ORIGINAL ARTICLE

# Amelioration of lead induced changes in ovary of mice, by garlic extract

Naureen Waseem, Shadab Ahmed Butt, Shabnam Hamid


## Abstract

**Objective:** To observe the effects of lead acetate and protective role of garlic extract on the histomorphology of the ovary in an animal model.

**Methods:** The experimental trial was conducted at the Department of Anatomy, Army Medical College Rawalpindi, in association with the National Institute of Health (NIH), Islamabad, from April to June 2013.

It comprised 30 adult non-pregnant female mice (BALBc strain) weighing 25-27gms. They were divided into three equal groups of 10 mice each. Group A, taken as control, was given normal diet. Group B was given lead acetate at a dose of 30mg/kg/day. Group C was given lead acetate 30mg/kg/day and garlic extract 500mg/kg/day through oral gavage tube for two months. Animals were dissected a day after the last dose. Size, shape, colour and consistency of the ovary was observed. The right ovary was processed, embedded and stained for histological study. Primary follicles were counted and noted. SPSS 18 wsa used for statistical analysis.

**Results:** The primary follicular count decreased significantly in Group B while it relatively increased in Group C. Morphology of the ovary was affected after exposure to lead acetate in Group B, while in Group C results were the same as in the Group A controls regarding gross architecture of the ovary.

**Conclusion:** Lead alters the normal histology and affects the physiology of the ovary. It interferes with the development of growing follicles in the ovary. Lead, being a reproductive toxicant, can be a cause of infertility in exposed females.


**Keywords:** Follicular count, Garlic extract, Lead acetate. (JPMA 64: 798; 2014)

## Introduction

Lead is a ubiquitous environmental pollutant. The female reproductive system and, therefore, human fertility may be affected by exposure to environmental toxicants like lead. Females are getting exposed to lead due to its presence in some leading cosmetics.

According to a 2008 study, most lipsticks contain lead.[1] It 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 at workplace, according to the National Institute of Occupational Safety and Health (NIOSH).[3] Central nervous system (CNS) and kidneys are affected in children exposed to lead.[4] Lead poisoning is defined by the American Academy of Paediatrics as blood lead levels higher than 10µg/dl.[5] Same levels were considered as a cause of concern by World Health Organisation (WHO).[6]

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]

In recent years, research work has thrown light on the use of plants on the reproductive health of man and animal.[11] Health benefits of garlic have been extensively reported such as regulating plasma lipid levels, lead and mercury intoxication, anti-carcinogenic, anti-microbial, anti-oxidant, and various other actions.[12,13] It exhibits anti-oxidant 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 the current study was to observe the effects of lead acetate on the ovary and the role of garlic extract.

## Material and Methods

The experimental trial was conducted at the Department of Anatomy, Army Medical College Rawalpindi, in association with the National Institute of Health (NIH), Islamabad, from April to June 2013.

Thirty adult, non-pregnant female mice (BALB/c strain) 6-8 weeks old weighing 25-27grams were used for the study. They were kept in the NIH animal house. Animals were divided into three groups and were fed with NIH

Department of Anatomy, Army Medical College, National University of Sciences and Technology, Rawalpindi.

**Correspondence:** Naureen Waseem. Email: drnaureen@amcollege.nust.edu.pk

laboratory diet for two months.

Animals in Group A served as control and were given normal saline by oral gavage tube. The experimental Group B was given lead acetate at a dose of 30mg/kg body weight[14] once daily for two months by oral gavage tube. Animals in Group C were given lead acetate at a dose of 30mg/kg body weight once daily along with garlic extract 500mg/kg[15] through oral gavage 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 dissected. Ovary was histomorphologically studied for size, shape, colour and consistency. The right ovary was taken to maintain uniformity and was placed in 10 per cent formalin. The ovary was processed and embedded. Tissues were cut into 5-micron thick sections using rotary microtome. The sections were stained using autostainer with haematoxylin and eosin (H&E) for routine histological study of the ovary.

Primary Follicles were counted and noted. One slide per specimen was observed. X10 objective was used to count the follicles. The classification used for noting down morphology of follicle took primary follicle (FI) as an oocyte completely surrounded by a single/double layer of cuboidal epithelium with zona pellucida in between.[16]

Data was by using SPSS 18. Descriptive statistics were used to describe the results. The significance difference was determined using analysis of variance (ANOVA) followed by post Hoc Tukey. Results were considered significant at $p<0.05$.

## Results

The ovaries of control Group A were ellipsoid, pale whitish in colour and firm in consistency with a beaded appearance (Figure-1). Histologically, the ovary was covered with a simple cuboidal layer of germinal epithelium. Underneath the germinal epithelium was tunica albuginea consisting of collagen fibres. 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. The mean number of primary follicles was 13.6±2.07 in the ovary of the control group (Table-1).

**Table-1:** Mean number of primary follicles in control Group A and experimental groups B and C.

| | Group A Mean±SD (n = 10) | Group B Mean±SD (n = 10) | Group C Mean±SD (n = 10) | p-value |
|---|---|---|---|---|
| **Primary follicles** | **13.6±2.07** | **10.80±2.25** | **13.10±2.42** | **0.026*** |

SD: Standard deviation.

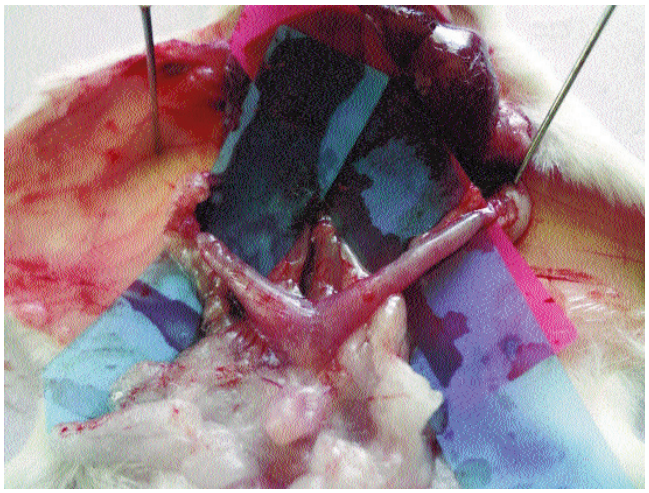

**Figure-1:** Normal ovary of control group (pin attached), with no haemorrhages, adhesions and congestion.

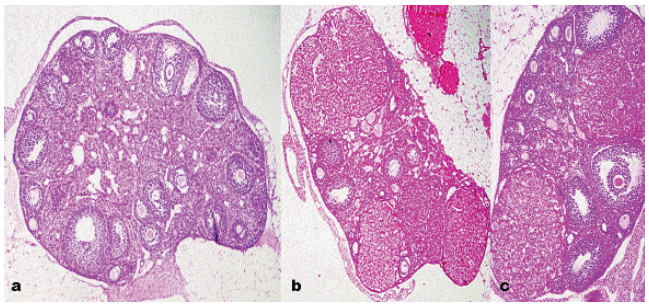


**Figure-2:** Photomicrographs showing ovary of control Group A (a), a decrease in the number of primary follicles of ovary of Group B (b) and an improvement in the follicular count in ovary of Group C (c).

The H&E stained slides of Group B showed slight disruption in the normal cytoarchitecture of the ovarian tissue. The size was smaller than the ovaries of the control group. A few ovaries were congested with a firm consistency. No adhesions were found on the ovary. Germinal epithelium was disrupted and vacuoles were observed in the tunica albuginea (Figure-2).

There was no inflammation observed. The mean number of follicles was 10.80±2.25. The number of primary follicles was less in experimental Group B when compared with controls ($p<0.026$), but the difference from Group C was insignificant ($p<0.075$). In group C, the mean number of follicles was 13.10±2.42. There was improvement of

**Table-2:** Comparison of number of primary follicles 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 |
| Primary Follicle | 0.026 | < 0.05* | 0.874 | > 0.05 | 0.075 | > 0.05 |

*p-value <0.05 significant.

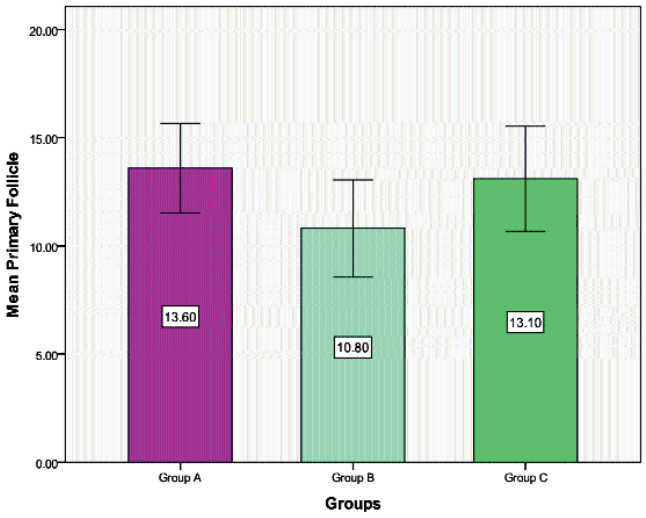


**Figure-3:** Comparison of number of primary follicles among groups.

primary follicular count, with similar results as Group A (p=0.874) (Table-2, Figure-3). The ovary architecture of experimental Group C was similar to the control group. The colour of ovaries was pale with a firm consistency and shape and size similar to control Group A. Garlic extract treatment resulted in marked attenuation of decreased follicular count induced by lead acetate.

## Discussion

Ovary is a vital organ for reproduction lying between the urinary and alimentary systems. It is liable to undergo morphological, histological and physiological changes when isolated either directly or indirectly. Ovaries are vulnerable to damage by exposure to reproductive toxicants such as lead. 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 noted atresia in all stages of folliculogenesis in rats.[2] Significant decrease in the number of ovarian follicles was observed by a study[8] on wistar rats' ovary. Similarly others[17,18] observed that lead causes reduction in the number of growing follicles. Qureshi et al. observed apparent damage of the ovary with follicular cells dispersion and vacuolar epithelial cells on microscopic examination with increase in number of atretic follicles after lead exposure.[19] The study also observed that lead causes reduction in the number of primary follicles and decrease the number of follicles that enter the growing phase.

In a study on effects of lead acetate on biomarkers of reproductive system in female rats, severe congestive and degenerative changes were seen in the ovary, with significant increase in lead levels in ovaries, fallopian tubes and uterus when compared with the controls.[9] Reduction in the number of primary follicles was observed in female rats exposed to lead acetate at a dose of 75mg/kg for two months.[20]

The present study showed decrease in size of ovaries in experimental Group B. Shape of the ovary was same as in control Group A. Congestion of the ovaries was seen in ovaries of experimental Group B. They were firm in consistency. Reduction in the number of ovarian follicles in Group B was noted 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 morphology of ovary induced by lead acetate in Group B. Our study showed that lead acetate disturbed the normal morphology of the ovary and decreased the number of primary follicles which improved after co-administration of garlic extract.

## Conclusion

Exposure to lead affects the histomorphology of ovary leading to decrease folliculogenesis. The garlic extract ameliorated the effects of lead acetate and played a protective role.

## References

1. Neman N. LIPSTICK INFORMATION: Dr.Nahid Neman Mt. Sinai Hospital. (Online) (2008). Available from URL: www.wingsandheros.com/articles/general-health-wellness-news-tips/lipstick-information-drnahid-neman-mt-sinai-

hospital.html.
2. Dhir V, Dhand P. Toxicological approach in chronic exposure to lead on reproductive functions in female rats. Toxicol Int 2010; 17: 1-7
3. Staudinger KC, Roth VS. Occupational lead poisoning. Am Fam Physician 1998; 57: 719-26.
4. Sangha JK., Kaur N and Singh I. Extent of heavy metal toxicity in the diet of school boy of Ludhiana. J Res 2001; 38: 124-9.
5. Ragan P, Turner T. Working to prevent lead poisoning in children: getting the lead out. JAAPA 2009; 22: 40-5.
6. Barbosa J F, Tanus-Santos J E, Gerlach RF, Parsons PJ. A critical review of biomarkers used for monitoring human exposure to lead: advantages, limitations and future needs. Environ Health Perspect 2005; 113: 1669-74.
7. Qureshi N, Sharma R. Lead toxicity and infertility in female swiss mice: A review. J Chemical Biological Physical Sci 2012; 2: 1849-61.
8. Eugenia D, Alexandra T, Diana A, Cristina RT. The consequences in utero exposure to lead acetate on exposure and integrity biomarkers of reproductive system in female rats. Medicina Veterinara. 2009; 2: 295-300.
9. Yousif WH, Adbullah ST. Reproductive efficiency of rats whose mothers treated with lead acetate during lactation: role of vitamin E. Iraqi J Veterinary Sci 2010; 24: 27-34.
10. Ait HN, Slimani M, Merad BB, Zaoiu C. Reproductive toxicity of lead acetate in adult male rats. Am J Sci Res 2009; 3: 38-50.
11. Raji L O, Fayemi OE, Ameen SA, Jagun, AT. The effects of aqueous extract of allium sativum (garlic) on some aspects of reproduction in the female albino rat (wistar strain). Global Veterinaria. 2012; 8: 414-20.
12. Sharma V, Sharma A, Kansal L. The effect of oral administration of allium sativum extracts on lead nitrate induced toxicity in male mice. Food Chem Toxicol 2010; 48: 928-36.
13. Asadaq SM, Inamdar MN. Pharmacodynamic and pharmacokinetic interactions of propanolol with garlic (Allium Sativum) in rats. Evid Based Complement Altern Med 2011; 2011: 824042.
14. Bomfim KM, Garia CAB, Reis FP and Palmeria JAV. Absorption levels and morphological features of fetal organs in wister rats treated with lead acetate. Int. J. Morphol 2012; 30: 119-24.
15. Assayed ME, Khalaf AA, Salem HA. Protective effects of garlic extract and vitamin C against in vivo cypermethrin- induced teratogenic effects in rat offspring. Food Chem Toxicol. 2010; 48: 3153-8.
16. Butt SA. Effects of ultrasound on the ovary and pregnancy. [PhD thesis]. University of Health Sciences; 2010.
17. Shirota M, Soda S, Katoh C, Asai S, Sato M, Ohta R, et al K. Effects of reduction of the number of primordial follicles on follicular development to achieve puberty in female rats. Reproduction 2003; 125: 85-94.
18. McMurry ST, Lochmiller RL, Chandra SA, Qualls CW. Sensitivity of selected immunological, hematological, and reproductive parameters in the cotton rat to subchronic lead exposure. J Wildl Dis 1995; 31: 193-204.
19. Qureshi N, Sharma R, Mogra S, Panwar K. Amelioration of lead induced alterations in ovary of Swiss mice by antioxidant vitamins. J Herbal Med Toxicol 2010; 4: 89-95.
20. Rajiha, A. and Al-Naimi. The therapeutic effect of silybum marianum on the lead acetate induced reproductive toxicity in both gender laboratory rats. Wasit J Sci Med 2012; 5: 144-55.