Promotion Effect of Extracts from *Ecliptae Herba* against Anti-Diabetic Drug Induced Bone Loss in Diabetic Rats

**Abstract:** *Ecliptae herba* (EH) has been used to strengthen bones for centuries. Evidence suggests that EH may have antosteoporotic properties. The goal of this study was to see how EH aqueous extract (EHE) affected diabetic rats that were also on pioglitazone (PGZ). In type 2 diabetes mellitus patients, pioglitazone may be associated to a decrease in bone density and an increased risk of fracture. Wistar albino rats were split into five groups, each with six rats: control (vehicle therapy), Streptozotocin (diabetes) group, EHE group, Pioglitazone (PGZ), and Pioglitazone +EHE group. Each medication was given by gastric gavage once a day for 35 days. Bone turnover markers were measured using ELISA assays. By preventing bone loss, EHE improves bone mass in diabetic rats. By increasing osteogenesis and modifying bone turnover markers, EHE may be able to prevent diabetic osteoporosis. This implies that EHE might be developed as an alternate treatment for osteoporosis caused by anti-diabetic medications and diabetes.

**Keywords:** *Ecliptae herba*, Diabetic osteoporosis, Streptozotocin, Pioglitazone.

**INTRODUCTION:**

Diabetes is the most prevalent chronic illness and the main cause of death in today's society (Stratmann, B., et al., 2007). It's a complicated metabolic condition characterised by high blood glucose levels produced by the body's cells' inability to utilise glucose properly (Ugochukwu, N. H., & Babady, N. E. 2002). Despite the fact that insulin therapy and other chemical therapies can help manage some aspects of diabetes, numerous problems still arise on a regular basis. Hyperglycemia is the major cause of severe diabetes complications because high glucose levels directly damage cells and increase lipid peroxidation (Davi, G. et al., 2005). Studies (Al-Azzawie, H. F., & Alhamdani, M. S. S. 2006; Valko, M. et al., 2007; Baynes, J. W. 1991; & Li, Z. K., & Li, D. D. 1997) have revealed that tissue antioxidant levels play a role in the origin of diabetes, and oxidative stress may be a common pathway linking many of the processes that produce diabetic complications (Baynes, J. W. 1991).

On oxidative stress, many therapeutic therapies, as well as therapeutic synergy, may be targeted. Diabetic osteoporosis (DOP) has become more common as the global incidence of diabetes has increased (Roy, B. 2013). Clinical studies have found that half to two-thirds of diabetics have reduced bone strength and/or an increased risk of fractures, with almost one-third of them being diagnosed with osteoporosis (Piscitelli, P. et al., 2015). In type 2 diabetes mellitus patients, pioglitazone may be associated to a decrease in bone density and an increased risk of fracture (Adil, M. et al., 2017). It mostly affects postmenopausal women and the elderly, and it has quickly become one of the most serious health concerns (Wang, X. et al., 2011; & Nakamura, T. et al., 2012).

In China, EH has been utilised to strengthen bones. The antosteoporotic activity of EH (Zhang, Z. G. et al., 2013) has been established in several investigations, however the potential therapeutic benefit of EH on osteoporosis caused by PGZ in diabetic rats is unclear. The goal of this study was to see how EHE affected diabetic rats co-treated with pioglitazone.
MATERIALS AND METHODS:

Animals:
The experiment was conducted with 30 male Sprague-Dawley rats weighing 100–120 g obtained from King Khalid University's Central Animal House in Abha, Saudi Arabia. The rats were maintained in a temperature-controlled facility (22± °C, 12 hour light/dark cycle) and fed standard rat chow with full access to water. The animal ethics committee at King Khalid University approved the experiment methods, which included diabetes induction and sacrifice, and they were carried out in compliance with the US National Institute of Health's standards for the care and use of laboratory animals (NIH Publication No. 85-23, revised 1996).

Induction of Diabetes:
To chemically induce diabetes in rats, a single intraperitoneal injection of 60 mg/kg STZ dissolved in 10 mM citrate buffer was given (pH 4.5). To avoid drug-induced hypoglycemia, the rats were given 5% glucose water for two days after receiving STZ. Twenty-seven rats with fasting blood glucose levels of greater than 11 mmol/L were categorized as diabetic after a week of injection. The control rats received the same dose of isotonic NaCl injection as the experimental rats.

Experimental Design:
The rats were split into five groups: Control (vehicle, Non-Diabetic control, n = 6), diabetic control (STZ treated), EHE (1.4 g/kg/day, n = 6), PGZ (10 mg/kg/day, n = 6), and combination (EHE 1.4 g/kg/day + PGZ 10 mg/kg/day, n = 6). The medication was administered by gastric gavage once a day for 35 days. Throughout the trial, the animals were examined daily for symptoms of illness. There were no animals that were really sick or died before the completion of the trial. The rats administered saline instead of streptozotocin in the control group (n=6) had normal blood glucose levels (120 mg/dL).

All of the animals were fasted overnight and their blood glucose levels were measured at the end of the trial. The animals were then given anesthesia with ketamine (80 mg/kg) and xylazine (8 mg/kg). Cutting at the stifle joint separated the femur andibia by heart puncture, the rats’ blood (10–15 mL) was collected into a simple red-top tube containing no anticoagulants. After centrifuging the blood samples at 4000 rpm for 15 minutes, the serum was kept in aliquots at -80 °C.

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.

Marker of Bone Formation and Bone Resorption:
Serum was used to assess all bone formation and resorption markers. The osteocalcin level was determined using a Rat-Mid Osteocalcin ELISA kit (IDS, UK), whereas the BALP level was determined using a rat BALP ELISA kit (Qayee, Shanghai). To assess bone resorption, a Rat deoxypyridinoline (DPD) ELISA Kit (Qayee, Shanghai) was used (Qayee, Shanghai). A microplate reader was used to measure the optical density at 450 nm (Epoch Microplate Spectrophotometer, BioTek, USA) (Hong, J. et al., 2004).

Statistical Analysis:
ANOVA was used to examine all of the data. Duncan's multiple comparison test was used to calculate the significance of the means. The analyses were all completed with a 95% confidence level.

RESULTS:
The glucose profiles of the positive control group (STZ) deteriorated over time (Table-1). However, EHE and PGZ, both alone and in combination, were demonstrated to protect against diabetes progression.

Table-1: Effect of EHE in Combination with PGZ on Fasting Blood Glucose Level

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Dose</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
<th>Day 35</th>
<th>Day 42</th>
<th>Day 49</th>
<th>Day 56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>5 mL/kg</td>
<td>74.22±3</td>
<td>72.32±2</td>
<td>75.71±3</td>
<td>75.40±1</td>
<td>78.30±1</td>
<td>82.46±1</td>
<td>84.40±1</td>
<td>85.40±1</td>
<td>83.40±1</td>
</tr>
<tr>
<td>Control</td>
<td>2 mL/kg</td>
<td>65 mg/kg</td>
<td>262.54±2</td>
<td>295.35±2</td>
<td>315.21±1</td>
<td>335.72±2</td>
<td>353.72±2</td>
<td>374.72±2</td>
<td>396.72±2</td>
<td>414.72±2</td>
</tr>
<tr>
<td>Positive</td>
<td>11.2*</td>
<td>6.3*</td>
<td>257.33±1</td>
<td>285.25±2</td>
<td>292.22±2</td>
<td>297.28±2</td>
<td>305.35±2</td>
<td>308.35±2</td>
<td>312.35±2</td>
<td>321.35±2</td>
</tr>
<tr>
<td>Control</td>
<td>65 mg/kg</td>
<td>EHE 25 mg/kg</td>
<td>267.33±1</td>
<td>285.25±2</td>
<td>292.22±2</td>
<td>297.28±2</td>
<td>305.35±2</td>
<td>308.35±2</td>
<td>312.35±2</td>
<td>321.35±2</td>
</tr>
<tr>
<td>PGZ</td>
<td>6.3*</td>
<td>25 mg/kg</td>
<td>234.32±2</td>
<td>216.24±2</td>
<td>207.26±2</td>
<td>193.23±2</td>
<td>174.35±2</td>
<td>142.31±2</td>
<td>125.37±2</td>
<td>96.36±8</td>
</tr>
<tr>
<td>EHE+ PGZ</td>
<td>6.3*</td>
<td>4 mL/kg</td>
<td>237.33±2</td>
<td>226.25±2</td>
<td>208.22±2</td>
<td>177.28±2</td>
<td>154.35±2</td>
<td>133.35±2</td>
<td>102.35±2</td>
<td>94.35±8</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard error of the mean (n=6)
*P<0.001 compared with normal control.
**Bone turnover markers:**
Blood osteocalcin levels were significantly lower, though serum DPD levels were considerably greater in the STZ group than in the NC group (Table 2). Despite the fact that BALP values were not substantially different across treatment groups, serum osteocalcin levels increased while DPD levels dropped after EHE treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Bone formation markers</th>
<th>Bone resorption marker</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Osteocalcin (ng/ml)</td>
<td>BALP (ng/ml)</td>
</tr>
<tr>
<td>NC</td>
<td>135.75 ± 6.8c</td>
<td>102.19 ± 7.69b</td>
</tr>
<tr>
<td>DC</td>
<td>14.14 ± 0.77a</td>
<td>64.06 ± 4.62a</td>
</tr>
<tr>
<td>EHE</td>
<td>57.12 ± 8.14b</td>
<td>80.18 ± 0.35a</td>
</tr>
<tr>
<td>PGZ</td>
<td>146.46 ± 4.01d</td>
<td>91.30 ± 8.21a</td>
</tr>
<tr>
<td>EHE+PGZ</td>
<td>147.56 ± 4.15d</td>
<td>93.10 ± 8.11a</td>
</tr>
</tbody>
</table>

**DISCUSSION:**
This study looked at the impact of EHE on bone abnormalities in STZ-induced diabetic rats. Diabetics and experimental animal models encounter significant levels of oxidative stress, resulting in increasing concentrations of oxygen free radicals, due to persistent and chronic hyper glycemia, which depletes the function of the anti oxidative defence system (Zhang, R. et al., 2012). In rats, EHE possesses anti osteoporosis effects, according to previous study (Zhang, Z. G. et al., 2013). These data suggest that the herbs might help in osteoporosis prevention and therapy.

Changes in articular cartilage, which is responsible for lubricating the ends of bones, induce osteoarthritis. STZ injection has also been linked to a reduction in chondrocyte counts, as well as an increase in tidemark roughness in the femoral articular cartilage. These data together imply that diabetic rats develop osteoarthritis-like symptoms. In both T1DM and T2DM rat, osteoarthritis-like symptoms have been reported (King, K. B., & Rosenthal, A. K. 2015; & Starup-Linde, J. 2013). Oxidative stress activation is considered to be a contributing component in these alterations.

Because oxidative stress may change the equilibrium between osteoblast and osteoclast activity, measuring bone turnover markers makes sense (Song, S. H. et al., 2016). Pioglitazone has been found in previous research to significantly improve aberrant urine calcium, AMPK mRNA expression, bone turnover indicators, fenur epiphysis micro-architecture, histology, and BMD in diabetic rats. Blood DPD levels increased in DC rats, but serum osteocalcin and BALP activity decreased, according to the findings of this study. Another interesting finding from this study is that after EHE therapy, serum osteocalcin levels increased but DPD levels fell (Table 2).

Similar effects have been observed with a number of osteoprotective herbs (Cheung, C. L. et al., 2013). In addition, prior research has demonstrated that EHE reduces IL-6 levels and there by inhibits bone resorption (Zhang, Z. G. et al., 2013). BALP activity is still low in EHE rats, indicating that mineral metabolism is still hampered. BALP is a bone-specific alkaline phosphatase isofrom that is produced by osteoblasts and reflects mineral metabolism (Adil, M. et al., 2017). The ratio of osteocalcin to DPD was virtually same in the EHE and NC groups, suggesting that EHE therapy nearly reached an equilibrium between bone production and bone resorption.

**CONCLUSION:**
Our findings show that EHE can help prevent bone loss in STZ treated rats co-treated with pioglitazone. EHE treatment lowered fasting blood glucose levels and enhanced DPD activity.

**ACKNOWLEDGMENTS:**
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**CONFLICTS 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.”

**REFERENCES:**


