of H_2O_2 at 240 nm and 25℃. The difference in absorbance per unit time was used as measure of CAT activity.

Determination of glutathion

GSG was determined in testes samples according to method of Ellman, 1959. Testis homogenates were mixed with PBS and 5,5, dithiobis 2-nitrobenzoic acid (DTNB) After 15 days of incubation, absorbance was taken 412 nm [17].

Determination of lipid peroxidation and NO level

Lipid peroxidation activity was determined by using TBA. Absorbance measured at 530 nm (Wills ED, 1987).

The role of nitric oxide synthase (NOS) was indirectly assessed by estimating the NO production. NO activity determined by using Gris reagent (equal volume of 1% sulphanilamide in 5% phosphoric acid and 1% NADH. Absorbance was measured at 550nm [18].

Serum count and sperm motility

After sacrifice the animals, caudal epididymis part of animals was removed and placed in small clean Petridis containing 1ml of phosphate buffer saline pH 7.4. The

caudal part was cut by sterilised blade into three pieces and squeezed gently by a fine forceps to release the sperm into PBS, and then sperm were count by Neubar Haemocytometer Chamber. Sperm suspension was placed on both sides of chamber and no. of sperm in squares of chamber was counted under the microscope at 100x magnification. Sperm count and motility was performed four times for each sample in accordance with WHO Laboratory Manual [19].

Statistical analysis

The obtained data were expressed as mean± SE. Kruskal – Wallis nonparametric one way analysis of variance (ANOVA) test was performed to establish whether or not scroes of different groups differed significantly and to test intergroup significant difference, Mann- Whitney U multiple comparison tests was performed by using stat Direct Software (UK). Differences were considered significant at (P<0.05).

Results and discussion

Results

Reproductive organ weights

As shown in table 1 injecting adult male rats with NIC caused statistically significant reduction in the weight of both right and left testis. However co-administration of Chlb root extract showed significant increase in weight and restored the weight of testes.

Table1. Sperm count and motility

Groups	Weight of Testis (per 100gm body weight)	Motility %	Count (10<6/ml)
Control	0.975	73	50.396
Nicotine	0.850	44.45	10.644
Plant extract	0.936	79	48.751
Nic+PE	0.896	68	31.786

Hormonal analysis (Gonadotrophic hormones & male reproductive hormone)

It is evident from the data in figure 1, 2 and 3 that i.p injection of NIC showed significant reduction in serum level of LH, FSH and testosterone. Co-administration of chlb plant extract revealed a significant elevation of these hormones. Thus Chlorophytum borivilianum root extract brought back the levels of LH, FSH and testosterone close to the control group.

Oxidant and antioxidant parameters

Data given in figures 4A and 4B elucidated that NIC treated rats resulted in significant increase in the level of NO and LPO, which are the hallmarks of lipid peroxidation. On the other hand, co-administration of Chlb root extract caused a marked decrease in oxidative stress parameters i.e. both NO and LPO production (P<0.05).

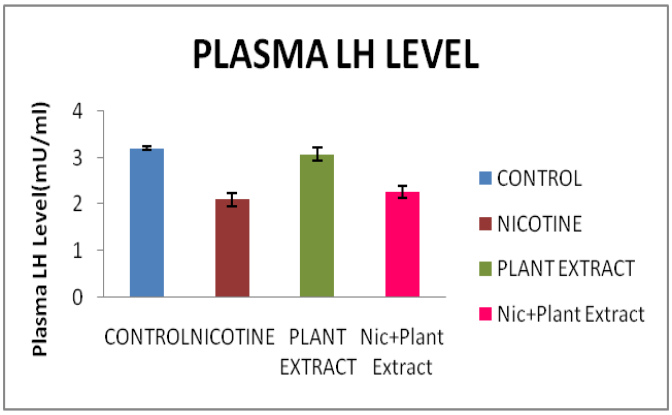

Figure 1. Effect of Nicotine (3mg/kg Body weight/21 days I.P injection) with or without plant extract Chlb and in Plasma LH level in male rats. Data expressed as mean± SE (P<0.05).

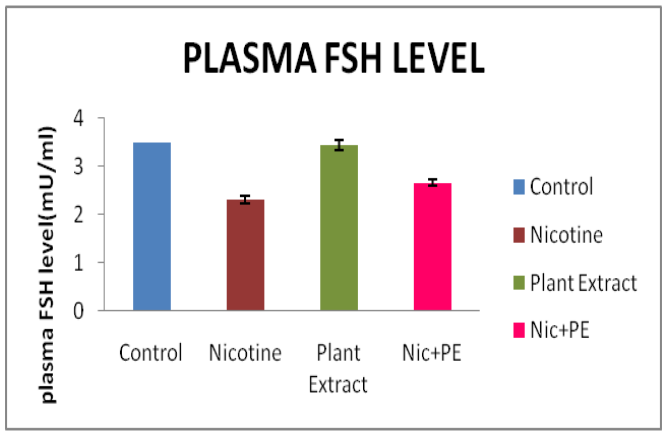

Figure 2. Effect of Nicotine (3mg/kg Body weight/21 days I.P injection) with or without plant extract Chlb and in Plasma FSH level in male rats. Data expressed as mean± SE (P<0.05).

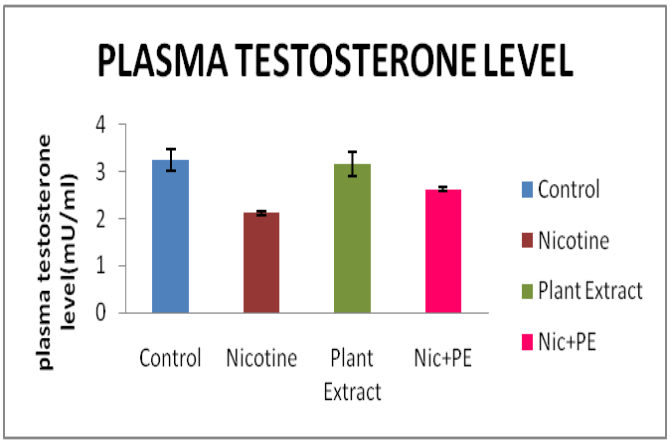

Figure 3. Effect of Nicotine (3mg/kg Body weight/21 days I.P injection) with or without plant extract Chlb and in Plasma testosterone level in male rats. Data expressed as mean± SE (P<0.05).

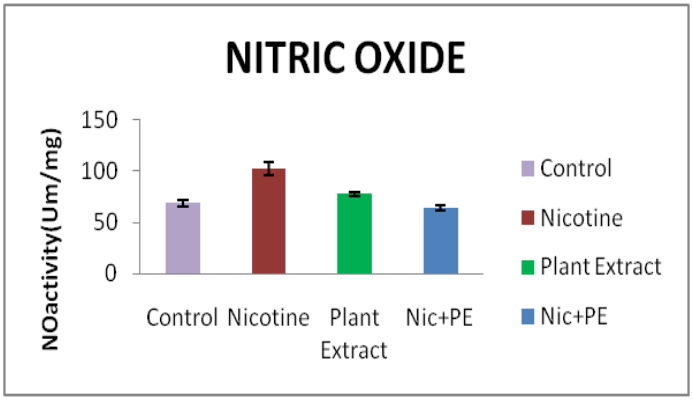

Figure 4A. Effect of Nicotine (3mg/kg Body weight/21 days I.P injection), induced change in NO level with or without Chlb Plant extract in the testis of male rat. Data expressed as mean± SE. (P<0.05).

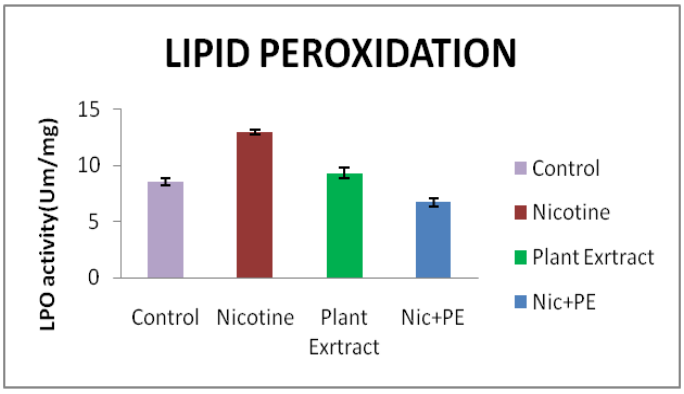

Figure 4B. Effect of Nicotine (3mg/kg Body weight/21 days I.P injection), induced change in LPO level with or without Chlb Plant extract in the testis of male rat. Data expressed as mean± SE. (P<0.05).

Chronic NIC administration also caused a significant decrease in tissue SOD ,CAT activities and GSH level in testicular tissue (Figure 5A, 5B and 5C) while plant extract supplementation restored the SOD, CAT and GSH level in testicular tissues. Thus Chlorophytum borivilianum (Chlb) root extract mitigated the adverse effect of nicotine on oxidant and antioxidant parameters.

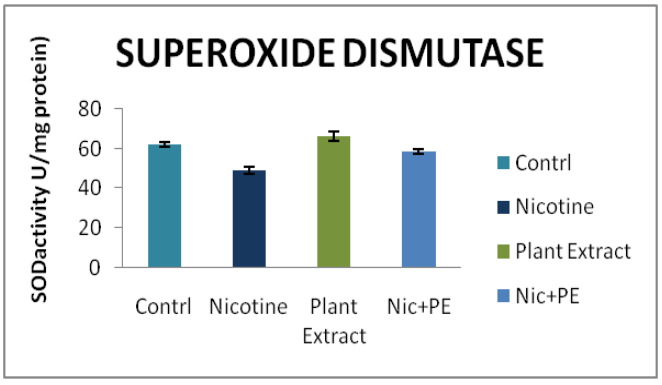

Figure 5A. Effect of Nicotine (3mg/kg Body weight/21 days I.P injection), with or without Chlb Plant extract on super oxide dismutase activity in the testis of male rat. Data expressed as mean± SE (P<0.05).

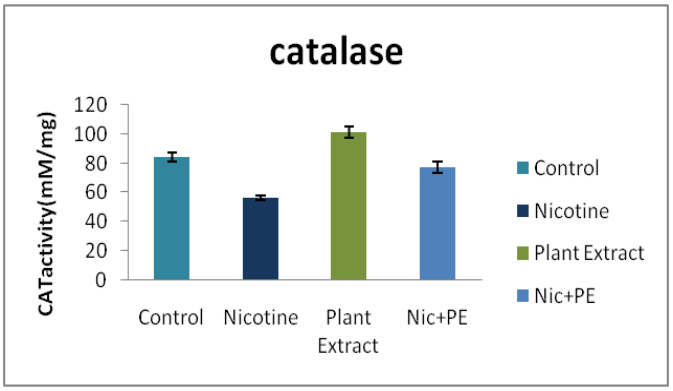

Figure 5B. Effect of Nicotine (3mg/kg Body weight/21 days I.P injection), with or without Chlb Plant extract on catalase activity in the testis of male rat. Data expressed as mean± SE. (P<0.05);

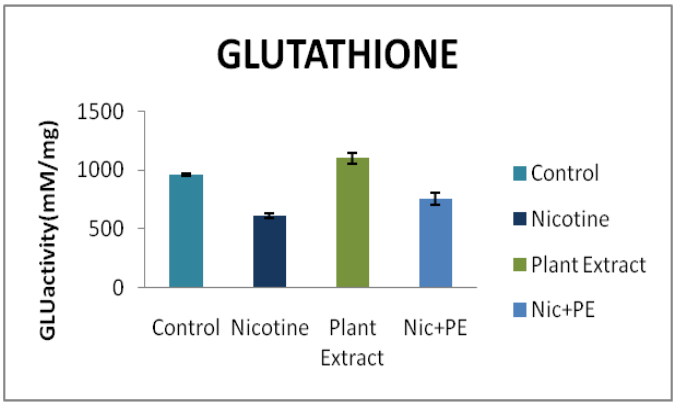

Figure 5C. Effect of Nicotine (3mg/kg Body weight/21 days I.P injection), with or without Chlb Plant extract on Glutathion level in the testis of male rat. Data expressed as mean± SE (P<0.05);

Sperm characteristics

As shown in table 1, the chronic treatment of male rats with NIC caused a significant decrease in sperm count and sperm motility as compared to normal. Supplementing Chlb extract to NIC treated rats significantly elevated sperm concentration and motility.

Discussion

Rats were chosen as the experimental animal in this study for their well-defined reproductive system and the fact that the compounds which could cause infertility in human males were also found to be active in rats. The finding of the study showed how root extract of C. borivilianum can reduce or reverse the toxic effects of nicotine on male reproductive system.

The male Wister rats treated with nicotine (3mg/kg body weight) for 21 days, showed a decrease in testicular weight. Testicular weight reduction is an important marker of gonadal toxicity. Jalil *et al* [20] reported NIC injection resulted in testosterone hormone and testis weight and impairment of reproductive variables which

supported the results of present study. Co- administration of Chlb root extract to NIC-injected rats enhanced the testicular weight in them

Decreased level of testosterone is one of the indicators of male reproductive toxicity as it is essential for growth of male secondary sex characters, sex organs and spermatogenesis. The observed decrease level of testosterone in NIC treated rats might be as a result of two reasons -a) decrease in pituitary output of LH and FSH which maintain testosterone level through hypothalamus-pituitary-testicular axis [21] and b) NIC may also cause disruption of cytoarchitecture of testis which consequently affect Leydig's cell number leading to decrease in testosterone [1]. The present study demonstrated supplementation of Chlb root extract in NIC-treated at significantly improved serum testosterone, LH and FSH suggesting that Chlb extract has stronger positive influence on testicular androgenesis. Testosterone and LH are essential for spermatogenesis and testes function. These two hormones significantly augmented after treatment with chlb extract suggesting that plant extract treatment improved spermatogenesis impairment and NIC-induced testicular toxicity. The restoration of sexual hormones activity rats with NIC by Chlb root extract may be due to significant protection of testicular cells by the root extract.

In the current study, injecting NIC in adult rats revealed a significant elevation of NO, and LPO as well significant decrease in SOD, catalase activities, and GSH level in testicular tissue as compared to control group. Nicotine has been found to disturb antioxidant defence mechanism, increased lipid peroxidation and lead to oxidative stress [9] which supported the results obtained in the present study. Thus, Chlb root extract supplementation restored the antioxidant parameters near to normal level. The protective effect of the plant extract may be due to the modulation of antioxidative systems, direct scavenging of ROS and decreased lipid peroxidation induced by NIC.

It is evident that i.p injection of NIC showed significant reduction in Sperm count and sperm motility. These findings were in line with other studies, which found that sperm count and motility of humans were adversely affected by smoking [1, 22]. The observed reduction in sperm indices may be due to action of NIC in generating reactive oxygen species. Reactive oxygen species (ROS), as a by product of nicotine acts in two ways. Firstly, it causes lipid peroxidation in spermatocyte membrane, resulting in decrease sperm motility and secondly, by damaging sperm nucear DNA reduces sperm number and fertilizing potential [23]. However, Present results showed that C. borivilianum treated rats had significantly increased weight, sperm count and motility in them.

Since, crude extract of C. borivilianum was used in present study, the findings obtained could be due to presence of antioxidant polyphenol as revealed by FTIR spectroscopy [11, 24] which may cause significant reduction in free radicals such as NO, and preservation of antioxidant capacity through maintaining endogenous antioxidant enzymes in testis.

Conclusion

Our study reinforced the prominent role of ROS as contributing factor in nicotin-induced testicular toxicity and also elicited that the root extract of Chlorophytum borivilianum could be protective in nicotine –induced testicular damage for its anti oxidative and cytoprotective effect caused by nicotine. Thus, the present study suggests that the C. borivilianum may have protective action against nicotine induced testicular toxicity and could be used as supplement, especially for cigarette smokers, for healthy reproductive life.

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