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Original Article

Astragalus membranaceus promotes bone formation in streptozotocin-induced diabetic rats

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ABSTRACT

In ovariectomized (OVX) rats, *Astragalus membranaceus* ethanol extract (*AMEE*), a traditional Asian herb, was shown to increase bone mineral density, biomechanical strength, and ash weight of the femur and tibia. The impact of *AMEE* on bone loss caused by diabetes is investigated in this study. The effect of *AMEE* on blood glucose, HBA1C levels, and bone mineral density was studied using a rat model. A total of twenty-four male Sprague Dawley rats were split into four groups (each with six rats): Normal control rats received saline (NC), diabetic control rats received saline (DC), diabetic rats received 1000 mg/kg of metformin (MET), and diabetic rats received 500 mg/kg of *AMEE*. Blood glucose, HBA1C levels, and bone mineral density were all improved after treatment with *AMEE*. These findings imply that *AMEE* may be more effective in preventing diabetes related bone loss.

Key words: *Astragalus membranaceus* extract, Diabetic osteoporosis, streptozotocin

INTRODUCTION

Diabetes and osteoporosis are two prevalent illnesses that can cause a variety of problems. Despite many therapeutic techniques, controlling and decreasing the burden of these diseases has proven difficult, especially when they arise one after the other.[1] Treatment options for diabetic-induced bone loss are less well researched. *AMEE* is one of the most often used therapeutic plants in Asian traditional medicine, and its main components include cycloartane triterpene glycosides, flavonoids, and notably isoflavones.[2-4]

The estrogenic action of *AMEE* extract[5], the inhibition of osteoclast formation in vitro[4], and the

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improvement of MG-63 cell proliferation[6] have all been documented. In addition, *AMEE* extract treatment reduced tibia and lumbar bone loss in ovariectomized (OVX) rats in an in vivo investigation. Some of these research show that combining *AMEE* extract with a calcium supplement may have a synergistic impact on bone mineral density. However, whether *AMEE* supplementation affects bone metabolism in diabetic rats has yet to be determined. As a result, the goal of this study was to see how *AMEE* affected bone mineral density in diabetic rats.

MATERIALS AND METHODS

Animals: Healthy male wistar albino rats weighing 180 to 240 g and aged 3 to 4 months were utilised in the investigation. The animals were taken from the Central Animal House of King Khalid University in Abha, Saudi Arabia. The animals were housed in cages throughout the experiment and fed a standard pellet meal and filtered water ad labium under standard conditions (light/dark cycle of 12 h/12 h, 50–70 percent humidity, 25 °C 3 °C). The animals were acclimatized to the laboratory environment for 14 days. The therapy was carried out with the consent of King Khalid University's animal ethics committee and in accordance with the National Institute of Health's standards for the care and use of laboratory animals in the United States. (NIH Publication No. 85-23, revised 1996).

Induction of diabetes: To induce diabetes in the animals, the pancreatic cell toxin streptozotocin (STZ) was administered intra peritoneally at a dosage of 65 mg/kg body weight (Sigma Chemical Co., freshly dissolved in sterile saline, 0.9 percent).[7,8] In the control group, all of the rats received the same quantity of vehicle. STZ was weighed individually for each animal, solubilized with 0.1 ml of freshly produced cold Na citrate buffer (NaB-0.1 M, pH 4.5), and given within 5 minutes to prevent deterioration. A volume of 1.0 ml/kg of STZ was estimated. Rats were administered a 5% glucose solution for 48 hours after the injection to offset the substantial acute hypoglycemia impact of STZ. Three days following STZ injection, blood was taken from the tail vein and analyzed for blood glucose using a glucometer (Aqua-Check, Roche). Animals having fasting blood glucose levels (FGLs) more than 250 mg/dL were classified as diabetic.

The rats were divided into four groups, each with six rats. Saline was administered to normal control rats (NC), diabetic control rats (DC), diabetic rats were given 1000 mg/kg body weight of metformin (MET), and the other diabetic rats were given 500 mg/kg body weight of *AMEE*. To evaluate the animals' hyperglycemic state, blood glucose levels were tested once a week for the duration of the experiment using a Roche Accu Chek advantage glucometer. The study excluded the animals which did not acquire blood glucose levels more than 250 mg/dL. Blood glucose levels were normal (120 mg/dl) in the rats in the control group (n=6) that were administered saline instead of streptozotocin.

Determination of fasting blood glucose: Blood samples were collected from the rats tail veins to measure blood glucose levels using a glucometer after they had been fasted for 12–14 hours. After the rats tails have been cleaned with 70% (v/v) ethanol, blood will be drawn using a 1-ml needle, placed on a glucose strip, and measured with a glucometer.

Determination of intra-peritoneal glucose tolerance test: All of the rats were fasted for 12–14 hours and blood were collected from the tail vein as a baseline. The rats were subsequently given 2 g/kg BW of a 40% (w/v) glucose solution intra peritoneally. Blood will be taken from the tail vein and analysed for blood glucose using a glucometer after 30, 60, 90, and 120 minutes after starting glucose treatment. Fasting blood sugar values of less than 250 mg/dl were used to prove diabetes in these rats.

Determination of hemoglobin A1c: After blood samples from the tail vein are collected and deposited on a test cartridge, haemoglobin A1c (HbA1c) will be analysed with a Clover A1cTM Self Analyzer. On the Clover A1cTM Self Analyzer's LCD screen, the percentage of HbA1c in the blood sample is shown.

Bone Mineral Density Measurement: After blood was taken, the BMD of the left femur and lumbar vertebrae (L1–L4) of rats was evaluated using dual energy X-ray absorptiometry (DEXA) scanning equipment (Lunar, WI, USA).

RESULT

The glucose profiles of the positive control group (STZ) deteriorated over time (Table-1). However, *AMEE* were demonstrated to protect against diabetes progression.

Table-1: Effect of *AMEE* on Fasting blood glucose level

Treatment Group	Dose	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	Day 56
Normal Control	5 mL/kg	74.82 ±4.2	75.22 ±3.3	76.81 ±4.5	77.70 ±2.7	78.70 ±2.5	82.56 ±2.2	83.50 ±2.05	85.60 ±1.32	86.30 ±1.42
Positive Control	65 mg/kg	251.54 ±9.2*	297.35 ±8.8*	324.21 ±14.12*	346.75 ±8.6*	371.74 ±8.4*	385.73 ±11.6*	378.76 ±9.5*	442.76 ±9.2*	475.77 ±10.6*
AMEE	500 mg/kg	264.33 ±7.3	276.25 ±9.4*	281.22 ±7.8*	286.28 ±8.2*	314.35 ±8.8*	317.35 ±9.8*	312.35 ±10.2*	328.35 ±8.2*	340.35 ±8.7*
Metformin	1000 mg/kg	265.33 ±8.3	275.25 ±9.4*	265.22 ±7.8*	242.28 ±8.2*	190.25 ±8.8*	160.55 ±9.8*	140.45 ±10.2*	105.15 ±9.2*	90.015 ±9.7*

Values are expressed as mean ± standard error of the mean (n=6)
*p<0.001 compared with normal control.

HBA1C levels were higher in the positive control group than in the normal control group (p<0.05), as indicated in table 2. In contrast to the positive control group, *AMEE* was shown to lower HBA1C levels, implying a favourable effect.

Table-2: Effect of *AMEE* on Glycoslyted Haemoglobin (HBA1C)

Treatment Group	Day 28
Normal Control	5.52±0.15
Positive Control	5.70±0.16*
AMEE	5.78±0.13*
Metformin	5.49±0.14*

Values are expressed as mean ± standard error of the mean (n=6); *P<0.001 compared with normal control.

Diabetic rats exhibited decreased lumbar (L1–L4) and femoral bone mineral density (BMD), which was improved by *AMEE* therapy (p<0.05). The positive group's BMD was considerably higher than the other treatment groups' (Table-3). *AMEE* may be able to protect bones against the consequences of hyper glycemia, according to these studies.

Table-3: Effect of *AMEE* on the bone mineral density of the lumbar vertebrae and femur bone

	Bone Mineral density(mg/cm3)	
Treatment Group	**Lumbar Vertebrae**	**Femur**
Normal Control	176 ± 2.6	225 ± 2.6
Positive Control	79 ± 2.7*	107 ± 2.4*
AMEE	168 ± 1.6*	205 ± 1.8*
Metformin	140 ± 2.2*	187 ± 2.6*

Values are expressed as mean ± standard error of the mean (n=6); *p<0.001 compared with normal control.

Statistical analysis: The data should be expressed as a mean and standard deviation (SD). Data from different groups will be statistically analysed using one way analysis of variance (ANOVA) and Tukey's multiple comparison test. A 'p' value of less than 0.05 indicates statistical significance.

DISCUSSION

AMEE 's effects on BMD (bone mineral density) in diabetic rats were investigated in this study. The BMD in the lumbar vertebrae and femur of diabetic rats was considerably enhanced by *AMEE*. Previous research has suggested that *AMEE* 's protective benefits in Cadeficient OVX *rat* might be due to a variety of mechanisms. Isoflavone, especially formononetin (Biochanin B, 7-hydroxy-4′-methoxyisoflavone), was the main component in the *AMEE* utilised in this investigation.[6] Furthermore, estrogen like compounds found in *AMEE*, such as isoflavones, may promote intestinal Ca absorption by an estrogen like mechanism, such as exogenous oestrogen treatment to ovariectomized rats, and boost intestinal Ca absorption.[9-11] However, the processes by which *AMEE* boosts intestinal Ca absorption are unknown, and more research is needed. Aside from increasing intestinal Ca absorption, there are a few potential direct pathways for *AMEE* 's improved

bone metabolism benefits. Isoflavones, for example, are phyto estrogens that may interact directly with oestrogen receptors in the bones, affecting bone metabolism.[12,13]

AMEE extract, for example, was found to suppress osteoclast formation in vitro and to decrease tibia and lumbar bone loss in OVX *rat* in vivo. In addition, 70 percent ethanol *AMEE* extract exhibited significant proliferative activity on MG-63 cells, an osteoblast like cell line in a prior research. *AMEE* appears to suppress osteoclasts while also enhancing bone osteoblasts, according to these findings. *AMEE* with or without high Ca reduced urine Ca excretion, one of the bone resorption indicators, in our investigation, which supports these direct processes in part. However, supplementing *AMEE* and Ca, either together or individually, had no effect on bone formation regulators such serum ALP and OC. *AMEE* extract treatment prevented tibia and lumbar bone loss in OVX rats, according to Kim and colleagues. Our research, on the other hand, convincingly shown that *AMEE* improves bone mineral density. In rats, 500 mg/kg body weight of *AMEE* was supplemented. *AMEE* supplementation had no toxicity in rats until a dosage of 2 g/kg body weight, according to a prior study. This supplementary quantity can be translated to 30 g/day for an adult with a 60 kg bodyweight if we apply our findings from experimental animals to humans. As a result, even in extract form, people may simply consume it. However, more research is needed before individuals can reap the benefits of the *AMEE* supplement's bone health benefits.

CONCLUSION

Finally, the results show that *AMEE* protects diabetic rats from bone loss. In addition, more research is needed to see how far *AMEE* can help bone metabolism.

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REFERENCES

1. Moein Ala, Razieh Mohammad Jafari and Ahmad Reza Dehpour*, "Diabetes Mellitus and Osteoporosis Correlation: Challenges and Hopes", Current Diabetes Reviews 2020; 16(9).
2. Hirotani M, Zhou Y, Rui H, Furuya T. Cycloartane triterpene glycosides from the hairy root cultures of Astragalus membranaceus. *Phytochemistry*.1994;37:1403–140714.
3. Zheng Z, Liu D, Song C, Cheng C, Hu Z. Studies on chemical constituents and immunological function activity of hairy root of Astragalus membranaceus. *Chin J Biotechnol.*1998;14:93–9715.
4. Kim C, Ka H, Lee JH, Kim JS, Song K, Park SW. Herbal extract prevents bone loss in ovariectomized rats. *Arch Pharm Res*. 2003; 26:917–92416.
5. Zhang CZ, Wang SX, Zhang Y, Chen JP, Liang XM. In vitro estrogenic activities of Chinese medicinal plants traditionally used for the management of menopausal symptoms. *J Ethnopharmacol*. 2005; 98:295–30017.
6. Kim HJ, Kim MH, Kang SC (2009) Proliferative and estrogenic effects of Astragalus membranaceus Bunge on the MG63 andMCF7-BUS cells. *Plant Science Conference*. 2009; 43:240.
7. Reddy GK, Stehno Bittel L, Hamade S, Enwemeka CS. The biomechanical integrity of bone in experimental diabetes. *Diabetes Res Clin Pract*.2001;54(1):1-8.
8. Krishnaraju Venkatesan et al. Geraniin prevents diabetes-induced bone loss in rats by reducing blood glucose and suppressing bone turnover. *International Journal of Research in Pharmaceutical and Nano Sciences*.2021;10(2):164-168.
9. Chen C, Kalu DN Modulation of intestinal estrogen receptor by ovariectomy, estrogen and growth hormone. *J Pharmacol Exp Ther*.1998;286:328–33325.
10. Liel Y, Shany S, Smirnoff P, Schwartz B, Estrogen increases1,25-dihydroxyvitamin D receptors expression and bioresponse inthe rat duodenal mucosa. *Endocrinology*.1999;140:280–28526.
11. Ten Bolscher M, Netelenbos JC, Barto R, Van Buuren LM, Van derVijgh WJ. Estrogen regulation of intestinal calcium absorption in the intact and ovariectomized adult rat. *J Bone Miner Res.*1999;14:1197–120227.
12. KuiperGG, CarlssonB, GrandienK, EnmarkE, HaggbladJ, Nilsson S, Gustafsson JA. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology.*1997;138:863–87028.
13. Onoe Y, Miyaura C, Ohta H, Nozawa S, Suda T. Expression of estrogen receptor beta in rat bone. *Endocrinology*.1997;138:4509–4512.