Dietary Exposure to Lead (Pb) Related with Biochemical and Histomorphological Changes in Testes and Kidney of Rabbit

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ABSTRACT: The experiment was conducted to observe the toxic effect of different doses of dietary lead (Pb) on biochemical, gross and histopathological changes in the testes and kidneys of male rabbits. A total number of 36 rabbits (4.5 months of age) were assigned in three dietary treatments with three replicates. Control group T₀ received only a basal diet and the other groups T₁ and T₂ received a basal diet supplemented with Pb at a dose level of 20 and 40 mg/kg bw respectively. By the end of the experiment (4 months), animals were slaughtered and blood and tissue samples were collected for biochemical and histopathological tests. Oral administration of Pb caused decreased weight and congestion of the kidney attributed to the glomerular shrinkage and reactive cell infiltration microscopically. Significant decrease in weight, degeneration of germ cells, and vacuolation of the seminiferous tubules were recorded in testes. Serum testosterone level was decreased in lead-treated groups which might be due to the cellular degeneration of Leydig cells. In conclusion, lead acetate had a serious dose-dependent biochemical and histomorphological effect in the male rabbit.

Keywords: Lead, Biochemical parameter (serum testosterone), Micromorphological.
1. Introduction

Lead (Pb) is a soft grey-blue toxic heavy metal found everywhere which causes poisoning in domestic animals and severely affected occupational problem associated with lead toxicity that takes place over the world (Ahmed et al., 2012). Various commodities like paints, water tape, cosmetics, fuel etc are integrated with lead due to certain properties including low melting point, resistance to corrosion, although it is toxic (Soliman et al., 2015. and Shotyket al., 2017). Paints, water, food, dust, soil, kitchen utensils, and leaded gasoline are the excellent sources of lead toxicity. They tend to accumulate in the food chain and in the body. As a result gastrointestinal, hematological, reproductive, immunological disorder have been recorded (Rotimi et al., 2007, Patrick et al., 2006 and Rana et al., 2017). It was reported that body weight and organ weight (testes) reduced due to lead exposure. Kidneys are indefensible to lead (Pb) exposure at a greater extent, as it is the major route of excretion from the body (WHO 2016, UNEP, WHO 1995) and alleviates kidney damage through oxidative stress. On histopathology, tubular lining cells of kidney revealed necrosis and fibrous tissue proliferation, reactive cell infiltration and hypertrophy of kidney (Milena Andjelkovic et al., 2019, Ramazan Durgut et al., 2008, Priya and Reddy 2012, Ayinde et al., 2012, and Jegede et al., 2015). Increased dietary intake of lead has been associated with cancers of different organ and all leukemia (Reddy et al., 2004). It was examined that higher polyunsaturated fatty acid content of testes enhances oxidative stress (Acharya et al., 2003) and degeneration of cells of seminiferous tubules including vacuolization (Ahmad 2003). It has also been shown that lead influences intracellular reactive oxygen species production and lipid peroxidation, which causing damage of tissue in the reproductive system of animal (Wang and Jia 2009) and impairment of the semen quality in the male reproductive system (Eibensteineret al., 2005; Telismanet al., 2007), decrease sperm quality and production (Ahmed et al., 2012). Lead can decrease the testosterone level of rabbit due to decrease the quantity of sulfated steroids excreted in the urine (El-Nattatet al., 2000). Although a number of studies have been focused on animal health risk associated with lead exposure, there is currently a lack of detail information about the biochemical effect of lead exposure. As a consequence, the present research designate to investigate the dietary exposure of lead acetate on the kidney and testis of rabbit as mammalian model. Evaluate the effect of lead on biochemical parameters of rabbit and observed histopathological changes in kidney and testes sample owing to lead toxicity in rabbit was our objective.

2. Objective

To observe the biochemical and histopathological changes in testes and kidney sample owing to lead toxicity in rabbits.

3. Study site and duration:

The research (rearing of rabbit, measurement of body weight, collection of blood and viscera) was carried out at Hajee Mohammad Danesh Science and Technology University (HSTU) animal shed, Basherhat, Dinajpur and histopathology was performed at the Histology laboratory of department of Anatomy and Histology, HSTU, Dinajpur.

4. Purchase Rabbit and Chemical:

Male Rabbits were collected from bahadur bazar market, Dinajpur, Bangladesh. Lead acetate (metal) powder was collected from Rahman Scientific Store, Dinajpur, Bangladesh.
5. Experimental design:

In this study 36 (4.5 months aged) male rabbits were distributed erratically into three groups T0, T1, and T2; each group contained 4 rabbits with three replications. Rabbits were randomly distributed to cages. The rabbits were handling carefully and observed intimately for 4 days with the maintenance of basal diet. Then treatment of rabbits with lead started. Group T0 were maintained as healthy control supplied only basal diet (cabbage, carrot, broiler grower). Lead powder @20 mg/kg bw in group T1, and @40 mg/kg bw in group T2 were given orally after mixing with broiler grower provided with basal diet. Adlibitum fresh drinking water were also supplied. Blood had collected from two rabbits from each group before started the treatment with lead and performed the testosterone hormone (TH) test. Blood was collected from ear and jugular vein of Rabbits. During the experiment all of the rabbits were observed regularly for abnormal physical and behavioral changes as well as mortality (if any) regarding lead toxicity.

6. Body weight measurement:

Body weight of each rabbits were taken before the treatment started and subsequently at monthly interval upto 4 months for all groups.

7. Collection of blood and viscera:

Two rabbits from all of the groups (T0, T1, and T2) were selected randomly and were slaughtered after fasting for 12 hours at the end of the experiment. Kidney and testes samples were collected to observe the gross changes of these organs. Blood samples were also collected from the jugular vein before the treatment started (1st day), and before slaughtered for analysis of blood including testosterone hormone test.

8. Histopathological study:

The collected samples were fixed in 10% formalin for 24 hours to preserve the sample for histopathological study. Dehydration of the tissues were then made by using ascending grade of alcohol (70%, 80%, 90%, 95%, 100% and 100%) for one hour in each. Then transferred the tissues into xylene-1 and xylene-2 for ninety minutes in each solution. Infiltration of the tissues were done at 600C temperature in the liquid paraffin for ninety minutes and continued once more. Then embedded the tissues in paraffin and paraffin blocks were made. Later on, sectioning of the paraffin blocks at 6 μm thickness were made by using microtome machine (Mu 509, Euromex, Japan). Then the slices were taken and placed on warm water in a water bath at 45oC for stretching. The sections were taken on a clean glass slide and stained with Hematoxylin and Eosin (H & E) stain for histological study. The slides were observed using a light microscope and taken the photographs with an automatic photo micrographic system.

9. Statistical Analysis:

Data were exposed as mean ± standard error (SE) and resolved by using one way analysis of variance (ANOVA) followed by Duncan’s test as a post-hoc test using IBM SPSS Statistics 22.0 software package and the table was made by Microsoft Excel 2007 software. Outcome were considered to be statistically momentous when P values are less than 0.01 (P<0.01).
10. Results:

In this experiment, all of the treatment groups provided with different dosage of dietary lead showed significant differences in biochemical, gross and histological changes which are delineated below: 10.1 Clinical findings In first month, there was no obvious clinical signs in rabbit of any treatment groups due to lead toxicity. After 1st month, clinical symptoms like mild depression, dullness, decreased feed intake, irritability, fatigue, myalgia, pale mucous membranes and gastrointestinal signs such as diarrhoea, difficulty in breathing, loss of appetite for food or water, lethargy, weakness was observed. Mortality was observed in T1(lead 20 mg/kg bw) and T2(lead 40 mg/kg bw) experimental group. 10.2 Blood parameter Rabbits exposed to lead had shown significantly lower testosterone hormone (TH) (P<0.01) concentrations at 4 months (last day of the experiment) compared to the control group (Table 1). Lowest testosterone hormone was found in group T2(0.51±0.05u/l) followed by group T1 (1.02±0.12u/l) compared to control T0(2.29±0.65u/l). 10.3 Gross findings Congestion of testes was grossly observed at postmortem examination (Fig. 2) and lowest testes weight was found in group T2(0.63±0.05 gm) followed by group T1(1.15±0.10 gm) against the control T0(1.53±0.09 gm) (Table 2). Similarly the congestion (Fig. 4) and weight loss (Table 2) of kidney in group T1(3.38±0.05) and T2(3.93±0.09) compared to control T0(4.35±0.33) was observed. 10.4 Microscopic findings Upon microscopic observation, testes of lead treated rabbits exhibited different types of vascular changes like reactive cell infiltration, degenerative changes including vacuolation in the seminiferous tubule, degeneration of germ cells, and absence of sperm in group T1(Fig. 6). Whereas in group T2(Fig. 7) highly vacuolated seminiferous tubule, the shape of seminiferous tubule were irregular and shrinkage and/or atrophied due to cytotoxicity with less germ cell population and spermatogenic cell necrosis in the seminiferous tubules. Testis of control group T0(Fig. 5) revealed no change and showed normal architecture of testis, the seminiferous tubules looked round or oval in shape enclosed by a thin basement membrane. Arrangement of the spermatogenic cells starting from spermatogonia, primary spermatocytes, secondary spermatocytes, spermatid till mature spermatozoa were observed from the basement membrane to the lumen of the seminiferous tubule. Microscopic examination of kidney of group T1(20 mg/kg bw) revealed no noteworthy changes in the proximal and distal convoluted tubules & loop of Henley, but phagocytic/reactive cell infiltration, and slight fibrous tissue proliferation was observed in glomerulus (Fig.9). Group T2(40 mg/kg bw) showed fibrous tissue proliferation in the tubular and peritubular structure and congested glomerulous with neutrophilic infiltration of kidney (Fig. 10). Kidney of control group T0(Fig.4.15) seems no change microscopically.

11. Discussion:

Chronic administration of lead acetate in the present study produced various clinical symptoms like mild depression, dullness, irritability, fatigue, myalgia, weakness, lethargy, and pale mucous membranes which supported previous findings from Ramazan Durgut et al., (2008) in rabbit.

The gross changes of the current study showed pale and congested kidney (Fig. 4) which confirms the findings of Ramazan Durgut et al., (2008). The weight of kidney, liver, testis was reduced (Table 2). The possible causes for the gross changes in liver, spleen, intestine and kidney are their involvement in lead metabolism as they are the primary target organ for the lead metabolism and kidney is the major root for excretion of these metabolites. However, gross examination of control group (T0) showed normal kidney (Fig. 3), and testes (Fig. 1). In this experiment, concentrations of serum testosterone decreased significantly in lead treated rabbits at 4 months compared to the
control group (table 4). Group T; and T: had lowered serum testosterone hormone (0.51±0.05 nmol/L) and (1.02±0.12 nmol/L) respectively compared to the control T0(2.29±0.65 nmol/L). Similar effects following chronic administration of lead were observed in male rabbits (El-Nattatet al., 2000), rats (Priya et al., 2012, Ayindeet al., 2012, Reshma Anjum et al., 2015 and Jegede et al., 2015). Lead had a interfering activity in the hypothalamic-pituitary axis in animal which exerted a detrimental effect on the testes (Sokol et al., 2002). Acharya et al., (2003) observed that serum testosterone decreased with an increase in semen lead concentration in Swiss mice although concentrations of blood lead were not correlated with serum testosterone. Observation of positive relationship between blood lead level and serum testosterone level had been reported by Kresovich et al., (2015); Lewis and Meeker (2015); Meeker et al., (2010); Telisman et al., (2007), whereas negative relationship or no effects had been reported at PbB>10 μg/dL. Dietary treatment with lead in this study significantly (P<0.01) decreased testes weight in the experimental rabbits (table 5) comparing to control group which is accord with some earlier experiment by Gupta (2003) and Predes (2010), and Amir Amanullah et al., (2016). The mean testicular weight in the lead acetate treated group were reduced significantly in comparison with the control group observed by Faiza Rao et al., (2016). Acharya et al., (2003) reported reduction in sperm production and testicular weight in mice due to excessive lead consumption. Durgesh et al., (2015) also reported that testis (p<0.01) and epididymis (p<0.01) weights were reduced significantly in lead acetate treated mice for 45 days comparing to control, whereas in vit.E + lead acetate treated group the weights of organs were not reduced significantly comparing to control. The loss of testicular weight may be due to the interference of steroid biosynthesis of leydig cell associated with the failure of formation of spermatogenic cells mass and spermatogenic arrest (Acharya et al., 2003). Microscopic study of testes in the present study showed normal architecture of testes in control group (T0), the seminiferous tubules looked round or oval in shape enclosed by a thin basement membrane. Arrangement of the spermatogenic cells starting from spermatogonia, primary spermatocytes, secondary spermatocytes, spermatid till mature spermatozoa were observed from the basement membrane to the lumen of the seminiferous tubule (Fig. 5). Group T; showed degeneration of germ cells, absence of sperm and vacuolation in the seminiferous tubule (Fig. 6) and ingroup T; the seminiferous tubules were irregular in shape and shrinkage and atrophied due to cytotoxicity and less germ cell population in the seminiferous tubule, vacuolation and spermatogenic cell necrosis in the seminiferous tubules (Fig. 7) was also observed. These findings are similar with some other findings such as Batra et al., (2001) who reported that lead exposed rats showed disorganisation and interruption of spermatogenesis in the lumen of seminiferous tubules. El-Shafaiet al., (2011) also mentioned that lead acetate (25 mg/kg bw) induced albino rats showed degeneration of cells of seminiferous like heterochromic nuclei, irregular basal lamina and vacuolization. Sperm density and activity were decreased, and malformation of sperm increased in lead acetate treated mice testes (Wang et al., 2013). Degeneration of testicular tissue of rats exposed with textile waste water consisting heavy metals above permissible levels which accord with the present findings (Gupta 2003). Similarly Ahmad (2003) suggested degeneration in the seminiferous tubule compared to control and also by Ahmed et al., (2012), El-Sayed et al., (2015), Fatma et al., (2015), Faiza Rao et al., (2016), and Amir Amanullah et al., (2016). These results indicated that lead targets testicular spermatogenesis to produce reproductive toxicity in adult male rabbits. Therefore chronic Pb exposure has a spermicidal effect that leads to degeneration and reduction of spermatogenic cell population resulting in shrinkage of the seminiferous tubules.
Microscopic examination of kidney of group T1 revealed no noteworthy changes in the proximal and distal convoluted tubules & loop of Henley, but phagocytic/reactive cell infiltration, and slight fibrous tissue proliferation was observed in glomerulus (Fig. 9). Group T2 showed fibrous tissue proliferation in the tubular and peritubular structure and congested glomerulous with neutrophilic infiltration of kidney (Fig. 10). Kidney of control group T0 (Fig. 8) seems no change microscopically. These findings are in agreement with Milena Andjelkovic et al., (2019), Ramazan Durgut et al., (2008), Mohammad et al., (2018), Zhang et al., (2017), Huang et al. (2017), Priya and Reddy, (2012), Ayinde et al., (2012), Reshma Anjum and Sreenivasula Reddy, (2015), and Jegede, et al. (2015). Samuel et al., (2017) reported massive lymphocytic infiltration with degenerative changes and necrosis of the renal cortical parenchymal cells and vacuolation of the peripheral interstitial cells of the cortex of lead acetate (60 mg/kg bw) induced rats kidney. Ahmed et al., (2012) also reported that renal tissues showed diffused degenerative changes of Bowman's capsule and renal glomeruli, advanced hyaline degeneration and necrosis of epithelial cells of kidney tubules in both cortex and medulla. Areas of coagulative necrosis and renal infarction was seen at high dose of lead, with infiltration of mononuclear inflammatory cells, also intranuclear inclusion bodies were seen in the epithelial cells of renal tubules.

12. Conclusions

In this experiment, lead acetate was supplied to rabbits at 20 mg/kg bw and 40 mg/kg bw orally, changes were observed on gross and microscopic study of testes and kidney, and biochemical parameters also. During the experiment two rabbits were died. As demonstrated in the current study, body weight as well as weight of organs (testes, kidney) decreased in lead treated rabbit is due to loss of appetite, malabsorption, impaired metabolism and gastrointestinal disturbances. There were different types of histological changes which accompanied with biochemical changes in the kidney and testes in lead treated groups compared to control group. According to the present study, lead supplementation at all doses had a noteworthy effect on microscopic structure of various organs including fibrous tissue proliferation in the glomerulous of kidney, degeneration of germ cells and vacuolation of the seminiferous tubules of testes. Serum testosterone level was decreased in lead treated groups which might be due to cellular degeneration of leydig/ interstitial cell. Due to lack of technical facilities, present findings cannot definitively ascertain fertility status. Therefore, future studies should aim to establish more concrete links between lead’s effects on reproductive dysfunctions and reduced fertility rates. Moreover, public health hazards due to lead exposure & its preventive strategies should also be undertaken.
TABLE

Table 1: Testosterone Hormone level of rabbit at 4 months fed varying levels of lead

<table>
<thead>
<tr>
<th>Parameters [Testosterone Hormone (nmol/L)]</th>
<th>Various treatment groups showing mean ± SE values</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T₀</td>
<td>T₁</td>
</tr>
<tr>
<td>Initial value</td>
<td>2.37±0.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.25±0.43&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>End value</td>
<td>2.29±0.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.02±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means on the same row with different superscripts are significantly different (P<0.01). NS: Non-significant and S: Significant at 1% level.

Table 2: The effects of different levels of lead on weight (gm) of organ of rabbit after slaughter (26.06.20)

<table>
<thead>
<tr>
<th>Name of organs</th>
<th>Testes weight of various treatment groups showing mean ± SE values</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T₀</td>
<td>T₁</td>
</tr>
<tr>
<td>Testes</td>
<td>1.53±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.15±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kidney</td>
<td>4.35±0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.38±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Liver</td>
<td>30.35±0.345&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.10±0.50&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means on the same row with different superscripts are significantly different (P<0.01). NS: Non-significant and S: Significant at 1% level.
GROSS AND MICROSCOPIC FIGURES

Fig 1: Normal Testes

Fig 2: Atrophied and Congested Testes in T₁ & T₂

Fig 3: Normal Kidney

Fig 4: Congested Kidney in T₁ & T₂
Fig. 5: Microscopic view of testis: showing normal histological structure (seminiferous tubules) (black arrow) in group T0 (H and E, Dimension- 947 619)

Fig. 6: Microscopic view of testis: showing vacuolation in the seminiferous tubule (white arrow head), degeneration of germ cells (black arrow head), and absence of sperm (blue arrow head) (seminiferous tubules) in group T1 (H and E, Dimension-947 619)
Fig 7: Microscopic view of testis: Showing highly vaculated seminiferous tubule (White arrow head), atrophied seminiferous tubule (Blue arrow head) with less germ cell population and necrosis of spermatogenic cells (Black arrow head) in the seminiferous tubules in group T2 (H and E, Dimension-947× 619)

Fig. 8: Microscopic view of kidney: Group T0 showed normal structure of kidney tubules and glomerulus and renal corpasol (white arrow head). (H and E, Dimension-947× 619)
Fig. 9: Microscopic view of kidney: Group T1 showed slight fibrous tissue infiltration (white arrow head), and shrinkage glomerulus (black arrow head). (H and E, Dimension- 947×619).

Fig. 10: Microscopic view of kidney: Group T2 showed fibrous tissue proliferation (White arrow head), shrinkage glomerulous (blue arrow head) with reactive cell infiltration of kidney. (H and E, Dimension- 947×619)
Acknowledgments

The author expresses her sincere appreciation, heartfelt indebtedness and profound regard to her respected teacher and supervisor, Professor Dr. Md. Toheder Rahaman and her co-supervisor, Dr. Khadija Al-Ferdous, Associate Professor, Department of Anatomy and Histology, Faculty of Veterinary & Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur for their scholastic guidance, generous help, keen interest, constructive criticism, valuable suggestions, constant encouragement and affectionate feelings from the beginning to the end of the research work and in the preparation of the thesis. The author also wishes to express her heartfelt indebtedness to Dr. Md. Najmul Hassan Parvez, Professor and Chairman, Department of Anatomy and Histology, HSTU, Dinajpur, for his valuable teaching and suggestions during the study period. The author expresses her regard and deepest sense of appreciation to her respective class teachers Dr. Md. Sadequl Islam, Dr. Md. Abu Hassan, Department of Anatomy and Histology for their good teaching and co-operation during the study period. The author would also like to thank to all staff of the department, her friend’s and Dean of the faculty for their sincere and active co-operation during the trial period.

Conflicts of Interest

There are no conflicts to declare.

Author’s contributions

All authors contributed equally to the preparation of the manuscript

REFERENCES


