

Curcumin Protective Potential for the Seminiferous Tubules of Mice Treated with Lead Acetate

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Received: 31 March 2023, Revised: 10 May 2023, Accepted: 6 June 2023, Published: 29 July 2023

Abstract

Curcumin as an antioxidant plays an important role in reducing lead toxicity. This research aimed to demonstrate the potential of curcumin in maintaining epithelial thickness and diameter of seminiferous tubules of mice treated with lead acetate. Thirty BALB/c mice (*Mus musculus*) were divided into 5 groups. Group N was the negative control group with no treatment. Group P was the positive control group, which was given 0.075 g/kgBW of lead acetate once in a day for 35 days. The treatment groups, T1, T2 and T3, were treated with curcumin 100, 200 and 400 mg/kg BW once a day for 3 days; after 3 days, they were treated with curcumin 100, 200 and 400 mg/kg BW; then, 1 h later, they were treated with lead acetate 0.075 g/kg BW once a day for 35 days. There was a significant difference in epithelial thickness of seminiferous tubule between group P and group T3, and a significant difference in the diameter of seminiferous tubules between group P and groups T2 and T3. Curcumin dosages of 200 and 400 mg/kgBW could significantly maintain the diameter of seminiferous tubules, and curcumin dosage of 400 mg/kgBW could significantly maintain the epithelial thickness of seminiferous tubules of mice treated with lead acetate.

Keywords: Curcumin, Epithelial thickness, Diameter, Seminiferous tubules, Lead acetate

Introduction

Lead causes health problems and environmental pollution in various parts of the world. Lead has been used widely and its use can accumulate in the body which can cause various problems in the body system, including the gastrointestinal, haematological, cardiovascular, immune, neurological and kidney functions. Lead exposure causes a significant disease burden [1]. Lead toxicity depends on dose and duration of exposure, it is a potent neurotoxin and even low-level exposure decreased IQ score [2]. Sources of lead exposure are industrial processes (production of lead-acid batteries, cable sheathing, plumbing materials and alloys, glazes, paints, ammunition, anti-knock and lubricating agents in petrol), food and smoking (the biggest contributors to daily lead intake are from ingestion of food, dirt and dust), drinking-water, domestic sources (contaminated dust and soil, some toys, some plastics or paints, some traditional medicines and makeup) [3]. Lead acetate reduced sperm count, motility and viability also altered testicular histopathology (testicular damage, seminiferous tubule necrosis and loss of sperm cells) compared to negative control [4].

One of the heavy metals that are toxic and often used in life is lead. Several studies on lead toxicity have been conducted. Common effects for men include: Decreased libido, infertility, spermatogenesis abnormalities, chromosomal damage, prostate dysfunction and changes in serum testosterone levels [5]. Lead acetate significantly increase malondialdehyde and decrease superoxide dismutase, glutathione peroxidase, total glutathione, testicular sperm count, epididymal sperm reserve, percentage of sperm motility and percentage of sperm survival [6].

A number of studies discovered role of antioxidants in preventing lead toxic effects. One of the herbal antioxidants available for use is curcumin, which is cheaper and causes less side effects [5]. Curcumin is a yellow-coloured polyphenolic compound from turmeric rhizome (*Curcuma longa* L.) [7]. It acts as antioxidant with a protective effect against metal exposure or toxicity [8,9]. It can also be an antioxidant against lead exposure [4]. It works by increasing status of antioxidants such as glutathione, glutathione peroxidase and superoxide dismutase [10]. Earlier research on lead-exposed mice showed that it could significantly increase the superoxide dismutase (SOD) activity in the cerebellum [11]. Administration of

curcumin to diabetic mice increased spermatozoa quantity and motility as well as the germline epithelial thickness and diameter of seminiferous tubules of the testes [12].

The aim of this study was to demonstrate the potential of curcumin in maintaining epithelial thickness and diameter of seminiferous tubules of mice treated with lead acetate.

Materials and methods

Chemicals

Lead acetate was purchased from Sigma-Aldrich Pte. Ltd. with a product number 215902, a CAS number 6080-56-4, an EC number 206-104-4, a linear formula $\text{Pb}(\text{CH}_3\text{CO}_2)_2 \cdot 3\text{H}_2\text{O}$, and a molecular weight 379.33 g/mol. It was then dissolved in aquabidest. Meanwhile, curcumin was purchased from Sigma-Aldrich Pte. Ltd. from powdered *Curcuma longa* (turmeric) with a product number C1386, a CAS number 458-37-7, an EC Number 207-280-5, a formula $\text{C}_{21}\text{H}_{20}\text{O}_6$ and a molecular weight 368.38 g/mol. It was then dissolved in carboxymethyl cellulose (CMC) 0.5 % solution.

Animals and experimental design

BALB/c mice (*Mus musculus*) were purchased from Pusat Veteriner Farma (Pusvetma) Surabaya, Indonesia. A total of 30 mice were divided into 5 groups. Group N was negative control group with no treatment. Group P was positive control group, which was given 0.075 g/kgBW of lead acetate once in a day for 35 days. The treatment groups, T1, T2 and T3, were treated with curcumin 100, 200 and 400 mg/kg BW once a day for 3 days; after 3 days, they were treated with curcumin 100, 200 and 400 mg/kg BW; then, 1 h later, they were treated with lead acetate 0.075 g/kg BW once a day for 35 days. Mice were placed at controlled ambient temperature following a standard procedure. At the end of the research, the mice were sacrificed; the testes were extracted for examination of the epithelial thickness and diameter of seminiferous tubules.

Seminiferous tubules preparation

Testes were preserved by insertion into a formalin buffer and made into paraffin blocks. Afterwards, testicular tissues were subjected to hematoxylin-eosin staining.

Measurements of epithelial thicknesses and diameters of seminiferous tubules

Histological preparations of testes were observed under a light microscope at 400× magnification to measure the epithelial thickness and diameter of seminiferous tubules using a micrometer. Epithelial thickness of seminiferous tubules was calculated based on mean epithelial thickness of seminiferous tubules by drawing a line from lateral side of seminiferous tubule to outer epithelium adjacent to lumen. Measurement was conducted with 24 repetitions for each sample. Diameter of seminiferous tubules was calculated based on mean of longest and shortest diameters. Measurement was conducted with 6 repetitions for each sample.

Statistical analysis

An analysis was conducted with the R (R Core Team) program. The descriptive analysis covered the mean, SD, normality testing and homogeneity testing. Analytical analysis used 1-way ANOVA. To figure out the most effective dosage, a post-hoc (post-ANOVA) test was carried out.

Results and discussion

Table 1 shows that mean epithelial thickness of seminiferous tubules in positive control group (group P) was relatively decreased to that of seminiferous tubules in the negative control group (group N). The mean epithelial thickness of seminiferous tubules in all treatment groups (group T1, T2 and T3) decreased in comparison to negative control group (group N), in contrast, increased in comparison to the positive control group (group P). The mean epithelial thickness of seminiferous tubules in group T3 had the smallest relatively decreased to group N, followed by group T2 and T1.

The same table also shows that mean diameter of seminiferous tubules in group P was relatively decreased to that of seminiferous tubules in group N. Mean diameters of seminiferous tubules in all treatment groups (group T1, T2 and T3) decreased in comparison to group N, in contrast, increased in comparison to group P. Mean diameter of seminiferous tubules in group T3 had smallest relatively decreased to group N, followed by groups T2 and T1.

Table 1 Mean epithelial thickness and diameter of seminiferous tubules.

Variables	Groups (μm ($\bar{x} \pm \text{SD}$))				
	N	P	T1	T2	T3
Epithelial thickness of seminiferous tubules	68.11 $\pm$ 3.77	55.75 $\pm$ 3.31	61.05 $\pm$ 3.77	61.25 $\pm$ 3.37	66.67 $\pm$ 3.61
Diameter of seminiferous tubules	207.00 $\pm$ 12.50	167.60 $\pm$ 7.44	170.30 $\pm$ 10.75	189.72 $\pm$ 11.97	191.48 $\pm$ 14.53

Table 2 1-way ANOVA results.

Variables	<i>p</i> -values
Epithelial thickness of seminiferous tubules	0.001*
Diameter of seminiferous tubules	0.001*

*Significant difference (significant at $p < 0.05$)

Table 3 Bonferroni post-hoc test on epithelial thickness of seminiferous tubules.

Groups	N	P	T1	T2
P	0.001*	-	-	-
T1	0.021*	0.165	-	-
T2	0.027*	0.132	1.000	-
T3	1.000	0.001*	0.115	0.144

*Significant difference (significant at $p < 0.05$)

Table 4 Bonferroni post-hoc test on diameter of seminiferous tubules.

Groups	N	P	T1	T2
P	0.001*	-	-	-
T1	0.001*	1.000	-	-
T2	0.168	0.030*	0.080	-
T3	0.299	0.016*	0.043*	1.000

*Significant difference (significant at $p < 0.05$)

Based on **Table 2**, the variable “epithelial thickness of seminiferous tubules” had a p -value 0.001 ($p < 0.005$), which means that there was a significant difference in the epithelial thickness of seminiferous tubules between groups (group N, P, T1, T2 and T3). Bonferroni post-hoc test results for the variable “epithelial thickness of seminiferous tubules.” **Table 3** shows that significant differences in the epithelial thickness with p -values < 0.05 were found between group N and group P, between group N and group T1, between group N and group T2 and between group N and group T3. A significant difference in the epithelial thickness of seminiferous tubules between treatment groups (group T1, T2 and T3) and control group P was found between group P and group T3, between group P and group T1 and between group P and group T2, there were insignificant increases in epithelial thickness of seminiferous tubules, with $p > 0.05$.

Based on **Table 2**, the variable “diameter of seminiferous tubules” had a p -value 0.001 ($p < 0.005$), which means that there was a significant difference in diameter of seminiferous tubules between groups (group N, P, T1, T2 and T3). The Bonferroni post-hoc test results for the variable “diameter of seminiferous tubules” in **Table 4** shows that significant differences in diameter of seminiferous tubules with p values 0.001 ($p < 0.05$) were found between group N and group P, between group N and group T1, between group P and group T2 and between group P and group T3. Significant differences in the diameter of seminiferous

tubules between treatment groups (group T1, T2 and T3) and the control group (group P) were found between group P and group T3 and T2. Between group P and group T1, there was an insignificant increase in diameter of seminiferous tubules, with $p > 0.05$.

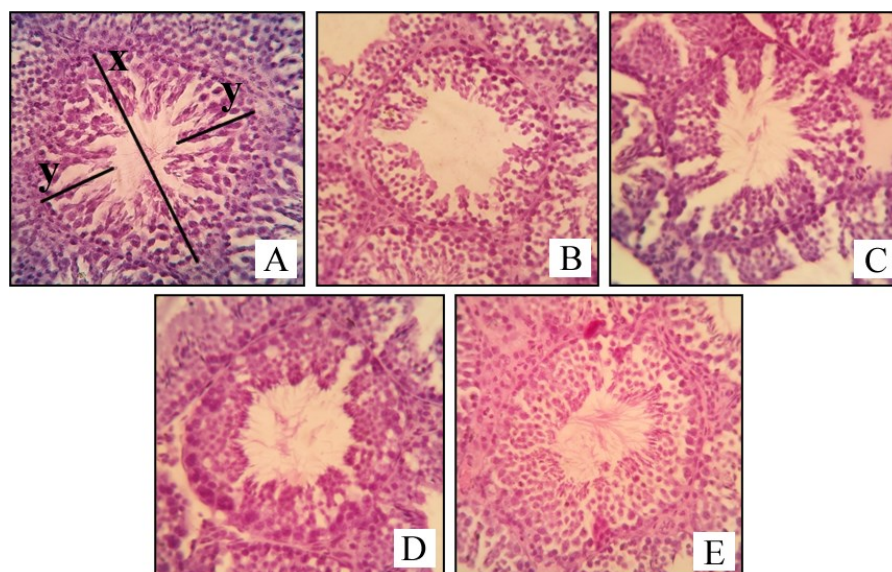


Figure 1 The epithelial thickness and diameter of seminiferous tubules. A) group N, B) group P, C) group T1, D) group T2 and E) group T3; x) diameter of seminiferous tubules, and y) epithelial thickness of seminiferous tubules.

Based on **Figure 1**, histological measurements of diameter and epithelial thickness of seminiferous tubules show that epithelial thickness of seminiferous tubules in group P was relatively decreased to that epithelial thickness of seminiferous tubules in group N. The epithelial thickness of seminiferous tubules in groups T1, T2 and T3 appeared thicker than group P. Lumen of seminiferous tubules in group P appeared wider than all other groups (group N, T1, T2 and T3).

In this research, there were decreases in diameter and epithelial thickness of seminiferous tubules in groups that were treated with lead acetate in comparison to control group (group N). Lead as a toxic substance penetrates the body and circulates through the blood vessels toward soft tissues such as those in testes and brain. Lead as a toxic substance in cerebral and testicular cells can influence the functioning and process involved in cerebral and testicular cells. It eventually causes excessive apoptosis in cerebral and testicular cells, also reduced diameter and epithelial thickness of seminiferous tubules.

A lead dosage of 0.075 g/kgBW that was administered once daily in this study could reduce the diameter and epithelial thickness of seminiferous tubules. The same was also discovered in another study in which lead acetate was administered at a dosage of 0.075 g/kgBW once in a day for 4 weeks, which caused epithelial thickness of seminiferous tubules to decrease significantly [13]. Administration of lead acetate also caused a significant disturbance to basement membrane of seminiferous tubules of testes of adult mice [14]. Administration of lead acetate at 0.1 % to mice reduced diameter of seminiferous tubules significantly [15].

A histological study revealed that lead could cause a significant change to germinal cells [16]. Reduction in the germinal cells led to the reduction in diameter and epithelial thickness of seminiferous tubules. This is in line with the finding that revealed thinning of spermatogonia, spermatocyte and spermatid in seminiferous tubules of lead-acetate-induced albino mice [17]. Another study also pointed out the degenerative changes occurring in seminiferous tubules and the decreasing number of spermatogenic cells in lead-nitrate-treated mice [18].

Administration of lead acetate also significantly lowered testicular and body weight of mice [19,20]. This is attributed to the apoptosis that occurred in the testes [21]. Increased apoptosis of spermatogonia, spermatocyte and spermatid also took place in mice that were treated with lead acetate 1,000 ppm/200 gBW for 8 and 16 weeks [22]. Lead acetate also reduced the sperm count, motility and viability, it also reduced testosterone serum and FSH (follicle stimulating hormone), Johnsen's score, Leydig cell count and Sertoli cell count [23].

This research showed that the administration of curcumin at 200 and 400 mg/kgBW could significantly maintain the diameter of seminiferous tubules of mice treated with lead acetate, and that administration of curcumin at 400 mg/kgBW could significantly maintain epithelial thickness of seminiferous tubules of lead-acetate-treated mice.

Administration of curcumin at 100 mg/kgBW was insignificant in maintaining diameter and epithelial thickness of seminiferous tubules, and administration of curcumin at 200 mg/kgBW was insignificant in maintaining the diameter. This was probably due to an insufficient dose of curcumin.

A similar finding showed that oral administration of curcumin at dosages of 100 and 200 mg/kgBW significantly increased diameter and epithelial height toward normal thresholds in methandienone-induced mice [24]. Administration of curcumin at 0.1 mg/gBW thickened epithelium of seminiferous tubules with methoxychlor (MXC) induction [25]. Administration of curcumin at 150 mg/kgBW for 8 weeks in mice fed with monosodium glutamate (MSG) could maintain diameter and epithelial thickness of seminiferous tubules, spermatozoa concentration, testosterone and LH (Luteinizing Hormone) levels [26].

Another study with gentamicin induction demonstrated that curcumin could maintain diameter of seminiferous tubules, Sertoli cell, spermatozoa, spermatid, Leydig cell count and serum testosterone level in pigs significantly [27]. Administration of 100 mg/kgBW curcumin for 8 weeks significantly reduced apoptotic rate of testicular cells and improved histological appearance of testes in obese and diabetic rats [28]. Ultraviolet exposure of mice and administration of curcumin could increase testicular weight, FSH (Follicle Stimulating Hormone) level and spermatozoa motility and decreased the spermatozoa mortality [29]. It was also found that administration of curcumin at 200 mg/kgBW for 7 days increased diameter and epithelial width of seminiferous tubules as well as the testicular weight of mice induced with titanium dioxide nanoparticles [30]. This increase in testicular weight was owed to the cessation of excessive apoptosis of cells in testes with administration of curcumin. It was proven that curcumin supplementation (2 weeks, 100 mg/kgBW) reduced apoptosis in testicular tissues of old mice [31].

Curcumin could lower apoptosis activity by, among others, chelating with lead. This is shown by the significantly decreased level of lead with the administration of curcumin at 100 and 200 mg/kgBW for 4 weeks to cerebellum of lead-acetate-treated mice [11]. Not only does it chelate with toxic metal (Pb), curcumin also serves as a ROS (reactive oxygen species) scavenger. Curcumin demonstrated a scavenging activity toward nitric oxide (NO), H₂O₂ and superoxide anion [33]. Being a scavenger of free radicals or ROS, curcumin causes ROS to decrease, preventing oxidative stress and in turn excessive cell death (apoptosis).

Conclusions

Curcumin dosages of 200 and 400 mg/kgBW could significantly maintain diameter of seminiferous tubules, and curcumin dosage of 400 mg/kgBW could significantly maintain epithelial thickness of seminiferous tubules of mice treated with lead acetate.

Acknowledgements

This research was funded by the Directorate General of Higher Education, the Ministry of Education and Culture of the Republic of Indonesia.

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