ORIGINAL ARTICLE

Toxic Effect of Nicotine on Leydig Cell Count and Testosterone Levels in Adult Albino Mice and its Protection by Date Palm Pit Powder

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ABSTRACT

Aim: To Evaluate The Protective Role Of Date Palm Pit Powder Against Toxic Effect Of Nicotine On Leydig Cell Count And Testosterone Levels In Adult Albino Mice.
Study design: Laboratory based randomized controlled trials.
Place and duration of study: The study was carried out at experimental research laboratory University of Health Sciences and Anatomy Department, Lahore from February to November 2012.
Methods: Thirty two male albino mice 6-8 weeks and weighing 30±5gm were used; these were divided randomly into groups A, B, C and D. Group A served as a control while groups B, C and D were experimental groups. The control group A was treated with 1.5ml/kg of normal saline orally for 15 days. Groups B, C and D were given daily of 0.5mg/kg of nicotine intraperitoneally dissolved in1.5ml/kg of saline for the first 15 days of experiment. Group B was sacrificed on 15th day to confirm toxicity. Groups C was given nicotine treatment till the 15th day and were then put on date palm pit powder (500mg/kg) for next 30 days. However for Group 'D' injections of 0.5mg/kg of nicotine dissolved in1.5ml/kg of saline was continued daily for next 30 days. Date palm pit powder was subsequently added orally on 16th day and was continued daily till the end of experiment (45th day).
Results: The results of present study showed that group B mice exhibited features of toxicity evident by statistically significant decrease in Leydig cell count and mean serum testosterone levels. Group C showed reversal of toxic effects. However; reversal of toxic effect is not evident in group D which was given nicotine and date palm pit powder together till the end of experiment.
Conclusion: The current work showed that date palm pit powder has a preventive effect on nicotine induced spermatotoxicity in adult albino mice only on stoppage of nicotine. .
Keywords: Nicotine, Date Palm Pit Powder (DPP), Mice, Leydig Cell Count, Testosterone Levels

INTRODUCTION

Cigarette Smoking is globally known to affect health. The Cigarette smoke contains lots of known toxins including nicotine[1] . Nicotine had been reported to produce toxic effects on Leydig cell count[2] .

Spermatogenesis is a highly controlled process under endocrinal and intrinsic mechanism. It results in transformation of spermatogonial stem cells to mature spermatozoa. Testosterone secreted from Leydig cells, inhibin from Sertoli cells and estradiol formed by conversion of testosterone control gonadotropin secretion on hypothalamus and pituitary by negative feedback, thus affecting spermatogenic method[3]. The Cigarette smoke exposed models had significantly decreased seminiferous tubules diameter, epithelial height and Leydig cell count and increased proportion of tubules with germ cell loss.

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Phoenix dactylifera, possess potent anti-oxidant and anti-mutagenic actions[4]. Date fruit had also been found to have gonadotrophic effects[5].

Literature also acknowledged the protective effect of date palm pit on toxicity produced by mercury & Cadmium which was manifested by amelioration of reduced weight of the reproductive organs, increased oxidative stress, reduced Johnson's score and decreased serum testosterone level [6,7].This antitoxic effect may be attributed to antioxidant properties of phoenix dactyliferia. Treatment with DPP can ameliorate the deleterious effects of Cadmium, probably by activating testicular endocrine and antioxidant system[8].

Nicotine suppress spermatogenesis due to the oxidative stress produced by free oxygen radicals and on promise that DPP possesses strong anti oxidant properties the present study was therefore designed accordingly.

MATERIAL AND METHODS

Date fruit was purchased from local market; its flesh was manually separated from the pit and pit was grinded to obtain its fine powder form. Thirty two

adult male albino mice 6-8 weeks old & weighing 30±5gm, of BALB/c strain were procured from National Institute of Health, Islamabad. Animals were provided with an optimium environment. Animals were fed on usual chow diet and were allowed to acclimatize. Thirty two mice were randomly divided into four experimental groups A, B, C & D each group consisting of eight animals.

At the end of experimental period each animal was anaesthetized with chloroform and blood samples were drawn by cardiac puncture and centrifuged. The clear serum was collected and stored at -20ºC for biochemical estimations. A vertical midline incision was given extending from xiphoid process to the pubic symphysis. The testes were pushed into the body cavity and removed by pulling the tail of epididymis. The tissue was immersed in Bouin's fixative immediately for 24 - 48 hours followed by processing in automatic tissue processor (Histotech III-USA). 4µm thick Sections were stained with Hematoxylin and eosin. Leydig cell count was measured using Leica 1000 DM microscope after calibrating 40X objective lens with ocular grid. Cells were counted in all square of grid superimposing the tubules excluding those lying on the upper and right edges of the grid. Counting was done in 5 randomly selected areas in each slide. Three slides were taken from each animal and mean number of Leydig cell was calculated for each animal and each group.

Statistical analysis: The collected information was analyzed using SPSS version 18. Mean±SD is given for quantitative variables. Any difference in the quantitative measurements was tested by one way ANOVA. Post-Hoc Tukey's test was applied to identify which group mean differed. The p values<0.05were considered statistically significant.

RESULTS

Fig**.** 1**:** Photomicrograph of testis from group A illustrating Leydig cells (dark blue arrow) with mean±S.D of 166.2±27.02 between two seminiferous tubule. H&.E. X1000

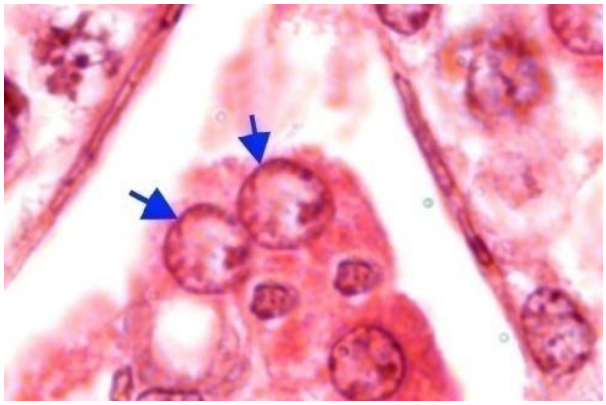

Fig**.** 2 Photomicrograph of testis from group B illustrating decrease in Leydig cellsnumber (dark blue arrow) with mean ± S.D of 83.3 ± 29.87 between two Seminiferoustubule. H&E. X1000.

Fig. 3 Photomicrograph of testis from group C illustrating greater increase in number of Leydig cells (dark blue arrows) with a mean ± SD of 172.5±36.92 b/w two seminiferous tubules. The increase is more than that of control group H&E. X1000.

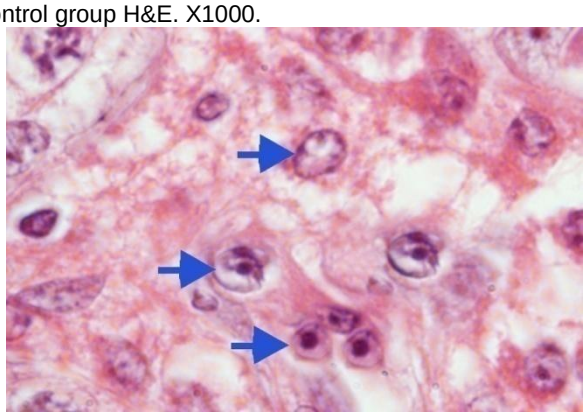

Fig. 4 Photomicrograph of testis from group D illustrating Leydig cells (dark blue arrow) with mean±SD of 100.1±16.7b/w two insignificant increase of Leydig cell number seen. H&E. X1000

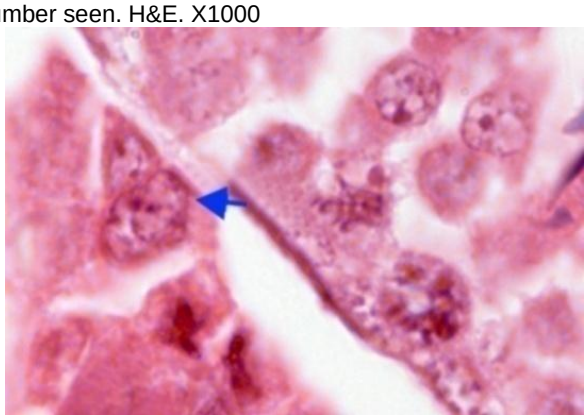

Table 1 Experimental Groups

Anmal G	Intervention	Dose	Route of administration	Duration
Group 'A'	Saline	1.5ml/kg	orally	15 DAYS
Group 'B'	Nicotine	0.5mg/kg dissolved in 1.5ml/kg saline	intraperitoneally	daily for 15 days
Group D	Nicotine	0.5mg/kg dissolved in 1.5ml/kg of saline	Intraperitoneally	daily for 15 days
	DPP	500mg/kg	Orally	16^{th} -45^{th} days
Group E	Nicotine	0.5mg/kg dissolved in 1.5ml/kg of saline	Intraperitoneally	daily for 45 days
	DPP	500mg/kg	Orally	16^{th} -45^{th} days

Table 2**:** One way ANOVA showing difference in the mean leydig cell count (cells/mm^2) & Serum testosterone levels (ng/ml) among control and experimental groups *p ≤ 0 .05 is considered statistically significant

Variables	Group A Mean ± SD n=8	Group B Mean ± SD n=8	Group C Mean ± SD n=8	Group D Mean ± SD n=8	p-value
Leydig cell count	166.2 ±27.07	83.3±29.9	172.5 ± 36.92	100.1 ± 16.7	0.01*
Serum testosterone	4.57 ±1.07	0.53 ±0.28	5.06 ±2.29	5.06 ±2.2	0.01*

DISCUSSION

Mean Leydig cell count showed statistically significant decrease ($p < 0.001$) in nicotine treated group B. It showed a statistically significant increase with administration of DPP. Decrease in group B and D had been found statistically significant when compared with groups A and C. Present work clearly depicts toxic effect of nicotine on Leydig cells in treated groups both for shorter (15 days) and longer duration (45 days). Similar results had been reported by Cohen and Duke (1984) who stipulated that nicotine produced specific intracellular reaction which resulted in cell death. However a statistically significant rise of Leydig cells is seen in group C when compared to that in groups B and D. Similar findings of increase in Leydig cell count and restoration of normal tubular architecture upon administration of date palm fruit to mercuric chloride toxicated rabbits were reported by Yasmina et al[9]. The suggested mechanism of action most likely is the presence of certain steroidal substances present in date palm pit powder responsible for increase of leydig cell count [10,11,12].

The results of present investigations showed a statistically significant reduction in serum testosterone levels with administration of nicotine in group B when compared with that of A. These findings agreed with those of Bose et al[13] who found that nicotine block transport of cholesterol across mitochondrial membrane by inhibiting the action of steroidogenic acute regulatory protein (STAR); cholesterol is a substrate consumed by STAR and contributes towards synthesis of Testosterone.

The current investigations showed that testosterone levels showed statistically significant increase upon administration of DPP in group C. Similar findings were also reported by Shariati[12] who documented that daily oral administration of pits of date palm caused statistically significant increase in testosterone level in serum of male albino mice.This finding was in accordance with the work done by Yasmin A et al.[9] who showed amelioration of testosterone levels upon administration of vitamin C and date palm to mercuric chloride intoxicated rabbits. These results were also in agreement with those reported by Ibrahim et al[14] who documented an increase in testosterone levels with administration of selenium, an important constituent in date palm, in testicular injury induced by high fat diet. Results of the present study have shown that nicotine induced damage in testis is ameliorated by DPP treatment as shown by significant improvement of number of Leydig cell count and Mean serum testosterone levels.

CONCLUSION

The result of present study suggest that DPP improves Leydig cell count and serum testosterone levels in nicotine induced testicular toxicity.

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