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The Effect of Low Dose of Lead Acetate on the Fallopian Tubes and the Role of Garlic Extract—A Histomorphologic Study on Mouse

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Abstract

This study aimed to investigate the effect of lead acetate on the fallopian tube of adult mice and the possible effect of garlic extract in a Laboratory Based Randomized Control Trial. In this study, 30 female BALBc mice were selected and randomly divided into three groups. Ten animals were placed in each group. Group A being the control received only the laboratory diet. Group B was given lead acetate at a dose of 30 mg/kg/day. Group C was given lead acetate at 30 mg/kg/day and garlic extract at 500 mg/kg/day. All treatments were given through oral gavage tube for 60 days. The mice were sacrificed and dissected at the end of 60 days. The fallopian tubes were carefully dissected out and fixed in 10% formalin for routine histological examination. The histological findings in experimental group B showed increase in epithelial height, whereas no such findings were observed in group A and there was slight increase in height in group C. The lead acetate affected the epithelial height in lead acetate treated group which improved when cotreated with garlic extract.

Keywords: Epithelial height; Garlic extract; Fallopian tubes; Mice.

1. INTRODUCTION

Lead is an environmental and industrial pollutant found in almost all phases of biological systems. It is one of the most ubiquitous toxic material to which we are exposed in our day to day life. It is used in the making of pipes, paints, enamels, soldered fillings, bullets, etc. Health risks due to lead toxicity are one of the world's current problems (Dhir and Dhand, 2010).

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. The amount of lead present in lipstick is six times the amount found in candy, approved by FDA. According to Neman (2008), most lipsticks contain lead who 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 (Dhir and Dhand, 2010). Depending on the level of exposure, lead can adversely affect the central nervous system, renal function, immune system, genital and cardiovascular system. Lead poisoning is defined by the American Academy of Pediatrics as blood lead levels higher than 10 µg/dl (Ragan and Turner, 2009). Same levels were considered as a cause of concern by World Health Organization (Barbosa *et al.*, 2005).

Lead being one of the reproductive toxicants can affect the gonadal structure and functions and can cause alterations in fertility (Qureshi and Sharma, 2012). Most of the available literature is related to the action of lead on male subjects. 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 (Dumitrescu *et al.*, 2009; Yousif and Adbullah, 2010; Ait *et al.*, 2009).

For centuries, fruits and vegetables have attributed to beneficial health effects. In recent years, research work threw light on the use of plants on the reproductive health of men and animals (Raji *et al.*, 2012). Garlic (*Allium Sativum*) is one of the studied plants, with a long history of therapeutic use. Health benefits of garlic have been extensively reported (Sharma *et al.*, 2010; Asdaq and Inamdar, 2010). It exhibits antioxidant properties due to rich organosulphur compounds. The preventive and curative effect of combined supplementation of garlic and vitamin B complex against lead toxicity in albino mice is reported before (Khan *et al.*, 2008). Data suggest that antioxidants have an important role in abating some hazards of lead.

Reports on the effects of garlic on female reproductive system are yet to be established (Raji *et al.*, 2012). Some previous studies have reported that garlic not only provides protection against lead toxicity but also can perform a therapeutical role against lead toxicity (Bhattercharjee *et al.*, 2003). The rationale of current study is to observe the effects of lead acetate on fallopian tubes and the protective role of garlic extract.

2. MATERIAL AND METHODS

This experimental trial was conducted at the Anatomy Department, Army Medical College, Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad from April to June 2013. Ethical considerations were fulfilled and ethical approval was taken from the Ethical committee of the Army Medical College, Rawalpindi prior to commencement of the study.

3. ANIMALS AND EXPERIMENTAL GROUPS

The animals were randomly divided into three equal groups. Thirty female BALB/c mice weighing 25-27 g were used in the experiment and were housed in controlled environment of Animal house of NIH, Islamabad. The mice were kept in cages at standard room temperature maintained on 12 h light/dark cycle. 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 at 11 AM 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 at 11 AM daily for two months.

3.1. Tissue Sampling and Histology

At the end of 60 days, the animals were euthanized 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. The fallopian tubes and ovaries were identified and right sided fallopian tube of each mouse (groups A, B, and C) was dissected out and put in 10% formalin for fixation. The tissue was further processed for paraffin embedding. The staining of sections was done with H&E for routine histological study.

3.2. Height of Epithelium

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 (X40 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 eyepiece was equal to 1 stage division (Figure 1).

$$\begin{aligned} 40 \text{ divisions of eyepiece} &= 1 \text{ stage division} = 100 \mu\text{m} \\ 1 \text{ division of eyepiece} &= 100/40 = 2.5 \mu\text{m} \end{aligned}$$

A calibration factor of 2.5 μm was obtained and was valid for optical combination. Three cells were chosen randomly for measuring epithelial height in different fields of slide at X40 objective, 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.

3.3. Statistical Analysis

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

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

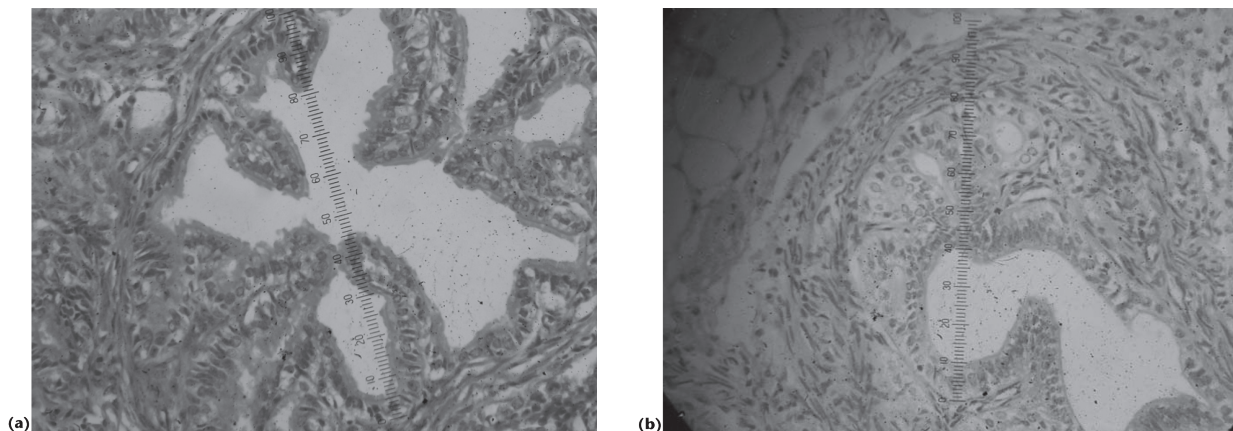


Table 1: Comparison of mean values of epithelium height between groups.

	Group A (n = 10)	Group B (n = 10)	Group C (n = 10)	p-value
Epithelium height (μm)	14.115 \pm 0.60	23.7 \pm 0.73	16.89 \pm 0.81	0.000

Values were described as Mean \pm SEM.

Table 2: Statistical significance of epithelium height in control group A and experimental groups 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)	0.000	<0.05	0.030	>0.05	0.000	<0.05

*p-value < 0.05 significant.

**p-value < 0.001 highly significant.

4. RESULTS

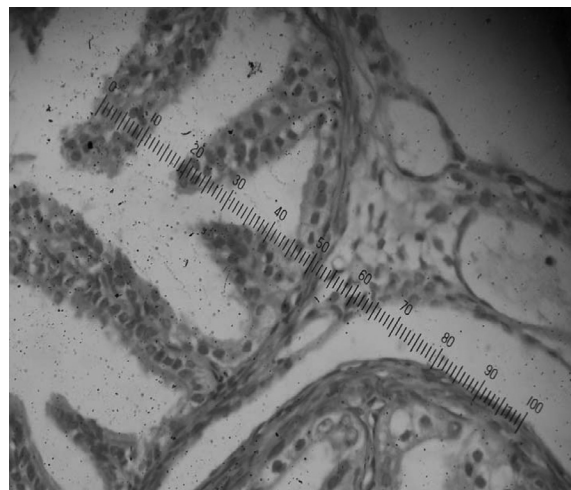
The mean epithelial height of fallopian tubes was found considerably increased in animals of lead acetate treated group B as compared to control group ($p < 0.05$). The increase in cellular height of the columnar epithelium of fallopian tube in lead acetate treated group might be due to altered hormone levels (Table 1).

In experimental group C, treated with lead acetate and garlic extract histological findings showed less increase in the epithelial height of fallopian tube as compared to group B (Table 2). This shows that garlic extract has played a protective role against the effects induced by lead acetate on fallopian tubes.

5. DISCUSSION

Lead is widely spread environmental pollutant reported to affect 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 and fallopian tube, environment is required for fertilization and implantation of zygote in the endometrium, any change in either component could be a cause of infertility (Kuć, 2012). The success of implantation process depends on a healthy zygote and endometrium as well as embryo – endometrial interface synchronization (Perrier *et al.*, 2006).

The objective of this study was to see lead acetate affects the epithelium of fallopian tubes of mice and to prove the protective role of garlic extract. In the present study, lead induced histological alterations in the epithelium height of fallopian tube and the changes were ameliorated with administration of garlic extract. The experimental

Figure 2: Decrease in height of epithelium at 40X (H&E) in experimental group C treated with lead acetate and garlic extract.

groups were compared with the control group, as well as with each other. The mean epithelial height of uterine tubes was normal in animals of control group. It was found considerably increased in animals of lead acetate treated group B as compared to control group ($p < 0.005$). The increase in epithelial height of fallopian tube in the lead acetate treated group might be due to hormonal imbalance. This is also established that estrogen treatment causes increase in epithelial height of uterine tube in mice (Lauschova, 1999). Lead acetate is found to cause the increase in epithelial height of not only fallopian tubes but also causes cellular hypertrophy of many other body organs (Tchernitchin *et al.*, 2011). In a previous study on effect of prenatal exposure to lead on estrogen action in prepubertal rat uterus observed estrogen induced luminal epithelial hypertrophy (Lauschova, 1999), but contrary findings were observed in a study that showed decrease in height of columnar cells after lead exposure (Qureshi and Sharma, 2012). The cells of luminal epithelium increased in height in rats exposed to 75 mg/kg body weight lead acetate for two months (Al-Naimi, 2012). Our data indicated increase in height of the cells of the lining epithelium, which might be because of altered estrogen and progesterone (Figure 2).

In experimental group C, treated with both lead acetate and garlic extract showed less increase in the epithelial height of the fallopian tube as compared to group B. This shows that garlic extract has protected the epithelium against the damaging effects induced by lead acetate on the fallopian tubes. Garlic is a medicinal plant that has been used in culinary for over 5000 years. Besides its use as a condiment, it has remarkable therapeutic and pharmacological properties. Senapati *et al.* (2004) reported the prophylactic efficacy of garlic extract in reducing the lead burden from many tissues. In another study, Pourjafar *et al.* (2007) also reported the ability of garlic to reduce the lead levels from the liver, kidney, blood, and bone. The protective efficacy of garlic extract was studied against lead induced hepatic injury in rats. The results clearly indicated the ameliorative ability of garlic toward hepatic injury caused by lead due to oxidative stress (Kilikdar *et al.*, 2011). The role of garlic on male reproductive organs has also been demonstrated before. It has been shown that treatment with garlic has no harmful effects when administered alone. However, the preventive effects of garlic extract against lead acetate are not yet studied on fallopian tube.

6. CONCLUSION

The study concludes that the lead acetate causes luminal cell hypertrophy in fallopian tubes. The study also concludes that garlic extract prevents the lead acetate induced changes on the fallopian tubes. As the change in the epithelium of fallopian tube may lead to infertility, these changes induced by lead acetate can be prevented by raw intake of garlic by females.

Author Contributions

All authors contributed equally to this work.

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