GARLIC AMELIORATES HISTOLOGICAL CHANGES IN THE UTERINE EPITHELIUM OF LEAD INDUCED MICE

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ABSTRACT

Objective: To evaluate the protective role of garlic extract on the histoloy of the uterine epithelium exposed to lead acetate 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 to June 2013.

Material and Methods: Thirty female BALBc mice were selected. Ten 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 30 mg/kg/day and garlic extract 500 mg/kg/day through oral gavage tube for 60 days. Animals were sacrificed and dissected at the end of 60 days. Right uterine horn was processed, embedded and stained for histological study. Height of epithelium was measured. It was taken from apical to basal end of the cells.

Results: There was increase in height of the lining epithelium of uterus in group B, mean value 19.70 ± 4.81 µm when compared to Group A, with mean value 13.25 ± 2.37 µm. The height of the epithelium was relatively reduced in group C, with mean value 14.50 ± 2.30 µm when compared with group B. In group C results were same as Group A. The *p* values were 0.001 when group A was compared to group B, 0.688 when group A was compared to group C and 0.005 when group B was compared to group C.

Conclusion: The height of epithelium was markedly increased in lead acetate treated group which returned to normal when co treated with garlic extract. Hence garlic ameliorated the changes induced by lead.

Keywords: Endometrium, Garlic extract, Lead acetate.

INTRODUCTION

Lead is an environmental and industrial pollutant found in the earth's crust. It is a metal which has been associated with human activities since 200 BC. From the view point of human reproduction, lead is known to cause a number of adverse consequences in both men and women. The female reproductive system and, therefore human fertility may be affected by exposure to environmental toxicants. Lead is even found in some popular brands of lipsticks. 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]. Lead poisoning is defined by the American Academy of Pediatrics as blood lead levels higher than 10µg/dl[3]. Same levels were considered as a cause of concern by World Health Organization[4].

Lead being one of the reproductive toxicant, can affect the gonadal structure and functions and can cause alterations in fertility[5]. The effects on the physiology, histomorphology, development and biomarkers have been observed on different organs of animals and humans. In many previous studies, the harmful effects of lead were noted[6-8].

In recent years, research work threw light on the use of plants on the reproductive health of man and animals[9]. Garlic (Allium Sativum) is one of the studied plants, with a long history of therapeutic use. Health benefits of garlic have been extensively reported[10,11]. Reports on the effects of garlic on female reproductive system are yet to be established[9]. The rationale of current study is to observe the effects of lead acetate on female reproductive organs and the protective role of garlic extract.

MATERIAL AND METHODS

This laboratory based randomized controlled trial was conducted in the

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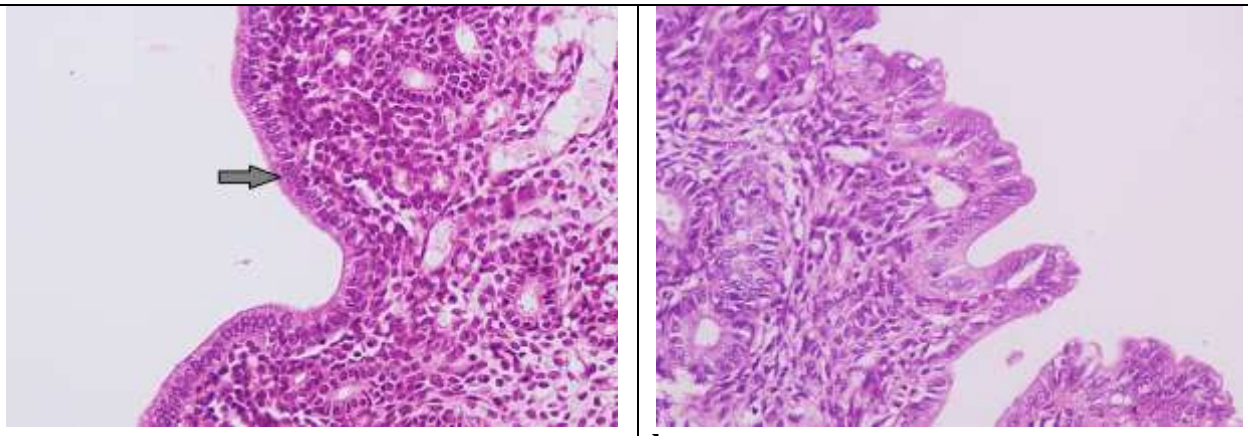
Department of Anatomy, Army Medical College Rawalpindi from April—June 2013. Animals were procured from National Institute of Health, Islamabad.



a. **b.**

Figure-1: Normal epithelium of control group seen in fig (a), arrow- simple columnar epithelium and increase in height of epithelium (b) at 40X (H&E) in experimental group B.

Table-1: Statistical significance of epithelium height in control group A and experimental group B and C.

	Group A vs. Group B		Group A vs. Group C		Group B vs. Group C	
	p-value	Significance	*p*-value	Significance	*p*-value	Significance
Epithelium Height (μm)	0.001	< 0.05*	0.688	> 0.05	0.005	< 0.05*

*p-value <0.05 significant, **p-value <0.001 highly significant

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

Animals in group A served as Control and were fed on normal diet. Mice in experimental group B were given lead acetate at a dose of 30 mg/kg body weight once daily for two months by oral gavage 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 through oral gavage tube once daily for two months. Lead acetate was purchased from Sigma Aldrich and garlic from the local market, and its extract was prepared. Solution of Lead acetate was made in 10ml water every day to deliver daily required dose of 30 mg/kg body weight. A total of 0.5 ml of this solution was given to each mouse in experimental groups B and C through oral gavage tube. Garlic extract was prepared and administered after dosage calculation. Fresh garlic extract (0.25 ml) was administered to each mouse in Group C experimental group.

At the end of 60 days, the animals were sacrificed and dissected. Right uterine horn was removed and was placed in 10 percent formalin in duly labeled plastic containers. Then the uterine horn was processed, embedded and sectioned. Histological study was done with H&E stained section.

The height of the epithelium was taken from the basement membrane up to the upper limit of the cell facing the lumen under high power field (40X objective) by Micrometry. First the calibration of the ocular micrometer scale was done with a calibrated stage micrometer. At 40X, it was noted that 40 divisions of eye piece was equal to 1 stage division.

40 divisions of eyepiece= 1 stage division= 100 μm

1 division of eyepiece= 100/40= 2.5 micron

A calibration factor of 2.5 micronmeter was obtained and was valid for optical combination. Three cells were chosen randomly for measuring epithelial height in different fields of slide, in one slide per specimen. Three observations per slide for each specimen were made and the average was noted as the final reading for that specimen.

The data was analyzed by using statistical package for social services (SPSS) version 18. Descriptive statistics were used to describe the results. The significance difference was determined using ANOVA and Post Hoc Tuckey test. Results were considered significant at $p<0.05$.

RESULTS

The uterine wall of control group showed three layers (endometrium, myometrium and perimetrium). The endometrium consisted of simple columnar epithelium and an underlying connective tissue stroma with glands (Fig-1a).

The H&E stained slides of group B showed pseudostratified epithelium with increase in height of columnar cells as compared to control (Fig-1b). Straitification of epithelium was seen in 6 animals. The uterus sections in experimental group C showed microscopic picture that closely resembled that of the control group. The epithelium was simple columnar with reduced epithelial height as compared to experimental group B. There was no straitification of epithelium seen in this group. The mean epithelium height was 13.25 ± 2.37µm, 19.70 ± 4.81µm and 14.50 ± 2.30 µm for control group A and experimental group B and C ($p<0.001$).

Significant difference was present between groups A and B regarding epithelium height. The results of group C were not significantly different when compared with control group. Results were statistically significant when group B was compared to group C (Table-2)

DISCUSSION

Lead is widely spread environmental pollutant known to affect both male and female reproductive systems in humans and experimental animals and causes infertility. Chronic exposure to lead induces hypertrophy of luminal epithelial cells, measured as increase in epithelial cell height. As an optimal uterine environment is required for the implantation of zygote in the endometrium, any change in either components of uterus can be a cause of infertility[12]. The success of implantation process depends on a receptive endometrium as well as embryo –endometrial interface synchronization (Perrier et al., 2006)[13]. Initial attatchment of the blastocyst to the uterus is mediated by the receptors on the uterine epithelium. Hence, a healthy uterine epithelium is necessary for implantation in the uterine mucosa[14].

The objective of this study was to see lead acetate affects the uterine epithelium of mice and to prove the protective role of garlic extract. In the present study lead induced histological alterations in the epithelium height of uterus and the changes were ameliorated with administration of garlic extract. In a previous study on effect of prenatal exposure to lead on estrogen action in prepubertal rat uterus observed estrogen induced luminal epithelial hypertrophy[15], but contrary findings were observed in a study that showed decrease in height of columnar cells after lead exposure[5]. The cells of luminal epithelium increased in height in rats exposed to 75 mg/kg body weight lead acetate for two months[16]. Our data indicated increase in height of the cells of the lining epithelium, which might be because of imbalance between estrogen and progesterone.

CONCLUSION

The results lead to the conclusion that persistence and exposure to lead in our environment affected the histomorphology of uterus which could be prevented after treatment with garlic extract. So it can also be concluded that garlic extract played a protective role, resulting in improvement of the tissue exposed to lead. Hence, use of raw garlic as a part of our daily diet may play a protective role against the effects of environmental toxicants on genital organs and we can contribute our part in reducing infertility.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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