ANTIENZYMETIC ACTION OF LEAD ACETATE AND ITS POSSIBLE REVERSAL BY ANTIOXIDANT IN TESTICULAR TISSUE OF SWISS ALBINO MICE DURING PUBERTAL PHASE OF LIFE.

Durgesh Nandini Sharma

Endocrinology and Physiology Unit, School of Studies in Zoology and Biotechnology, Vikram University, Ujjain (M.P.) 456010 India

Corresponding author: dr.durgeshnandini@rediffmail.com

ABSTRACT: Lead intoxication has been associated with male reproductive toxicity in experimental animals and lead may have the potential to produce adverse effects on enzymatic activity in testicular tissue of Swiss albino mice. The present study was undertaken to investigate the ability of antioxidant (Vitamin E) to protect against lead acetated (LA) induced testicular enzymatic toxicity in male albino mice during pubertal phase of life. The weight of testis, caput epididymidis, cauda epididymidis, vas deferens and testicular enzymatic activity (Glutathione peroxidase (GSH-Px), Succenate dehydrogenase (SDH), 65–3β hydroxysteroid dehydrogenase (65–3β–HSD) and 17β–hydroxysteroid dehydrogenase (17β–HSD) were studied. Administration of LA at a dose of 1.25mg/kg body weight for 45 days lowered the weights of testes, caput epididymidis, cauda epididymidis, vas deferens and decreased the activities GSH-Px, SDH, 65–3β–HSD and 17β–HSD. Coadministration of vitamin E (2 mg/kg BW) to the LA group restored all the parameters cited above to near the control values. Therefore, this study revealed that vitamin E has beneficial effects against LA induced enzymatic toxicity in testicular tissue of mice.

Key words: Albino mice, Enzymes, Lead Acetate, Testes, Vitamin E.

INTRODUCTION

The diverse deleterious health effects upon exposure to heavy metals in the environment are a matter of serious concern and a global issue. Lead is the most abundant toxic metal in the environment (Patra et al., 2011). Lead does not have any detectable beneficial biological role however on the contrary its detrimental effect on physiological, biochemical and behavioral dysfunctions have been documented in animals and humans by several investigators (Ruff et al., 1996). Lead is a male reproductive toxicant (Sallmen, 2001). Toxicity is manifested in male reproductive function by deposition of lead in testes, epididymis, vas deferens, seminal vesicle and seminal ejaculate. Lead has an adverse effect on sperm count, sperm motility and retarded the activity of alive sperm (Chowdhury, 2009). Clinical and animal studies indicate that abnormalities of spermatogenesis result from toxic exposure (Ati Hamadouche et al., 2009). The mechanism behind lead toxicity is the oxidative stress and it develops when there is an imbalance between the generation of reactive oxygen species and the scavenging capacity of antioxidants in the reproductive tract. Reactive oxygen species (ROS) have been shown to have an important role in the normal functioning of a reproductive system and in the pathogenesis of infertility (El-Tohamy, and El-Nattat 2010). Accumulated evidence has revealed that testicular enzymology which is basically characterized by steroidogenesis process, gets disrupted, at least in part, by oxidative stress mechanisms (Biwas and Ghosh, 2004). Studies in male rats have shown that lead intoxication disrupts testicular steroidogenesis by inhibiting the activities of testicular steroidogenic enzymes (Liu et al., 2008). Enzymes are one of the major targets for metalloid action. Measurement of certain patterns of cellular enzymes under different conditions of treatments with various types of toxicants could provide good evidence for the cytotoxicity and hence the impairment of cell function (Lavitschka et al., 2007). Reproductive toxicity is the adverse effects of chemicals on gonadal structure and functions, alterations in fertility and impaired gamete function (Timbrell, 1995).
The treatment of lead poisoning, especially at sub clinical level is equally important. Most of the chelating agents tend to have adverse side effects and the benefits are usually transitory, since, blood lead can be rapidly replaced from the bone store (Mahaffey et al., 2000). Oxidative damage associated with the presence of lead has been illustrated as one possible mechanism involved in lead toxicity (Adoneylo and Oteiza, 1999), which suggests that antioxidant might play a role in the treatment of lead poisoning (Gurer et al., 2001). Animals have protective mechanism in the form of antioxidant nutrients, vitamins and several enzymes. Antioxidant may play an important role in abating some hazardous effects of lead. The body consists of an elaborate antioxidant defense system that depends on dietary intake of antioxidant vitamins and minerals. Chow (1991) reported vitamin E and occupies an important and unique position in the overall antioxidant defense. The antioxidant function of vitamin E is closely related to the status of many dietary components. Antioxidative properties of vitamin E is believed to prevent reproductive disease associated with oxidative stress (Brigelius-Flohe et al. 2002). Vitamin E interacts with oxidizing radicals and terminates the chain reaction of lipid peroxidations (Jones et al., 1995). In many studies, vitamin E neutralizes lipid peroxidation and unsaturated membrane lipids because of its oxygen scavenging effect (Kalender et al., 2006) El-Shenawy et al., (2010) showed that vitamin E has a preventive and reducible role against the oxidative stress induced by a toxic substance in the testes. The effect of lead acetate on testicular enzymes and its mechanism of action on the male gonads have not been studied. Therefore, the present study has been undertaken on Swiss albino mice to investigate the effects of lead acetate on testicular enzymatic activities and weight of accessory reproductive organs and their protection by antioxidant.

MATERIALS AND METHODS

Animals
Healthy adult male swiss albino mice (*Mus musculus*) weighing 35 to 40 g. were used for the experiment. Animals (80 to 90 days) were maintained under standard laboratory condition and provided them balance diet and water ad-libitum daily.

Treatments
Animals were divided into control, experimental and recovery groups. The control group was given vehicle only. The experimental groups were given lead acetate (1.25 mg/kg) daily for 45 days by gavage (0.2 ml/animal). The recovery groups received lead acetate (1.25 mg/kg) and vitamin E (2 mg/kg) for the same period by the same route. The treatment duration of 45 days was selected as the length of the complete spermatogenic epididymal maturation cycle in mice. The doses selected were based on previous work in our laboratory (Sharma and Bhattacharya, 2014). Animals were sacrificed after their respective treatments and their testis, caput epididymidis, cauda epididymidis and vas deferens weights were recorded with an automatic balance (AND GX-600, Japan).

Measurement of testicular enzymes

Glutathione peroxidase (GSH-Px)
The testicular enzyme activity was assayed by the modified technique of Paglia and Valentine (1976). A known tissue weight was homogenized in the required volume of 0.01% digitonin and centrifuged at 12,000 g for 30 min at 4°C. This supernatant (0.1 ml) was used in the reaction mixture of 0.8 ml. The reaction was initiated by addition of 0.1 ml H₂O₂. The decrease in absorbance at 340 nm was recorded for 3 min at an interval of 1 min to calculate enzyme activity.

Succinate dehydrogenase (SDH)
The activity of SDH in the testis was assayed by the method of Beatty et al., (1966). To the sample tube containing 0.4 ml of tissue homogenate in cold distilled water, 1ml sodium succinate and 1ml 2,4-iodophenyl-3,4-nitophenyl-5-phenyl tetrazolium chloride (INT) was added and incubated at 370C for 15 min. In the blank, INT was replaced by 1 ml distilled water. The reaction was terminated by 0.1 ml of 30% trichloroacetic acid. The resulting formazan was extracted in 7 ml ethyl acetate and colour intensity was measured at 420 nm in a Spectronic 106 colorimeter. The activity was expressed as µg formazan/100 mg fresh tissue weight.

3β–HSD and 17β–HSD
One testis from each animal was used for studying the activities of 65–3β–hydroxysteroid dehydrogenase (65–3β–HSD) and 17β– hydroxysteroid dehydrogenase (17β–HSD). Testicular 65–3β –HSD and 17β–HSD were measured in UV spectrophotometer according to the procedure of Talalay (1962) and Jarabak et al., (1962) and subsequently modified by Biswas et al., (1983, 2001). One unit for both 65–3β – and 17β–HSD was defined as the amount causing a change in absorbance of 0.001/min at 340nm.
Statistics analysis
For all biochemical estimation a minimum of 10 to 12 replicates were used for each parameter and tissue. The data were statistically analyzed using ANOVA followed by Scheffe’s test for multiple pairwise comparisons (Gad and Weil 1989). A significant level of $p \leq 0.05$ was accepted.

RESULTS
Organ weights
The weights of testis ($P < 0.01$), caput epididymis ($P < 0.05$), cauda epididymis ($P < 0.05$) and vas deferens ($P < 0.05$) were significantly lower in mice treated with LA for 45 days compared to control, while vit.E + LA group did not show any significant difference in organ weights compared to control (Table 1).

Assessment of biochemical changes
To determine the testicular damage caused by LA and the protective effect of vitamin E, the activities of some testicular enzymes (GSH-Px, SDH, 65–3β – HSD and 17β– HSD) were used as biomarkers of the testis. After 45 days of LA administration, several changes of the parameters have been observed to indicate the occurrence of testicular injuries by comparing to control group. In this investigation, a highly significant ($P < 0.001$) decrease in GSH-Px and SDH activities were observed after LA intoxication. LA treatment also resulted in a reduction in 65–3β – HSD and 17β– HSD activities in LA intoxicated mice. These enzymes activities did not differ from control value when vitamin E was co administered with LA (Table 2).

Table 1: Organ weights (mg) control and experimental groups of mice.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Treated (LA) for 45 days</th>
<th>Treated (LA+Vit.E) for 45 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testes weight</td>
<td>123.5+4.2</td>
<td>87.5+6.2**</td>
<td>119.1±1.0NS</td>
</tr>
<tr>
<td>Caput epididymidis</td>
<td>27.8+0.7</td>
<td>19.7±0.5*</td>
<td>28.5±0.1NS</td>
</tr>
<tr>
<td>Cauda epididymidis</td>
<td>19.5+0.7</td>
<td>10.3±1.3*</td>
<td>17.2±0.6NS</td>
</tr>
<tr>
<td>Vas deferens</td>
<td>14.0±0.4</td>
<td>7.1±0.2*</td>
<td>12.9±0.6NS</td>
</tr>
</tbody>
</table>

All values are expressed + SEM, Significant level, NS= Non significant, * = ($P<0.05$), **= ($P<0.01$), compared with control, treated (LA) and control treated (LA+Vit.E).

Table 2: Biochemical parameters of control and experimental groups of mice.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Treated (LA) for 45 days</th>
<th>Treated (LA+Vit.E) for 45 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione peroxidase (mU/mg/min)</td>
<td>0.036±0.01</td>
<td>0.05±0.001***</td>
<td>0.039±0.004NS</td>
</tr>
<tr>
<td>Succinate dehydrogenase (µg formazan formed/15 min/100 mg)</td>
<td>465±12.13</td>
<td>233±19.33***</td>
<td>435±12.80NS</td>
</tr>
<tr>
<td>65–3β – HSD (unit/mg tissue per h)</td>
<td>26.39±0.141</td>
<td>20.09±0.140*</td>
<td>24.35±0.124NS</td>
</tr>
<tr>
<td>17β– HSD (unit/mg tissue per h)</td>
<td>27.82±0.58</td>
<td>21.62±0.43*</td>
<td>25.98±0.79NS</td>
</tr>
</tbody>
</table>

All values are expressed + SEM, Significant level, NS= Non significant, * = ($P<0.05$), **= ($P<0.01$), ***= ($P<0.001$), compared with control, treated (LA) and control treated (LA+Vit.E).

DISCUSSION
Lead is multifactorial and directly interrupts enzyme activation, competitively inhibits trace mineral absorption, binds to sulfhydryl proteins (interrupting structural protein synthesis), alters calcium homeostasis, and lowers the level of available sulfhydryl antioxidant reserves in the body (Sharma et al., 2009). In the present study, we observed that lead acetate administration caused testicular dysfunction by disturbing various biochemical parameters such as GSH-Px, SDH, 65–3β – HSD and 17β– HSD.
The present study demonstrated that the activities of GSH-Px and SDH in the testicular tissue were significantly declined in LA-group comparing with controls. Several studies reported alterations in antioxidant enzyme activities such as SOD, catalase and glutathione peroxidise (GPX) and changes in the concentrations of some non-enzymatic antioxidant molecules, such as glutathione (GSH) in lead exposed animals (McGowan et al., 1986) and workers (Gayathri et al., 2007; Mohammad et al., 2008). These findings suggest a possible involvement of oxidative stress in the pathophysiology of lead toxicity. Testicular steroidogenenic enzymes (65–3β–HSD and 17β–HSD) activities decreased after LA treatment. Our result is in agreement with Biswas and Ghosh (2004) who observed low activity of Δ5–3β–hydroxysteroid dehydrogenase (Δ5–3β–HSD) and 17β– hydroxysteroid dehydrogenase (17β–HSD) in the testicular tissue of rats exposed to lead at dose of 8.0mg /kg i.p. over a period of 14 days. Batra et al., (2001) observed a dose dependent reduction in the activity of two major enzymes in the testis, Alkaline phosphatase and Na-K ATPase, in lead exposed animals which is another probable mechanism of lead induced reproductive toxicity. During this investigation, testis and other accessory sex organ weights were significantly reduced after LA treatment. Macroscopic changes in accessory sex organs such as diminished weight of testes, seminal vesicles, epididymis, and ventral prostate have been demonstrated in various studies using experimental animals (Ronis et al., 1996). Microscopic changes, histological as well as macroscopic ones, have been induced by increasing lead levels in lead exposed male rats (Adhikari et al., 2001) including changes in the testicular tissues morphology (Bonde et al., 2002), and decreased germ cells layer population (Batra et al., 2001). Moreover testicular size and weight are normally regulated by fluid secretion from Sertoli cells and the production of sperm in the seminiferous tubules (Waites and Gladwell 1982). Reduction of testicular weight less than control values after 45 days of lead administration is in support of degeneration of germ cells and sertoli cells in lead-treated rats. Our studies indicate that lead causes disturbances in metabolism of reproductive organs by alterations of biochemical parameters due to oxidative stress. Antioxidants provide a defense mechanism through 3 levels of protection- prevention, interception and repair. In a normal situation, the cellular antioxidant mechanisms present in almost all tissues and their secretions are likely to quench those reactive oxygen species (ROS) and protect against oxidative damage (Jones et al., 1979). In the present study, it was observed that vitamin E is a potent antioxidant or free radical scavenger which reduces the lead toxicity in Swiss albino mice testes. The beneficial effects of vit. E can be attributed to the antioxidant effects of this vitamin; it is scavenger of oxygen-free radicals which are toxic byproducts of many metabolic processes (Yousef 2010). Oda and El-Maddawy (2012) reported that the beneficial effect of vit. E is mostly due to its antioxidant properties. Vit. E protects critical cellular structures against damage caused by oxygen-free radicals and reactive products of lipid peroxidation (Yousef 2006). Moreover, vit. E is essential in maintaining the physiological integrity of testis, epididymis and accessory glands (Cerolini et al., 2006). Conversely, deficiency of vit. E may lead to detrimental effects on the reproductive organs, such as degenerative spermatogonium, testicular damage and degeneration of the seminiferous tubules (Yousef 2010). Treatment of vit. E with LA caused a significant (P < 0.05) increase in activity of testicular enzymes GSH-Px, SDH and also increases in activity of testicular steroidogenenic enzymes 65–3β– HSD and 17β–HSD. These results are in agreement with the findings by Mishra and Acharya (2004) who found that supplementation of vitamin E and C (100 mg/kg/body weight) along with lead acetate (10 mg/kg/body weight) prevents the lead induced oxidative damage of germinal cells of male mice. Similarly Chinoy and Sharma (1998) reported amelioration of fluoride toxicity by vitamin E and D in reproductive organs of male mice. Ghosh et al., (2002) reported that vitamin C and E ameliorate oxidative stress related testicular impairment in animal tissue. The present study showed that treatment of Vit. E with LA did not show any significant difference in the weight of testes and other accessory reproductive organs weight and activities of testicular enzymes indicating the protective role of vitamin E as an antioxidant.

CONCLUSION

From the current results, it can be concluded that concurrent administration of vitamin E to LA treated animals ameliorated the induced weight and testicular enzymes damage. This is consistent with a vital role of vitamin E in antioxidant systems that protect against LA damage, possibly by preventing oxidative damage to testes. The present studies suggest therapeutic effects of vitamin E to minimize the testicular enzymatic toxicity of LA exposure.

ACKNOWLEDGEMENT

Author is thankful to School of Studies in Zoology and Biotechnology, Vikram University, Ujjain (M.P.), for providing necessary laboratory facilities during this investigation.
REFERENCES


