

Protective Role of Vitamin C against Teratogenic Effects of Lead Acetate on Mouse Kidney

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ABSTRACT

Background: Lead (Pb) poisoning has been known to produce congenital abnormalities in humans and experimental animals, it has also been reported to produce oxidative damage in developing adult rats. Supplementation of diet with vitamins C and E have been found to reduce toxic effects of lead acetate on adult animals and human being.

Methods: Twenty one female pregnant mice were used as an experimental model. They were divided into three groups, Group A as control group and Group B and C as an experimental group. Group A (control group) was given 0.02 ml distilled water as a vehicle, group B was given lead acetate 10 mg / kg dissolved in 0.02 ml of distilled water throughout the pregnancy. Group C was given lead acetate 10 mg / kg and Ascorbic Acid 140 mg / kg each dissolved separately in 0.02 ml of distilled water simultaneously throughout the pregnancy. The drug was administered daily in single dose orally to all the pregnant mice. The female mice were sacrificed on 18th day of gestation and fetuses were then dissected out to observe their kidneys.

Results: The mean thickness of nephrogenic zone was in range of 100-105µm in groups A and C. It was 99-105µm in group B. Statistical analysis by ANOVA showed insignificant results among three groups. The cortical thickness of kidneys was in range of 576-581µm, 499-522µm and 575-580µm in groups A, B and C respectively. ANOVA showed significant difference between mean cortical thickness of three groups.

Conclusion: In current investigation nephrotoxicity by lead acetate was observed and its prevention by vitamin C was documented.

Key words: Lead acetate, Ascorbic Acid (vitamin C)

INTRODUCTION

Lead is one of the most harmful heavy metals which is able to induce renal, hepatic and testicular injury. This toxicant can be stored in the bone and this body burden may be mobilized during pregnancy and lactation. In gestational period, the metal alters the normal fetal development. Like other heavy metals lead is known to induce oxidative stress in animals indicated by a sharp rise in lipid peroxidation products (LPP). Numerous animal studies have confirmed that exposure to low levels of lead acetate either during early development or during postweaning periods can produce long lasting changes in the fetus.

In vitro and vivo studies, in rat upon administration of low to moderate concentrations

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(10nM – 1µM) of lead acetate, showed apoptosis in retina; selective loss of rods and bipolar cells was observed during development and in adult rats; the dying cells exhibited signs of apoptosis; the cell death was reported to be age- and dose-dependent¹.

Lead acetate was reported to produce neurotoxic effects by increasing lipid per oxidation, which can be assessed indirectly by analysing lipid fluorescence products. In the brain, lead accumulated preferentially in the parietal cortex, corpus striatum, thalamus and hippocampus as compared to that in the control group; the lipid fluorescence products were significantly increased in the corpus striatum, thalamus, and hippocampus indicating lipid per oxidation is directly related to lipid and lead contents of tissue².

The natural antioxidants check the negative influence of free radicals and its associated reactions^{3,4} conventional therapeutic management includes chelation of lead from accumulated tissue, but chelating agents are contraindicated in pregnancy⁵. However recent implication of oxidative stress contributing to lead associated tissue injury suggests incorporation of antioxidants for better

therapeutic management⁶. Use of antioxidants in vivo proved beneficial in protecting cells from oxidative damage⁷.

Lead acetate intoxication was reported to decrease the normal hepatocytes count, indicating the damage to membranes of cells and organelles which were damaged by lipid per oxidation due to reactive oxygen species (ROS); it was also observed that Vitamin C treatment prior to lead intoxication prevented the damage to hepatocytes. Vitamin C is a powerful scavenger of ROS, which stopped the process of lipid per oxidation, thereby preserving membrane integrity⁸.

Antioxidant property of ascorbic acid is well established but has not been studied sufficiently for its protective effects against lead acetate induced toxicity on kidney of pups. The present work was, therefore, designed to investigate the nephrotoxic effects of lead acetate and the protective role of vitamin C on the developing kidney, using mouse as an experimental model.

MATERIAL & METHODS

The study was conducted on 21 female mice (BALB/c strain) weighing about 30-34 g, maintained on standard pellet diet and drinking water ad libitum. The mice were purchased from National Institute of Health, Islamabad. The mice were kept under close observation in order to maintain good health for conducting experiment properly. The room lighting consisted of alternate 12 hours light and dark periods. All the mice were randomly divided into three equal groups and were marked as groups A, B, C.

Three females along with a male mouse were housed in a single cage for mating purpose. The female mice were examined every morning for the presence of vaginal plug and were separated from the male and housed in separate cages after confirmation of pregnancy; appearance of vaginal plug was regarded as day zero of pregnancy. Thereafter, the mice were divided randomly into three groups of equal number, containing seven mice each.

Lead acetate trihydrate of E-Merck and Ascorbic acid of Riedel-de Haen (RdH) were procured through University of Health Sciences, Lahore. The dose selection of lead acetate and ascorbic acid was based on previous studies^{9,10}.

Group A (control group) was given 0.02 ml distilled water as a vehicle, group B was given lead acetate 10mg/kg dissolved in 0.02ml of distilled water throughout the pregnancy. Group C was given lead acetate 10mg/kg and Ascorbic Acid 140mg/kg each dissolved separately in 0.02ml of distilled water simultaneously throughout the pregnancy. The drug

was administered daily in single dose orally to all the pregnant mice.

Pregnant female mice were sacrificed to deliver the fetuses on 18th day of gestation. The animal was anaesthetized by chloroform and then dissected to deliver the fetuses.

The fetuses were dissected by giving a vertical mid line incision under the dissecting stereo microscope (Carolina Biological- MOTIC ST-39 series – Wolfe microscope) and the kidneys were removed; these were inspected for their color and any visible deformity. Each kidney was washed with distilled water, weighed and fixed in a 10% formalin solution for 72 hours. The specimens were dehydrated in ascending series of ethanol, cleared in xylene and infiltrated with filtered paraffin with melting point of 56-58 °C. The paraffin blocks were made and five micron thin sections were obtained using a rotary microtome (Leica RM 2125).

Hematoxylin and eosin stains were used for histological study using light microscope (Leica DM 1000). Measurement of nephrogenic and cortical thickness was made after calibrating eyepiece graticule with stage micrometer at various magnifications using X10 and X40 objectives as described below:

- X10 eyepiece and X40 objective were adjusted to focus the stage micrometer scale.
- Exact number of divisions of eyepiece micrometer scale coinciding with an exact number of divisions of stage micrometer scale was determined.
- 19 eyepiece divisions were equal to 5 stage division.
- 1 stage division = 10 μ m
- 19 divisions of eyepiece micrometer = 5 stage division = 50 μ m

1 division of eyepiece micrometer = $50/19 = 2.63\mu\text{m}$

Statistical analysis: The statistical analysis was carried out using computer software Statistical Package for Social Sciences (SPSS) version 15.0. Mean \pm S.D. were given for normally distributed quantitative variables. ANOVA technique was applied to observe mean differences among all groups and post hoc Scheffe test was used to observe mean difference between two groups. A p-value < 0.05 was considered statistically significant.

RESULTS

On gross examination the kidneys were bean-shaped, reddish brown in color and had a smooth texture; these were located in the lumbar region with the left kidney situated slightly cranially than the right kidney. No gross abnormalities of shape, size or

location were observed in the control or the treated groups.

The kidneys showed clearly defined cortex and medulla. The capsule having fibrous tissue was well developed in the treated as well as in control group. The mouse fetal kidneys differed significantly from the adult kidneys due to the presence of a distinct subcapsular nephrogenic zone. The cortex was distinguishable into two zones, an outer nephrogenic zone and inner, deep part of cortex (Fig. 1,2,3). Nephrogenic zone was present inside the capsule, composed of nephrons in different stages of development, and undifferentiated tissue (Fig. 1,2,3). The cortical portion of the kidneys contained convoluted tubules, renal corpuscles, straight tubules and medullary rays. They also contained interlobular vessels. The medullary rays comprised of straight parts of nephric and collecting tubules.

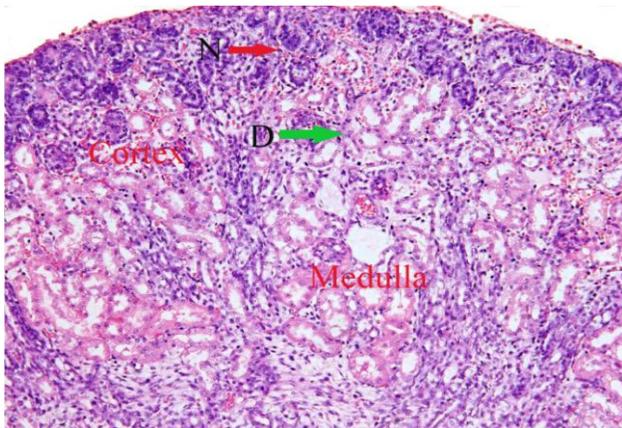


Figure 1, Photomicrograph of kidney (group A) showing cortex and medulla, deep part of cortex (D) and nephrogenic zone (N) of cortex. H&E stain x200

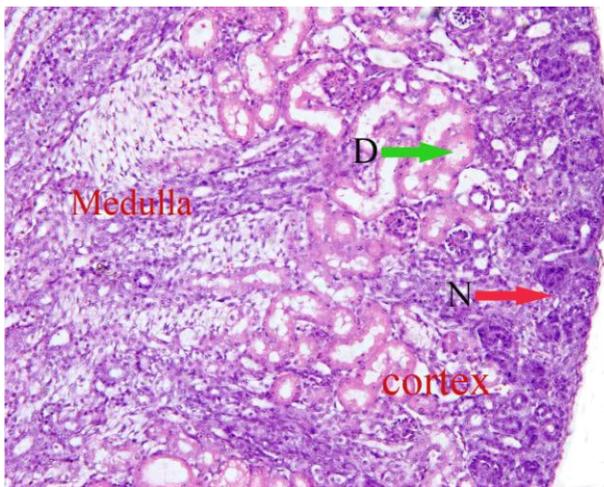


Figure 2, Photomicrograph of kidney (group B) showing cortex and medulla, deep part of cortex (D) and nephrogenic zone (N) of cortex. H&E stain x200

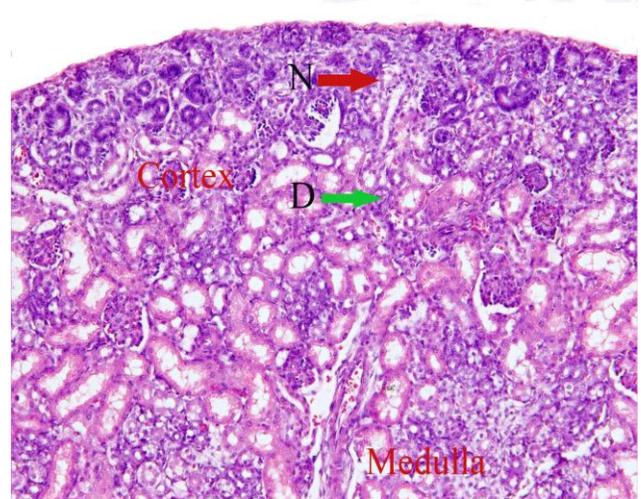


Figure 3, Photomicrograph of kidney (group C) showing cortex and medulla, deep part of cortex (D) and nephrogenic zone (N) of cortex. H&E stain x200

Nephrogenic Zone: The mean thickness of nephrogenic zone was in range of 100-105µm in groups A and C. It was 99-105µm in group B (Fig 1,2,3). Statistical analysis by ANOVA showed insignificant results among three groups (Table 1).

Table 1: Comparison of mean nephrogenic zone thickness of kidneys among three groups by ANOVA.

Variables	Nephrogenic zone thickness (µm)
Group A Mean ±S.D	102±1.7
Group B Mean ±S.D	101±1.6
Group C Mean ±S.D	102±1.7
P value	0.062**

*p-value<0.05 is statistically significant

**Statistical analysis showing insignificant difference among groups.

Cortex of kidney: The cortical thickness of kidneys was in range of 576-581µm, 499-522µm and 575-580µm in groups A, B and C respectively. The fetal kidneys from lead exposed group B exhibited thin cortex as compared to groups A and C (Fig.1,2,3). ANOVA showed significant difference between mean cortical thickness of three groups ($p < 0.0001$, Table 2). Multiple comparison using post hoc Scheffe test showed significant difference between groups A and B ($578.6 \pm 1.4 \mu\text{m}$ vs $515.9 \pm 5 \mu\text{m}$) illustrating that mean cortical thickness was less in group B as compared to group A ($p < 0.0001$, Table 2a). Groups B and C ($515.9 \pm 5 \mu\text{m}$ vs $577.6 \pm 1.4 \mu\text{m}$) showed significant difference ($p < 0.0001$, Table 2a) using Scheffe test indicating that cortical thickness was less in group B as compared to group C. Scheffe test also showed that difference was statistically insignificant ($p = 0.49$, Table 2a) among the groups A and C ($578.6 \pm 1.4 \mu\text{m}$

vs 577.6±1.4µm) revealing protection by vitamin C against lead acetate toxicity in group C.

Table 2: Comparison of mean cortical thickness of kidneys among three groups by ANOVA.

Variables	Cortical thickness(µm)
Group A Mean ±S.D	578.6± 1.4
Group B Mean ±S.D	515.9±5
Group C Mean ±S.D	577.8±1.4
P value	0.0001*

*p-value<0.05 is statistically significant

Table 2a: Multiple comparison of cortical thickness of kidneys among three groups by ANOVA using Scheffe test.

Comparison among groups		Mean difference (α-β)	Level of significance p- value Group
Group (α)	Group compared (β)		
A	B	62.7	0.0001*
	C	0.81	0.49
C	B	61.9	0.0001*

*p-value<0.05 is statistically significant

DISCUSSION

Lead acetate has been reported to be associated with growth retardation in human being.¹¹ Although limited exposure to lead acetate during pregnancy, it may have considerable lead burdens from the earlier exposure to it during their childhood. Bone stores of lead acetate are considered to be its main source in blood even if environmental exposure is low during pregnancy¹².

In present work, group B showed reduced cortical width (M.D= 61.9µm, p<0.0001, Table 2 and 2a) as compared to group A. The findings were statistically highly significant (p<0.0001) and corroborate similar findings from the previous studies^{13,14}. Nephrogenic zone thickness (p>0.05, Table 1) were comparable in three groups. Effects on kidney might be due to direct and indirect action of reactive oxygen species (ROS) produced by lead acetate and ROS causing lipid peroxidation of membranes of cells and organelles, damaging their structural integrity¹⁵. Schwartz et al. (2000)¹⁶ reported the indirect action of ROS by decreasing glutathione reductase and increase in oxidized glutathione in blood resulting oxidative damage to tissue. Lead deposited predominantly in the proximal tubule, considered to be the main reason for stronger deleterious effects on the cortex than medulla of the kidney.¹⁷ Retarded growth observed in current investigation may be considered to be due to reduced cell proliferation.

The current work showed an increase in cortical thickness in group C (M.D.=61.9µm, p<0.0001, Table 2 and 2a).

The data presented in the present studies showed that Vitamin C protected the fetus in a manner similar to that previously reported in adults^{3,10,15}. These findings in the current project were statistically significant and corroborated with findings of Mishra M et al.(2004)¹⁸ lead acetate in an experimental study on albino mice (10 weeks old) produced oligospermia, they found that intraperitoneal administration of vitamin C increased the sperm count indicating protection by it. Ascorbic acid was reported to decrease the concentration of lead acetate in blood of adult mice preventing anemia¹⁹. Supplementation of diet with ascorbic acid during lead exposure was also reported to reduce spermatotoxicity by inhibiting generation of lead related reactive oxygen species (ROS)²⁰. The present studies finding imply that differences in treated animals with lead acetate and vitamin C plus lead acetate are due to protective effects of vitamin C as it is known to scavenge free radical and decreasing concentration of lead acetate in blood. Similar effects of vitamin C were reported by protecting lipid and protein in biological fluids and oxidative DNA damage in human and animals²¹. It was supposed that vitamin C provided protection to developing kidney by decreasing concentration of lead acetate in blood.

CONCLUSION

In current investigation nephrotoxicity by lead acetate was observed and its prevention by vitamin C was documented.

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