Research Square

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Acute Bone Damage Through Liver-bone Axis Induced by Thioacetamide in Rats

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Abstract

Background: Thioacetamide（TAA）is used in various fields, such as synthetic drugs, organic chemical synthesis, and materials chemistry. In the medical field, TAA is mainly used to establish animal liver injury models and other organ damage models to explore their mechanisms for helping patients with liver disease, however, TAA caused bone damage is barely understood. Therefore, the aim of our study consisted in building a rat model reflecting the TAA-treated caused acute bone damage.

Results: Serum samples collected from 5-times TAA-treated rats and were used in biochemical test, we found the level of aspartate aminotransferase (AST), alanine aminotransferase (ALT), uric acid (UA), total bile acid (TBA), alkaline phosphatase (ALP), carbamide (UREA) and creatinine (CREA) exhibited sharply rise, while the level of serum content of total protein (TP), lactate dehydrogenase (LDH), calcium (Ca) and phosphorus (P) were severely reduced. At the same time, we obtained some data about cortical bone and trabecular bone by Micro-CT analysis, it revealed significantly decreased bone surface, tissue surface, bone volume, tissue volume in TAA-treated rats, moreover, we used a static biomechanical test system to test the femoral force range of the hind limbs of SD rats, we found bone can resist less pressure and it is easy to take fracture.

Conclusions: Summarizing, our rat model presents possible mediators of liver damage, liver damage and changes in bone structure and mineralization are already visible by Micro-CT analysis after five-times of TAA treatment. The fast response and easy building possibly make it an ideal model to investigate bone metabolism in liver damage after they were affected by TAA.

Background

Thioacetamide (TAA) is a widely used commercial chemical, it has been used as an organic solvent, textile, and paper industries (Thioacetamide 2002). In 1948, Fitzhugh found liver tumors in rats fed TAA (Fitzhugh and Nelson 1948). The study exhibited a number of thiono-sulfur containing compounds including TAA have toxic properties, these effects include bone marrow depression, liver damage and lung damage (Neal and Halpert 1982). In recent years, some researchers pay attention to the reason of TAA caused liver damage, TAA mediated by microsomal CYP2E1 to TAA-S or S-dioxide that initiates cellular necrosis (Chilakapati et al. 2005), Karantonis suggested that reducing the levels of reactive oxygen species may improve liver damage (Pallottini et al. 2006), the platelet-activating factor had implicating participation in liver fibrotic process (Karantonis et al. 2010). Then, other researchers explored how to inhibit TAA toxicity in liver, hepatic irradiation preconditioning enhances the effect of bone marrow-derived mesenchymal stem cell improving TAA-induced liver fibrosis in rats (Shao et al. 2014), a study demonstrate that a small-molecule inhibitor of connexin 32 can protect against liver failure and death in wild-type mice when co-administered with TAA (Patel et al. 2012), as well as miR-34a-5p that is microRNA was the most suitable early and sensitive biomarker for TAA-treated hepatic carcinoma (Dweep et al. 2017). In addition, metabolic bone disease is common among patients with chronic liver disease (Compston 1986; Rouillard and Lane 2001), chronic liver disease is associated with alterations in receptor

activator of nuclear factor kB ligand and osteoprotegerin serum levels, which could affect bone metabolism (Moschen et al. 2005). When the liver is unusual, bone will be damaged, it is result in chronic hepatitis C, calcium, vitamin D, vitamin K2 and hepatic lipids (Jackson et al. 2006; Lwamoto et al. 2011; Orsini et al. 2013). We also know TAA-treated caused liver damage, however, TAA caused bone damage are barely understood. Bone damage was discovered as early as 1984. Japanese scholar Lassila V proposed that TAA-induced liver injury accompanied changes in serum proteins and alveolar bone, mainly in alveolar bone, around teeth, during occlusal stress and trauma, osteoblast Significantly reduced activity, reduced bone mass, and reduced formation of new bone (Lassila and Virtanen 1984a). In 1996, Nakano also proposed that using carbon tetrachloride (CCL_4) and TAA to prepare liver cirrhosis models, TAA and CCL_4 induced liver cirrhosis can cause osteodystrophy, which is mainly manifested in bone volume reduction (Mirkova 1996). Skeletal system includes bone of the skeleton, cartilages, ligaments, and connective tissues, the femur that is weight-bearing bone transfers weight from hip joint to knee joint (Markings 1995). we wonder if the bone damage caused by TAA is related to liver damage or TAA directly causes bone damage,because our experiment took less than 20 days.

Methods

Animal

All the animal experiments were warranted by the Institute animal ethics committee and experiments were performed in accordance with institutional policies. Sprague-Dawley rats (18 Male; 200–250 g) were obtained from Shanghai BK company and bred in the Animal Experimental Center of the Zhejiang Institute of Traditional Chinese Medicine in Hangzhou. The laboratory diet was based on a standard AIN-93 laboratory diet (Xietong Biotechnology, Nanjing, China) and kept in an ambient room with the following conditions: temperature (20 ± 2 °C), humidity 60%～65% and light (12 h light–dark cycle). They were acclimatized for four days before the study started, 12 rats were randomly divided into two groups(n = 6/group) treated with TAA in different concentration (200 mg/Kg, 400 mg/Kg), 6 rats were the control group treated with Normal Saline. The treatment groups were: (1)normal control, in which the rats received tap water and were treated with salt solution, (2)low dose group, in which treated with TAA of 200 mg/Kg/2 day, (3) high dose group, in which treated with TAA of 400 mg/Kg/2 day. At the end of the experiment, rats were anesthetized with pentobarbital sodium to prepare a 2% concentration solution, 1 mL/400 g.

Histopathological Examination

Immediately after sacrifice, the liver tissues were removed and instantly fixed in 10% neutral buffered formalin for 24 h. After fixation, the tissue samples were dehydrated with gradient ethanol of low to high concentration in turn, dewaxed with xylene, soaked in wax and then embedded to make tissue wax blocks by the Biological tissue embedding machine (KeDee, China), those were all processed by the conventional paraffin embedding technique. To detect collagen deposition, paraffin-embedded liver sections were cut to 4 μm thickness by the Paraffin slicer (Thermo, the USA), the wax strips were placed on the water

surface of the Biological tissue spread baking machine (KeDee, China), and scooped up with glass slides after leveled, which were baked for Masson's trichrome (MT) stain and examined under a light microscope.

Chemical

TAA was obtained from Sangon Biotech Co., Ltd. (Shanghai, China) ,analysis of purity > 98%. It was dissolved in normal saline.

Treatment of animals

Adult male Sprague-Dawley rats of body weight 200–250 g were acclimatized in the laboratory for a week before starting experiment. They were fed freely with food and water. Rats were divided separately into three experimental groups (n = 6 per group). The groups formed were: control group (normal saline), medium dose group (TAA 200 mg/kg), high dose group (TAA 400 mg/kg). The groups were intraperitoneally injected once two days. Before rats were intraperitoneal injection, we had weighted every rat. After 5 times, fasting for 24 hours, rats were anesthetized and blood samples were collected for the separation of serum. The rats were then euthanized by the approved protocol, livers, kidneys, and bones were collected. The animal experiments were conducted according to the Guidelines for Animal Experimentation in our animal center. At the end of the experiment, all SD rats were anesthetized and killed within one day. The anesthetic used was sodium pentobarbital. Pentobarbital sodium was formulated into a 2% solution, using a sterile syringe, according to Rats were intraperitoneally injected with 1 mL/400 g, waiting for the rats to lose consciousness and the pain reaction had disappeared, then the rats were laparotomy, and the blood of the rats was drawn with a sterile syringe. The rats eventually died due to excessive blood loss in the whole process, the rats did not feel severe pain.

Serum Biochemical Analysisi

Activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and serum content of total protein (TP), total bile acid (TBA), calcium (Ca), phosphorus (P), carbamide (UREA), creatinine (CREA), uric acid (UA) were analyzed via analyzer (in the Animal Experimental Center of the Zhejiang Institute of Traditional Chinese Medicine in Hangzhou) and present data.

Micro-computed tomography (μCT) analysis

Bone structure of rat left posterior femurs were determined by μCT scans (Bruker, Kontich, Belgium, Institute of Orthopaedic and Traumatology of Zhejiang Province, Hangzhou, Zhejiang, China), to assess the cortical bone and the trabecular bone, which was scanned with standard parameters (70 kV, 357 μA, power 25 W, exposure time 270 ms, resolution 18 μm). Continuous planar Micro-CT images were obtained by scanning along the sagittal direction of the distal femur of the rat, and 3D images were reconstructed. Next, the range of interests (ROI) were 100 slices beginning with the 100th slice at the distal area of the femur. Total surface area (TS), bone surface area (BS), bone volume (BV), total volume (TV) of cortical and trabecular bone were determined, and draw the 3D stereogram of ROI.

Statistical analysis

All experimental data are expressed as the means ± S.D. Statistical significance differences between groups were assessed by one-way analysis of variance (Anova) followed by the Dunnett and LSD post hoc test for multiple comparisons. All data were analyzed using the SPSS statistical package version 22.0 for Windows (IBM, Armonk, NY, USA). $P < 0.05$ indicated a statistically significant difference.

Results

General observations

Prior to TAA administration, SD rats have normal physiology, smooth coat color, sensitive activity, and no other abnormalities. After adapting to the new one-week feeding environment, the control group and different doses of TAA group (200 mg/Kg and 400 mg/Kg group) were injected intraperitoneally, and injected five times every other day. 24 hours after the last injection, blood was taken under the maxillofacial, and serum was separated for biochemical detection related biochemical indicators, and observed the changes.

TAA-induced liver injury

The Fig. 1 (a) is represent Chemical structure of Thioacetamide, after the injection of normal saline and different doses of TAA, the relevant biochemical indicators changed significantly. Following TAA administration, Serum AST, ALT and UA levels were increased in two doses of TAA groups after TAA injection. In Fig. 1 (b), the AST level in the 400 mg/Kg group (400TAA) increased by 34.7% compared with the control group, and the AST level in the 200 mg/Kg group (200TAA) increased by 23.8% compared with the control group. In Fig. 1 (c), the ALT level in the 400TAA group was increased by 34.8% compared with the control group, whereas the ALT level in the 200TAA group was not much different from the control group. In Fig. 1 (d), the UA level in the 400TAA group increased by 52.1% compared to the control group, but there was no significant difference between the 200TAA and the control group.

Figure 2 (a) and (d) show that serum TP and LDH in SD rats indicated a downward trend after intraperitoneal injection, and TP in the 200TAA and 400TAA groups decreased by 26.4% and 27% compared with the control group. The LDH decreased by 51.6% compared with the control group. However, (b) and (c) in Fig. 2 represented an increase in serum TBA and ALP, 200TAA and 400TAA. The TBA of the group huge increased more than twofold, respectively, compared with the control group, and the ALP of the 200TAA and 400TAA groups increased by 33.5% and 70% respectively.

The Fig. 3 reflected the differences of level that exist between serum Ca, P, UREA and CREA. The first point to note is the different degrees of reduction in the number of serum level of Ca and P, Ca in the 200TAA and 400TAA groups decreased by 7.5% and 10.6% compared with the control group (Fig. 3a), P in the 200TAA and 400TAA groups decreased by 3.2% and 28.7% compared with the control group (Fig. 3b), however, the number of UREA (Fig. 3c) and CREA (Fig. 3d) in the 200TAA and 400TAA groups respectively

increased by 42% and 30%, There was a slight increase from control group to 200TAA group on UREA and CREA level.

Figure 4 shows Masson staining of liver pathological sections of the control group and different doses of the TAA group.

After five times of intraperitoneal injection of the drug, SD rats were uniformly anesthetized and sacrificed. Each group was selected as a representative. It can be seen that the body of SD rats was affected by the drug at Fig. 5 (a). With the increase of TAA dose, these body's growth and development was more affected. The Fig. 5 (b) indicate SD rats were gradually increasing weight through intraperitoneal injection of saline. A very noticeable trend was the steady decrease in weight of 200TAA (Fig. 5c) and 400TAA (Fig. 5d) group. The data showed that the number of weights in control group increased by 13.7%, but the number of weights in 200TAA and 400TAA group respectively dropped by 10.2% and 22.9%. To objectively compare the three groups of body weight, we subtracted the body weight after the first intraperitoneal injection from the bodyweight after the last intraperitoneal injection, and obtained a difference, which was compared and analyzed with the change of the difference, the diagram fold a clear comparison between control group and different dose of TAA-treated (Fig. 5e).

To test the possibility that bone injury in SD rats is associated with TAA-treated, we examined the three-dimensional structure of the femur using the NRecon software (Fig. 6, 7), at the same times, we obtained plane Micro-CT image of distal femur about sagittal plane that we interested (Fig. 8). From the three diagram it can be safely concluded that bone volume has dropped dramatically from control group to 400TAA group, and we could see obvious changes in cortical bone and trabecular bone.

According to the bar chart, when we compare control group and 400TAA group, we see the data that BS, TS, BV and TV of cortical bone has dropped dramatically (Fig. 9), the number of BS, TS, BV, and TV of 400TAA group respectively dropped by 24.3%, 26.5%, 20.9%, and 22.5% compared with the control group. Except for the difference between the BV and the control group in the 200TAA group, there was no significant difference in other data (Fig. 9). In addition, we also examined BS, TS, BV, and TV of trabecular bone (Fig. 10). When 400TAA group compared with control group, it can be seen from the chart that significantly smaller than control group. the number of BS, TS, BV, and TV of 400TAA group respectively dropped by 30.9%, 31.2%, 37.3%, and 41.8%.

At last, we used a static biomechanical test system to test the femoral force range of the hind limbs of SD rats (Fig. 11), as well as choosing three-picture represented that femoral stress was affected. The general trend appears to be decreases, the femoral force range of 200TAA decreased by 11.7% compared with control group, while the femoral force range of 400TAA decreased by 42.1% compared with control group. To sum up, we see a different trend emerging.

Discussion

Metabolic bone disease is related to patients with chronic liver disease,represented as osteoporosis, osteopenia, or osteomalacia, which increase bone pain and fracture risk (Collier 2007; Rouillard and Lane 2001). The pathogenesis of hepatic osteodystrophy (HOD) is complexed and likely is multifactorial, after its formation, HOD is hard to treat (Liu et al. 2018; Moschen et al. 2005; van der Merwe et al. 2003). To allow an early diagnosis and find the appropriate treatment plan, it is important to understand the mechanisms causing HOD. Therefore, our aim consisted in building a rat model mimicking the human situation and easily transferable to other mouse strains for looking into specific proteins or genes.

In 1948, scholars suggested that TAA has hepatotoxicity, can lead to liver fibrosis, and even produce liver tumors in a certain drug concentration and within a certain time, at the same time, the researchers did not find that TAA had an effect on other organs, and suggested that the extent to which certain organs were affected may not be discovered, and further research is needed(Fitzhugh and Nelson 1948). Then, TAA has been shown to cause cholangiocarcinoma(Gupta 1955). Some scholars have also found that TAA not only damages the liver of animals, but also affects the kidneys, brain, spleen, and bone of animals(Al-Bader et al. 2000; Kleinfeld 1957; Lassila and Virtanen 1984b; Saran et al. 2004). When the liver of animal model was treated by TAA, the enzyme metabolism of the liver, protein, adipose, amino acid and the messenger RNA were changed to varying degrees compared with the normal group (Dweep et al. 2017; Fontana et al. 1996; Nozu et al. 1992; Okuyama et al. 2005; Waters et al. 2005). However, most scholars are concerned about the role of TAA in the liver and its mechanism. Few scholars are concerned about the bone damage caused by TAA.

Our data underline that rats with severe liver diseases resulting in osteopenia, especially weight-bearing bones. In this study, the increased concentration of AST and ALT represented severe liver injury, in addition, the increased concentration of UA, UREA, CREA, and TBA represented severe kidney injury, which correlates with other studies (Al-Hashem et al. 2019; Jeong et al. 2015; Waters et al. 2005). We also found the decreased concentration of TP and increased concentration of ALP, which indicated liver disease(Li et al. 2017). The levels of rat serum ALT, AST, and ALP exposed to 200 mg/Kg TAA and 400 mg/Kg TAA group were significantly increased, which indicated that the liver cell membrane was impaired and the release of them into blood was increased after TAA administration. Our data showed serum calcium concentration of 200 mg/Kg TAA and 400 mg/Kg TAA group was decrease compared with control group, the reason of decreased serum calcium is associated with the progression of cell injury since alterations in cell signaling play a determinant role in the toxicological processes (Diez-Fernandez et al. 1996). Serum phosphorus is primarily in the form of inorganic phosphate, which is maintained within the physiological range by regulation of bone formation, dietary absorption, and renal excretion, as well as equilibration with intracellular stores, long-standing phosphorus deficiency will increase risk of osteomalacia (Takeda et al. 2012), then, the concentration of serum phosphorus in our data was decrease compared TAA-treated group with control group, which is as expected in other literature. LDH is an insensitive index of all types of hepatic necrosis except hypoxic (Burke 1978), the level of LDH was not increase compared TAA-treated group with the control group in our studies. In our rats model, TAA-treated animals developed severe liver fibrosis as demonstrated by Masson staining which correlates with other studies (Okuyama et al. 2005; Tuñón et al. 2009), In the Masson-stained picture of the liver, the fibrosis of

liver was indicated the pathological sections stained with blue dye, the more blue staining, the more severe the liver fibrosis. In conclusion, we successfully established a rat model of liver injury caused by TAA, so we examined the changes in the bones of the rats by μCT, we obtained 3D reconstruction data of bone area that we interested. Firstly, we can see the change of weight level in three group, a decrease dramatically of weight in 200 mg/Kg TAA and 400 mg/Kg TAA group was observed, which indicated TAA-treated rats was affected by intraperitoneal injection of TAA, Neal have reported that thiono-sulfur compounds exhibit toxic properties in mammals, these effects include bone marrow depression, liver damage (Neal and Halpert 1982). Pauli Virtance considered an increase in osteoclastic resorption in the alveolar bone around the occlusal stressed tooth simultaneously with a horizontal bone loss (Virtanen and Lassila 1986). Secondly, μCT analysis of the bone revealed that the bone structure significantly changed in TAA-treated rats, in the cortical bone, BMD were not affected, strong decreases in BV, TV, BS, and TS were observed, at the same time, most significant changes were observed in the trabecular bone, where reduced BV and TV came along with decreased BS and TS. Furthermore, a decrease in the diameter of femur was observed on the Fig. 5, especially 400 mg/Kg TAA group compared with control group. In addition, a decrease in the cortical bone thickness was observed on the Fig. 7 when 400 mg/Kg TAA group compared with control group. In the last, force analysis of femur represented gradually down trend form normal group to TAA-treated group, TAA administration is leading to bone fragility and increase risk of fracture. From μCT data, we can see that the main part of skeletal injury caused by TAA is in cortical bone, but the effect of TAA on trabecular bone is not so obvious, the administration time of TAA may too short because of only had five intraperitoneal injections. Therefore, our results showed TAA administration have effect on bone metabolism of SD rats, it may relate to liver injury, but no literature suggests that acute liver injury can quickly affect bone metabolism. In this rat model, significant changes in bone metabolism were observed only 20 days, it can quickly build a rat model to represent bone disease for studying mechanism of liver damage. The liver is a multifunctional organ that occupies a key position in the modulation of protein, lipid, and carbohydrate metabolism, and it plays a significant role in mineral metabolism and growth (Nussler et al. 2014), In the literature, there has been a convergence of evidence suggesting bone loss is the primordial bone disorder when found patients was in the early stage of hepatic disease (Guanabens et al. 2013), Therefore, after we speculate that TAA is injected intraperitoneally, TAA or TAA complex will directly damage the bones of rats through the rats' own metabolism. Osteoblasts have affected on osteoclast formation, differentiation, or apoptosis through several pathways, such as OPG/RANKL/RANK, RANKL/LGR4/RANK, Ephrin2/ephB4, and Fas/FasL pathways (Chen et al. 2018), when patients with chronic liver disease were observed, the literature demonstrate that it is associated with alterations in OPG/RANKL pathway, which could modulate bone loss (Moschen et al. 2005; Orsini et al. 2013). In addition, in rats with TAA-induced or carbon tetrachloride-induced cirrhosis, there were reduction in bone volume and were histologically similar to osteoporosis in humans, Atsushi Nakano suggested chronic parenchymal liver injury itself causes osteoporosis due to a combination of low bone formation rates and high resorption rates, the principal pathogenesis of HOD seems to be intestinal Ca malabsorption due to lower serum albumin and villous atrophy, serum levels of vitamin D metabolites have little influence on the pathogenesis of HOD (Nakano et al. 1996), however, as healthy postmenopausal women, vitamin D supplementation resulted in a small but significant

improvement in hip bone density, did not significantly reduce hip fracture, while increased the risk of kidney stones (Jackson et al. 2006). Although vitamin D has a critical effect on liver injury and it is related to 25-hydroxyvitamin D that is the major circulating metabolite of vitamin D, the association in liver injury of TAA-treated and bone disease suggest that further research is necessary to clarify potential links. Osteoporosis was associated with primary biliary cirrhosis (PBC) and was a risk factor for vertebral fracture (Guanabens et al. 2010), further studies that the links of TAA, PBC, and vertebral fracture will be necessary to establish their role in the pathogenesis of liver injury. Then again, some studies suggested vitamin K2, Zinc and selenoprotein P have important effect on bone metabolism (Lwamoto et al. 2011; Pietschmann et al. 2014; Sun et al. 2011), insulin-like growth factor-1 (IGF-1) is a main determinant of low cortical but not trabecular bone mass (Liu et al. 2018). We guess IGF-1 associated with metabolic bone disease.

Conclusions

Our data of experiment suggest that as a widely used drug, TAA can damage the liver and even cause cancer, it can also damage other organs, when we choose a certain dose for intraperitoneal injection, it can also cause acute damage to the bone, which may pass through the liver bone axis. While the experimental time is short, and there may be other faster ways to damage the bone. According to our experimental data, we suggest that TAA is the most serious damage to the weight-bearing bone of rats in a short time, especially the cortical bone, the high dose of TAA caused the cortical bone to be significantly thinner, and the ability of withstand huge force was significantly reduced, the inside structure of trabecular bone had not Seriously affected, in the end, we will continue to explore more in-depth pathological mechanism between TAA, liver, and bone, and build a stable animal model to research TAA caused bone disease.

Abbreviations

TAA
Thioacetamide; AST:aspartate aminotransferase; ALT:alanine aminotransferase; UA:uric acid; TBA:total bile acid; ALP:alkaline phosphatase; UREA:carbamide; CREA:creatinine; TP:serum content of total protein; LDH:lactate dehydrogenase; Ca:calcium; P:phosphorus; μCT:Micro-computed tomography

Declarations

Author contribution statement

JX and YLconceived and designed research. YL, JHL□YTY and LYC conducted experiments. HS□BLW and JR contributed new reagents or analytical tools. HY and JX analyzed data. YL wrote the manuscript. All authors read and approved the manuscript. Our data were generated in-house and Each author's contribution did not use a paper mill.

Acknowledgments

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Compliance with ethical standards

I confirm that our animal experiment according to the audit of the laboratory animal management and ethics committee, the animal experiment process of this project conforms to the principles of animal protection, animal welfare, and the ethics as well as the related stipulation on national experimental animal welfare ethics. During the experiment, there was no excessive bleeding, abuse, indiscriminate killing of experimental animals, and no meaningless repetitive tests. Ensure the natural and healthy life of laboratory animals, prevent unnecessary stress, pain, and harm to animals, and use the least painful method to dispose of animals at the end of the experiment. Our experimental protocols were approved by the Animal Experimental Center of the Zhejiang Institute of Traditional Chinese Medicine. Approval No. IACUC-20181029-11, Number of Animal Use Permit: SYXK (□) 2018-0012.

Competing interests

We have no conflict of interest.

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Figures

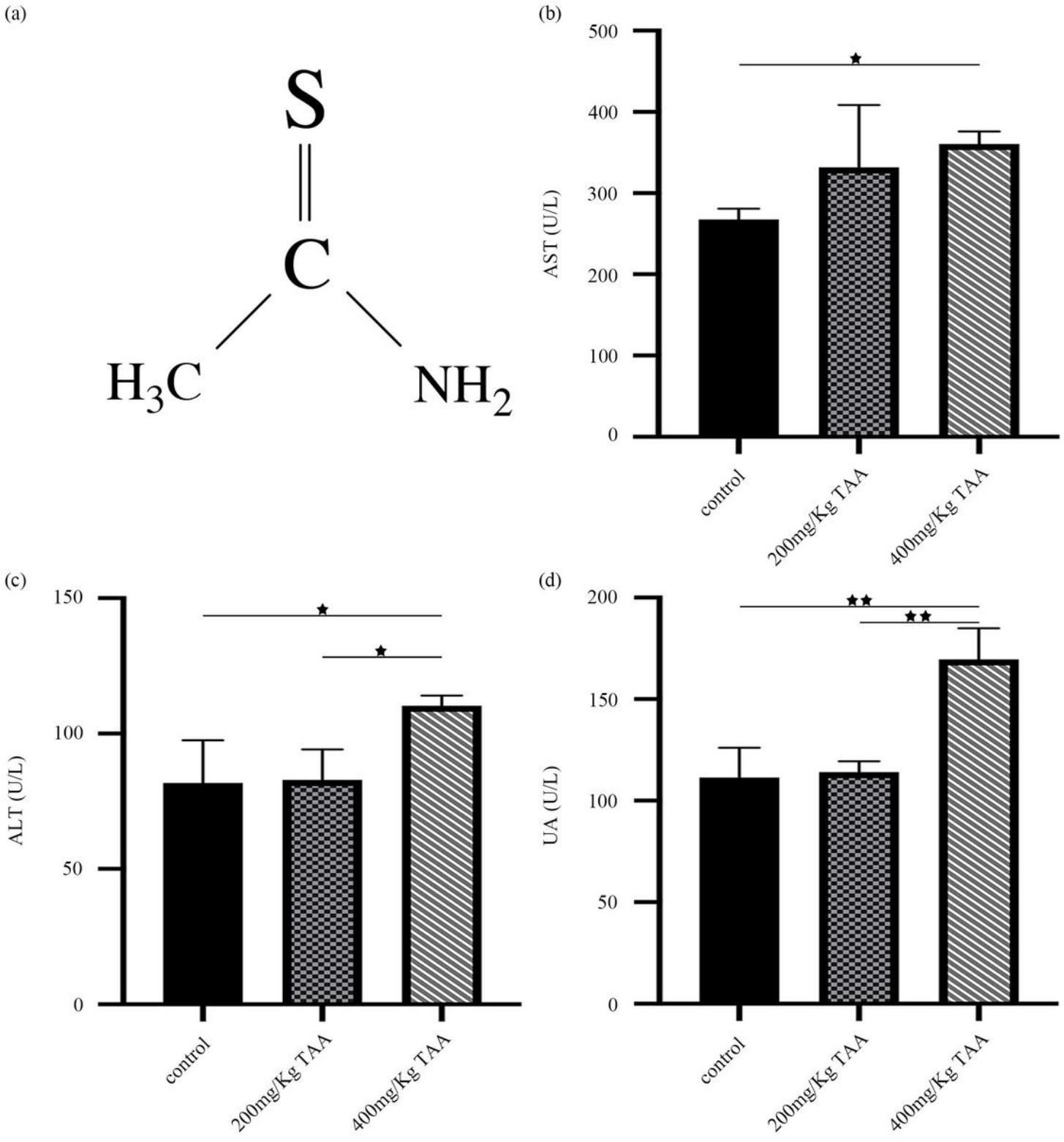

Figure 1

TAA Chemical structure and change of serum concentration of AST, ALT and UA.

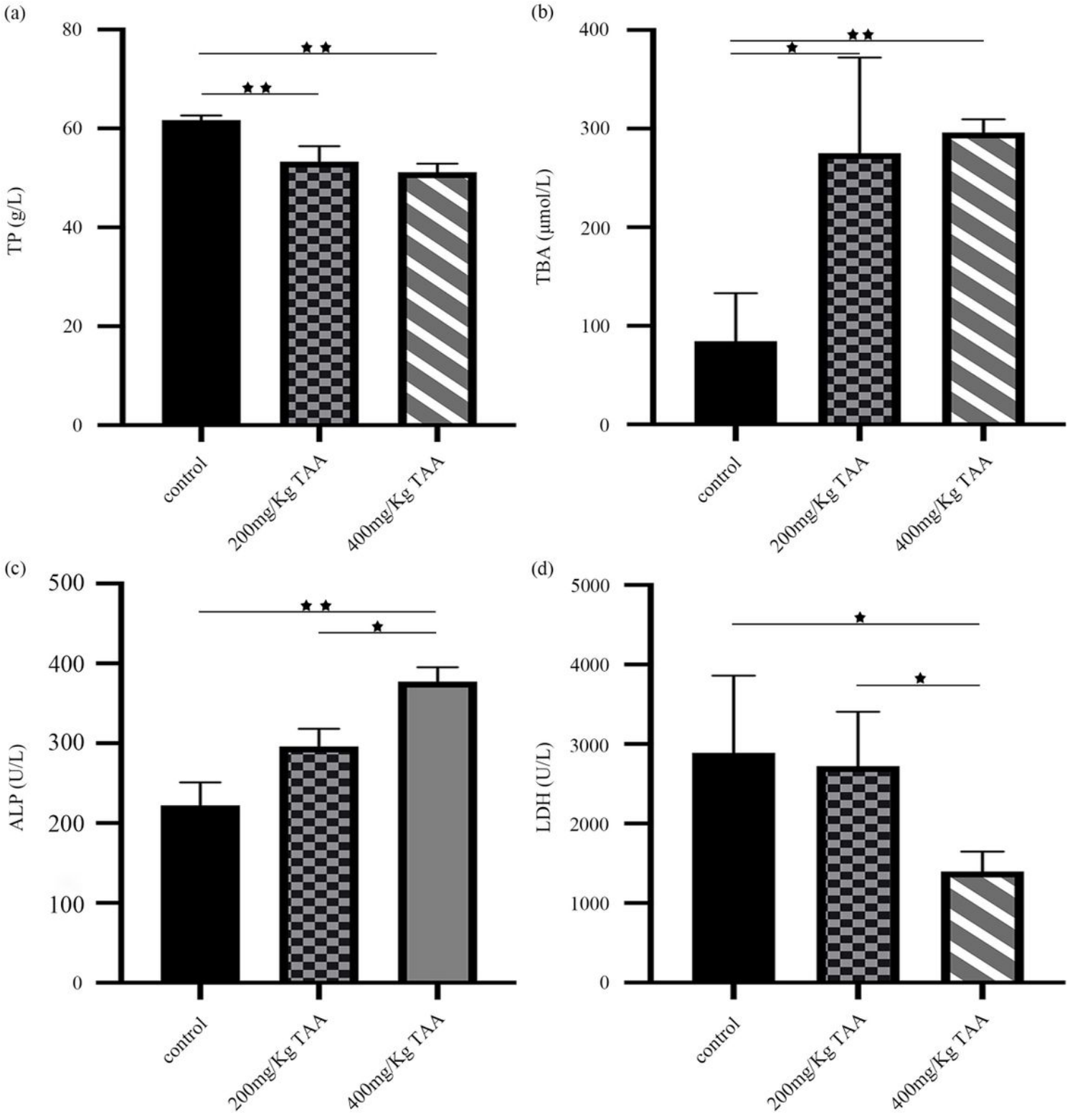

Figure 2

Change of serum concentration of TP, TBA, ALP and LDH.

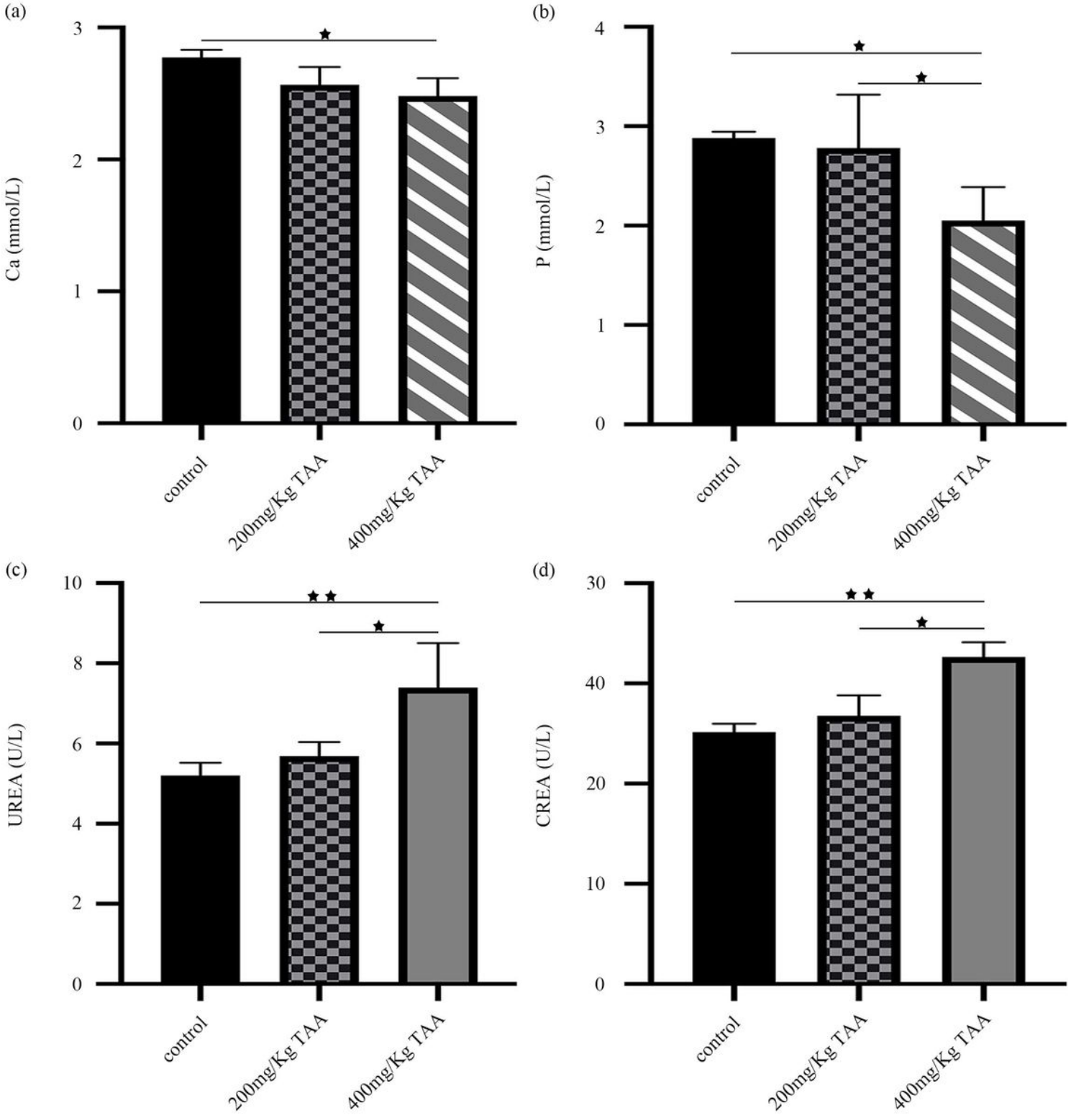

Figure 3

Change of serum concentration of Ca, P, UREA and CREA.

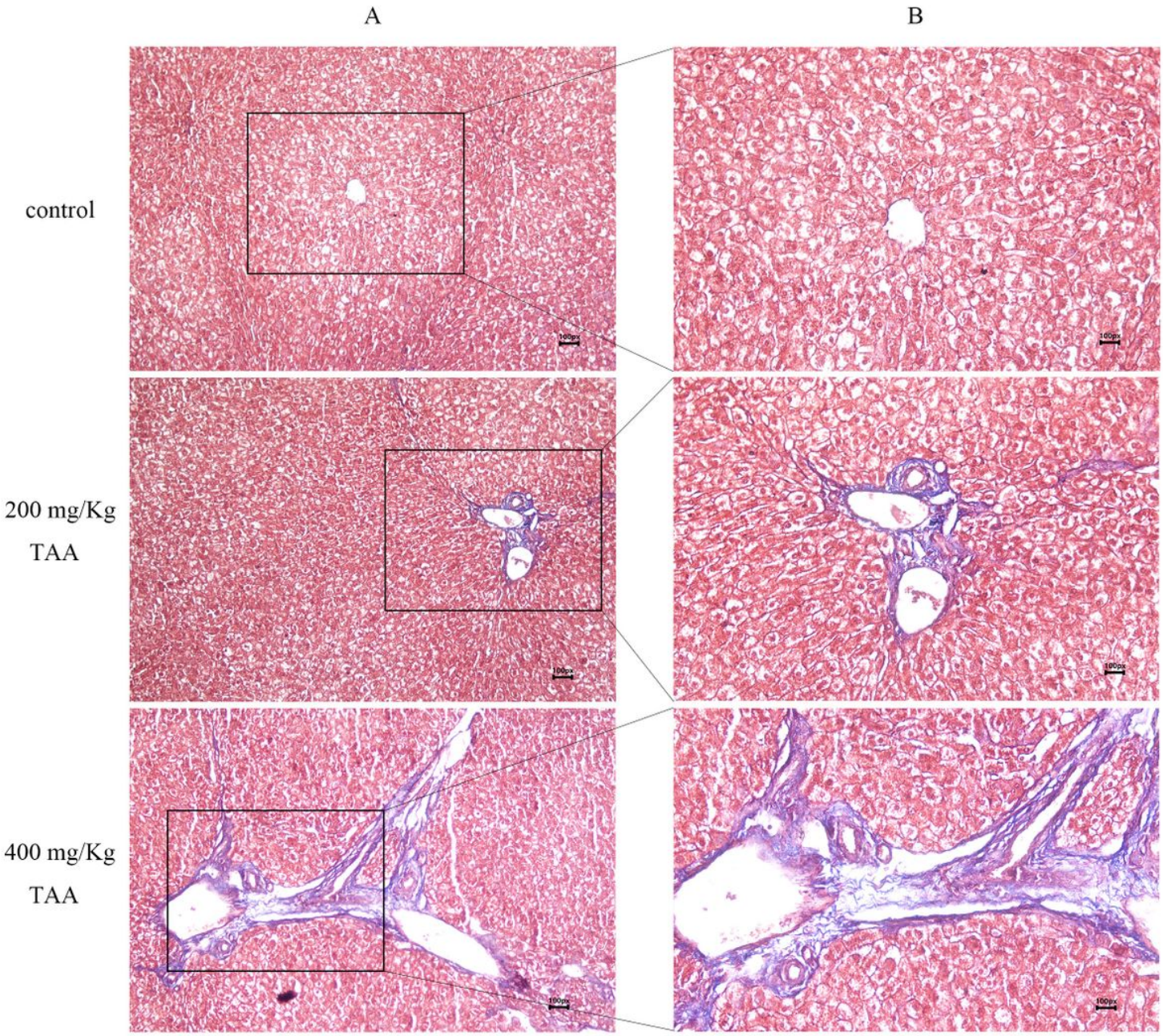

Figure 4

Masson staining of liver pathological sections of the control group and different doses of the TAA group.

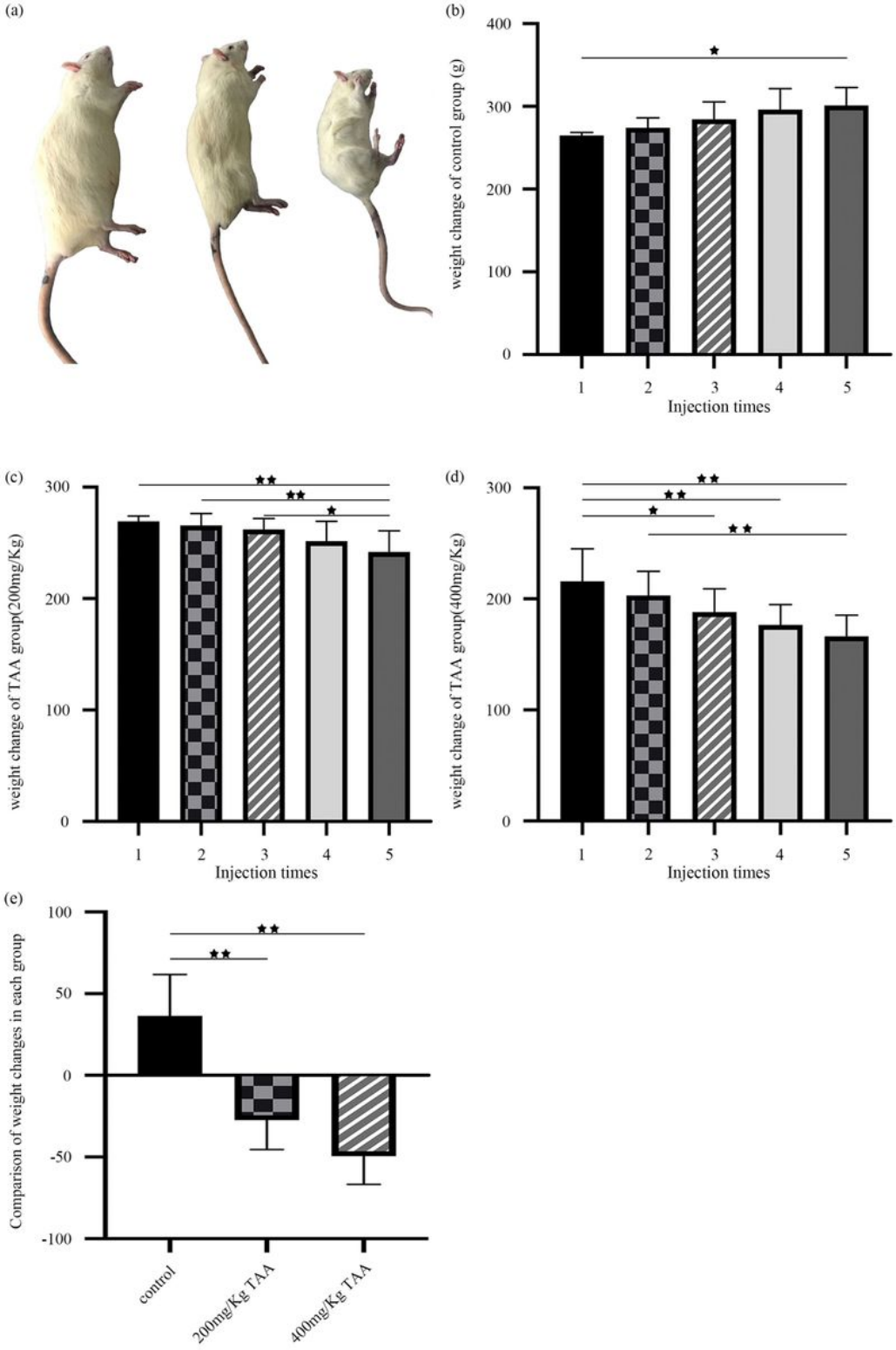

Figure 5

The change trend of rat body weight.

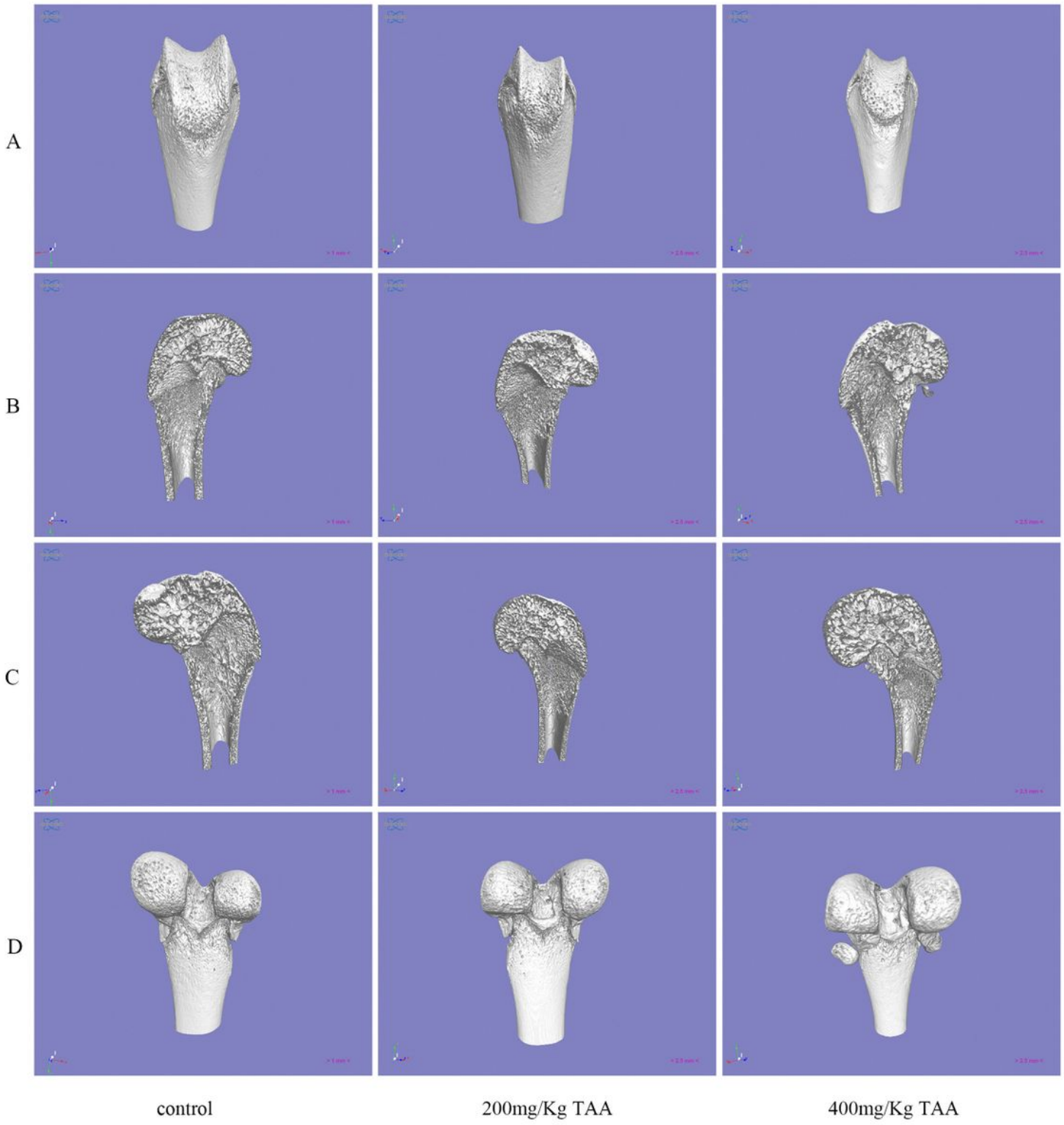

Figure 6

Three-dimensional image reconstruction of rat femur with control group、200mg/Kg TAA and 400mg/Kg TAA group.

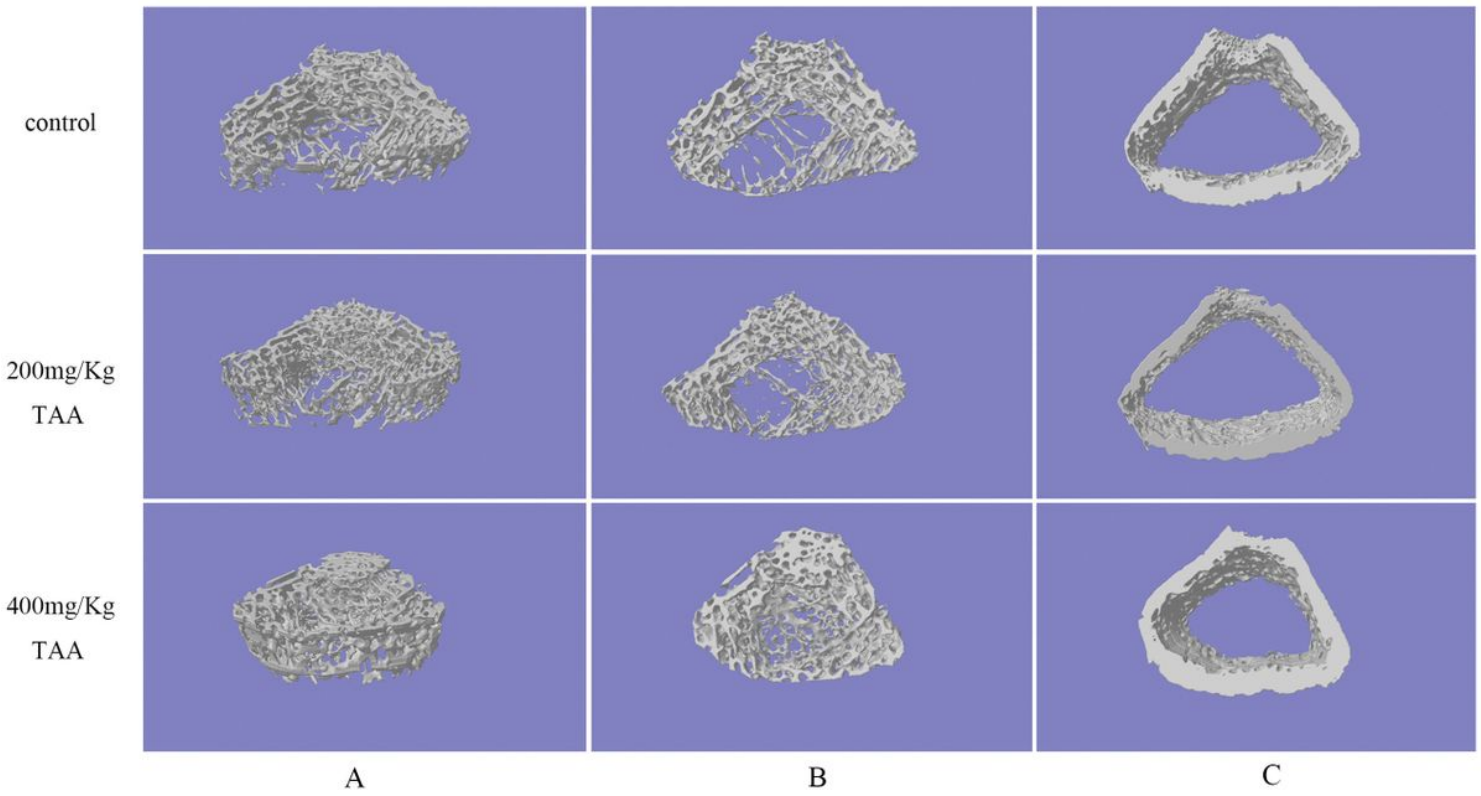

Figure 7

Three-dimensional image reconstruction of rat femur in cortical bone and trabecular bone with control group，200mg/Kg TAA and 400mg/Kg TAA group.

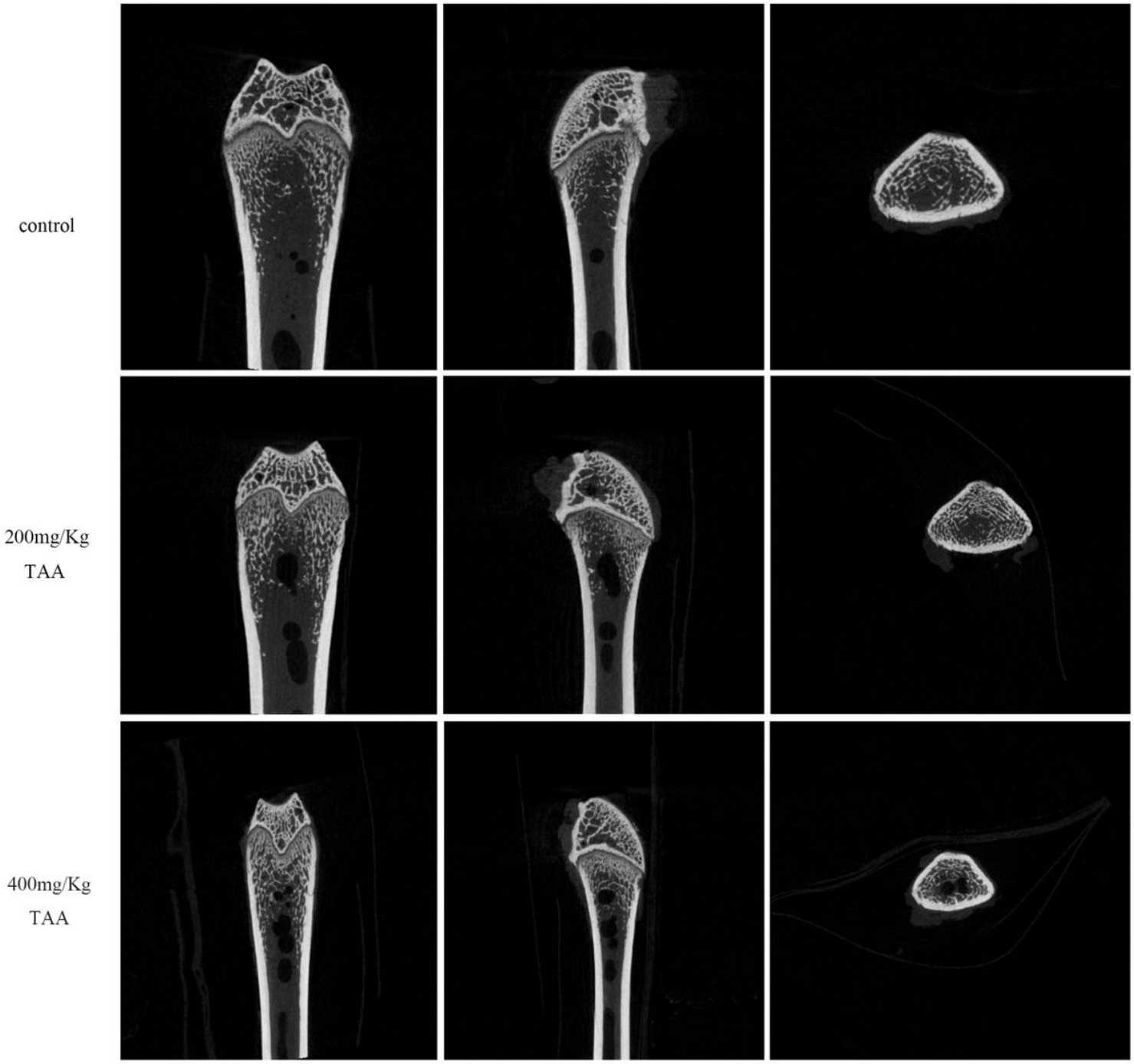

Figure 8

Scanning along the sagittal direction of the distal femur of the rat to obtain a continuous planar micro-CT image with control group、200mg/Kg TAA and 400mg/Kg TAA group.

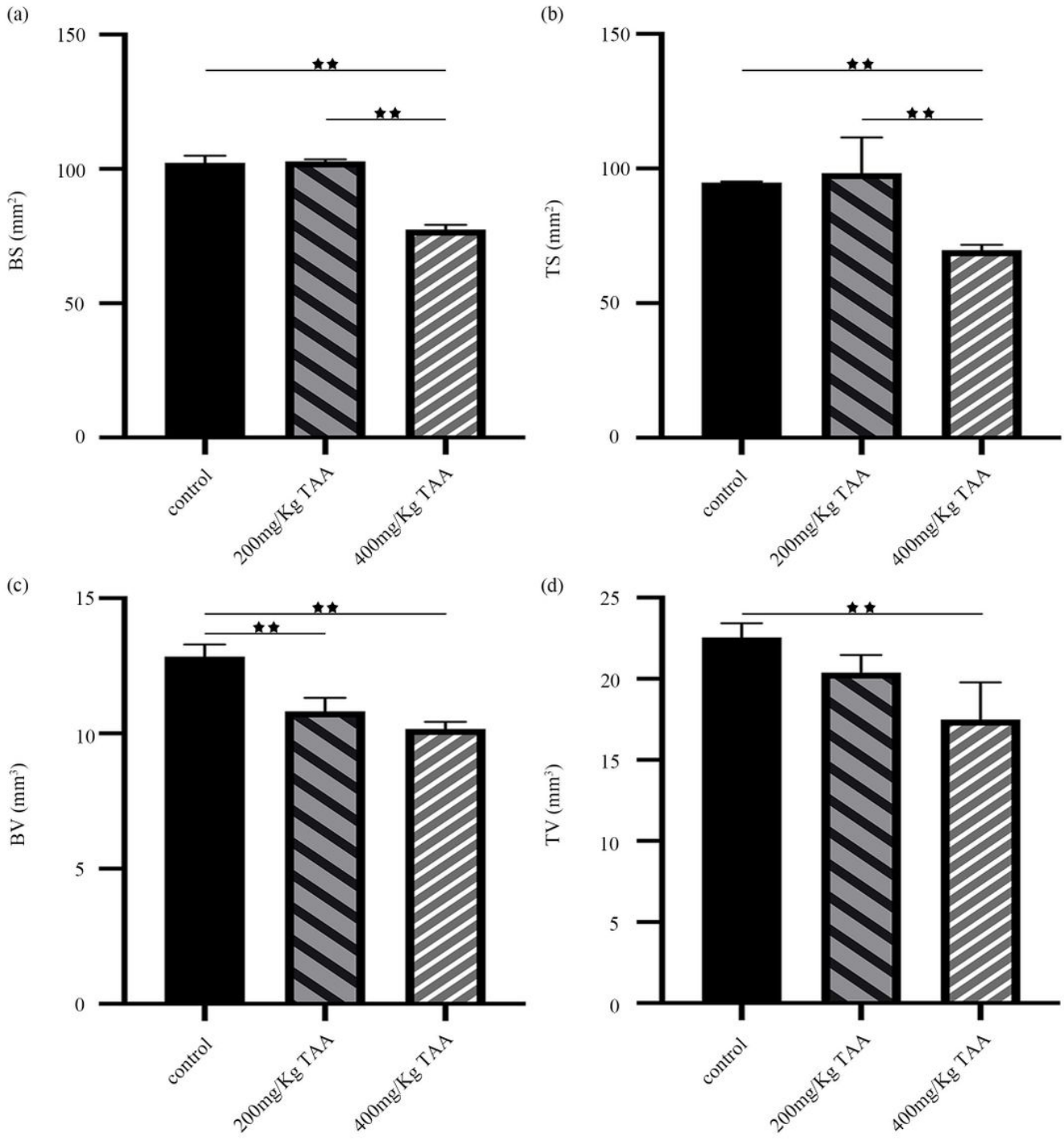

Figure 9

Change trend of BS, TS, BV and TV in cortical bone.

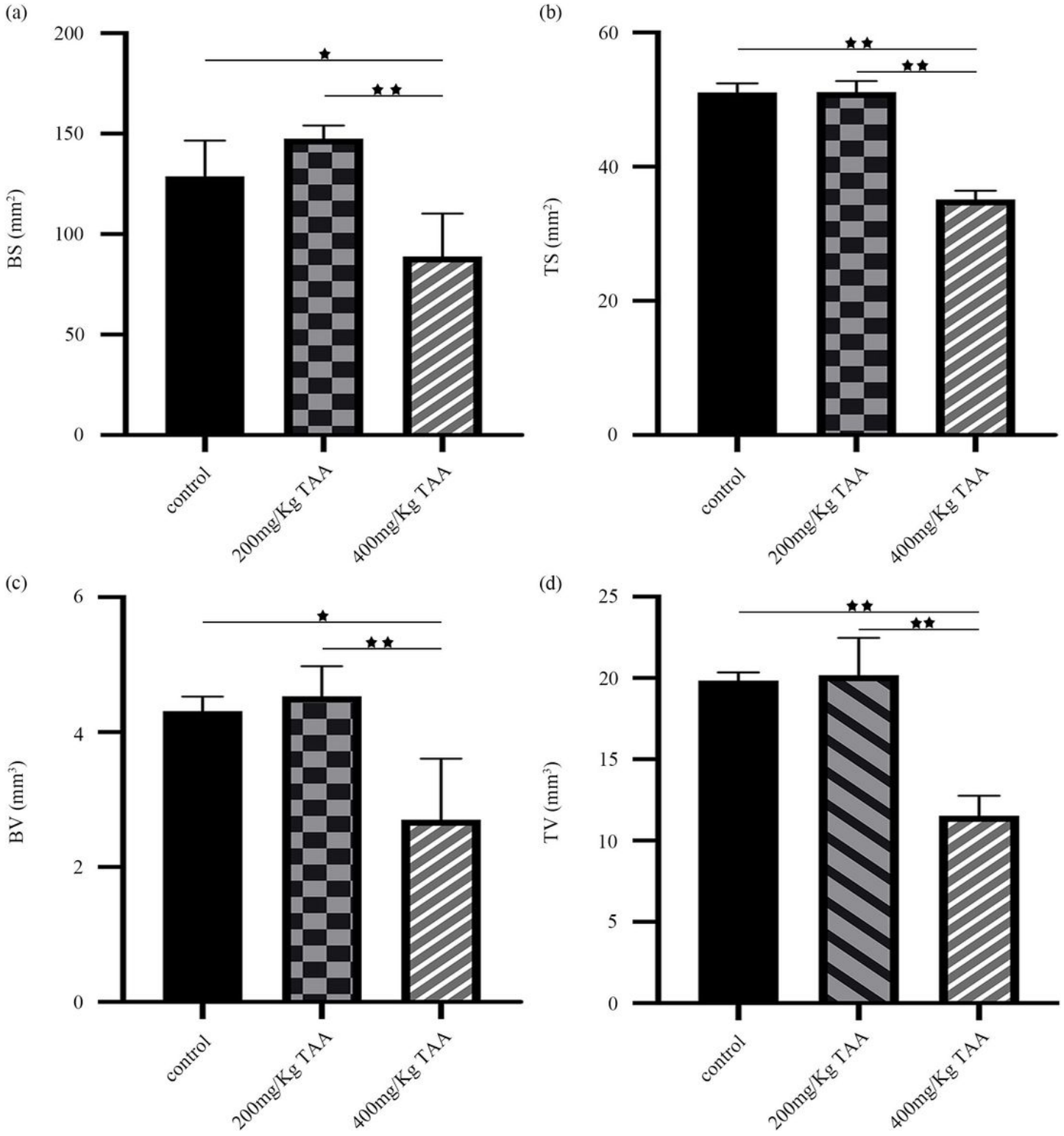

Figure 10

Change trend of BS, TS, BV and TV in trabecular bone.

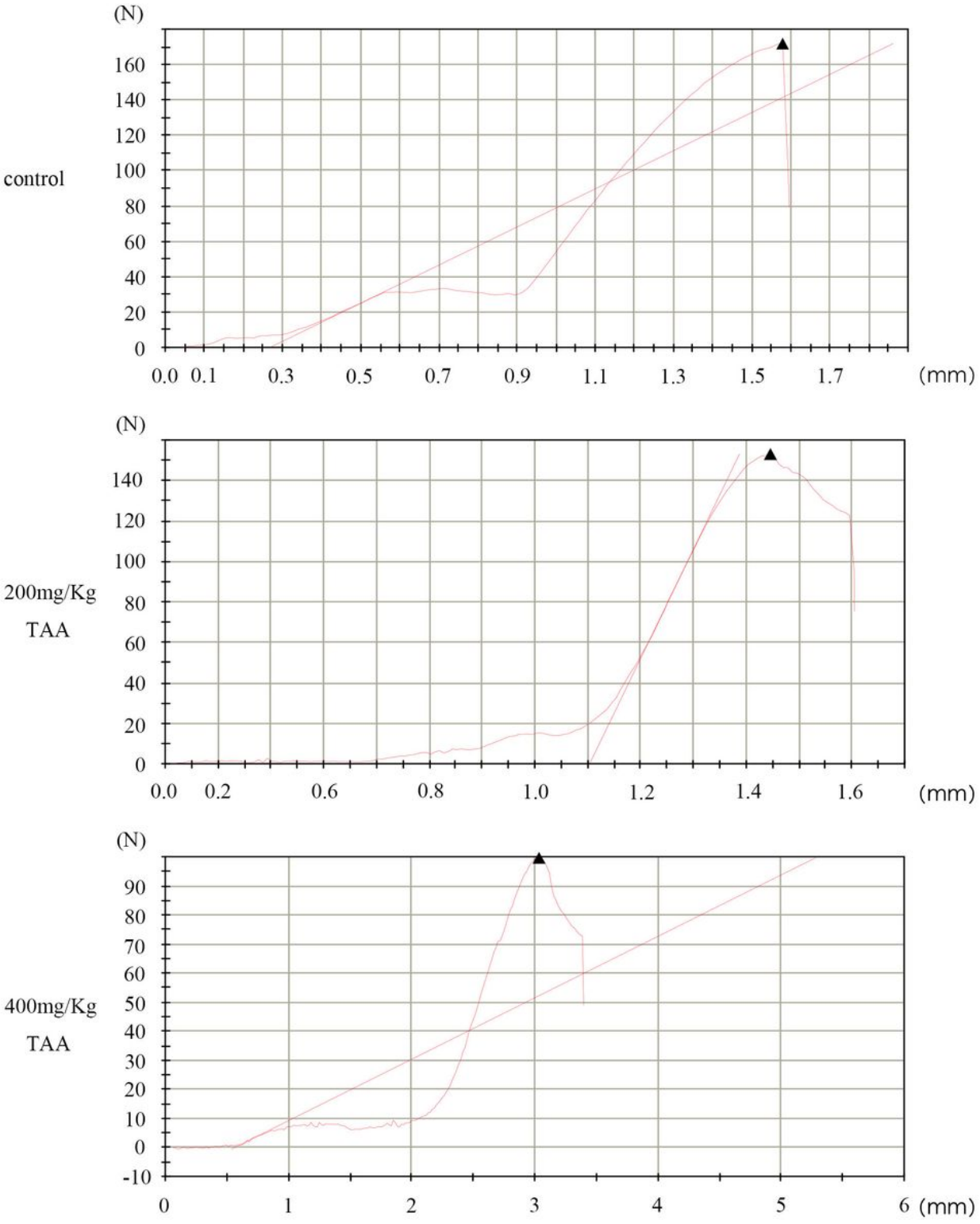

Figure 11

The field of load of the femur after intraperitoneal injection of saline and intraperitoneal injection of different doses of TAA (200mg/Kg TAA and 400mg/Kg TAA) in rats.

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