ORIGINAL ARTICLE

Effects of Honey on Lead Induced Changes in Spermatogenesis

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ABSTRACT

Objective: The objective of this study was to observe changes in spermatogenesis testes of albino rats exposed to intraperitoneal lead acetate and to look for the reversibility of these changes after cessation of lead acetate and subsequent oral administration of honey.

Study Design: Experimental animal study.

Place and Duration of Study: National Institute of Health Islamabad from January to June, 2009.

Materials and Methods: Animals were obtained from the animal house of N.I.H and were divided into three groups A, B and C. Group A was subdivided into two groups A-I and A-II. Group B was also subdivided into two sub groups; B-I & B-II. Group C was not subdivided into subgroups. The animals in group A were used as control, while those of groups B and C were treated with lead acetate that was given intraperitonially in the dose of 4mg/kg body weight, 5 days a week for 6 weeks. The animals in group B-I were sacrificed at the end of six week to observe the toxic changes while animas in group B-II were kept alive for another 6 weeks on normal diet. The animals in group C were given honey in dosage of 10ml/100ml water with normal diet for further 6weeks. These groups (B-II and C) were then sacrificed after 12 weeks to observe the effects of honey on spermatogenesis.

Results: The **h**istological comparison of testes of both groups of animals showed that after six weeks, the width of germinal epithelium and the number of spermatogenic cells had decreased in lead toxic groups as compared to the control rats ($p<0.05$) and in majority of the seminiferous tubules, the basement membrane was disrupted. The width of germinal epithelium, and the number of spermatogenic cells were improved after oral administration of honey.

Conclusion: This study provides evidence that lead has toxic effects on testis which are partially reversible on oral intake of honey.

Key Words: *lead, testes, rat, honey.*

Introduction

In Pakistan people are specially exposed to lead pollution through three main sources i.e., air, soil, and water.[1] Lead toxicity induces rupture of nuclear membrane accompanied by fragmentation of nucleus in testis (Karyorrhexis).[2] In the females, lead toxicity results in irregular menstrual periods, decreased ovarian weight, decreased corpora leutea.[3] The common sources of lead are:

1. When the lead rich gasoline comes in contact with the soil, it gets contaminated.[4]
2. Workers exposed to high levels of lead in refineries and smelters. They also come in contact while manufacturing of lead batteries and cables, rubber and (PVC) plastic products.[5]
3. The center for disease control (CDC) discovered that there is no toxic threshold for lead. This means that there is no measurable level of lead in the body below which no harm can occur.[6] Lead toxicity has shown to disrupt both spermatogenesis and steoidogenesis.[7]Thus; it becomes imperative to find out measures, by which our body can maintain and regulate healthy living and homeostasis even if exposed to high levels of lead toxicity. The standard treatment of lead toxicity is chelation therapy which has many side effects.[8] Honey is a sweet, golden

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coloured, viscous liquid food produced in the honey sac sofvarious honey bees. Its value in treating burns, infected surgical wounds and ulcers is established. Its viscosity enables it to absorb water from surrounding inflamed tissue. Honey is remedial in cases of persistent coughs and sore throat.[9] It provides an important part of energy needed by the body to combat infections, and for blood formation.[10] Honey is cheap, easily available, that's why I have use honey for my research project.

Materials and Methods

A total of 50, eight weeks old healthy adult male albino rats of Sprague Dawley strain, weighing 200±10 gm were used in the study. These animals were randomly divided into three groups; one was labeled as Control (A), while the other was labeled as Experimental (B and C) group. These animals were randomly divided into three groups. Day 0 was considered to be the starting day of experiment.

1. Group A was further subdivided into two subgroups; A-I &A-II (each group having 10 animals)
2. Group B was also subdivided into two sub groups; B-I & B-II (each group having 10 animals)
3. Group C with 10 animals was not subdivided into subgroups.
4. A-I Group was control group for lead toxic group B-I. Both groups were sacrificed at six weeks.
5. A-II Group was control group for B-II and C. Both groups were sacrificed at twelve weeks. All animals were kept in the animal house under standard conditions at a room temperature ranging between18o C to 26o C for six weeks. They were maintained on 12 hours light and dark cycle. The rats were fed ad libitum. Day 0 was considered to be the starting day of experiment. The animals in group B-I, B-II and C were treated with intraperitoneal lead acetate in the dosage of 4mg / Kg body weight / day, 5 days a week for a period of 6 weeks. The animals in group B-I were sacrificed at the end of six week to observe the toxic changes while animals in group B-II were kept alive for another 6 weeks on normal diet. The animals in group C were given honey in dosage of 10ml/100ml water with normal diet for further 6weeks. These groups (B-II and C) were then sacrificed after 12 weeks to observe the effects of honey on spermatogenesis. After sacrifice, each animal was taken out of the jar and placed on the dissecting board. The scrotal sac was then opened with the help of forceps and scissors. Testes were examined with the help of hand lens and their colour, consistency and gross appearance was noted. The testes were fixed in Formalin and then processed for paraffin embedding. Five micrometer thick section were cut, stained with Hematoxylin & Eosin, and observed microscopically for germinal epithelium thickness and to study the cells of spermatogenic series.

Statistical Analysis

The data was entered into SPSS version 13.0. Analysis of variance (ANOVA) was used to compare the change in variables between the groups. Mean and standard deviation of the parameters were calculated and results of different study groups were compared. P-value of < 0.05 was considered significant.

Results

All the rats in control group A remained

active and healthy with normal feeding behavior. After six weeks, the animals in group C were relatively more active than Group B-II animals. The testes of rats exposed to toxic dose of lead (Group B-I & B-II) showed reduction in size; they were pale looking, tough in consistency and showed resistance on cutting. It was difficult to pluck out any tubule from the testes and stringing out phenomenon was absent. The testes of the rat in group C were light pink in color and firm in consistency. Their blood vessels were visible under magnifying glass and showed mild resistance on cutting. The animals in this group gained significant weight increase in the testes i.e. 1.27 gm. (SD ± 0.040) in comparison with group B-II.

Germ Cell Count / Cross Section of Seminiferous Tubule:

The average germ cell count in group A-I was 304.53 cells/cross section of seminiferous tubule (SD + 28.46), in group A-II it was 323.53 cells/unit (SD = 28.46), in group B-I it was 116.91 cells/unit (SD + 32.66), in group B-II it was 136.24 cells/unit (SD + 33.51) and in group C it was 214.58 cells/unit (SD + 33.51). The difference between all the groups was significant ($p < 0.001$). The germ cell count was significantly higher in group A-I as compared to groups B-I, B-II and C ($p < 0.001$) (Figure 1). The difference between groups A-I and A-II was insignificant ($p > 0.467$). In group A-II, the germ cell count was significantly higher as compared to groups B-I, B-II and C ($p < 0.001$) but the difference from group A-I was insignificant ($p > 0.467$). The germ cell count was significantly lower in group B-I as compared to groups A-I, A-II and C ($p < 0.001$) but the difference from group B-II was insignificant ($p > 0.449$) Figure 2. In group B-II, the germ cell count was significantly lower as compared to groups A-I, A-II and C ($p < 0.001$) but insignificant from group B-I ($p > 0.449$).

The germ cell count in group C was significantly lower as compared to groups A-I and A-II but significantly higher as compared to groups B-I and B-II, ($p < 0.001$). (Figure .3) & Table No.II

The testes of rats in the control group A were easily pushed out of the scrotal sac, well vascularized and were soft in consistency. They were pink in colour and on cutting, gave little resistance. The seminiferous tubules had normal plucking and stringing out phenomenon of the tubules. The mean weight of pairedtestes was 1.28 gm (SD + 0.04).The testes of rats exposed to toxic dose of lead (Group B) showed reduction in size; they were pale looking, tough in consistency. It was difficult to pluck out any tubule from the testes. The mean weight of paired testes was 1.16 gm (SD ± 0.029) in Group B .

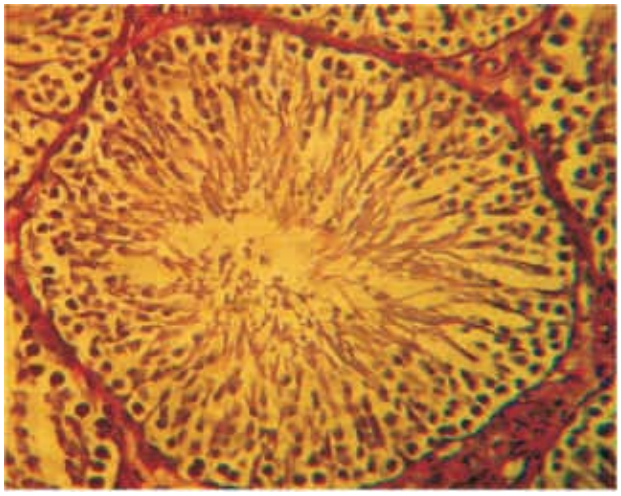

Figure-1: Photomicrograph; testis cross section, seminiferous tubule of control group A- I, animal1 number 1. H & E x 400

The epithelial height was significantly

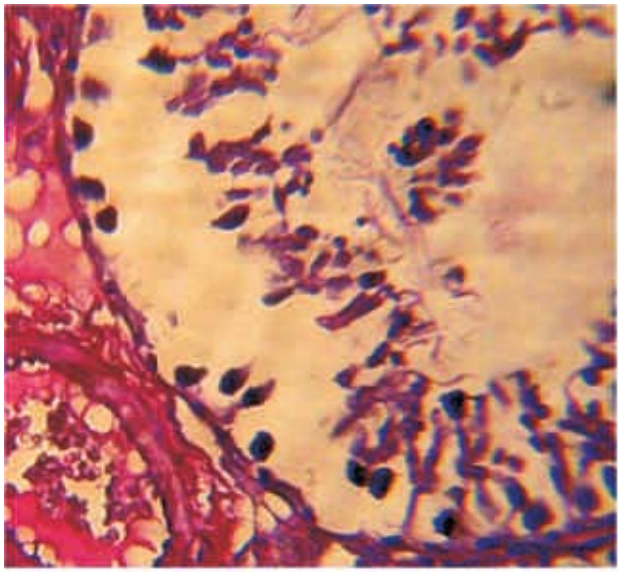

Figure-2: Photomicrograph-Section of testis of experimental group (Group B-I) animal number 10, showing reduced germ cell count and reduced height of germinal epithelium. H&E stain. x 420.

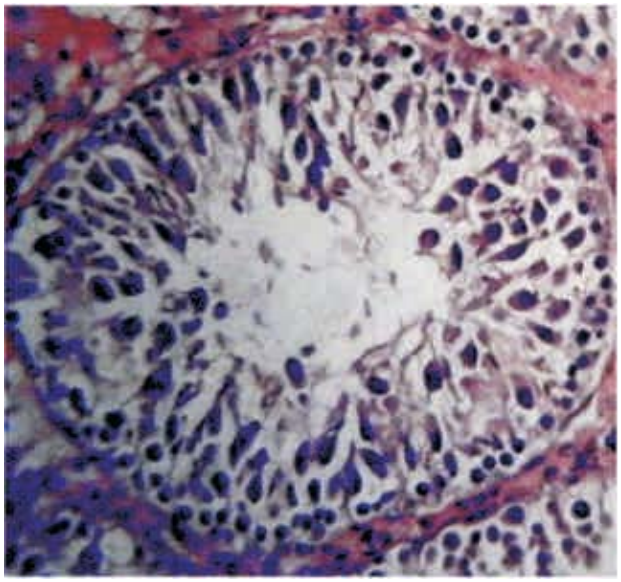

Figure - 3: Photomicrograph; cross section of testis; honey treated group C, animal number 10, seminiferous tubule showing basement membrane and spermatogenesis H&E stain x 400

higher in group A as compared to groups B, ($p < 0.001$) Figure 1.

Table No-I: Table showing description of Germ Cell Count (Cells/ Cross Section of Seminiferous Tubule) in all the study groups (error bars ± S

Group	Sub Group	Lead Toxic	Treated with Honey	Sacrificed after period of	Germ Cell Count
A (N =20)	A-I (N=10)	--	--	6 Weeks	304.53
	A-II (N=10)	--	--	12 Weeks	323.53
B (N= 30)	B-I (N=10)	6 Weeks	--	6 Weeks	116.91
	B-II (N=10)	6 Weeks	--	12 Weeks (6 Weeks withdrawal of Lead Acetate)	136.25
C (N=15)	--	6 Weeks	6 Weeks	12 Weeks	214.58

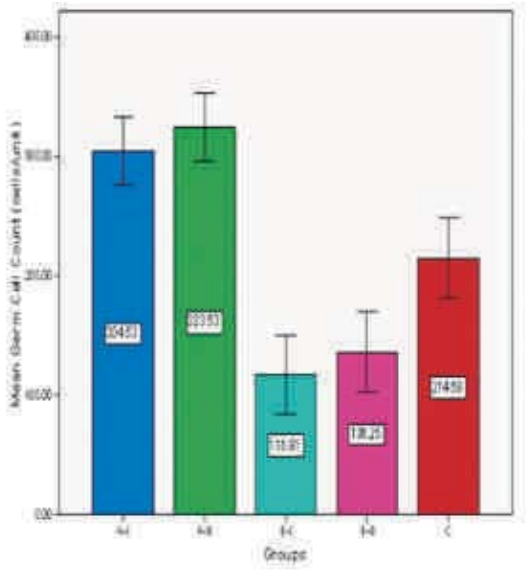

Figure 4: Bar chart showing description of Germ Cell Count (Cells/ Cross Section of Seminiferous Tubule) in all the study groups (error bars ± SD

Discussion

The present study was conducted to evaluate the protective role of honey on lead induced histological changes in the rat's testes. Lancranjan et. al, (1975) and Cullen et al., (1983)conducted a study on men exposed to lead at their workplace. They showed abnormalities of spermatogenesis.[11,12] Roshandel (2006) found that after 8 weeks of lead exposure, there was decrease in the height of germinal

epithelium and the number of sertoli cells in test group, whereas spermatogonia and primary spermatocytes remained unchanged . This study showed that lead intoxication induced some changes in the adult testes which were irreversible even after D-Penicillamine treatment which is contrary to our results, which showed that honey caused partial reversible changes in the testes.[13]Another study conducted by Sokol (1985).[14] revealed that lead acetate exposure in 52 days old male wistar rats is toxic to the reproductive axis and caused abnormalities of spermatogenesis which is in accordance to the present study. There was no testicular weight change found in that study as 0.3% lead acetate was given in distilled water. However rats lost overall weight.[14] Manlay (1995) administered lead acetate to rats in the dose of 8mg/kg body weight, 5 days a week for 35 days.[15] The study concluded that the germ cells and Sertoli cells were not affected by such a high dose of lead and this is in contrary to the present study but it did affect accessory sex glands by reducing the intertubular tissue volume in testes which indicate Leydig cell function impairment.[15]During past three decades, the decline in male reproductive health and fertility has been linked with environmental toxicants and xenobiotics.[16] A study conducted by Mohammed (2011) showed that honey could improve the toxic effect of lead on testicular function partly by improving testicular blood flow and spermatogenesis via the oestrogenic activity of its phenolic compounds.[17]My present study validated the previous studies results and further added a new dimension to the existing literature by investigating the effect of honey on lead-induced toxicity in the testes of Sprague Dawley rats.

Conclusion

This study provides evidence that lead has toxic effects on testis which are partially reversible on oral intake of honey.

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