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The effects of berberine hydrochloride on the bone quality in methamphetamine-addicted rats after 3 weeks of withdrawal

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Abstract: Animal studies have shown that berberine hydrochloride reduces osteoporosis and has anti-inflammatory effects. The aim of this study was to evaluate the therapeutic effect of berberine hydrochloride on the quality of new bone and the severity of bone inflammation in methamphetamine-addicted rats after 3 weeks of withdrawal. A total of 21 male Wistar rats were divided into 2 groups: control (n = 7), methamphetamine (n = 7), and methamphetamine + berberine groups (n = 7). The 2 addicted groups received water-soluble methamphetamine up to 12 mg/kg for 2 weeks. Subsequently, they were abstinent for 3 weeks. Only 1 group received 100 mg/ kg/ day of berberine. After 3 weeks, the mandibular bone of rats was removed for histopathological evaluation of the severity of inflammation and the quality of new bone via hematoxylin and eosin (H&E) staining. Data were analyzed using Shapiro-Wilk and Mann-Whitney U tests. The software used was SPSS version 24, and the significance level was 0.05. The new bone formation was more mature in the methamphetamine + berberine group than in the methamphetamine group (P < 0.05). The bone inflammation was more severe in the methamphetamine group than in the methamphetamine + berberine group (P < 0.05). Because of the beneficial effects of berberine on the mandibular jaw, it can reduce the oral side effects of methamphetamine addiction.

Keywords: Methamphetamine, Berberine Hydrochloride, Bone, Inflammation, Maturity

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1 Introduction

Drug addiction is a chronic and recurrent disorder in which the tendency to use drugs continues despite serious negative consequences (Cami and Farré, 2003). Addictive substances cause a pleasant feeling or relieve anxiety. Among drugs, methamphetamine has a high potential for addiction in a person, making methamphetamine addiction a global concern (Tomita et al., 2014).

Methamphetamine abuse increases crime, accidents, and physical and psychological damage. Methamphetamine not only affects the central nervous system but also damages other organs. An increasing number of clinical and autopsy studies have reported that methamphetamine use is associated with angina, tachycardia, hypertension, myocarditis, cardiomyopathy, arrhythmia, and sudden death (Volkow et al., 2010). Oral examinations performed on some convicts showed that methamphetamine abusers had a higher risk of tooth decay and periodontal disease compared with other prisoners (Nassar and Ouanounou, 2020). Numerous studies have shown that methamphetamine reduces bone regeneration by suppressing the activity of mesenchymal stem cells and osteoblasts and can lead to decreased bone density and thus increased osteoporosis (Shen et al., 2018; Tomita et al., 2014).

Berberine is an alkaloid plant with a long therapeutic history in traditional Chinese and Indian medicine (Arayne et al., 2007). Berberine has antimicrobial, antiviral, anti-diarrheal, antipyretic, and anti-cancer effects and regulates the immune system (Kalmarzi et al., 2019). Berberine plays an effective role in bone regeneration by inducing bone formation in mesenchymal stem cells, increasing bone calcification, and reducing bone resorption (Han et al., 2018; Lee et al., 2008).

Based on the facts mentioned above, it can be hypothesized that berberine can improve the destructive effects of methamphetamine on bone. The aim of this study was to evaluate the effect of berberine hydrochloride on mandibular bone quality in methamphetamine-addicted rats after 3 weeks of withdrawal.

2 Materials and Methods

This study was performed on 14 male Wistar rats (200-250 g) purchased from the Pasteur Institute of Iran. Before the experiments, 1 week was considered to adapt the animals to the new laboratory conditions. The rats were kept in transparent cages (1 rat per cage) with wood chip bedding and had free access to pellet food and water. The conditions were similar in both rooms during the study and maintained constant conditions (temperature: 22 ± 2 °C; humidity: 60% ± 5%). The animals were classified into 2 groups: methamphetamine + vehicle (n = 7) and methamphetamine + berberine (n = 7) groups.

Rats received water-soluble methamphetamine for 2 weeks (up to 12 mg/kg body weight). In the first week, they received methamphetamine at doses of 1, 2, 4, 8, and 12 mg/kg/day, and in the second week, they received a fixed dose of 12 mg/kg/day. For each rat, it was measured in terms of rat weight and dissolved in the rat's daily water intake. After that, 1 group was left for 3 weeks with only water and food, and the other group received berberine hydrochloride by

oral gavage for 3 weeks daily (100 mg/kg/day). At the end of the experiment, the animals were placed under anesthesia, and trans cardiac perfusion was accomplished with 0.9% saline, continued by 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). Their mandibular bones were sampled for histopathological evaluation of the severity of inflammation and the quality of new bone. The samples were fixed in 10% formalin solution; then, they were embedded in paraffin after being extracted and post-fixed for 3 days using the same fixative. Longitudinal sections (5-10 μm thicknesses) were prepared from bone tissue. After staining with hematoxylin and eosin (H&E), they were examined under a light microscope. The inflammation was graded using table 1.

Table 1. Grading of inflammation

Grade	Inflammation
Grade 0	No inflammation
Grade 1	(10-30%) Mild inflammation
Grade 2	(30-50%) Moderate inflammation
Grade 3	(> %50) Severe inflammation

Data were analyzed by SPSS version 24 using Shapiro-Wilk and Mann-Whitney tests. The significance level was 0.05.

3 Results and Discussions

The Maturity of new bone

The distribution of the maturity of new bone in the 2 groups is shown in figure 1. The maturity of new bone in the 2 groups was significantly different ($p < 0.05$), indicating that the maturity of new grown bone was more in the berberine hydrochloride treated group than methamphetamine treated group ($p < 0.05$).

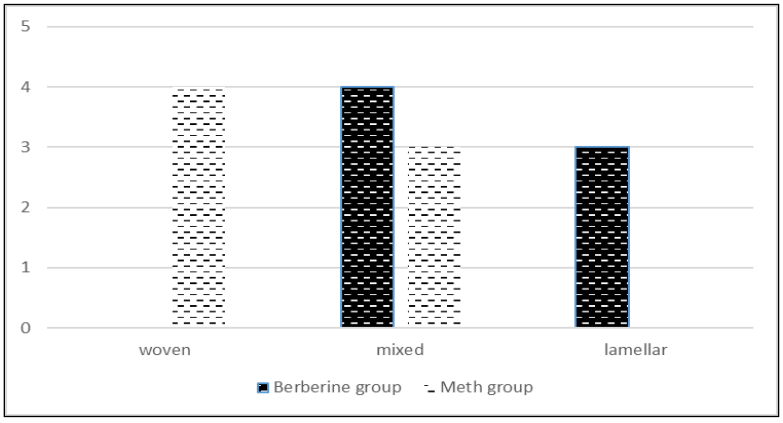

Figure 1: The distribution of the quality of new bone in the 2 groups. According to figure 1, in the berberine hydrochloride group, new bone was mostly mixed and lamellar, and no woven bone was found in any of the slides, while in the methamphetamine group, the new bone was more mixed and woven, and no lamellar bone was found in any of the slides.

The severity of bone inflammation

distribution of bone inflammation severity between the 2 groups is shown in figure 2. The severity of bone inflammation was significantly different in the 2 groups ($p < 0.05$), indicating that the severity of bone inflammation was significantly lower in the berberine hydrochloride group than in the methamphetamine group ($p < 0.05$).

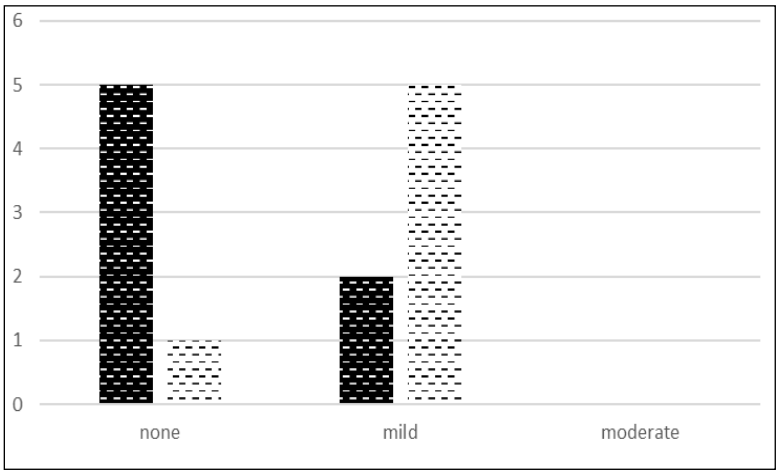

Figure 2: The distribution of bone inflammation severity between the 2 groups. No significant inflammation was observed in most of the slides in the berberine hydrochloride group. Mild inflammation was observed in some slides, while inflammation was seen in all slides of the methamphetamine group, which was mild or severe. There was no moderate inflammation in either slide of the 2 groups.

Figure 3 shows the maturity of new grown bone and figure 4 shows the inflammation severity in the study groups.

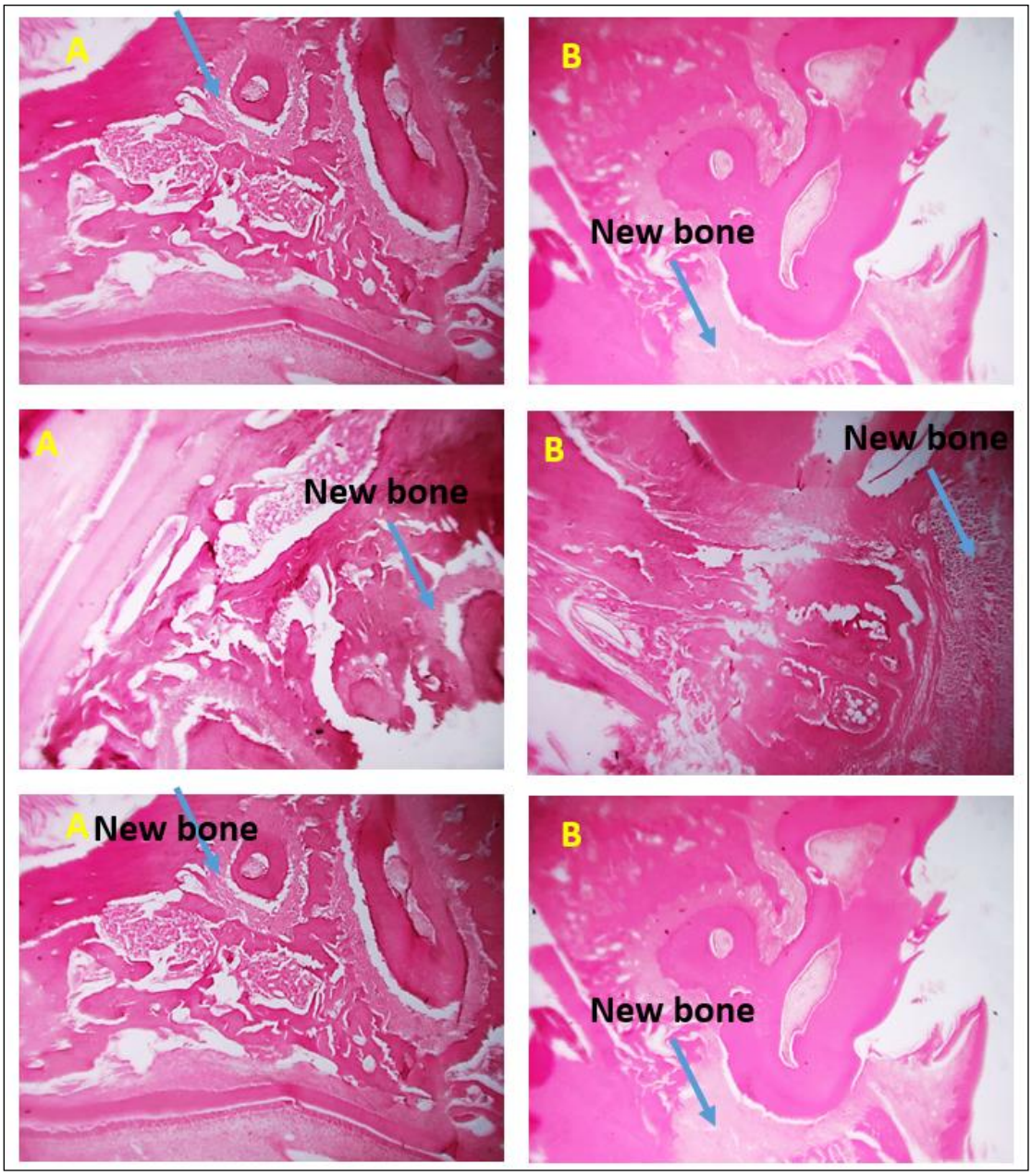

Figure 3: The maturity of new bone in methamphetamine and berberine hydrochloride treated groups**.** The quality of new bone was more mature in the berberine hydrochloride group than methamphetamine treated group (A: methamphetamine treated group, B: berberine hydrochloride treated group).

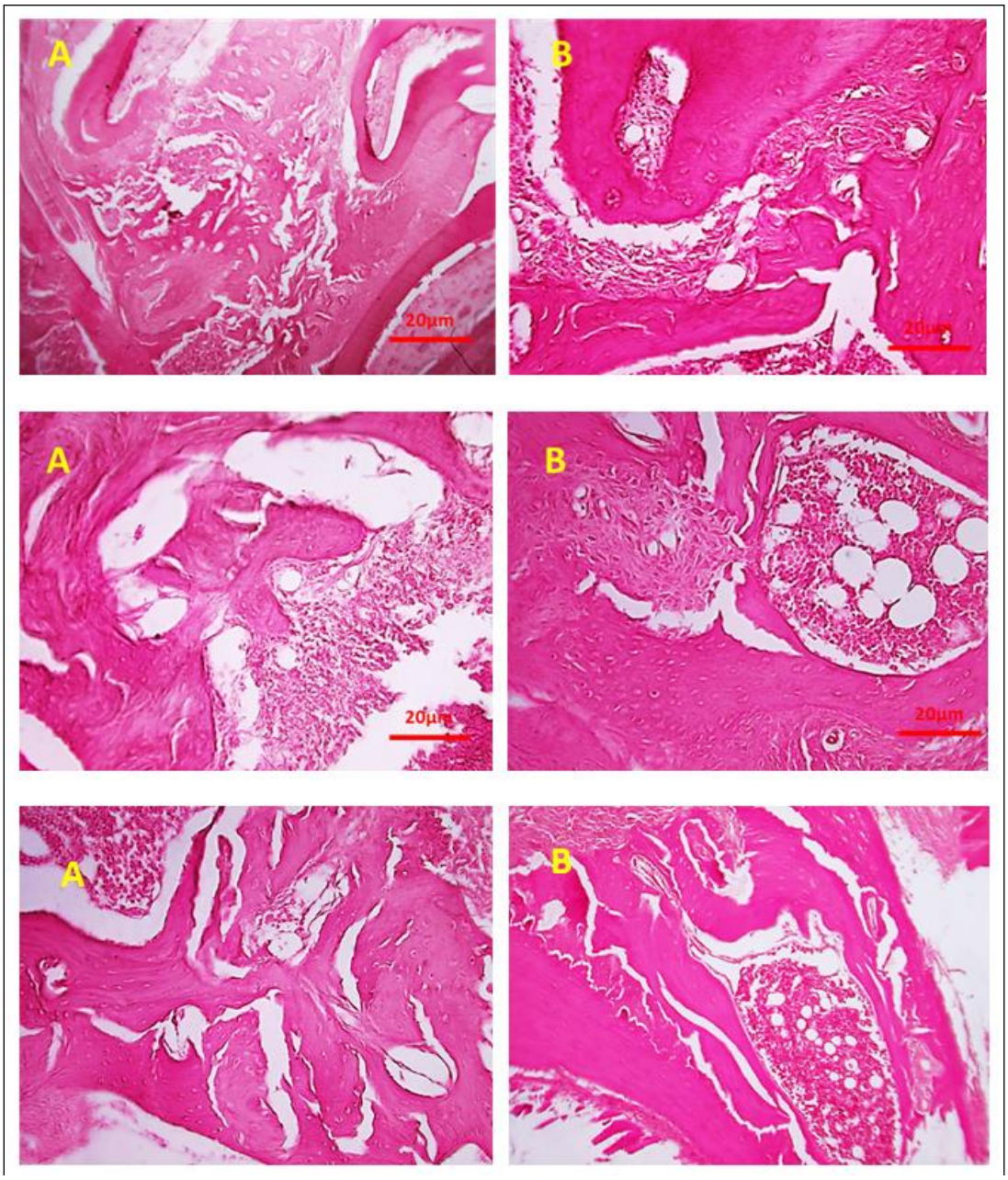

Figure 4: Bone inflammation severity in methamphetamine and berberine hydrochloride treated groups. The severity of bone inflammation was significantly lower in the berberine hydrochloride group than methamphetamine treated group (A: methamphetamine treated group, B: berberine hydrochloride treated group).

Based on the findings, berberine hydrochloride can increase the quality of new bone and reduce the severity of inflammation in the mandibular bone in methamphetamine-addicted rats after 3 weeks of withdrawal. Bone is a dynamic organ that is constantly rebuilding itself. Bone resorption and ossification are regulated by osteoclasts and osteoblasts (Tomita et al., 2014). Osteoblast differentiation is regulated by the normal Wnt/β-catenin signaling pathway (Zhang et al., 2021). Osteoclast differentiation is regulated by essential factors that can activate multiple intracellular signaling pathways. The receptor activator of NF-κB ligand (RANKL) is one of the major regulators. RANKL and RANK binding activate several primary signaling pathways, such as p38, mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase

(ERK), c-Jun N-terminal kinase (JNK), Akt, and NF-κB, which are essential for osteoclast differentiation. Among them, NF-κB and Akt are highly sensitive to berberine treatment, and berberine inhibits osteoclastogenesis and the survival of RANKL-induced osteoclasts by suppressing NF-κB and activating Akt, thereby preventing bone resorption (Zhang et al., 2021). Berberine hydrochloride reduces inflammation in periodontal tissues, including bone, by reducing the expression and secretion of inflammatory cytokines, such as tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), IL-17, RANK, matrix metalloproteinases (MMP-2), MMP-9, and monocyte chemoattractant protein 1 (MCP-1) (Mohammadian Haftcheshmeh and Momtazi-Borojeni, 2021). Methamphetamine increases IL-1β and plays an important role in bone resorption (Tipton et al., 2010). Berberine decreases IL- 1β and prevents bone resorption (Mercado et al., 2002).

Bone alkaline phosphatase is an enzyme that plays an important role in bone calcification (Vimalraj, 2020). Methamphetamine reduces bone calcification by reducing this enzyme (Shen et al., 2018). Berberine also increases bone calcification by increasing alkaline phosphatase activity (Han et al., 2018). RUNX2, osteopontin, and osteocalcin proteins are effective in the ossification of mesenchymal stem cells, and methamphetamine suppresses ossification by reducing their expression. Berberine induces the ossification of mesenchymal stem cells by increasing the expression of these proteins (Lee et al., 2008).

4 Conclusion

Because of the beneficial effects of berberine on the mandibular jaw, it can reduce the oral side effects of methamphetamine addiction.

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Conflict of interests

The authors declared that there is no conflict of interests regarding publication of this paper.

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