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Article

Protective role of exercise and curcumin on regional BMD and oxidative stress induced by lead

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Abstract: The current study aimed to assess the impacts of 8-week non-pharmacological strategies on the regional bone mineral density (BMD) and the oxidative stress among rats regarding lead acetate (Pb) exposure. Randomly, we divided 40 rats into 5 groups: Pb, SHAM, curcumin+Pb, exercise+Pb, and curcumin+exercise+Pb. The rats received Pb (20 mg/kg), curcumin solution (30 mg/kg), and/or treadmill running 5 times/week during an eight-week research protocol. The femur and tibia regional BMD were measured by the DEXA system. Additionally, blood collections were performed to measure oxidative/antioxidant markers. It was demonstrated that BMD lessened significantly in the femur and tibia of rats exposed to Pb, particularly in their distal epiphysis. Whereas TBARS remarkably elevated, TAC dropped in the Pb group. On the other hand, the curcumin supplementation alone did not affect BMD, while performing the weight-bearing exercise resulted in a significant elevation of BMD in spongy tissue (i.e., the proximal and distal epiphysis of femur and tibia bones), specifically a combination of exercise and curcumin consumption protocols. Therefore, exercise training and consuming curcumin supplements may provide osteoprotective benefits against Pb-induced toxicity.

Keywords: osteoporosis; oxidative stress; weight-bearing exercise; curcumin; lead acetate

1. Introduction

In 2018, during the first WHO global conference on air pollution and health, air pollution was called “the silent public health emergency” and “the new tobacco” by the WHO’s general director, Dr. Tedros Adhanom Ghebreyesus [1]. Lead acetate, known as Pb, has been known as an air pollutant having harmful influences on biological organs in humans and animals, such as bone mineral density (BMD) [2,3]. Substantial research shows that Pb disrupts bone metabolism and increases the risk of fracture and osteoporosis. Studies have reported that Pb is inferred to inhibit osteoclastic activity and stimulate bone resorption, which leads to reduced BMD [4]. Growing research evidence suggests that cellular injury and oxidative stress can also stem from exposure to Pb, which results in an imbalanced status between the generated free radicals and the antioxidant defense system [5]. In contrast, recently, biological studies related to bone cells have expressed that some healthy natural elements and molecules can help the body to eliminate the toxins [6] and to prevent the bone loss by either inhibiting the osteoclasts’ activities for the bone resorption or stimulating osteoblasts’ activities for bone formation [7].

Considering all Pb-related toxic effects and physiological changes, diverse approaches have been recommended to eliminate or reduce the negative effects of long-term exposure to Pb on the regional BMD of the tibia bone. One of the valuable

strategies is to do weight-bearing exercises [8–10] and/or antioxidant supplementation. The available data has suggested that the Pb takes part in the formation of oxidative stress via generating ROS [5,11]. Similarly, the use of plant drugs has received attention again as such substances in the plants do not accumulate in the organs of the body, which stems from their biological balance and lack of having side effects and/or fairly low side effects. It has been illustrated that curcumin [1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], which is called diferuloylmethane, plays the main coloring role marker factor existing in the rhizomes of Curcuma longa (i.e., turmeric) [12]. This material has been widely applied in classical medical science [12]. An accurate investigation reported that Curcuma longa stimulates the apoptosis process in osteoclast cells of rabbits, which indicates the influential role of curcumin to provoke the osteoclast apoptosis, which results in inhibiting the bone resorption [13]. Evidence illustrated that there is a positive relationship between BMD and antioxidant supplement intake (i.e., vitamin C, vitamin E, carotenoids, and flavonoids) among premenopausal and postmenopausal women populations [14,15]. Whereas, based on our knowledge, the advantages of antioxidant consumption for bone tissue are still argumentative. Similarly, several clinical studies have supported the theory that reducing stress on weight-bearing bones affects BMD [8,16]. Previous studies expressed that immobilization (i.e., 60-day bed resting at a 6° head-down tilt position) and oxidative stress have a positive correlation with osteoporosis. However, this study reported that antioxidant consumptions do not influence bone remodelling factors following the 60-day bed resting at 6° head-down tilt position [16].

Moreover, interestingly, the beneficial impacts of antioxidants [17] and exercise training [18,19] on both BMD and the risk of bone loss alter based on the anatomic site of the bone tissues. While several studies have supported the positive effects imposed by regular exercises and antioxidant supplements on BMD, a relatively small number of studies utilized the impact of weight-bearing exercise and herbal turmeric supplements during chronic exposure to Pb on regional BMD of weight-bearing bones in rats. Even though the previously conducted studies have majorly focused on either estimating the density of the total bone minerals or examining the bone mass in the middle part of the long bone, several recent studies have demonstrated that osteoporosis affects the bone microstructures, especially in the spongy bone tissue, such as in the proximal and distal regions of the long bones [20]. However, the available data are insufficient regarding the individual and concomitant effects of the weight-bearing exercise and the herbal antioxidant supplementation (i.e., curcumin) on the regional BMD of the tibia bone during chronic exposure to Pb.

As an investigation about the toxicity impacts of Pb on BMD is harmful among human subjects and also it is not practical to monitor the people's diet completely, the lab assessment has been recommended for evaluating the regional bone mass density following an eight-week chronic Pb exposure while considering two non-pharmacological strategies [i.e., weight-bearing training and curcumin consumption (as an antioxidant and anti-inflammatory supplement) protocols]; which would explain some of the concerns regarding the topic.

Therefore, the present study hypothesized that curcumin supplements and weight-bearing exercises are positively associated with the regional BMD of the femur

and tibia bones during concomitant exposure to lead acetate (Pb). Thus, the current research was conducted to examine how the 8-week resistance training with curcumin supplementation affects the regional BMD of the femur (i.e., femoral neck, femur diaphysis, and distal femur epiphysis) and tibia bones (i.e., proximal epiphysis, diaphysis, and distal epiphysis) in rats that were chronically exposed to Pb. Plus, we assess which BMD of various regional bones (femoral neck, femur diaphysis, distal femur epiphysis, tibia proximal epiphysis, tibia diaphysis, and tibia distal epiphysis) is more vulnerable regarding the specific rat group following the eight-week intervention protocols. In addition, we assessed thiobarbituric acid reactive substances (TBARS) and the total antioxidant capacity (TAC) levels in serum regarding the impact of Pb on oxidative stress [5,11], and also the oxidative stress association with BMD [14,15].

2. Methods

2.1. Ethical considerations and the experimental environment

In this experimental investigation, all methods and data collection were confirmed by the Department of Physiology, University of Mazandaran. Plus, all protocols were applied regarding the caring and usage guidelines of animals published by the Council of the American Physiological Society [21]. In this case, we took care of the rats according to established laboratory states, including a temperature of 22 ℃ ± 2 ℃, humidity of 50% ± 5%, a natural day-night cycle (12 h light), and a daily diet of 10/100 g body weight. In addition, water supplies were accessible as reported earlier [22]. The pollutant standard index (PSI) was based on the accepted range revealed by the Iranian Meteorological Organization.

2.2. Classification of the animals

We procured 40 male Wistar rats (200–250 g weight) from the Iran Pasture Institute. Then, they were adjusted with the standard status of the laboratory for one week. After that, at random, we divided them into 5 groups, including (1) SHAM (n = 8); (2) Pb (n = 8); (3) curcumin+Pb (CUR+Pb, n = 8); (4) exercise training+Pb (EXE+Pb, n = 8); (5) curcumin+exercise training+Pb (CUR+EXE+Pb, n = 8).

Needless to say, the SHAM group was set as the control group (the sham-operate group), which was given 30 mg/kg of ethyl oleate three days/week during an eight-week period. As for the Pb group, the rats received 20 mg/kg of lead acetate three days/week during an eight-week period. Also, the CUR+Pb was given 20 mg/kg of lead acetate and 30 mg/kg of curcumin three days/week during an eight-week period. As for the EXE+Pb group, the rats received 20 mg/kg of lead acetate three days/week and performed a 25-to-64-minute workout of an accelerated-running load-bearing on a treadmill five times/week during an eight-week period. The CUR+EXE+Pb group was given 20 mg/kg of lead acetate and 30 mg/kg of curcumin three days/week and also performed a 25-to-64-minute workout of an accelerated-running load-bearing on a treadmill five times/week during an eight-week period.

It also should be noted that the weights of animals were measured at two different times, including (1) before the beginning of the study (at the end of a one-week

adjustment period); and (2) before starting tissue sampling. **Table 1** summarizes the weight reports of all five groups.

Table 1. Weight reports (mean + standard deviation) of animals in five experimental groups.

Groups	Weight (g) at Beginning of study	Weight (g) Before sampling
SHAM (*n* = 8)	268 ± 25	333 ± 25
Pb (*n* = 8)	285 ± 22	317 ± 22
EXE+Pb (*n* = 8)	295 ± 20	314 ± 20
CUR+Pb (*n* = 8)	270 ± 43	284 ± 43
EXE+CUR+Pb (*n* = 8)	297 ± 24	331 ± 24
N = 40		

2.3. The intervention of Pb and curcumin supplement

Based on previous studies, we followed a dosage of 20 and 30 mg/kg of lead acetate and curcumin, respectively [23]. Pb was dissolved in the Milli-Q water, while we solubilized curcumin in 50% ethanol. Therefore, we dissolved 30 mg/kg of curcumin in ethyl oleate to inject it by the intra-peritoneal (IP) method. It should be noted that curcumin was kept away from light in this study.

2.4. Exercise intervention

All the rats categorized into EXE+Pb and/or EXE+CUR+Pb groups performed a regular five-day-a-week running exercise for an eight-week training protocol on a motor-operated treadmill specialized for rodents. At the first week of the exercise protocol, the running started at a speed of 15 m/min for 25 min, which moderately reached the speed of 22 m/min for 64 min at the last week of exercise protocols [22].

The static wire loops were located at the end of the treadmill. In order to encourage the animals to continue the exercise on the motor-operated treadmill, those wire loops were charged with electricity that generated a mild shock (0.75 mA, 500 ms duration, 0.5 Hz rate). Therefore, the rats were motivated to keep performing the exercise in order to avoid those mild foot shocks. An expert activated those loops during all the training sessions while supervising the duration of the training. Needless to say, the animals swiftly learned to keep walking on the treadmill while avoiding the shock, except one rat that did not will to perform the exercise and, therefore, was omitted from the exercise group.

2.5. Tissue collection and DEXA assay

In the current study, ketamine and xylazine were used as the anesthetic for all animals. After an overnight fast of 12 to 14 h, all animals were decapitated. Plus, it should be noted that all bone-tissue samples were collected at least 24 h following exercise and curcumin supplement protocols. Subsequently, we split the soft tissues of the right femur and tibia bones, and froze them in liquid nitrogen. Then, the samples were stored at the temperature of −70 ℃ for later examination. In addition, it should be expressed that the regional bone mass density of the tibia was assessed by the Dual Energy X-Ray Absorptiometry (DEXA) system.

2.6. Blood sampling and measurement of TAC and TBARS

After being anesthetized by ketamine and xylazine, we applied a syringe to collect blood samples from the cardiac puncture. Immediately, the blood was transferred into the disposable glass tubes. Subsequently, within 30 min after collecting, the blood samples were centrifuged at 3000 rpm for 15 min by a refrigerated centrifuge. Next, they were kept at −80 ℃ for later evaluation.

Additionally, we split the serum for biochemical assessment of either TAC or TBARS. According to the recommended method, we evaluated the lipid peroxidation amounts by the thiobarbituric-acid reaction [24]. In this regard, the TAC was assessed by applying an accessible kit (Randox Laboratories, Crumlin, UK), which the method has been reported earlier [25]. Accordingly, hydroxyl radical was generated, which is known as the most reactive oxygen radical. Then, we mixed a ferrous ion solution with hydrogen peroxide. Subsequently, follow-up radicals (i.e., brown-colored dianisidine radical cations) were made by the hydroxyl radical, which were potent as well. Then, we evaluated the antioxidative impact of the sample against those radical reactions. It should be noted that the lab outcomes are reported by μmol/ml. Also, the lab analysis had an acceptable precision value that was under 3%.

2.7. The statistical analysis

We used SPSS software (version 28 for Windows, IBM, Armonk, NY, USA) for statistical data analyses. Also, GraphPad Prism®, version 8.0.2 (GraphPad Software, Inc., La Jolla, CA, USA) was applied to draw the figures. Firstly, the collected data of BMD, TAC and TBARS were assessed, and they were determined to be at normal distribution after applying log formation. Subsequently, the parametric statistical test of variance analysis (ANOVA) and the Bonferroni test were conducted to assess the possible alternations among all five rat groups. Additionally, the data are reported as mean and standard deviation. The significant value was settled at $p < 0.05$.

3. Results

3.1. Impact of treatment interventions on the femur regional BMD

Regarding the exposure to the Pb (20 mg/kg) during the 8-week period, statistical analysis showed a remarkably lessened BMD of the femoral neck in the Pb group compared to SHAM (−41.7%, $p = 0.013$), EXE+Pb (−58.2%, $p = 0.001$), and EXE+CUR+Pb (−68.6%, $p < 0.001$). Whereas an increased BMD of the femoral neck was noted in the EXE+Pb group compared with the CUR+Pb group (+20.9%, $p = 0.037$) (**Figure 1A**).

Additionally, no differences in BMD were noted in the femur diaphysis among all five groups following the 8-week period (i.e., SHAM, Pb, CUR+Pb, EXE+Pb, and CUR+EXE+Pb) ($p > 0.05$, **Figure 1B**). As for distal femur epiphysis, there was a mild elevation in BMD levels in the EXE+CUR+Pb group in comparison to the SHAM group (+21.41%, $p = 0.041$). Whereas a significant dropped BMD of distal femur epiphysis was noted in the Pb group compared to EXE+Pb (−53.35%, $p = 0.006$) and EXE+CUR+Pb (−73.64%, $p < 0.001$). Plus, there was a decrease in BMD levels in the

CUR+Pb group in comparison to the EXE+CUR+Pb group (−32.77%, p = 0.02) (**Figure 1C**).

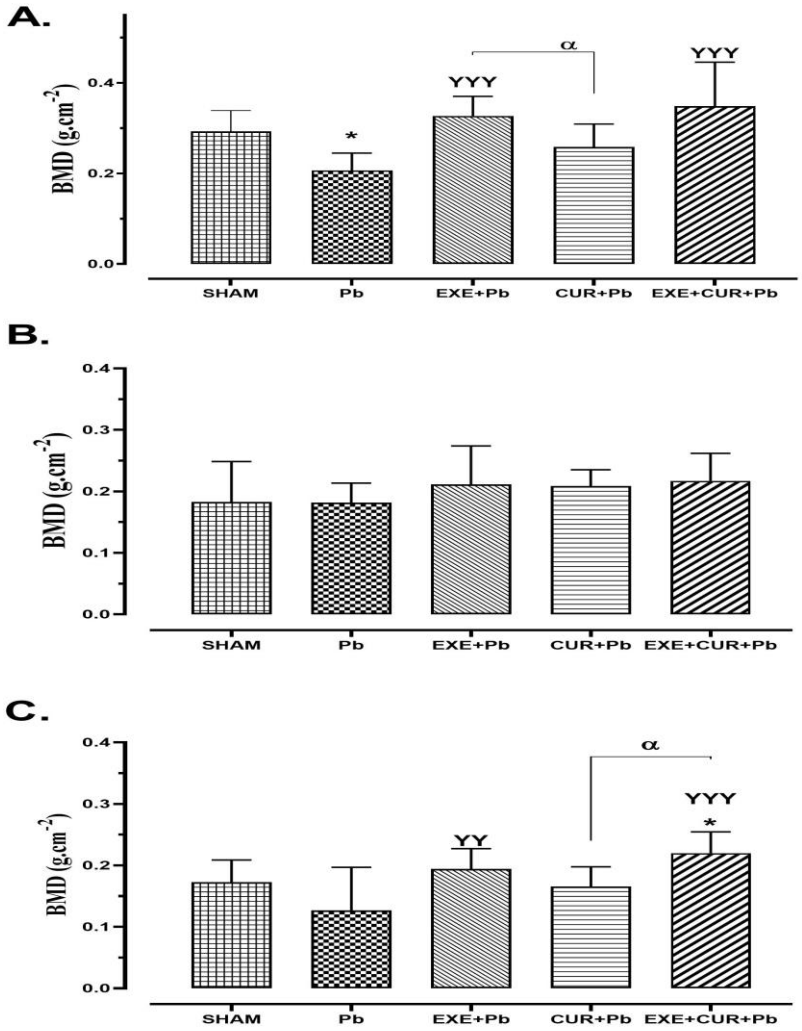

Figure 1. The impact of chronic exposure to lead acetate on the femur regional BMD (mean ± standard deviation) among five groups of rats. **(A)** The influences of different treating interventions (i.e., curcumin supplement consumption and/or exercise training protocols) on rats' femoral neck following chronic exposure to lead acetate; **(B)** the influences of different treating interventions (i.e., curcumin supplement consumption and/or exercise training protocols) on rats' femoral diaphysis following chronic exposure to lead acetate; **(C)** the influences of different treating interventions (i.e., curcumin supplement consumption and/or exercise training protocols) on rats' distal femoral epiphysis following chronic exposure to lead acetate.

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, Differences between the exact group and SHAM group; $^{Y}p < 0.05$, $^{YY}p < 0.01$, $^{YYY}p < 0.001$, Differences between the exact group and Pb group; $^{\alpha}p < 0.05$, $^{\alpha\alpha}p < 0.01$, $^{\alpha\alpha\alpha}p < 0.001$.

3.2. Impact of treatment interventions on the tibia regional BMD

Subsequent to the exposure to the Pb (20 mg/kg) during the 8-week period, statistical analysis showed a lessened BMD of the proximal tibia epiphysis in the Pb group compared to EXE+CUR+Pb (−53.94%, p = 0.016). Whereas a mild increased BMD of the proximal tibia epiphysis was noted in the EXE+CUR+Pb group compared with the SHAM group (+37.45%, p = 0.047) (**Figure 2A**).

Additionally, no differences in BMD were noted in the tibia diaphysis among all five groups following the 8-week period (i.e., SHAM, Pb, CUR+Pb, EXE+Pb, and CUR+EXE+Pb) ($p > 0.05$, **Figure 2B**). As for distal tibia epiphysis, there was a remarkable elevation and dropped BMD levels in the SHAM group in comparison to the Pb group (+44.6%, $p < 0.001$) and the EXE+CUR+Pb group (−27.9%, $p = 0.007$), respectively. Whereas a significant dropped BMD of distal tibia epiphysis was noted in the Pb group compared to the EXE+Pb (−109.4%, $p < 0.001$), CUR+Pb group (−102.61%, $p < 0.001$), and EXE+CUR+Pb (−130.84%, $p < 0.001$) (**Figure 2C**).

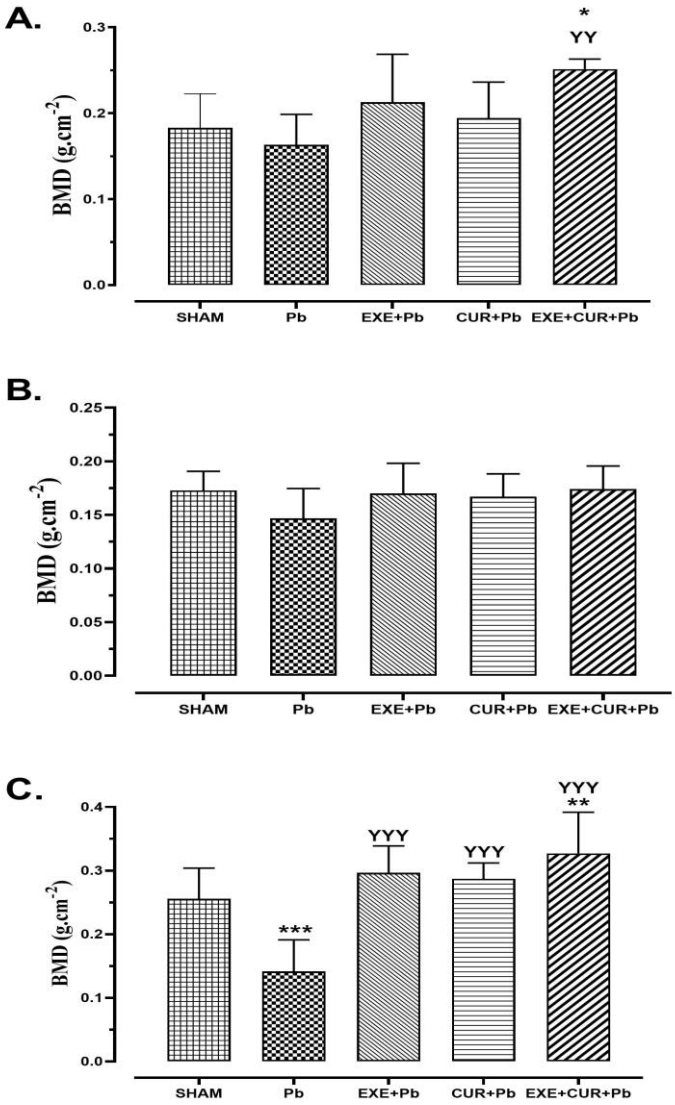

Figure 2. The impact of chronic exposure to lead acetate on the tibia regional BMD (mean ± standard deviation) among five groups of rats. **(A)** The influences of different treating interventions (i.e., curcumin supplement consumption and/or exercise training protocols) on rats' proximal tibia epiphysis following chronic exposure to lead acetate; **(B)** the influences of different treating interventions (i.e., curcumin supplement consumption and/or exercise training protocols) on rats' tibia diaphysis following chronic exposure to lead acetate; **(C)** the influences of different treating interventions (i.e., curcumin supplement consumption and/or exercise training protocols) on rats' distal tibia epiphysis following chronic exposure to lead acetate.

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, Differences between the exact group and SHAM group; $^{Y}p < 0.05$, $^{YY}p < 0.01$, $^{YYY}p < 0.001$, Differences between the exact group and Pb group.

3.3. The differences between various regional BMD of the femur and tibia in the exact group

Following the eight-week exposure to the Pb (20 mg/kg), during the 8-week period, a median difference was noted between femoral neck BMD and distal tibia epiphysis BMD in the Pb group ($p = 0.012$, **Figure 3A**). Also, a mild difference was found between femur diaphysis and tibia diaphysis in the Pb group ($p = 0.024$, **Figure 3A**).

As for the EXE+Pb group, following the eight-week exposure to the Pb (20 mg/kg) and performing the load-bearing exercise, femoral neck BMD had significant alternations with other regional bones, including femur diaphysis ($p = 0.004$), distal femoral epiphysis ($p = 0.002$), proximal tibia epiphysis ($p = 0.012$) and tibia diaphysis ($p = 0.001$; **Figure 3B**). In addition, the median differences were noted between distal tibia epiphysis BMD and three other regional BMDs such as distal femoral epiphysis ($p = 0.011$), proximal tibia epiphysis ($p = 0.01$), and tibia diaphysis ($p = 0.001$, **Figure 3B**).

On the other hand, following the eight-week exposure to the Pb (20 mg/kg) and curcumin consumption, femoral neck BMD had significant alternations with distal femoral epiphysis ($p = 0.002$), proximal tibia epiphysis ($p = 0.046$), and tibia diaphysis ($p = 0.005$) in the CUR+Pb group (**Figure 3C**). Plus, the median to remarkable differences were noted between the femoral diaphysis BMD with two other regional bones, including tibia diaphysis ($p = 0.017$) and distal tibia epiphysis ($p = 0.002$, **Figure 3C**). In addition, the significant differences were noted between distal tibia epiphysis BMD and three other regional BMDs such as distal femoral epiphysis ($p < 0.001$), proximal tibia epiphysis ($p = 0.003$), and tibia diaphysis ($p < 0.001$, **Figure 3C**).

As for the EXE+CUR+Pb group, following the eight-week exposure to the Pb (20 mg/kg) and the combination of exercise and curcumin supplement, femoral neck BMD had significant alternations with femoral diaphysis ($p = 0.019$), distal femoral epiphysis ($p = 0.002$), and tibia diaphysis ($p = 0.003$; **Figure 3D**). Plus, the median to remarkable differences were noted between the femoral diaphysis BMD with two other regional bones, including tibia diaphysis ($p = 0.018$) and distal tibia epiphysis ($p = 0.021$; **Figure 3D**). In addition, the BMD differences were noted between distal femoral epiphysis and two other regional bones such as tibia diaphysis ($p = 0.009$) and distal tibia epiphysis ($p = 0.011$; **Figure 3D**). Also, a significant alternation was found between BMD of tibia diaphysis and distal tibia epiphysis ($p = 0.003$; **Figure 3D**).

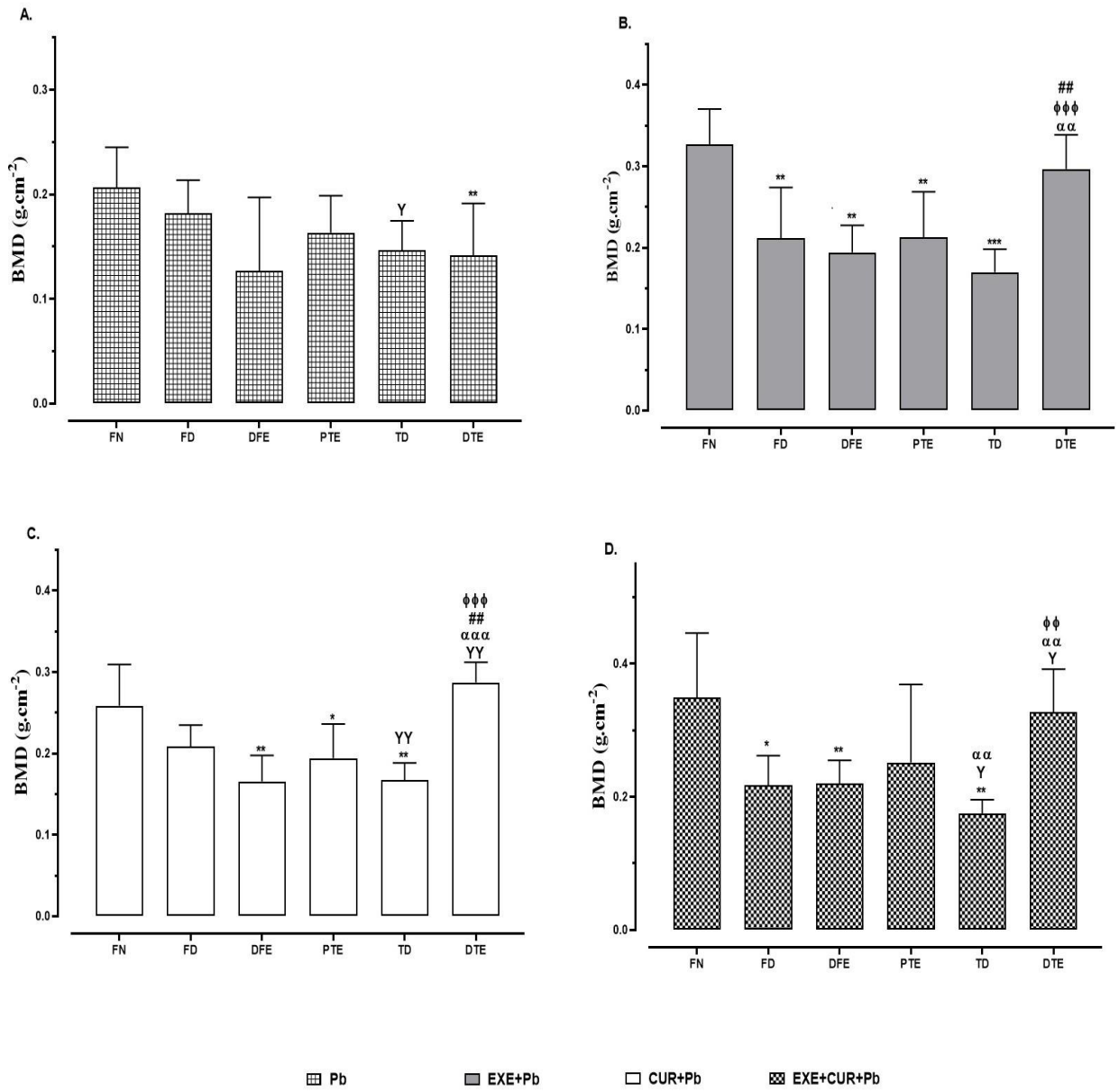

Figure 3. The differences between various regional BMD (mean ± standard deviation) of femur and tibia in rats during chronic exposure to lead acetate, and both exercise and curcumin interventions. **(A)** The differences between various regional BMD of the femur and tibia in the Pb group; **(B)** the possible differences between various regional BMD of the femur and tibia in the EXE+Pb group; **(C)** the differences between various regional BMD of the femur and tibia in the CUR+Pb group; **(D)** the differences between various regional BMD of the femur and tibia in the EXE+CUR+Pb group.

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, Differences between the bone regional and FN; $^{Y}p < 0.05$, $^{YY}p < 0.01$, $^{YYY}p < 0.001$, Differences between the bone regional and FD; $^{\alpha}p < 0.05$, $^{\alpha\alpha}p < 0.01$, $^{\alpha\alpha\alpha}p < 0.001$, Differences between the bone regional and DFE; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, Differences between the bone regional and PTE; $^{\phi}p < 0.05$, $^{\phi\phi}p < 0.01$, $^{\phi\phi\phi}p < 0.001$, Differences between the bone regional and TD.

3.4. Impact of treatment interventions on the oxidative stress

Figure 4 illustrates the TAC changes among all groups. During the 8-week intervention protocols, a remarkable TAC drop was noted in the Pb group compared to SHAM (−37.85%, $p < 0.001$), EXE+PB (−47.1%, $p < 0.001$), CUR+Pb (−47.1%, $p < 0.001$), and EXE+CUR+Pb (−60.94%, $p < 0.001$). Interestingly, the TAC values

were lower in the SHAM group in comparison with the EXE+Pb (−6.74%, $p = 0.001$), CUR+Pb (−6.74%, $p = 0.002$), and EXE+CUR+Pb (−16.75%, $p < 0.001$) groups. Also, it was noted that the combination of exercise and curcumin (EXE+CUR+Pb) was more efficient than either the solely treatment of exercise (EXE+Pb group; +9.4%) or curcumin (CUR+Pb group; +9.4%) ($p < 0.001$, **Figure 4**).

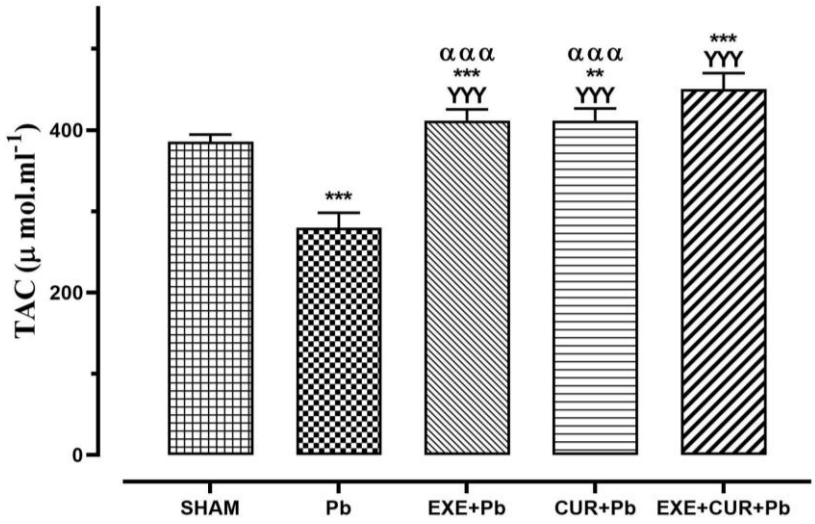

Figure 4. The impact of chronic exposure to lead acetate on TAC concentrations (mean ± standard deviation) in rats.

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; Differences between the exact group and SHAM group; $^{Y}p < 0.05$, $^{YY}p < 0.01$, $^{YYY}p < 0.001$; Differences between the exact group and Pb group; $^{\alpha}p < 0.05$, $^{\alpha\alpha}p < 0.01$, $^{\alpha\alpha\alpha}p < 0.001$; Differences between the exact group and EXE+CUR+Pb group.

Furthermore, **Figure 5** shows the TBARS levels among all groups. It was noted that the 20 mg/kg Pb exposure resulted in a sharp elevated TBARS level in both Pb (+71.65%, $p < 0.001$) and CUR+Pb (+33.6%, $p = 0.002$) groups in comparison with the SHAM group, especially the Pb group. Interestingly, the TBARS values were lower in the EXE+Pb (−31.34%, $p = 0.002$) and EXE+CUR+Pb (−50%, $p = 0.002$) groups in comparison with the SHAM group.

On the other hand, all three treatment groups, including EXE+PB (−60%, $p < 0.001$), CUR+Pb (−22.2%, $p = 0.001$), and EXE+CUR+Pb (−70.9%, $p < 0.001$) had significantly lower TBARS levels than the Pb group. Plus, it was noted that performing exercise in both the EXE+PB (−48.6%, $p < 0.001$) and EXE+CUR+Pb (−62.6%, $p < 0.001$) groups was more efficient than the sole treatment of curcumin (CUR+Pb group) (**Figure 5**).

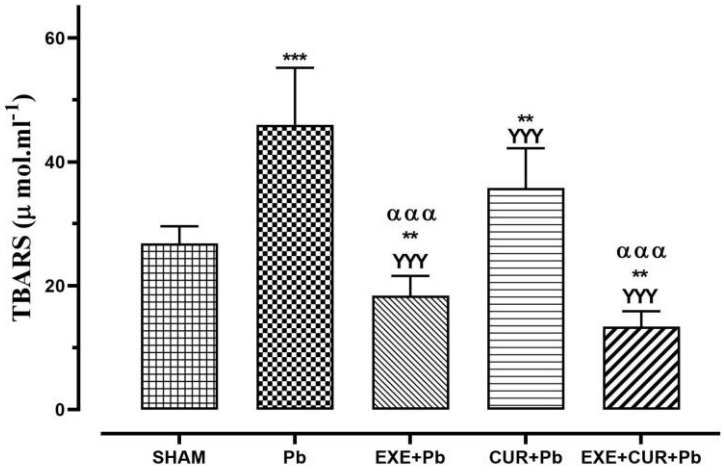

Figure 5. The impact of chronic exposure to lead acetate on TBARS concentrations (mean ± standard deviation) in rats.

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; Differences between the exact group and SHAM group; $^{Y}p < 0.05$, $^{YY}p < 0.01$, $^{YYY}p < 0.001$; Differences between the exact group and Pb group; $^{\alpha}p < 0.05$, $^{\alpha\alpha}p < 0.01$, $^{\alpha\alpha\alpha}p < 0.001$; Differences between the exact group and CUR+Pb group.

4. Discussion

As far as we know, our study is the first investigation that measures the impacts of the weight-bearing exercise and the turmeric antioxidant extract on femur and tibia regional BMD and oxidative stress among rats following an eight-week exposure to Pb (20 mg/kg). Mainly, the current study noted that the chronic 8-week exposure to 20 mg/kg Pb causes a sharp drop in the BMD of the distal epiphysis of the tibia and a mild drop in the BMD of the femoral neck compared to the control group (SHAM). On the other hand, the combination treatment of exercise and curcumin (EXE+CUR+Pb group) was more efficient compared to the sole treatment of exercise (EXE+Pb) or curcumin (i.e., CUR+Pb group), which means the combination treatment significantly induced an inhibitory influence on a decrease in lead acetate-induced BMD levels, specifically in the spongy bone tissue (i.e., femoral neck, femur distal epiphysis and tibia proximal and distal epiphyses).

Even though the present study indicated that the EXE+Pb, CUR+Pb, and/or EXE+CUR+Pb treatments induced an increase in the regional BMD of the femur and tibia bones, the mentioned interventions were more effective in inducing an increase in the femoral neck and femur and tibia distal epiphysis BMD. Also, our study demonstrated that exposure to Pb significantly raises the TBARS amounts and drops the TAC levels.

This result concerning oxidative/antioxidant imbalance induced by the eight-week Pb exposure, which likely led to the lessened regional BMD, particularly the tibia distal epiphysis, which has spongy bone tissue. The pieces of evidence have indicated some metals, such as lead acetate, may cause lipid peroxidation, DNA damage, and depletion of the body's antioxidant defense through reactive oxygen production (ROS) [5]. Oxygen radicals produced by the Pb stimulate lipid peroxidation, which causes significant damage to some tissues, particularly the bone tissue that is the major center for storing the lead acetate. Furthermore, this

phenomenon may change the protein structures and deactivate the responsible hormones for osteogenesis processes. It may consequently provide a condition such as aging in the bone mineral density [26]. In the current study, it has been proved that the curcumin supplement treatment with/without exercise increases the body's TAC during the eight-week Pb exposure. Whereas the curcumin supplement treatment with/without exercise increases the body's TAC during the eight-week Pb exposure, it decreases the TBARS (as an oxidative stress index). These changes probably prevent a lead acetate-induced decrease in the regional BMD of the femur and tibia bones. The recent results indicate that nutrition has a preventive role in the lead acetate-induced negative effects in BMD. Consequently, it maintains and improves the BMD against lead and its detrimental effects resulting from oxidative stress.

In this research, treadmill running was applied, as a weight-bearing exercise, in the rat population. The results expressed that the exercise treatment with/without a curcumin supplement, especially the combination of exercise and curcumin treatment, was effective in inducing the elevation of the femur and tibia regional BMD compared with the curcumin treatment (CUR+Pb group). In addition, these strategies were more effective in inducing an increase in the BMD in the tibia distal epiphysis in comparison with other regional bones of the femur and tibia. The recent results suggest that the BMD changes are related to the bone mechanism following the mechanical load status.

Although it is uncertain which mechanism is responsible for such a great response of the femoral neck and tibia distal regions compared to other bone regions of the femur and tibia, it may stem from the location and diameter of the bone tissue. During the running exercise, the femoral neck and distal tibia bear more mechanical loading in comparison with other bone parts of the femur and tibia. Therefore, these locations boost the total load on the femoral neck and distal part of the tibia compared to the femur and tibia diaphysis and their other ends. In addition, the femoral neck and tibia distal end have a smaller diameter than the other regions, which means these two bone regions bear more weight/surface during the treadmill exercise [27]. Accordingly, the intake of antioxidant supplements may adjust the BMD positively by decreasing oxidative stress, although it is not a determinant factor to the highest level of the BMD and it is necessary for the bone to be exposed to weight-bearing.

Exercise can affect BMD through various mechanisms. During the exercise, force generation by the muscle increases bone metabolism improving osteogenesis. Weight-bearing activities especially those that include striking and jumping activities can increase bone mass and density [8].

The imposed-load nature of the physical activity (PA) is one of the factors defining how bone tissue responds [18,28]. Since the threshold-driven has been suggested as a mechanical stimulant for the physiological reaction of bone tissues [29,30], the intensity parameters of the training protocol are more highlighted, particularly load intensity [18]. In this regard, the training protocols that include either gravitational-driven force [31–33] or muscular-driven loads [34–36] have beneficial impacts on the bone mass density [18].

The increase in the trabecular bone mass through endurance or resistance exercises is a result of the thickness increase, the number increase, and the decrease in the distance among the trabecular or the increase in the trabecular density, which principal reason is the trabecular thickness [37]. Furthermore, our findings revealed

that exercise impacts on the BMD within femur and tibia spongy regions were more obvious than their diaphysis since these regions were expected to be exposed to Pb more. Results also suggested that the Pb affected the spongy tissue because the diaphysis BMD during 8 weeks of exposure to lead acetate did not indicate a significant effect. Also, lead-induced BMD changes on the distal epiphysis of the tibia with the spongy tissue were more obvious. It is not clear why lead affects BMD in spongy tissue. On the other hand, various reports have revealed that the mechanical load-induced adaptation on the dense tissue volume is smaller than the spongy tissue. Due to the structural differences, the ratio of the surface to the volume is much more among the spongy bone tissues compared with the dense bone tissues [38]. In addition, its reversible rate is higher than the dense tissue [38]. It is found that, due to more blood flow in the spongy tissue, its metabolic activity is higher and it has a better response to loading, hormones, or drugs. For example, it is reported that the bloodstream in the tibia metaphysis is 50 to 100 times more than in the shaft (diaphysis) among rats, mice, and dogs. The interstitial fluid pressure of the proximal region to the distal region in special organs increases, affecting the response of the bone tissue to the catabolic and anabolic stimuli [38]. Therefore, in the present study, better responsibility of the regional density in the spongy tissue, specifically the distal epiphysis of tibia, can probably be attributed to the abovementioned topics.

5. Conclusion

In summary, an eight-week exposure to Pb caused the oxidant/antioxidant imbalance and significantly dropped the BMD levels, particularly within the femur and tibia spongy bone tissues. Thus, chronic exposure to Pb may be a risk factor for skeletal diseases. Meanwhile, the BMD changes in the bone are related to regional mechanical loading. Thus, a healthy lifestyle is required to maintain the BMD during chronic exposure to air pollution, so exercise training with a curcumin supplement may provide osteoprotective benefits against Pb-induced toxicity. Although our findings expressed that there is a negative biochemical relationship between lipid peroxidation and BMD, we recommend that future studies elucidate the possible mechanism and impacts related to the roles of pro- and antioxidants that can modulate the osteoporosis.

Highlights:

- Chronic exposure to Pb has drawbacks and impacts on bone mineral density, which can threaten the bone health.
- Performing regular exercise training and curcumin can assist to modulate the air pollution consequences.
- Exposure to air pollution leads to increasing lipid peroxidation, which seems to have a negative biochemical association with bone mineral density.

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Abbreviations

SHAM	control group
EXE	exercise training
CUR	curcumin
Pb	lead acetate
BMD	bone mineral density
FN	femoral neck
FD	femoral diaphysis
DFE	distal femoral epiphysis
PTE	proximal tibia epiphysis
TD	tibia diaphysis
DTE	distal tibia epiphysis
TBARS	Thiobarbituric Acid Reactive Substances
TAC	Total Antioxidant Capacity

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