

ORIGINAL ARTICLE

The Therapeutic Effect of Honey on Dexamethasone-Induced Osteoporosis in Rats

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ABSTRACT

Background: The effect of glucocorticoids on calcium balance and bone growth may lead to osteoporosis and currently glucocorticoid-induced osteoporosis is one of the recognized types of osteoporosis. The present study aimed at evaluating the therapeutic effect of honey on dexamethasone-induced osteoporosis in rat model.

Methods: Thirty-two male rats were randomly divided into four groups including dexamethasone receiving group, physiological serum receiving group, dexamethasone and honey receiving group, and dexamethasone and alendronate group. All rats were treated for 4 weeks. At the end of the treatment period, blood was collected and the changes in blood phosphorus, calcium and alkaline phosphatase (ALP) levels were compared on the first day. Animals were sacrificed and femurs were separated for histological evaluation, while specimens were obtained from the epiphysis and metaphysis.

Results: The positive effect of honey on prevention of osteoporosis was demonstrated, although there were no significant differences between groups regarding serum calcium. Histomorphometric parameters revealed the effective role of honey in prevention of dexamethasone-induced osteoporosis.

Conclusion: Prescription of dexamethasone was illustrated to reduce the histomorphometric parameters of rat femur that caused osteoporosis. On the other hand, the administration of honey with dexamethasone could largely prevent more reduction for osteoporosis. Therefore, honey is suggested as a potential treatment for glucocorticoid-induced osteoporosis.

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Introduction

Osteoporosis is a skeletal disorder that primarily occurs with aging and is characterized by a significant reduction in bone strength, putting the affected individual at risk of fractures (1). There are two main types of osteoporosis including primary

and secondary osteoporosis. Primary osteoporosis is the most common type and can occur at any age and in both males and females, although it is more frequently observed in postmenopausal women and elderly men (2, 3). Glucocorticoids are commonly used for the treatment of various diseases (4). One

of the side effects of these drugs is their impact on calcium balance, bone growth, and the development of secondary osteoporosis (5). Glucocorticoid-induced osteoporosis is recognized as the most prevalent drug-induced form of osteoporosis (6).

Honey is a natural product in the form of a solution that contains a high concentration of complex carbohydrates and has been widely utilized for its therapeutic effects (7). Honey primarily consists of carbohydrates such as monosaccharides, disaccharides, oligosaccharides, and polysaccharides, as well as enzymes like glucose oxidase, diastase, catalase, and peroxidase. Other chemical compounds present in honey include organic acids, ascorbic acid, vitamins, amino acids, proteins, flavonoids, and phenolic acids. These chemical compounds have contributed to honey's recognition as an essential factor in human health (8). Nutrition with honey alone has been shown to affect animal bone mineral density positively (9). Recent studies have indicated the positive impact of honey on wound healing and bone repair in ovariectomized mice (10, 11). However, to date, no study has been conducted to assess the effect of honey on glucocorticoid-induced osteoporosis; so the present study aimed to evaluate the therapeutic effect of honey on dexamethasone-induced osteoporosis in rats.

Materials and Methods

Thirty-two male albino rats, aged less than 2 months with an average weight of approximately 150 g, were purchased from Lorestan University, Khorramabad, Iran. Throughout the study period, the animals were maintained under conditions of 12-h light/dark cycles and a temperature of 20–22°C, and they were fed with standard food and provided with ample water for 10 days to acclimatize to the new environment before the experiments commenced. After 10 days, the rats were randomly divided into four equal groups as (i) control group (C), while animals in this group received daily subcutaneous injections of physiological saline in an equal volume to dexamethasone; (ii) group two (D), while animals in this group were injected daily with 0.1 mg/kg dexamethasone subcutaneously to induce osteoporosis (5); (iii) group three (DH), that in addition to the daily subcutaneous dexamethasone injection at 0.1 mg/kg, honey was administered orally daily, while a honey dosage of 1 g/kg was administered by a gavage needle (12); and (iv) group four (DA), that in addition to the daily subcutaneous dexamethasone injection at 0.1 mg/kg, sodium alendronate was administered orally daily at 0.2 mg/kg using a gavage needle too.

All rats were subjected to 4 weeks of study, and

on the final day, blood samples were collected from the animals' hearts using a 5 mL syringe to assess changes in blood phosphorus (P), calcium (Ca), and alkaline phosphatase (ALP) levels. The collected blood samples were maintained under laboratory conditions for 20 minutes and then centrifuged at 2000 rpm for 15 minutes. Subsequently, the serum of each sample was collected in 1.5 mL microtubes and stored at -20°C until biochemical measurements to be conducted.

ALP enzyme activity was performed using a biochemical analyzer, employing the DGKC method by the standards of the German Biochemistry Society, and utilizing a commercial kit (Pars-Azmoon, Iran). The calcium level was determined using the Arsenazo method in conjunction with a commercial kit (Pars Azmoon, Iran) via a biochemical analyzer. Phosphorus quantification was achieved through a biochemical analyzer utilizing a kit manufactured by Pars-Azmoon too. According to the procedure outlined in this laboratory kit, phosphate reacted with molybdates to generate phosphomolybdate, which was subsequently reduced in the presence of a reducing agent (paramethylaminophenol) to produce a blue color that can be measured at a wavelength of 340 nm. Additionally, the buffer in the kit prevented protein precipitation and minimized turbidity formation.

Subsequently, animals in a deeply anesthetized state were euthanized humanely through the intramuscular injection of 25 mg of ketamine and 5 mg/kg of diazepam, followed by the immediate separation of their femurs. After femur extraction, the left femur bone samples were immersed in 10% formalin for three weeks. Subsequently, they underwent decalcification in 5% nitric acid (HNO₃), enabling the tissues to become suitable for sectioning. The epiphyseal and metaphyseal regions of the femur bone were segregated and processed using a tissue preparation apparatus to produce paraffin blocks and a microtome, and these sections were later stained with Hematoxylin and Eosin (H&E).

Following staining, parameters related to trabecular bone, including the thickness of epiphyseal and metaphyseal trabeculae, along with the bone area-to-tissue area ratio in the femoral sections, were determined. Measurements of trabecular thickness involved assessing the thickness of 10 epiphyseal trabeculae or 10 metaphyseal trabeculae in each sample, and the result was calculated as the average thickness of these 10 trabeculae per sample. To calculate the bone area-to-tissue area ratio, the total area of trabeculae within a rectangle measuring 0.8×0.4 mm was measured and expressed as a percentage by dividing it by 0.32.

All these parameters were measured using a digital photomicroscope connected to a personal computer equipped with Axio Vision LE 4.8.2 software (13). Data analysis was conducted using GraphPad Prism software (Version 5). One-way ANOVA and Tukey post hoc tests were applied to analyze data in various groups of the study. The results were considered significant at the p value <0.05 level.

Results

The results of the serum ALP activity evaluation in the examined groups were presented in Figure 1. The mean ALP level in the control group exceeded those in the other investigated groups. Notably, a statistically significant difference in ALP activity was observed between the control group and those receiving dexamethasone-alendronate and dexamethasone-honey ($p < 0.05$). The average serum phosphorus level in the dexamethasone-honey group was more than other examined groups. Furthermore, the groups receiving dexamethasone-alendronate and dexamethasone-honey showed a statistically significant difference for phosphorus level. The results regarding serum calcium level in the examined groups indicated no significant difference in calcium level among the groups ($p > 0.05$, Figure 2).

Figures 3 and 4 depict histomorphometric evaluation and photomicrographs from various groups' epiphyseal and metaphyseal regions. As observed, osteoporosis was evident in the groups treated with dexamethasone due to larger spaces lacking trabeculae. The prescription of dexamethasone significantly reduced the thickness of epiphyseal trabeculae in the femur bones of rats compared to the control group. Furthermore, the administration of dexamethasone in conjunction with honey also led to a significant decrease in the

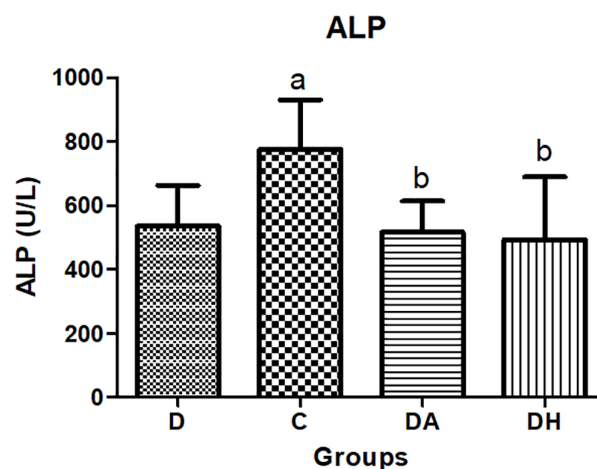


Figure 1: Serum ALP activity (mean \pm standard deviation) in the examined groups. D: Dexamethasone group, C: Control group, DA: Dexamethasone-alendronate group, DH: Dexamethasone-honey group. ALP: Alkaline phosphatase. Dissimilar letters indicate a significant difference ($p < 0.05$).

thickness of epiphyseal trabeculae in the femur bones of rats compared to the control group. However, statistically, this parameter resulted in a significant difference compared to the dexamethasone-receiving group and was higher. The co-administration of dexamethasone with alendronate had no significant effect in this regard ($p < 0.05$, Table 1).

The administration of dexamethasone resulted in a significant reduction in the thickness of metaphyseal trabeculae in the femoral bone of rats compared to the control group. The co-administration of dexamethasone with honey did not significantly reduce the thickness of metaphyseal trabeculae in the femoral bone of rats compared to the control group. Statistical analysis revealed that the thickness of metaphyseal trabeculae in the group treated with dexamethasone along with alendronate was similar to that in the control group ($p > 0.05$, Table 2).

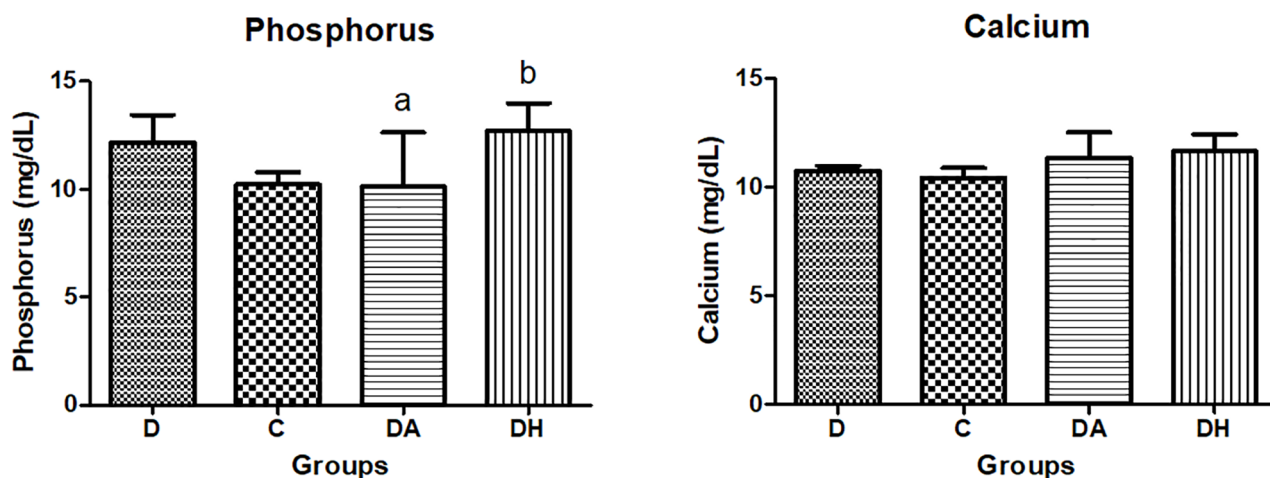


Figure 2: Serum phosphorus and calcium levels (mean \pm standard deviation) in the examined groups. D: Dexamethasone group, C: Control group, DA: Dexamethasone-alendronate group, DH: Dexamethasone-honey group. Dissimilar letters indicate a significant difference ($p < 0.05$).

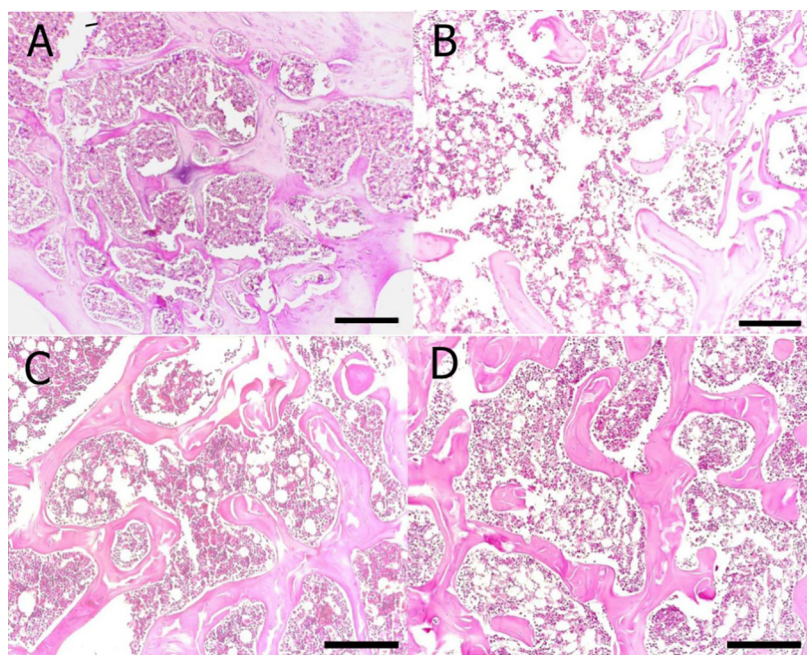


Figure 3: Photomicrographs obtained from the central section of the femoral epiphysis in different groups. A: Control, B: Dexamethasone, C: Dexamethasone-Honey, and D: Dexamethasone-Alendronate. The increased spacing between trabeculae indicates the development of osteoporosis, particularly noticeable in the dexamethasone-receiving group. Additionally, an increase in the number of bone marrow adipocytes, a characteristic of glucocorticoid-induced osteoporosis, is evident in these groups (H&E staining, bar=125).

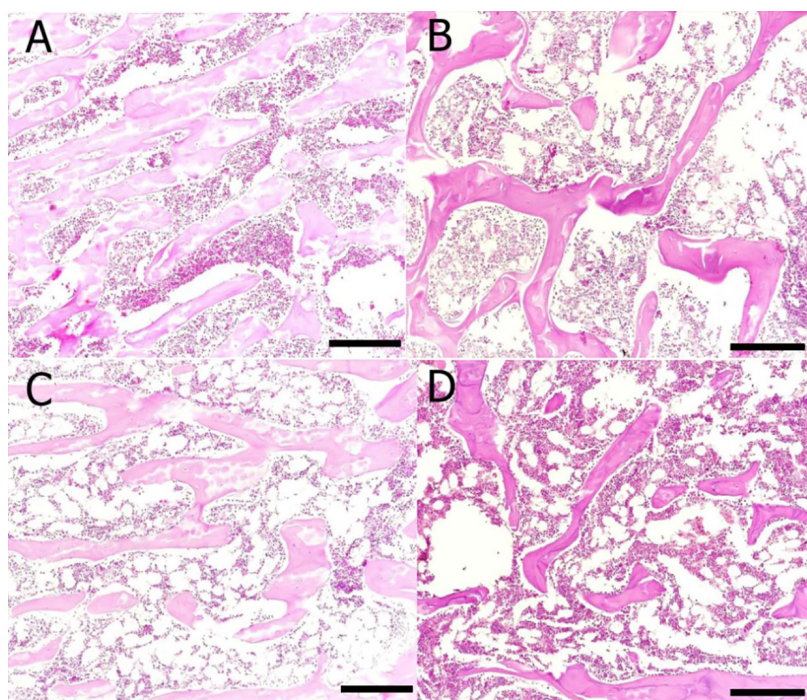


Figure 4: Photomicrographs obtained from the central section of the femoral metaphysis in different groups. A: Control, B: Dexamethasone, C: Dexamethasone-Honey, and D: Dexamethasone-Alendronate. The increased spacing between trabeculae indicates the development of osteoporosis, particularly noticeable in the dexamethasone-receiving group ((H&E staining, bar=125).

The administration of dexamethasone led to a significant decrease in the ratio of trabecular area to total tissue area in the epiphyseal region when compared to the control group. However, the co-administration of dexamethasone with honey did not significantly decline this parameter compared to the control group. Furthermore, the administration of

dexamethasone along with alendronate did not result in a significant change in the ratio when compared to the control group ($p < 0.05$, Table 3).

The prescription of dexamethasone significantly declined the ratio of metaphyseal trabecular area to total tissue area when compared to the control group. Additionally, the administration of dexamethasone

Table 1: The thickness of epiphyseal trabeculae (mean±standard deviation).

Variable	Dexamethasone-alendronate group	Dexamethasone-honey group	Dexamethasone group	Control group
Thickness of epiphyseal trabeculae (µm)	79.02±14.42 Ac	72.58±1.78 Dc	54.41±13.36 B	91.08±8.46 A

Dissimilar letters indicate a significant difference ($p<0.05$).

Table 2: The thickness of metaphyseal trabeculae (mean±standard deviation).

Variable	Dexamethasone-alendronate group	Dexamethasone-honey group	Dexamethasone group	Control group
The thickness of metaphyseal trabeculae (µm)	36.75±4.46 Ac	35.84±4.7 Ad	29.7±3.06 Bd	41.16±5.99 A

Dissimilar letters indicate a significant difference ($p<0.05$).

Table 3: The ratio of trabecular area to total tissue area in the epiphyseal region (mean±standard deviation).

Variable	Dexamethasone-alendronate group	Dexamethasone-honey group	Dexamethasone group	Control group
The ratio of trabecular area to total tissue area (%)	31.11±4.95 A	28.06±3.35 Ab	21.37±4 B	36.02±11.23 A

Dissimilar letters indicate a significant difference ($p<0.05$).

Table 4: The ratio of metaphyseal trabecular area to total tissue area (mean±standard deviation).

Variable	Dexamethasone-alendronate group	Dexamethasone-honey group	Dexamethasone group	Control group
Percentage of trabecular area to total tissue area (%)	33.77±4.71 Ad	30.91±8.05 Cd	19.13±3.78 B	37.5±5.97 A

The dissimilar letters indicate a significant difference ($p<0.05$).

along with honey also led to a significant difference in this parameter in comparison to the control group, despite a significant difference noticed in this group when compared to the group treated with dexamethasone alone, which exhibited a higher value in this regard. The administration of dexamethasone along with alendronate did not induce any significant change in this parameter when compared to the control group ($p<0.05$, Table 4).

Discussion

From a mechanistic perspective, the most significant effect of glucocorticoids on bone is the reduction of bone formation, although these drugs also increase the process of bone resorption (14-16). Histopathologically, the most notable feature in glucocorticoid-induced osteoporosis is the reduction in the thickness of bone trabeculae (17). Regarding histomorphometry, it should be noted that the most pronounced consequence of dexamethasone administration in rats is the thinning of trabeculae in cancellous bone analysis without altering their mode of connection to each other. This bone analysis results from intensified bone degradation by osteoclasts and reduced bone formation (18).

Reduced trabecular bone area relative to tissue area and decreased thickness of bone trabeculae in osteoporosis induced by the administration of glucocorticoids to rats indicate a decline in bone formation and an increase in its resorption (18, 19). In the current study, the administration of dexamethasone also led to a significant decrease in histomorphometric parameters which suggests the development of osteoporosis. The administration of dexamethasone with honey resulted in a significant decline in histomorphometric variables when compared to the control group. However, there were no significant differences in other histomorphometric parameters. Furthermore, when comparing dexamethasone alone to dexamethasone with honey, it was observed that dexamethasone alone significantly reduced histomorphometric variables. Nutrition with honey alone demonstrated positive effects on bone mineral density of animals (9). Additionally, honey administration, compared to calcium, improved trabecular bone structure and prevented bone resorption in ovariectomized rats (11, 20).

Recently, it has been reported that consuming honey with moderate-intensity jumping exercise can impact bone health in young rats (12). Honey, due to its antioxidant properties and anti-inflammatory

effects, can act as a free radical scavenger, reducing oxidative stress levels and inhibiting pro-inflammatory cytokines. These factors lead to the survival of osteoblasts and a reduction in osteoclast activity, ultimately resulting in improved bone density. Honey may serve as an alternative treatment for postmenopausal osteoporosis in women with minimal side effects (21).

In rats, serum ALP activity results from the activities of hepatic/bone and intestinal isoenzymes (22). Hepatic and bone isoenzymes are produced from a single gene, and their differences manifest after translation. Unlike most animal species in which hepatic isoenzyme predominates, intestinal and bone isoenzymes are predominant in rats. Therefore, changes in serum ALP in rats can be influenced by nutrition (via the intestinal isoenzyme) and body growth and mass (via the bone isoenzyme). An increase in serum ALP due to the bone isoenzyme is usually 2-3 times, but an increase due to the hepatic isoenzyme can be much more significant (23, 24). According to available data, serum ALP activity in juvenile animals is often higher than in adults, attributed to the bone isoenzyme and the activities of bone formation and mineralization by osteoblasts during prepubertal periods (25). Bone-specific alkaline phosphatase (BALP) is influenced by various factors, including age, gender, hormonal status, and diet, and, in general, it is higher in juvenile animals than mature ones (26, 27). Considering that skeletal maturation in rats occurs slowly and bone formation processes extend for over a year, the elevated serum ALP activity in the control group may have occurred for this reason.

The steroid ALP is induced by several drugs, such as corticosteroids and phenobarbitals, that can increase the serum ALP level. Steroid ALP exhibits significant activity in canine serum and can elevate ALP activity in the serum. However, it does not appear that this isoenzyme plays a prominent role in ALP elevation in the blood of rats through drug-induced mechanisms or steroid compounds (25). In the present study, serum ALP activity decreased in the dexamethasone-receiving group compared to the control group. This reduction in ALP activity was also observed in the groups receiving dexamethasone in combination with alendronate and dexamethasone with honey, which did not significantly differ from the dexamethasone-alone group. Based on these findings, it can be concluded that dexamethasone leads to a reduction in serum ALP activity in rats, possibly due to a decrease in the number or activity level of osteoblasts. Furthermore, alendronate and honey could not enhance bone formation in dexamethasone-receiving rats, as evidenced by

the lack of prevention in the decrease in osteoblast activity (25).

Serum phosphate concentration may be influenced by age, race, gender, and pregnancy (28, 29). Generally, growing animals have higher concentrations that decrease with age (30, 31). In rats, serum phosphate decreases with age and is higher in males than females (32). Depending on dietary habits, phosphorus concentration may increase after food intake (33). In the present study, the serum phosphorus concentration in the group receiving dexamethasone was higher than in the control group. This result may be attributed to reduced osteoblast numbers due to dexamethasone administration. Additionally, the group receiving alendronate and dexamethasone illustrated the lowest, while the group receiving honey and dexamethasone displayed the highest mean serum phosphorus level. These findings show that alendronate performs better than honey in preventing osteoblast destruction by dexamethasone, thereby preserving these cells, facilitates the shift of phosphorus from the blood stream to the bones, and enhances bone mineralization. Furthermore, there is a possibility that honey improves intestinal phosphorus absorption, leading to an increase in its concentration in the blood stream.

Calcium is influenced by mediators such as parathyroid hormone (PTH), vitamin D, and calcitonin (34). Calcitonin and PTH indirectly affect the intestine by stimulating 1α -hydroxylase in the kidneys, which increases the production of $1,25(\text{OH})_2\text{D}$. Dexamethasone leads to osteoblast destruction, limiting calcium utilization in bone formation. Since blood calcium levels are tightly controlled by various factors, including parathormone, calcitonin, and vitamin D, the excess calcium in the blood is likely rapidly excreted by the kidneys, leading to no significant differences in calcium concentration among the groups.

Conclusion

Overall, the study findings demonstrated that dexamethasone administration reduced histomorphometric parameters related to the femur bone in rats, resulting in osteoporosis. Concurrent administration of organic honey with dexamethasone significantly mitigates this reduction in histological tissue examinations. Therefore, honey could potentially be used as a supplementation for glucocorticoid-induced osteoporosis prevention.

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Authors' Contribution

F.P.: Conception and design of the work; acquisition and interpretation of the data. G.F.: Drafting the manuscript; copy-editing; revising the final version and final approval of the version to be published. M.K.: Analysis and interpretation of the data; and manuscript review. A.R.: Preparing figures and tables, and manuscript review.

Conflicts of Interest

None declared.

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