ORIGINAL ARTICLE

Effect of phoenix dactyliferia (date palm) pit powder on nicotine induced spermatotoxicty in adult albino mice

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Abstract

Objective: To study the effect of Phoenix Dactyliferia pit powder on nicotine-induced spermatotoxicty in adult albino mice.

Methods: The study was conducted at the University of Health Sciences, Lahore, from February to November 2012, and comprised adult male albino mice aged 6-8 weeks. The animals were divided into five equal groups. Group A consisted of controls who were treated with 1.5ml/kg of normal saline for 15 days, while nicotine 0.5mg/kg was administered intraperitoneally daily to experimental groups B, C and D for the first 15 days. Group B was sacrificed on the 15th day to confirm toxicity, whereas nicotine treatment was stopped in groups C and D. Group C was given normal saline (1.5ml/kg) whereas group D was given date palm pit powder 500mg/kg for the next 30 days. However, Group E was given nicotine 0.5mg/kg for 45 days and date palm pit powder was added orally from the 16th day and it continued daily till the end of the experiment. SPSS 18 was used for statistical analysis.

Results: The mean weight of each of the 40 animals in the study was 30±5gm, and all the five groups had 8(20%) mice each. Group B exhibited features of toxicity evident by statistically significant decrease in Johnsen score ($p<0.001$) and diameter of seminiferous tubule ($p<0.001$). Group C showed partial reversal of toxic effects but these positive effects were less compared to group D which showed complete reversal of toxicity evident by statistically significant increase in Johnsen score ($p<0.001$) and diameter of seminiferous tubule ($p<0.001$). However, reversal of toxic effect was not evident in group E.

Conclusion: Partial recovery from nicotine-induced spermatotoxicity occurred after withdrawal of nicotine treatment whereas near normal restoration of structure was seen with administration of date palm pit powder after the stoppage of nicotine.

Keywords: Nicotine, Toxicity, Phoenix Dactyliferia, Spermatogenesis. (JPMA 65: 43; 2015)

Introduction

Nicotine is colourless, volatile and highly toxic alkaloid which constitutes 0.3% to 5% of the tobacco plant by dry weight.[1] Nicotine can be absorbed directly into the blood through the skin or alveolar membrane.[2]

Nicotine has been found to yield harmful effects on the gonads both through direct and indirect mechanisms. Direct impairment could be disruption of blood testicular barrier producing detrimental effects on the spermatogenic cell lineage.[3] Nicotine has also been reported to exert indirect toxic effect on gonad through its action on hypothalamic-pituitary-gonad axis.[4] Deleterious effects of nicotine are partly due to increased production of reactive oxygen species.[5]

There has been a surge in usage of herbal medicine both in developed and under-developed countries. One such herbal product is Phoenix Dactylifera (date palm). Though date palm has since ages been considered a complete food in some countries, there are few reports supporting its medicinal values, including promotion of spermatogenesis.[6]

It has been reported that the aqueous and ethanolic extracts of the date palm fruit and date palm pits are effective in ameliorating the severity of gastric ulceration; the effect is assumed to be due to anti-oxidant properties of date palm.[7]

Literature also documents the protective effect of date palm pit on toxicity produced by methylprednisolone on testis shown by significant increase in testosterone level in serum of male albino rats and increased spermatogenesis.[8] Similarly, administration of date palm on account of its antioxidant characteristics reverses spermatotoxicity produced by mercury.[8] Date palm pollen have shown protective effect on cadmium-induced toxicity probably by activation of testicular, endocrine and antioxidant system.[9]

Toxic effects of nicotine on spermatogenesis are well known. Effect of date palm pit powder to promote

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spermatogenesis both in the presence and absence of toxic substances is also well known. However, there is hardly any study using date palm pit powder to combat nicotine-induced toxic effects on spermatogenesis. The present study was designed to study these effects in albino mice.

Materials and Methods

The study was conducted at the experimental research laboratory of the University of Health Sciences, Lahore, for a period of one year from February to November 2012 and comprised adult male albino mice aged 6-8 weeks procured from the National Institute of Health, Islamabad. The healthy animals were weighed and housed in 10 cages of appropriate size. Animals were kept in a controlled environment at room temperature of 23±2°C, humidity of 50±5% and light and dark cycles of 12 hours each. Animals were fed on normal chow and given water ad libitum. They were allowed to acclimatise for a period of two weeks before the experiment started.

The mice were divided randomly into five groups. The control group A was treated with 1.5ml/kg of normal saline orally for 15 days and was then sacrificed. Groups B, C and D were given daily intraperitoneal injections of 0.5mg/kg10 of nicotine dissolved in normal saline for 15 days to confirm toxicity. Group C was then put orally on normal saline 1.5ml/kg and group D on date palm pit powder 500mg/kg for the next 30 days. Group E was given nicotine 0.5mg/kg for 45 days continuously and date palm pit powder 500mg/kg was added on the 16th day and was continued for the next 30 days i.e. the 45th day. The animals were sacrificed under chloroform anaesthesia to remove both testes.

Each testis was sectioned along midline and immersed immediately in Bouin's fixative for 24-48 hours. Each half of the testis was processed in automatic tissue processor (Histotech III-USA) and paraffin blocks were prepared. Sections 4μm thick were obtained and stained with haematoxylin and eosin (H&E) and Periodic acid Schiff method in a usual way.

Colour, shape, texture and paired testicular weight were recorded and compared between the control and experimental groups. Mice were weighed weekly and observed daily for their appearance, activity and signs of morbidity and good health.

Johnsen findings of H&E-stained sections at X400 were recorded, interpreted and quantified in line with literature.[11] According to criteria, 10 is complete spermatogenesis with many (≥5) spermatozoa; 9 means many (≥5) spermatozoa present but germinal epithelium disorganised with marked sloughing or obliteration of lumen; 8 is only a few (<5) spermatozoa; 7 is no spermatozoa but many spermatids (≥5); 6 is no spermatozoa only a few (<5) spermatids; 5 is no spermatozoa or spermatids but many spermatocytes (≥5); 4 is no spermatozoa or spermatids and only a few spermatocytes (< 5); 3 is spermatogonia, only germ cell present; 2 is no germ cells but sertoli cells are present; 1 no cells in tubular section.

The scoring was performed at X40 objective exposing several tubules. In case of doubt the presence of spermatozoa was checked at higher magnification. Tubules in one field at one corner were chosen, scored and the slide was moved in figure of 'S' to bring adjacent area within the field and the scoring continued. Damaged tubules at the edges of section were excluded. Minimum of 10 seminiferous tubules from each of the slides were studied and scored. Three stained slides from each animal were examined and total of 1200 observations were made.

The diameters of seminiferous tubules were measured using Leica 1000 DM microscope using objective power of X100. The ocular micrometer was calibrated with stage micrometer. Transversely sectioned profiles of seminiferous tubules were used, and diameter of each was recorded twice at right angle to each other; the mean diameter of each of these seminiferous tubules was multiplied with the calibration factor. Six seminiferous tubules were used from each slide and three slides from each animal were used.

Data was analysed using SPSS 18. Mean ± standard deviation (SD) was given for quantitative variables, including animal body weight, testicular weight, Johnsen score and diameter of seminiferous tubule. The outcome in quantitative measurements was tested by one way analysis of variance (ANOVA). Post-Hoc Tukey's test was applied to identify which group mean differed. $P \leq 0.05$ was considered statistically significant.

Results

The mean weight of each of the 40 animals in the study was 30±5gm, and all the five groups had 8(20%) mice each. All animals of control group A remained active and healthy. However, nicotine treated animals of experimental groups B, C, D and E showed variable degree of restlessness, irritability and weakness. These symptoms ceased after stoppage of nicotine administration and treatment with date palm pit powder. Group B exhibited features of toxicity evident by statistically significant decrease in Johnsen score ($p<0.001$) and diameter of seminiferous tubule ($p<0.001$). Group C showed partial reversal of toxic effects but these positive effects were less

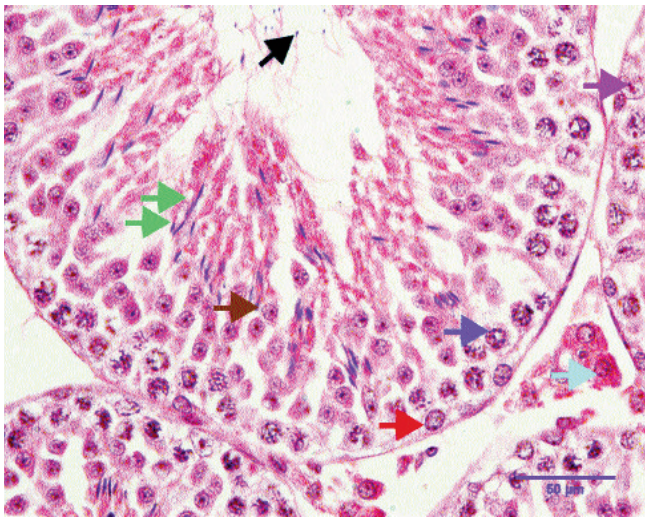

Figure-1: Photomicrograph of testis from group A illustrating the typical structure of seminiferous tubule at Johnsen score 10 showing all stages of spermatogenesis, spermatogonia close to the basement membrane (dark red arrow) primary spermatocytes (dark blue arrow), round spermatid (brown arrow),elongated spermatid (green arrows) and mature spermatozoa(black arrow) seen. Interstitial cells of Leydig (light blue arrow) conspicuous between intertubular area. Sertoli cells seen (pink arrow). Hematoxylin and eosin stain.

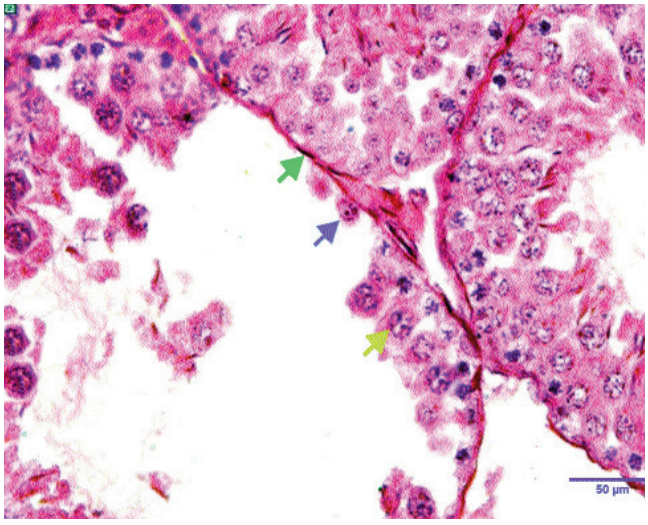

Figure-2: Photomicrograph of testis from group B treated with nicotine for 2 weeks illustrating structure of seminiferous tubule showing Johnsen of 5. Tubule contains spermatogonia (blue arrow), primary spermatocytes (yellow arrow). Spermatids and spermatozoa are not observed. Myoid cells (green arrow) seen outside the basement membrane and disruption of normal arrangement of seminiferous epithelium. Hematoxylin and eosin stain.

compared to group D which showed complete reversal of toxicity evident by statistically significant increase in Johnsen score ($p<0.001$) and diameter of seminiferous tubule ($p<0.001$). However, reversal of toxic effect was not

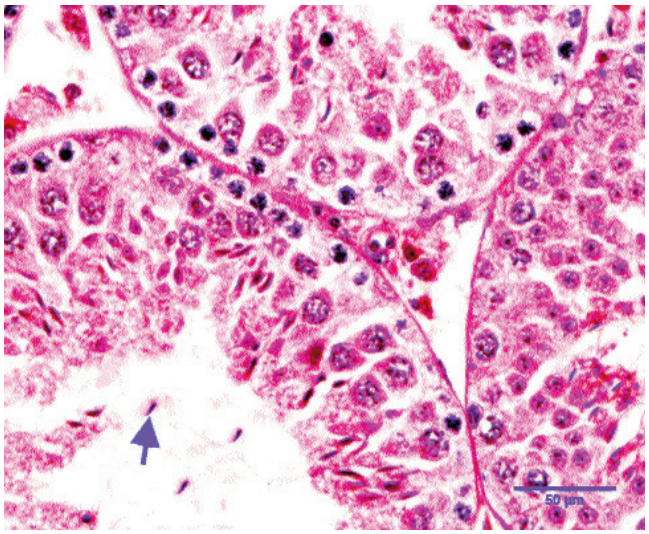

Figure-3: Photomicrograph of testis from group C illustrating typical structures of a Seminiferous tubule with a Johnsen of 8 with partial restoration of spermatogenic cell line. Elongated spermatids seen (dark blue arrow). Hematoxylin and eosin stain. X400.

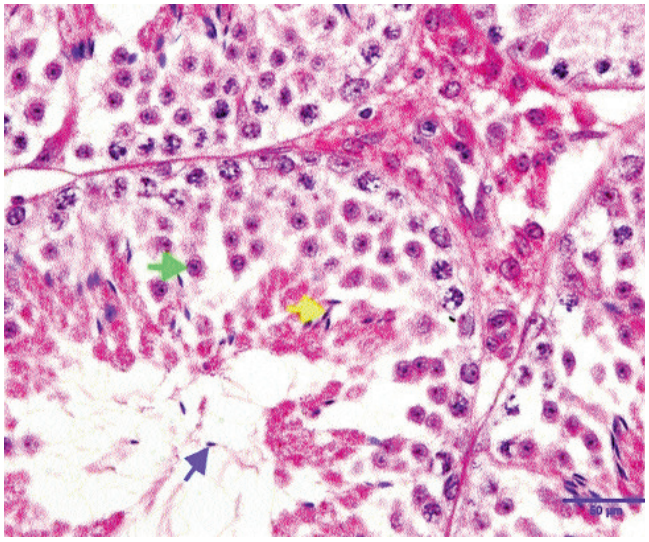

Figure-4: Photomicrograph of group D illustrating part of seminiferous tubule with a Johnsen score of 9. All stages of spermatogenic cell line are seen. Round spermatids (green arrow), elongated spermatid (yellow arrow) and mature spermatozoa in large number (dark blue arrow) seen. Haematoxylin and eosin stain. X400.

evident in group E (Table).

At the end of experimental period animals were sacrificed under anaesthesia. There was no significant difference in colour, shape and texture of testis in the different groups.

Histological examination of testes from control group revealed that normal seminiferous epithelium with all

Table: Comparison among groups.

Parameter	Group A Mean±SD n=8	Group B Mean±SD n=8	Group C Mean±SD n=8	Group D Mean±SD n=8	Group E Mean±SD n=8	P-value
Animal weight in gm at the start of experiment	31.50±2.44	31.63±1.76	32.50±2.20	32±1.77	31.25±1.83	0.762
Animal weight in gm on 14th day of the experiment	37.38 ± 1.68	24.88±3.44	26.13±2.53	26.25±1.38	26.38±1.4	0.001*
Weight of paired testis in gm.	0.21 ± 0.03	0.14 ± 0.03	0.16±0.03	0.21±0.03	0.19±0.02	0.01*
Diameter of seminiferous tubule in microns	188.3±29.2	127.4±18	175.4±17.9	177.9±23.1	142.4±24.4	0.001*
Johnsen Score	9.5±0.5	5.6±0.8	7.1±0.9	8.7±0.4	7.2±1.3	0.001*

P value <0.05 is considered statistically significant*
n = number of the animal.

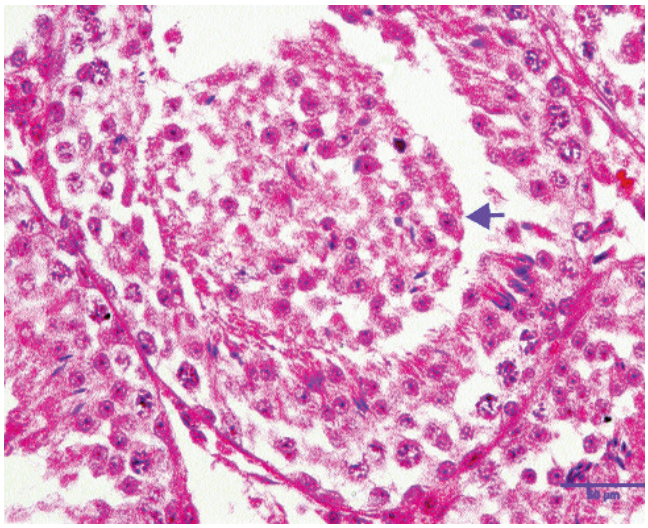

Figure-5: Photomicrograph of group E showing section of seminiferous tubule with Johnsen score of 6. Mature spermatozoa are not discernible. Few elongated spermatids with many rounded spermatids seen. There is sequestration of epithelium towards the center of lumen (dark blue arrow). Haematoxylin and eosin stain. X400.

stages of spermatogenesis was present (Figure-1). However, group B showed disruption of normal epithelium with absence of mature sperms (Figure-2). Group C showed partial reversal of these effects with presence of round and elongated spermatids (Figure-3). Group D showed restoration of near normal spermatogenic lineage on nicotine withdrawal and subsequent treatment with date palm pit powder (Figure-4). However, group E exhibited features of toxicity still evident by absence of mature spermatozoa and sequestration of spermatogenic cell towards centre of lumen, an evidence of loss of coherence between the cells (Figure-5).

Discussion

The study revealed a statistically significant reduction in mean Johnsen score in testes of nicotine-treated mice, showing toxic influence of nicotine on spermatogenesis. Johnsen criteria offer a convenient and rapid method for quantitative analysis of spermatogenesis.[11] Mean Johnsen score in control group (A) was 10 implying complete spermatogenesis with ≥5 spermatozoa per tubule, whereas Johnsen score dropped to 5 in B group after 15 days of nicotine treatment, indicating that neither spermatid nor spermatozoa were present but tubules contained ≥5 spermatocytes per tubule. This finding also corroborated the previous finding related to the effects of nicotine on spermatogenesis resulting in degeneration of germ cells and accumulation of lipid droplets in sperm.[12]

Similar effect of significant reduction in the number of spermatogenic cell lineage has been shown previously.[5] Both reduction in spermatogenic cell lineage and abnormalities of sperm head, affecting sperm count and motility were seen. Our results are also in agreement with a study[13] in which the administration of nicotine resulted in disappearance of elongated and round spermatids. There are also reports that show nicotine acts as an endocrine disruptor for the male hormone profile and testicular function probably by inhibiting the release of follicle-stimulating and luteinising hormones through its action on the hypothalamo-hypophyseal-gonadal axis.[14]

Our observations on experimental groups revealed that administration of date palm pit powder to nicotine-treated albino mice resulted in improvement of spermatogenesis as confirmed by increase in mean Johnsen score.[11] Group C, given nicotine for 15 days followed by normal saline for one month, also showed some improvements in the score but this was not statistically significant when compared with that of group A. However, the results of groups C showed statistically significant difference in mean Johnsen score when compared with that of group B, indicating a partial restoration of spermatogenesis after withdrawal of nicotine.

Administration of date palm pit powder for 30 days had ameliorated toxic effects of nicotine, given for 15 days, on spermatogenesis, as was evident by significant improvement in mean Johnsen score to 9 in group D.

However, there was partial improvement in Johnsen score in group E wherein the animals were given nicotine for 45 days but pit powder duration was only 30 days. It then appeared that long-term nicotine treatment would cause severe deleterious effects and would need extended treatment with pit powder. Detailed studies are needed in this direction. Our investigations also agreed with those reported by a study[15] which showed that administration of date palm pit powder resulted both in an increase in sperm density and testosterone level, indirectly promoting spermatogenesis.

Administration of green tea to nicotine-treated animal model resulted in amelioration of toxicity, probably through an antioxidant effect, as evident by improved spermatogenesis, restoration of tubular diameter and increase in Leydig cell count.[15] Administration of vitamin E, another antioxidant, to nicotine-treated mice resulted in recurrence of many elongated spermatids and mature spermatozoa in previously nicotine affected seminiferous tubule.[13] Taking a lead from these studies, we can postulate that amelioration seen in our study was most likely related to an antioxidant effect of date palm pit powder on nicotine-induced spermatotoxicity.

Our results indicate that the average diameter of seminiferous tubule had statistically decreased in group B when compared with that of A group ($p<0.05$). The decrease in tubular diameter was consistent with the findings reported earlier[16] when nicotine was injected intraperitoneally for 2 weeks. In the current investigation the diameter of seminiferous tubule returned to near normal in groups C and D. This finding is consistent with earlier ones[17] reporting restoration of tubular diameter to near control level upon administration of green tea to nicotine-treated mice. The earlier study attributed this to an antioxidant nature of green tea to combat nicotine toxicity for restoration of spermatogenesis. The present investigation also showed restoration of tubular diameter. This increased in tubular diameter was more in group D (date palm pit powder) when compared to that of group C (saline treated), implying that amelioration of toxic effect is more in group D when compared with group C.

Diameter of seminiferous tubule was used as an indirect measure of spermatogenic function as increased surface area of spermatogenic epithelium anchors more Sertoli cells and spermatogonia.[18] Relative increase in diameter was likely indicative of androgenic effect as reported earlier.[19] Furthermore, an increase in sperm production without any change in tubular diameter was reported earlier.[20] He attributed it to an antioxidant effect of Tribulus terrestris whereas date palm was reported to possess both androgenic and antioxidant properties.[21]

The result of present data showed that nicotine administration resulted in both reduced body and testicular weight. This reduction in body weight was in agreement with that reported by study[22] that showed that blocking nicotinic receptor by mecamylamine, a nonselective nicotinic acetylcholine receptor antagonist, present in brainstem would promote food intake by blocking dopaminergic pathway.

Statistically significant difference in mean weight loss on 14th day on nicotine administration in groups B, C, D and E was observed when compared with that of group A. This finding was consistent with earlier work[23] that attributed weight alteration with reduced amount of intake of food. This weight-loss was ameliorated with the administration of date palm pit powder as observed in group D. Group C also showed improvement in weight after withdrawal of nicotine. However; group E exhibited weight-loss in the earlier period of experiment only. At the end of experiment, the difference was statistically insignificant between groups D and E, suggesting that administration of date palm pit powder had ameliorated the weight-loss produced by nicotine treatment when given simultaneously or when nicotine was stopped after 15th day.

Our study also demonstrated that nicotine administration resulted in statistically significant reduction in paired testicular weight in groups B and E. The decreased in testicular weight was attributed to reduced size of seminiferous tubules due to decreased number of spermatogenic cells. These findings were in agreement with an earlier report[24] in which it was stated that a drop in testosterone level was responsible for decreased spermatogenesis and hence decrease in testicular weight. These results were also in accord with those of a study[10] which reported that prolonged nicotine used in mice produced reduction in testicular weight and atrophy of male accessory sex glands, due to the androgenic depletion.

However; testicular weight in groups C and D showed a statistically significant increase, indicating amelioration of toxicity as confirmed by increase of spermatogenesis in groups C and D. Increased weight-gain of testis upon administration of date palm pit powder in group D was in agreement with previous investigations in which goat milk (antioxidant action) was given to nicotine-treated mice[25] which resulted in improved fertility in male rats evident by increased sperm count and number.

The present work clearly depicts that administration of nicotine resulted in statistically significant reduction in

animal weight, paired testicular weight, tubular diameter and reduced generation of spermatogenic cell line which significantly improved with the administration of date palm pit powder.

Conclusion

There was reduction in Johnsen score for spermatogenesis and reduction in diameter of spermatogenesis in nicotine-treated mice. It appears that date palm pit powder improves the process of spermatogenesis and diameter of seminiferous tubules in nicotine-induced testicular toxicity. The findings of current investigation may provide a cheap and natural source of treatment in cases of male infertility induced by persistent consumption of nicotine.

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