EFFECT OF LEAD ACETATE ON FOLLICULAR COUNT OF MICE OVARY AND THE PROTECTIVE ROLE OF GARLIC EXTRACT

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ABSTRACT

Objective: The study was conducted to evaluate the effects of lead acetate and protective role of garlic extract on the histomorphology of the ovarian follicles in an animal model.

Study Design: Laboratory based randomized control trial

Place and Duration of Study: Department of Anatomy, Army Medical College in collaboration with National Institute of Health from April—June 2013

Material and Methods: Thirty female BALBc mice were selected. Mice were randomly divided into three groups. 10 animals were placed in each group. Group A being the control was given normal diet. Group B was given lead acetate at a dose of 30 mg/kg/day. Group C was given lead acetate 30mg/kg/day and garlic extract 500 mg/kg/day through oral lavage tube for 60 days. Animals were sacrificed and dissected at the end of 60th day. Length and width of the ovary were measured, right ovary was processed, embedded and stained for histological study. Primary, secondary and graafian follicles were counted and noted.

Results: There was reduction in the number of primary and graafian follicles in group B when compared to group A. In group C there was relatively increase in number of follicles, when compared to group B. Number of secondary follicles was almost same in all the groups. The length of ovary was higher in group A as compared to group B. In group C results were same as group A regarding length of the ovary. Width of ovary was same between the respective groups.

Conclusion: The follicular count was markedly affected in lead acetate treated group which improved when co treated with garlic extract in experimental group C. Hence, garlic had a protective role against lead induced changes in mice ovary.

Keywords: Follicular count, Garlic extract, Lead acetate.

INTRODUCTION

Lead has been one of the oldest known and widely studied occupational and environmental pollutant. Lead is a known toxic heavy metal for the past 6000 years. It is used in pipes, paints, enamels and glazes. Water, air and food are sources of lead exposure to general population.

According to Neman most lipsticks contain lead[1]. She reported that chance of breast cancer increases with increasing dose of lead in lipsticks. Lead poisoning due to occupational exposure is very common in adults leading to reversible changes in mood and personality[2]. More than 3 million workers in the United States are potentially exposed to lead in the workplace according to National Institute of Occupational Safety and Health (NIOSH)[3]. Central nervous system and kidneys are affected in children exposed to lead[4]. Lead poisoning is defined by the American Academy of Pediatrics as blood lead levels higher than 10µg/dl[5]. Same levels were considered as a cause of concern by World Health Organization[6].

The female reproductive system is greatly affected by exposure to environmental toxicants. Lead being one of the reproductive toxicant, can affect the gonadal structure and functions and can cause alterations in fertility[7]. The effects on the physiology, histomorphology, development and biomarkers have been observed on different organs of animals and humans. In most of the previous studies, the harmful effects of lead were noted[8-10].

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Received: 15 Jul 2013; Accepted: 10 Sep 2013

In recent years, research work threw light on the use of plants on the reproductive health of man and animals[11]. Garlic (Allium Sativum) is one of the studied plants, with a long history of therapeutic use. Health benefits of garlic have been extensively reported such as regulating plasma lipid levels, lead and mercury intoxication, anticarcinogenic, antimicrobial, antioxidant, and various other actions[12,13]. It exhibits antioxidant properties due to rich organosulphur compounds such as allicin. Reports on the effects of garlic on female reproductive system are yet to be established[11]. The rationale of current study is to observe the effects of lead acetate on female reproductive organs and the role of garlic extract.

MATERIAL AND METHODS

This laboratory based randomized controlled trial was conducted in the department of Anatomy, Army Medical College Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad from April—June 2013. The experiment was carried out with permission of ethical committee on animal experiments, of the Army Medical College, Rawalpindi.

Thirty female BALB/c mice weighing 25-27 grams were used in the experiment and were housed in the controlled environment of animal house of the NIH, Islamabad. The animals were randomly divided into three equal groups using random number table. Mice were fed with NIH laboratory diet for two months.

Animals in group A served as control and were given normal saline by oral lavage tube. Mice in experimental group B were given lead acetate at a dose of 30 mg/kg body weight[14] once daily for two months by oral lavage tube. Animals in group C were given lead acetate at a dose of 30 mg/kg body weight once daily along with garlic extract 500 mg/kg[15] through oral lavage tube once daily for two months. Lead acetate was purchased from Sigma-Aldrich (product number. 11504) and garlic from the local market and the required solutions were prepared according to the doses.

At the end of 60 days, the animals were anaesthetized by placing ether soaked cotton in the jar. The animals were placed on a clean sheet of paper on a dissecting board. The midline incision was made on the skin of the abdomen by scalpel. The flaps in the body wall were spread open by making lateral incisions and were pinned back to expose the organs. Right ovary was taken to maintain uniformity and was placed in 10% formalin in duly labeled plastic containers, was processed and embedded. Tissues were cut into 5 microns thick sections using rotary microtome. The sections were stained with autostainer with hematoxylin and

Table-1: Comparison of ovary weight, length & width between control group A and experimental group B and C.

n	Group A (n = 10)	Group B (n = 10)	Group C (n = 10)	*p*-value
Ovary length gm	0.011 ± 0.0032	0.010 ± 0.000	0.011 ± 0.0032	0.612
Ovary length µm	2370 ± 246.02	1770 ± 488.31	1925 ± 394.58	0.005
Ovary width µm	1245 ± 159.34	1097.5 ± 164.34	1135 ± 126.49	0.095

Values were described as Mean ± SD

Table-2: Comparison of secondary and graafian follicles between control group A and experimental group B and C.

	Group A (n = 10)	Group B (n = 10)	Group C (n = 10)	*p*-value
Number of secondary follicles	6 (4 – 6.25)	3.5 (2 – 4.5)	5 (3 - 6)	0.189
Number of graafian follicles	2 (0.75 - 2)	0.5 (0 - 1)	1.5 (0.75 - 2)	0.012

Values were described as Median (Inter-quartile Range), *p-value <0.05 siginificant, **p-value <0.001 highly siginificant

eosin (H and E) for routine histological study of ovary.

Histomicroscopic study: length of the ovary, it was taken from one pole to the other using X4 objective. Three readings were taken and their average as final reading for that specimen.

Width of the ovary: it was taken from the hilar margin to the free margin using X 4 objective. Three observations were taken and their average as final reading for that specimen.

Number of follicles: follicles were counted and noted. One slide per specimen was observed. X 10 objective was used to count the follicles. The morphology of follicle was noted according to the following classification[16]:

Primary follicle (FI) (an oocyte completely surrounded by a single/double layer of cuboidal epithelium with zona pellucida in between).

Secondary follicle (FII) (an oocyte surrounded by more than one layer of cuboidal cells with antrum and zona pellucida in between)

Graafian follicle (FIII) (an oocyte surrounded by zona pellucida, stratified layers of granulosa cells with a large antrum).

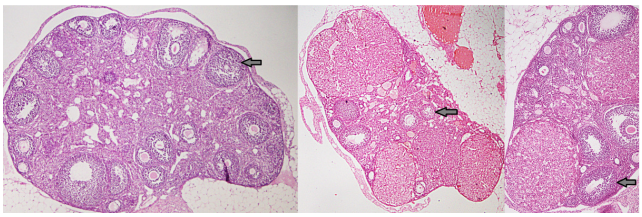
Statistical analysis

The data was analyzed by using Statistical Package for Social Sciences (SPSS) version 18. Descriptive statistics were used to describe the results. The significance of difference was determined using ANOVA followed by post Hoc Tukey for normal data and median test for non-normal data. Results were considered significant at $p < 0.05$.

RESULTS

The ovaries of control group A were covered with a simple cuboidal layer of germinal epithelium. Underneath the germinal epithelium was tunica albuginea consisting of collagen fibers. The ovarian tissue was divided into two parts, the inner medulla which contained stroma and blood vessels and an outer cortex which contained the follicles at different stages of the development (fig-1a).

The haematoxylin and eosin (H and E) stained slides of group B showed slight disruption in the normal cytoarchitecture of the ovarian tissue. Germinal epithelium was disrupted and vacuoles were observed in the tunica albuginea.



a b c

Figure-1: Normal ovary of control group seen in (a), reduced ovarian follicles in (b) in experimental group B and relatively increase in the follicular count in experimental group C at 10X (H and E).

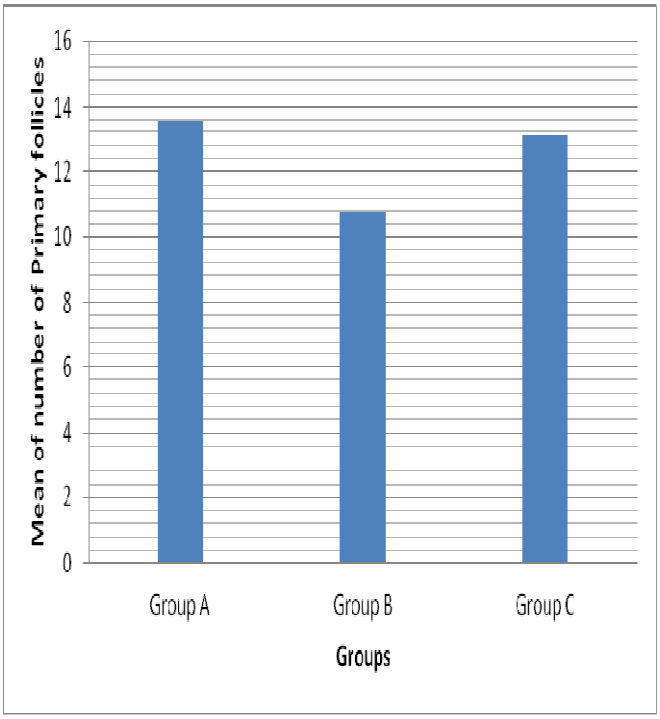


Figure-2: Comparison of primary follicles between control group A and experimental group B and C.

There was no inflammation observed. The number of primary follicles (figure–1b, 2) was less in experimental group B when compared

with control (p=0.026) but the difference from group C was insignificant (p=0.075). In group C there was improvement of primary follicular count, with similar results as group A (p=0.874). The number of graafian follicles in experimental group B was lower than the control group (Table-2). Number of graafian follicles were more in the group C in comparison to experimental group B. Secondary follicles were similar among the three groups with statistically insignificant results (p=0.189).

The ovary architecture of experimental group C was similar to control group. Garlic extract treatment resulted in marked attenuation of decreased follicular count induced by lead acetate (figure-1c).

Length of the ovary (table-1) was significantly more in group A as compared to the group B (p=0.005) and the group C (p=0.042) but difference between group B and group C was insignificant (p = 0.651). Regarding width of the ovary there was insignificant difference among the three groups.

DISCUSSION

The objective of this study was to see the effects of lead acetate on the histomorphology of mice ovary and the protective role of garlic extract. In the present study lead induced histological alterations in various components of the ovary and the changes were ameliorated with administration of garlic extract. The experimental groups were compared with the control group, as well as with each other. The results of group B were compared with group C and of group A with group C. In previous studies on the effect of lead acetate, histological studies of ovary showed atresia in all stages of folliculogenesis in rats[2]. Significant decrease in the number of ovarian follicles was observed by Dorostghoal[8] in wistar rats' ovary. Similarly Shirota et al[17] and McMurry et al[18] noted that lead causes reduction in the number of growing follicles.

This study showed reduction in the number of ovarian follicles in group B as compared to the control group. Upon concurrent treatment with garlic extract in group C, there was marked attenuation of decreased ovarian follicular count and disturbed follicular structure induced by lead acetate in group B. Our study showed that lead acetate decreased the number of primary, secondary and tertiary follicles which improved after co administration of garlic extract.

CONCLUSION

The results lead to the conclusion that exposure to lead in our environment has effects on the histomorphology of ovary leading to decrease folliculogenesis. The garlic extract ameliorated the effects of lead acetate.

Acknowledgements

Authors express gratitude to Lt Col Khadija Qamar (Associate Professor, Anatomy Department, Army Medical College) for assistance and help.

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