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EFFECT OF *ECLIPTAE HERBA* ON BONE FORMATION IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Traditional uses of *Ecliptae herba* (*EH*) include bone strengthening. *Ecliptae herba* may have antiosteoporotic properties, according to accumulating data. The purpose of this research was to see how aqueous *Ecliptae herba* extract (*EHE*) affected diabetic osteoporosis. The effect of *Ecliptae herba* extract on blood glucose, HBA1C levels, and bone mineral density in rats was studied using a rat model. Twenty four male Sprague Dawley rats (n=6) were split into four groups: Saline was administered to normal control rats (NC), diabetic control rats (DC), diabetic rats were given 1000mg/kg body weight of metformin (MET) and1000mg/kg body weight of *Ecliptae herba* extract respectively. When compared to normal controls, *Ecliptae herba* extract therapy significantly enhanced bone mass. This implies that *Ecliptae herba* extract might be developed as an alternative treatment for diabetes induced osteoporosis.

KEYWORDS

Ecliptae herba, Diabetic osteoporosis, Streptozotocin induced diabetes.

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INTRODUCTION

The evaluation of bone health in diabetic patients should be part of their care. The increase in the prevalence of osteoporosis has coincided with an increase in the average life expectancy of individuals with diabetes, owing to advances in medical treatment. Diabetes may weaken bone health, in addition to the traditional reasons of

osteoporosis, such as age. Because of the disease's pathogenetic complexity, studies on bone involvement in people with diabetes mellitus have yielded mixed findings. Type 1 diabetes patients had reduced bone mineral density (BMD) and a greater risk of fractures, according to new research. Despite having a greater BMD than patients with type 1 diabetes, there is growing evidence that people with type 2 diabetes who have comorbidities are at an increased risk of certain forms of osteoporotic fractures.

Although a variety of factors impact the likelihood of fractures, including the frequency and kind of falls, visual impairment, neuropathy, and diminished muscular strength, the bone's strength appears to be the most important determinant[1,2]. *Ecliptae herba* extract (*EHE*) Radix has been shown in mice and rats to enhance bone density and change bone histomorphology[1]. However, there is no direct evidence that *Ecliptae herba* extract has an inhibitory impact on diabetes induced bone loss. The goal of this study was to see if *Ecliptae herba* extract had an osteoprotective impact on bone mineral density (BMD) in STZ treated rats.

MATERIAL AND METHODS

Animals

Healthy male wistar albino rats weighing 180 to 240g 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 libitum under standard conditions (light/dark cycle of 12 h/12 h, 50-70 percent humidity, 25°C 3°C). The animals were acclimatised to the laboratory environment for 14 days. The treatment was carried out in accordance with the approval of King Khalid University's animal ethics committee and the National Institute of Health's guidelines 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) (Sigma Chemical Co., freshly dissolved in sterile saline, 0.9 percent) was administered intraperitoneally at a dosage of 65mg/kg body weight[3,4]. The control rats received the same quantity of vehicle. STZ was weighed individually for each animal, solubilized with 0.1ml of freshly produced cold Na citrate buffer (NaB-0.1 M, pH 4.5) and given within 5 minutes to prevent deterioration. The volume of STZ injection per kilograms was determined to be 1.0ml/kg. Rats were administered a 5 percent glucose solution for 48 hours after receiving STZ to counteract the drug's substantial acute hypoglycemia impact. Three days following STZ injection, blood was taken from the tail vein and examined using a glucometer for blood glucose (Aqua-Check, Roche). Fasting blood glucose levels (FGLs) more than 250mg/dL were considered diabetic in animals.

The rats were divided into four groups, each of which had six rats. Normal control rats received saline (NC), diabetic control rats received saline (DC), diabetic rat groups received 1000mg/kg of metformin (MET), and 1000mg/kg of *Ecliptae herba* extract respectively. The animals' hyperglycemic state was determined by measuring blood glucose levels once a week during the study using a Roche Accu-Chek advantage glucometer. The study excluded the animals which did not acquire blood glucose levels more than 250mg/dL. Blood glucose levels were normal (120mg/dl) in the control group (n=6) that were administered saline instead of streptozotocin.

Determination of fasting blood glucose

The rats were fasted for 12-14 hours before blood samples from their tail veins were obtained to determine blood glucose levels using a glucometer. 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 before blood was collected from the tail vein as a baseline. The rats were subsequently given 2g/kg body weight (BW) of a 40% (w/v) glucose solution intraperitoneally. Blood will be taken from the tail vein and analysed for blood glucose using a glucometer after 30, 60, 90, and 120 minutes after glucose treatment. Fasting blood sugar values of less than 250mg/dl were used to diagnose diabetes in these rats.

Determination of hemoglobin A1c

After blood samples from the tail vein are collected and placed on a test cartridge, haemoglobin A1c (HbA1c) will be analysed using a Clover A1cTM Self-Analyzer. The Clover A1cTM Self-Analyzer's LCD screen will show the percentage of HbA1c in the blood sample.

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).

RESULTS AND DISCUSSION

The glucose profiles of the positive control group (STZ) deteriorated over time (Table No.1). However, *Ecliptae herba* extract were demonstrated to protect against diabetes progression.

HBA1C levels were higher in the positive control group than in the normal control group ($p<0.05$), as indicated in Table No.2. In contrast to the positive control group, *Ecliptae herba* extract was shown to lower HBA1C levels, implying a favourable effect.

The findings of bone mineral density study revealed that diabetic rats had lower lumbar (L1-L4) and femoral bone mineral density (BMD), which was recovered by *Ecliptae herba* extract treatment ($p<0.05$). The BMD of the positive group and the other treatment groups differed significantly (Table No.3). These findings imply that *Ecliptae herba* extract may able to protect bones from the effects of hyperglycemia.

Statistical analysis

The data should be expressed as a mean and standard deviation (SD). To statistically analyse data from different groups, one way analysis of variance (ANOVA) and Tukey's multiple comparison test will be employed. A “p” value of less than 0.05 is considered statistically significant.

Discussion

Traditional bone disorders have been treated with *Ecliptae herba* extract for many years. *Ecliptae herba* extract has antiosteoporotic properties, according to certain research[1,5]. However, it is unclear whether the mixture's antiosteoporotic impact is comparable to that shown in diabetics. In the case of osteoporosis, the mechanism of action of this extract is uncertain. The impact of *Ecliptae herba* extract on STZ induced diabetic osteoporosis in rats was studied for the first time in the current study. *Ecliptae herba* extract has been shown to prevent rats from developing osteoporosis like symptoms after exposure to OVX (Ovariectomy). *Ecliptae herba* extract has a stronger inhibitory impact on both bone formation and resorption. Furthermore, in ovariectomized rats, *Ecliptae herba* extract was found to alter blood levels of IL 6 and CT. *Ecliptae herba* extract's ability to prevent OVX induced bone loss was linked to a decrease in RANKL expression. Another study found that wedelolactone, a chemical derived from the *Ecliptae herba*, has the ability to boost osteoblastogenesis.

The active chemical wedelolactone was isolated from E.coli. Through ERK and JNK-mediated BMP2 production and Smad1/5/8 activation, Herba has demonstrated clear efficacy in promoting osteoblastic differentiation and bone mineralization. This research adds to our understanding of how wedelolactone affects osteoblastogenesis. Diabetic rats showed decreased lumbar (L1–L4) and femoral bone mineral density (BMD), which was restored by *Ecliptae herba* extract therapy ($p< 0.05$). The positive group's BMD differed considerably from the other treatment groups (Table No.3). These data suggest that *Ecliptae herba* extract may be able to protect bones against hyperglycemia consequences.

The effects of *Ecliptae herba* extract on bone quality in a STZ induced animal model of type 2 diabetes are investigated in this work. Previous research has looked at the effects of RSG on bone loss, formation, and resorption, as well as bone structure and content, as well as bone quality as measured by mechanical characteristics[6-10]. The use of *Ecliptae herba* extract had a positive effect on unloading, as shown by increases in BMD. *Ecliptae herba* extract's bone-protective properties have been suggested to be owing to its direct action on bone production and suppression of osteoclast development[6].

Reduced levels of bone turnover markers and changes in urine calcium and phosphorus excretion were the primary indicators of *Ecliptae herba* extract's bone loss prevention efficacy. *Ecliptae herba* extract therapy has also been shown to improve bone biomechanical strength and prevent the degradation of trabecular bone micro-architecture in previous studies. *Ecliptae herba* extract also suppressed osteoclastogenesis *in vitro* by increasing osteoprotegrin and decreasing NF-kB ligand expressio[11]. *Ecliptae herba* extract are often utilised in individuals with fractures and osteoporosis[11].

Furthermore, their osteoprotective properties have been demonstrated, as well as *Ecliptae herba* extract's protective impact against mortality in hip fracture patients. *Ecliptae herba* extract has been shown to play a protective effect in reducing RANKL induced osteoclastogenesis *in vitro* by decreasing bone resorption related gene expression.

Table No.1: Effect of *Ecliptae herba* extract 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	5mL/kg	74.32± 4.2	75.42± 3.3	77.81± 3.7	79.40± 2.7	78.30± 2.5	82.46± 1.8	84.40± 1.15	82.40± 1.12	86.40± 1.62
Positive Control	65mg/kg	262.54± 9.2*	298.35± 8.8*	316.31± 11.62*	334.74± 8.6*	355.78± 9.4*	379.72± 10.5*	393.72± 10.6*	418.72± 12.2*	434.76± 9.8*
Ecliptae herba extract	1000mg/kg	268.36± 8.3	287.27± 9.6*	294.26± 8.8*	293.26± 9.2*	314.34± 7.8*	308.34± 10.8*	312.36± 9.2*	321.34± 9.4*	334.37± 8.7*
Metformin	1000mg/kg	267.35± 8.3	248.15± 10.4*	236.24± 8.8*	211.29± 8.5*	188.28± 8.6*	153.54± 8.8*	123.45± 10.4*	102.14± 9.5*	92.135± 8.7*

Values are expressed as mean ± standard error of the mean (n=6)

*p<0.001 compared with normal control.

Table No.2: Effect of *Ecliptae herba* extract on Glycoslyted Haemoglobin (HBA1C)

S.No	Treatment Group	Day 28
1	Normal Control	5.52±0.24
2	Positive Control	5.82±0.16*
3	*Ecliptae herba* extract	5.78±0.13*
4	Metformin	5.49±0.14*

Values are expressed as mean ± standard error of the mean (n=6)

*p<0.001 compared with normal control.

Table No.3: Effect of *Ecliptae herba* extract on the bone mineral density of the lumbar vertebrae and femur bone

S.No	Treatment Group	Bone Mineral density(mg/cm3)	
		Lumbar Vertebrae	Femur
1	Normal Control	179 ± 2.8	227 ± 2.8
2	Positive Control	79 ± 2.9*	106 ± 2.4*
3	*Ecliptae herba* extract	158 ± 1.5*	206 ± 1.9*
4	Metformin	138 ± 3.1*	187± 2.9*

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

CONCLUSION

Our study provides evidence that aqueous *Ecliptae herba* extract may have potential use in the complementary and alternative treatments of diabetic osteoporosis.

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CONFLICT OF INTEREST

"The authors state that they have no competing interests. The funders had no involvement in the study's design, data collection, analysis, or interpretation, manuscript preparation, or the decision to publish the findings".

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