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The Efficiency of Garlic Extract in Prevention of Lead Acetate Toxicity on Fallopian Tube-A Hormonal Study

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Abstract

(i) Objective: This study was conducted to analyze the effect of lead acetate on the serum estrogen and progesterone levels in adult mice and the role of garlic extract. (ii) Study Design: Laboratory based Randomized Control Trial. (iii) Place and duration of study: Anatomy Department, Army Medical College, Rawalpindi in collaboration with National Institute of health from April-June 2013. (iv) Material and Methods: In this study 30 female BALBc mice were selected and randomly divided into three groups.10 animals were placed in each group. Group A being the control received only the laboratory diet prepared at NIH. 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. The mice were sacrificed and dissected at the end of 60 days. Using intracardiac route 5ml blood was taken from each animal for hormonal assay. (v) Results: Hormone assay of the serum in the experimental group B showed decrease in serum estrogen and progesterone levels as compared to the group A and there was minor decrease in the hormonal levels in group C. (vi) Conclusion: Lead acetate causes decrease in the serum estrogen and progesterone levels in adult female mice and garlic extract prevents this effect.

Keywords: Garlic extract; Fallopian tubes; Estrogen; Progesterone

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. Water, air and food are sources of lead exposure to general population.

According to Neman [1] most lipsticks contain lead1. 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 10 µg/dl [5]. Same blood lebels 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]. Lead exerts toxic effects by multiple modes of action, including inhibition of enzymatic function, generation of oxidative stress, intervention with the action of essential cations, particularly calcium, zinc and iron, disruption of the integrity of membranes in cells and organelles and modification in cell signaling [8]. 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 [9-11]. Some studies suggested that low doses of lead affect reproduction and sexual development in small mammals either directly or indirectly [12]. Reproduction is likely to be of utmost importance in ecotoxicological assessment because of importance on population dynamics [13].

In recent years, research work threw light on the use of plants on the reproductive health of man and animals [14]. 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 [15,16]. Garlic preparations exert antioxidant action of scavenging reactive oxygen species, enhancing the cellular antioxidant enzymes glutathione peroxidase, superoxide dismutase and catalase, and increasing their concentration in cells The antioxidant compounds contained in garlic are known to exhibit antioxidant and metal chelating properties [17]. 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 [14]. The preventive and curative effect of combined supplementation of garlic and vitamin B complex against lead toxicity in albino mice is reported before [18]. Some previous studies have reported that garlic not only provides protection against lead toxicity but it can perform a therapeutical role against lead toxicity [19]. The rationale of current study is to observe the effects of lead acetate on serum estrogen and progesterone and the role of garlic extract.

Material and Methods

This laboratory based randomized controlled trial was conductedin the department of Anatomy, Army Medical College Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad from April-June 2013. Ethical approval for the experiment was taken from the Ethical committee of Army Medical College, Rawalpindi prior to commencement of the study (Ethical approved number 02/CREAM-A, Feb 2013).

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 gavage tube. 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 30mg/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 (product number. 11504) and garlic from the local market and the required solutions were prepared according to the doses [20].

At the end of 60 days, the animals were euthanized by ether anaesthesia and 5 cc was drawn through cardiac puncture. The blood was collected in non-heparinized test tubes and centrifuged at 5000 rpm (3556 RCF) for 5 min to separate serum. Serum was then placed in freezer at -20°C for hormone assay.

The data was analyzed by using statistical package for social sciences (SPSS) version 18. Descriptive statistics were used to describe the results. Mean and Standard deviation (SD) were calculated for serum estrogen and progesterone levels.The significance difference was determined using paired sample t test. Results were considered significant at $p<0.05$.

Results

The mean serum estrogen level of control group A was 80.35 ± 10.72, for group B it was 42.55 ± 4.94 and for group C it was 62.67 ± 4.37. The difference in the mean of serum estrogens levels between three groups were found statistically significant. (Table 1)

The mean serum progesterone level of control group A was 24.47± 2.11, for group B it was 16.41 ± 1.0, and for group C it was 24.46 ± 2.12. The difference in the mean serum progesterone levels between three groups was found statistically significant (Table-2).

Parameter	Groups			t	p-value
	A	B	C		
	Mean ± SD	Mean ± SD	Mean ± SD		
Estrogen level (p mol/l)	80.35 ± 10.72	42.55 ± 4.94		12.12	0
		42.55 ± 4.94	62.67 ± 4.37	-8.7	0
	80.35 ± 10.72		62.67 ± 4.37	4.3	0.002

Table 1: Comparison of estrogen levels between the groups

Parameter	Groups			t	p value
	A	B	C		
	Mean ± SD	Mean ± SD	Mean ± SD		
Progesterone level (ng/ml)	24.47 ± 2.11	16.41 ± 1.01		11.39	0
		16.41 ± 1.01	24.46 ± 2.12	-0.97	0
	24.47 ± 2.11		24.46 ± 2.12	0.036	0.97

Table 2: Comparison of progesterone levels between the groups

Discussion

The objective of this study was to see the effects of lead acetate on the serum estrogen and progesterone levels and the protective role of garlic extract. In the present study lead induced alterations in serum estrogen and progesterone levels 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 mean serum estrogen levels were significantly lower in lead acetate treated group B (42.55 ± 4.94) compared to control group (80.35 ± 10.72) ($p<0.05$) and the group C (62.67 ± 4.37) ($p<0.05$). This implies that lead acetate decreases serum estrogen levels and garlic extract protects the mice from lead acetate induced decrease in estrogen level. The increase in estrogen in group C compared to group B might be due to increase of LH hormone that leads to increase of its binding with the receptors on theca cells[21].

Similar to the findings regarding estrogen levels, the mean serum progesterone levels were also significantly lower in lead acetate treated group compared to controlgroup ($p<0.05$) and the group treated with lead acetate and garlic extract ($p<0.05$). The elevation of steroid hormones after treatment with lead acetate and garlic extract (group C) may be due to role of garlic components to reduce the negative effects of toxicity of lead acetate [22].The increase of LH hormone is considered as a principle factor to stimulate the theca interna to elevate production of pregnenolone compound which is converted by granulose cells to progesterone [21].

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 pharamcological properties. Senapati et al. [23] reported the prophylactic efficacy of garlic extract in reducing the lead burden from many tissues. In another study, Pourjafar et al. [24] also reported the ability of garlic to reduce the lead levels from the liver, kidney, blood and bone.

These findings of our study are similar to the findings of Arak [25] and Abdou [26] who found decrease in levels of estrogen and progesterone levels after lead acetate exposure. Arak found a decrease in levels of serum estrogen in mice which were exposed to 10mg/kg of lead acetate for 8 weeks. The findings were similar to the findings of some other researchers who reported decrease in levels of serum estrogen and progesterone in mice exposed to 200mg/L lead acetate for 3 weeks [22]. Eugenia [9]on his study on female reproductive organs after long term exposure to low lead levels also found decrease in the levels of estrogen and progesterone. The reason for decrease in these hormone levels in group B might be the decrease in the levels of gonadotrophic hormones due to lead exposure.

Conclusion

From the present study it is concluded that treatment of mice to lead acetate results in a decrease in serum levels of estrogen and progesterone which can therefore affect fertility, while co-administration of garlic extract has protective role against the fall in the levels of estrogens and progesterone.

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