A high molecular weight protein Bengalin from the Indian black scorpion (Heterometrus bengalensis C.L. Koch) venom having antiosteoporosis activity in female albino rats

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Abstract  
This study reports the presence of a high molecular weight protein (Bengalin) from the Indian black scorpion (Heterometrus bengalensis) venom having antiosteoporosis activity in experimental osteoporosis developed in female albino Wister rats. Bengalin was purified through DEAE-cellulose ion exchange chromatography and high performance liquid chromatography. The molecular weight of the Bengalin was found to be 72 kDa and the first 20 amino acid sequence was found to be G-P-L-T-I-L-H-I-N-D-V-H-A-A/R-F-E-Q/G-F/G-N-T. Bengalin exhibited significant antiosteoporosis activity in experimental female rats, which was confirmed through analysis of urine Ca\(^{2+}\), PO\(_4\)^{3-}/C0, CRE and OH-P. Bengalin (3 \(\mu\)g and 5 \(\mu\)g/100 g rat/i.p.) antagonized osteoporosis by restoring urinary Ca\(^{2+}\), PO\(_4\)^{3-}/C0, CRE and OH-P, serum/plasma Ca\(^{2+}\), PO\(_4\)^{3-}/C0, ALP, TRAP, PTH, T3, TSH, Osteocalcin, IL1, IL6 and TNF\(\alpha\), as compared with the sham operated control rats. Bone minerals density of osteoporosis female rats was improved due to Bengalin, observed through DEXA scan. Subacute toxicity studies in male albino mice, Bengalin showed cardiotoxicity. In vivo experiments, Bengalin showed cardiototoxicity on isolated guinea pig heart, guinea pig auricle, and neurotoxicity on isolated rat phrenic nerve diaphragm preparation. Further detail studies on the toxicity, antiosteoporosis and structural identity of Bengalin are warranted.

1. Introduction

Osteoporosis is a worldwide socio medical problem, with a high prevalence not only in the western countries but also in Asia and Latin America (Delmas, 2002). In United States, forty four million men and women aged fifty years and older have a low bone mass and osteoporosis (Follin and Hansen, 2003). Conventional therapy recommended for the treatment of osteoporosis includes supplementation with estrogen, progesterone, calcitonin, bisphosphonates etc. that possesses several shortcomings including cancer in breast or uterus. Selective estrogen receptor modulators such as raloxifene, bisphosphonates are used for beneficial effects on bone mineral density.
Scorpion envenomation, alter several hormone level including estrogen, prostaglandin etc in clinical (Ben Nasr et al., 2007) and in experimental condition (Nassar et al., 1990). Scorpion venom also alters the serum mineral constituents like calcium, phosphate, potassium (Omran et al., 2007) and in experimental condition (Nassar et al., 2007). Such alteration in blood mineral may affect bone mineral composition. American dental association reported that a compound (kaliotoxin) found in the venom of the scorpion *Androctonus mauretanicus*, could significantly inhibit the bone loss resulting from advanced periodontal disease (Valverde et al., 2004). Mercer et al. (1992) reported that contertrostatin, a homodimeric snake venom disintegrin (a small disulfide-rich protein containing an Arg–Gly–Asp sequence near their carboxyl terminus), is a potent inhibitor of β3 integrin-mediated osteoclast attachment. Sato et al. (1990) worked out with the snake venom protein Echistrin derived from saw scale viper and was found to be a potent inhibitor of bone resorption on isolated osteoclast. Oursler and Spelsberg (1993) made an extensive review on the use of Echistatin as a potential drug for osteoporosis.

Attempts have been taken to identify newer therapeutic agent active against osteoporosis from natural resources. Gomes et al. (2009) reported for the first time that the venom of the Indian black scorpion (*Heterometrus bengalensis*) possessed antioestrogen activity in experimental female albino rats. The present communication identified the presence of a high molecular weight protein (Bengalin) from the *H. bengalensis* venom and established its antioestrogen activity in female albino rats.

2. Materials and methods

2.1. Chemicals

All chemicals and solvents used were of analytical grade unless otherwise stated. And following kits were used calcium, phosphorous, magnesium, creatinine (Merck, India), osteocalcin (Biosource, Belgium), PTH (Biomerica, Germany), rat IL1, rat IL6 and rat TNF α kit (R & D, USA).

2.2. Purification and characterization of antioestrogen factor (Bengalin)

2.2.1. Collection of scorpion and scorpion venom

Adult live scorpions (*H. bengalensis*) of both sexes were collected and scorpion venom (SV) was extracted once in a month by applying square wave electrical stimulation (25 V, 1 ms) to the telson. The venom was pooled, lyophilized and stored at 4°C in amber colour bottle until further use. Before use, SV was weighed, dissolved in phosphate buffer saline 0.01 M, pH 7.2 and expressed in terms of dry weight.

2.2.2. DEAE-cellulose ion exchange chromatography

Lyophilized scorpion (*H. bengalensis*) venom (200 mg) was dissolved in phosphate buffer (pH 7.2) over night and centrifuged (15 min × 1800 g). The supernatant was subjected to DEAE-cellulose column (20 × 80 mm). The flow rate was adjusted at 25 ml/h. Phosphate buffer (0.02 M, pH 7.2) containing increasing molarities of NaCl (0.02, 0.05, 0.1, 0.2, 0.5 and 1.0 M) was used to elute the proteins from the column. Fractions (5 ml) were collected at room temperature (24 ± 2°C) and protein levels were estimated (Lowry et al., 1951). The antioestrogen activity of the fractions were examined in female albino osteoporosis rats.

2.2.3. High performance liquid chromatography

DEAE-cellulose purified venom protein (P3) was further purified through HPLC (WATERS 600, USA). Protein-Pak 300 SW column (7.5 × 300 mm) and 2487 γ Absorbance detector, using 10 mM sodium phosphate buffer containing 0.1 M sodium chloride (pH 7.5) with a flow rate 1 ml/min. Detection of protein peak was done at 280 nm and the retention time of the protein was calculated.

2.2.4. Homogeneity testing

Slab gel (7.5%) was performed according to Davis (1964) with HPLC purified venom fraction (20 μg protein) that possessed the antioestrogen activity. Tris–glycine buffer (pH 8.8) and 15 mA current was used for 3 h at 4°C. The gel was stained with 0.01% coomassie blue, destained with 7% acetic acid and 10% methanol. Protein bands were visualized and photographed.

2.2.5. Determination of molecular weight

Molecular weight of the purified protein was determined by SDS-PAGE (10% acrylamide containing 1% SDS), using standard molecular weight marker proteins (50–160 kDa) after the method of Laemmilli (1970).

2.2.6. Determination of amino acid sequence

The HPLC purified fraction were concentrated and = 15 μg protein (following SDS-PAGE) was electro-transferred to PVDF membrane (0.4 μm pore size) using 10 mM CAPS transferring buffer (pH 11) containing 10% (v/v) methanol at 100 mA for 6 h. The PVDF membrane was stained with 0.2% (w/v) Ponceau S dried and used for N-terminal amino acid sequence using Applied Biosystem precise protein sequencer.

2.2.7. Development and confirmation of osteoporosis

Female Wister albino rats (28–30 week old, 150–160 g) were collected from M/S B N Ghosh & Company, Calcutta, India. Bilateral ovariectomy was done and osteoporosis was confirmed through urine markers (Gomes et al., 2009).

2.2.8. Evaluation of antioestrogen activity

After confirmation of osteoporosis, all the osteoporosis rats were divided into three groups (n = 12 in each group). Sham operated control Gr I was kept different from the osteoporosis group. Osteoporosis Gr II received vehicle (0.9% saline), osteoporosis Gr III received Bengalin (3 μg/100 g rat) × 15 i.p. dose alternate days, osteoporosis Gr IV received Bengalin (5 μg/100 g rat) and osteoporosis group V received standard drug vitamin D3, arachitol (Vieth, 2005), 200 mg/kg and calcium, 1500 mg/kg (Flynn, 2003) × 15 i.p. dose alternate days. At day 60, urine was collected and subjected to urine analysis. The rats were anesthetized,
blood was collected, serum/plasma was separated by centrifugation (2500 rpm × 30 min) for estimation of interleukins, hormones and enzymes and kept at −20 °C until further use. Femur and spine bone was collected for bone minerals content and bone mineral density by DEXA scan.

2.2.9. Analysis of bone mineral density (BMD) by DEXA scan

All the groups of animals were evaluated for bone mineral density of their dual femur and lumbar spine using the Prodigy LUNAR DEXA System, software version 8.80 (GE Medical Systems) in normal scan mode with 3.70 mrem. A crossed step-wedge (comprised of epoxy based materials that mimic the radiographic properties of tissue and bone) was used to calibrate the system as per the protocol reported earlier (Holdsworth et al., 2000).

2.2.10. Toxicity studies

2.2.10.1. Subacute toxicity studies. Swiss albino male mice were injected Bengaline (50 μg/kg, i.p.) × 30 days for subacute toxicity study. On 31st day blood was collected, serum was separated from which different hematological and biochemical parameters were estimated using standard protocol.

2.2.10.2. Isolated guinea pig heart and auricle. Isolated guinea pig heart was prepared after Langendorff (1895) and isolated guinea pig auricle was prepared after Burn (1952). Spontaneous contractions of the heart and auricle were recorded after Bengalin exposure.

2.2.10.3. Isolated rat phrenic nerve diaphragm. Isolated rat phrenic nerve diaphragm (RPND) was prepared after Bulbring (1946). The preparation was stimulated with a square wave electronic stimulator (Grass, USA) and contractions were recorded after Bengalin exposure.

2.2.11. Animal ethical committee permission

All animal experiments were approved by University animal ethics committee, and were in accordance with the guideline of the Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA), Government of India.

2.2.12. Statistical analysis

All the results were expressed in terms of mean ± SE, n = 12 at each dose level. The level of significance was determined through one way ANOVA, p < 0.05 was considered significant.

3. Result

3.1. Purification of scorpion venom by ion exchange chromatography

Crude scorpion venom (200 mg), applied on a DEAE-cellulose column (20 × 80 mm), eluted with phosphate buffer (0.2 M, pH 7.2) and stepwise NaCl (dissolved in 0.2 M phosphate buffer, pH 7.2) gradient, produced four protein peaks (P1, P2, P3, P4). P3 (tube no. 38) was eluted with 0.02 M NaCl, posses antosteoporosis activity in female albino rats. Yield of P3 was found to be 3.35 ± 0.08%, protein loss was 4.33 ± 0.12% and 95.66 ± 2.44% protein was recovered by this process (Fig. 1).

3.2. HPLC and homogeneity

HPLC elution pattern of the protein peak P3 showed one sharp peak (retention time 8.6 min) and two minor humps (Fig. 1). For the sake of convenience, this peak having antosteoporosis activity was provisionally designated as ‘Bengalin’ (originated from bengalensis).

3.3. Molecular weight determination and amino acid sequence

On SDS-PAGE the molecular weight of Bengalin was found to be 72 kDa. N-terminal amino acid sequence of Bengalin determined by automatic Edman degradation was found to be – G-P-L-T-I-L-H-I-N-D-V-H-A-A/R-F-E-Q/G-F/G-N-T.

3.4. Antosteoporosis activity of Bengalin through urine and serum analysis

At day 60, urine Ca²⁺, Po₄³⁻, CRE, OH-P was significantly increased in Gr II OST rats as compared with Gr I sham control rats (214.3%, 123.1%, 52.6%, and 157.4% respectively). The urinary constituents were significantly decreased in Gr III (131.6%, 81.2%, 34.9%, and 75.6% respectively), Gr IV (120%, 61.1%, 26.1%, and 72.7% respectively) and Gr V (109.5%, 81%, 20.8%, and 65.9% respectively), as compared with the Gr II OST rats (Table 1).

At day 60, serum Ca²⁺, Po₄³⁻ and TRAP level increased significantly (125.9%, 44.8% and 33.5% respectively), while serum ALP was significantly decreased (30.8%) in Gr II OST rats, as compared with Gr I sham control rats. The serum Ca²⁺, Po₄³⁻ and TRAP decreased significantly in Gr III (66.8%, 35.4%, and 21.3% respectively), Gr IV (67.8%, 29.8%, and 23.1% respectively) and Gr V (100.6%, 33.4%, and 20.7% respectively), as compared with Gr II OST rats. Serum ALP level was significantly increased in Gr III (21.5%), Gr IV (21.2%) and Gr V (20.5%), as compared with Gr II OST rats (Table 1).

At day 60, plasma T3, TSH and PTH level was significantly increased (22.9%, 38.4%, and 15.6% respectively) in Gr II OST rat as compared with Gr I sham control rats. These serum hormones were significantly decreased in Gr III (12.2%, 20.1% and 12.4% respectively), Gr IV (11.9%, 15.8% and 11.4%, respectively) and Gr V (12.7%, 17.1% and 13.4% respectively) as compared with Gr II OST rats. T₄, FSH, EST and osteocalcin level significantly decreased (65.7%, 95.1%, 130.2% and 146.02% respectively) in Gr II OST rat as compared with Gr I sham control rats. Only osteocalcin level significantly increased in Gr III, Gr IV and Gr V (112.2%, 92.8% and 100.6% respectively) as compared with Gr II OST rats. However, T₄, FSH and EST level of Gr III, Gr IV and Gr V rats did not showed any change compared with Gr II OST rats (Table 2).
Serum interleukins IL1, IL6 and TNF-α level was significantly increased (30.57%, 20.65% and 166.5% respectively) in group II OST rats, as compared with Gr I sham control rats. Serum interleukins IL1, IL6 and TNF-α level were significantly decreased in Gr III (21.08%, 16.54% and 111.3% respectively), Gr IV (14.7%, 15.7%, and 120.1% respectively) and Gr V (18.34%, 15.8% and 107.7% respectively), as compared with the Gr II OST rats (Table 2).

3.5. Antiosteoporosis activity of Bengalin through bone minerals analysis

At day 60, bone Ca$^{2+}$, P, Mg$^{2+}$, and Zn$^{2+}$ level was significantly decreased in Gr II OST rats (129.8%, 55.3%, 49.2%, and 60.25% respectively), as compared with Gr I Sham control rats. These bone minerals were increased significantly in Gr III (28.8%, 27.2%, 19.6%, and 24.27% respectively), Gr IV (33.3%, 24.3%, 15.9%, and 26.35% respectively) and Gr V (42.7%, 24.1%, 18.9%, and 18% respectively), as compared with Gr II OST rats. The Na$^+$ content of bone was significantly increased in Gr II OST rats (97.2%), as compared with Gr I sham control rats. The Na$^+$ content was significantly decreased in Gr III, Gr IV and Gr V (26.7%, 31.5%, and 24.6% respectively), as compared with Gr II OST rats (Table 3).

3.6. Antiosteoporosis activity of Bengalin through bone minerals density

DEXA scan data was analyzed for consistency and reproducibility by calculating the coefficient of variation at the thoracic and lumbar spine (1.14 ± 0.02%), femur neck (0.53 ± 0.06%) and condylar femur (1.17 ± 0.1%). BMDs observed using the modified protocol for the dual femoral and twelfth thoracic and lumbar vertebrae showed a higher level of statistical homogeneity compared to the other sets and have been found to correlate well with their chemically assessed bone ash mineral content.

3.7. Toxicity studies

Subacute toxicity studies in male albino mice, Bengalin (50 μg/kg, i.p. × 30 days, n = 6), did not produced significant changes in hematological parameters (total RBC count, total WBC count, differential WBC count). Bengalin (50 μg/kg, i.p. × 30 days, n = 6) significantly increased the serum biochemical markers as compared with control mice AST (control: 55.43 ± 2.1 IU/l, Bengalin treated: 75.58 ± 3.9 IU/l, p < 0.05) and CK (control: 31.2 ± 3.4 IU/l, Bengalin treated: 55.6 ± 2.5 IU/l, p < 0.05).

On isolated guinea pig heart, Bengalin (0.1 mg) decreased the rate (42.5 ± 6%), amplitude of contraction (30 ± 2%) within 5 ± 1 min of observation and produced irreversible blockage of within 90 ± 8 min of observation (n = 4). On isolated guinea pig auricle, Bengalin (0.1 mg) produced irreversible blockage of contraction within 65 ± 5 min of observation (n = 4). On isolated rat phrenic nerve diaphragm preparation, Bengalin (0.1 mg) significantly decreased (50 ± 3%) electrical stimulation induced twitch response within 60 ± 2 min of observation (n = 4).
Table 1
Effect of Bengalin and STD drug on urine and serum biochemical constituents in different groups of female albino rats.

<table>
<thead>
<tr>
<th>Gr. of animal</th>
<th>Urine parameters (μg/12 h)</th>
<th>Serum parameters (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cà²⁺</td>
<td>PO₄³⁻</td>
</tr>
<tr>
<td>SHAM (Gr I)</td>
<td>0.14 ± 0.03</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>OST (Gr II)</td>
<td>0.44 ± 0.04***</td>
<td>0.29 ± 0.03***</td>
</tr>
<tr>
<td>OST + Bengalin (3 μg/100 g rat) (Gr III)</td>
<td>0.19 ± 0.03***</td>
<td>0.16 ± 0.01**</td>
</tr>
<tr>
<td>OST + Bengalin (5 μg/100 g rat) (Gr IV)</td>
<td>0.2 ± 0.04***</td>
<td>0.18 ± 0.01**</td>
</tr>
<tr>
<td>OST + STD (Gr V)</td>
<td>0.21 ± 0.03***</td>
<td>0.16 ± 0.01**</td>
</tr>
</tbody>
</table>

Values shown are mean ± SE (n = 12), *p < 0.05, **p < 0.01, ***p < 0.001 significant (Gr. I vs. Gr II; and Gr II vs. Gr III, Gr IV and Gr V). OST = Osteoporosis. All the rats were sacrificed at day 60.

Table 2
Effect of Bengalin and STD drug on serum hormones and interleukins in different groups of female albino rats.

<table>
<thead>
<tr>
<th>Gr. of animal</th>
<th>T₃ (ng/dl)</th>
<th>T₄ (μg/dl)</th>
<th>TSH (μIU/ml)</th>
<th>FSH (m IU/ml)</th>
<th>EST (pg/ml)</th>
<th>PTH (pg/ml)</th>
<th>Osteocalcin (ng/ml)</th>
<th>LL (pg/ml)</th>
<th>IL6 (pg/ml)</th>
<th>TNF α (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM (Gr I)</td>
<td>107.5 ± 3.36</td>
<td>5.67 ± 0.13</td>
<td>53.12 ± 2.72</td>
<td>8.41 ± 0.61</td>
<td>19.41 ± 1.72</td>
<td>136.5 ± 2.36</td>
<td>15.77 ± 0.74</td>
<td>14.03 ± 0.5</td>
<td>61.58 ± 1.72</td>
<td>21.15 ± 0.81</td>
</tr>
<tr>
<td>OST (Gr II)</td>
<td>132.2 ± 5.02**</td>
<td>3.42 ± 0.14*</td>
<td>73.54 ± 3.07**</td>
<td>4.31 ± 0.53**</td>
<td>8.43 ± 0.34**</td>
<td>157.8 ± 5.11**</td>
<td>6.41 ± 0.48***</td>
<td>18.32 ± 0.21**</td>
<td>74.3 ± 1.6**</td>
<td>56.38 ± 0.84***</td>
</tr>
<tr>
<td>OST + Bengalin (3 μg/100 g rat) (Gr III)</td>
<td>117.8 ± 4.95*</td>
<td>4.7 ± 0.19</td>
<td>61.21 ± 2.2*</td>
<td>4.61 ± 0.56</td>
<td>6.83 ± 0.2</td>
<td>140.3 ± 6.84*</td>
<td>13.54 ± 0.58**</td>
<td>15.13 ± 0.23*</td>
<td>63.75 ± 1.3*</td>
<td>26.68 ± 0.71***</td>
</tr>
<tr>
<td>OST + Bengalin (5 μg/100 g rat) (Gr IV)</td>
<td>118.1 ± 3.36*</td>
<td>4.25 ± 0.16</td>
<td>63.48 ± 1.6*</td>
<td>4.92 ± 0.62</td>
<td>6.44 ± 0.38</td>
<td>141.6 ± 4.84*</td>
<td>12.36 ± 0.52**</td>
<td>15.96 ± 0.31*</td>
<td>64.2 ± 1.4*</td>
<td>25.56 ± 0.7***</td>
</tr>
<tr>
<td>OST + STD (Gr V)</td>
<td>117.3 ± 3.42*</td>
<td>4.77 ± 0.12</td>
<td>62.77 ± 2.14*</td>
<td>4.23 ± 0.44</td>
<td>9.65 ± 1.03</td>
<td>139.2 ± 6.55*</td>
<td>12.86 ± 0.37***</td>
<td>15.48 ± 0.24*</td>
<td>64.12 ± 0.67*</td>
<td>27.14 ± 1.22***</td>
</tr>
</tbody>
</table>

Values shown are mean ± SE (n = 12), *p < 0.05, **p < 0.01, ***p < 0.001 significant (Gr. I vs. Gr II; and Gr II vs. Gr III, Gr IV and Gr V). OST = Osteoporosis. All the rats were sacrificed at day 60.
Values shown are Mean ± SD (n = 12); *p < 0.05, **p < 0.01, ***p < 0.001 significant (Gr. I vs. Gr. II; and Gr II vs. Gr III, Gr IV and Gr V). OST = Osteoporosis. All the rats were sacrificed at day 60.

4. Discussion

In our earlier studies, it was established that the crude scorpion (*H. bengalensis*) venom possess the anti-osteoporosis activity in female albino rats by restoring urinary, serum and bone markers of osteoporosis (Gomes et al., 2009). In the present communication, a high molecular weight protein (Bengalin) has been isolated from Indian black scorpion (*H. bengalensis*) venom by ion exchange chromatography followed by high performance liquid chromatography that posses antiosteoporosis activity. The SDS MW of Bengalin was 72 kDa and the first 20 amino acid sequence has been determined. However, it was very difficult to comment on the structural configuration of Bengalin, until complete amino acid sequence is done.

Bengalin restored the urinary Ca²⁺, PO₄³⁻, CRE and OH-P, serum/plasma Ca²⁺, PO₄³⁻ which were decreased due to bone minerals (Ca²⁺ and PO₄³⁻), and organic matrix and collagen (CRE and OH-P) degradation and were released into extracellular fluid thus increased the urinary and serum constituents. Bone tissue consists of mainly Calcium Hydroxyapatite [Ca₁₀(PO₄)₆(OH)₂], different matrix proteins such as type I collagen, osteocalcin, osteonectin, osteopontin, sialoproteins and proteoglycans (Young et al., 1992; Robey et al., 1993; Mundlos and Olsen, 1997). At the time of bone resorption, calcium, phosphate was released into extracellular fluid followed by resorption of organic matrix. In osteoporosis, the serum TRAP was increased due to extracellular fluid followed by resorption of organic matrix.

Thyroid hormones are crucial for cartilage growth and differentiation thereby increased bone turnover (Bassett et al., 2007). Prolonged sub clinical hyperthyroidism due to i-thyroxine treatment has been associated with reduced bone mass and thus with the potential risk of premature development of osteoporosis (Nuzzo et al., 1998). In the present study, the serum T3 and TSH were increased in osteoporosis rats, as compared with sham control rats. Bengalin significantly decreased both T3 and TSH level as compared with osteoporosis rats. Osteocalcin is a non collagenous bone matrix protein synthesized by the mature osteoblasts is a specific biochemical marker of bone formation (Bullon et al., 2007). In the present study, the osteoblastic activity was inhibited as well as osteoclastic activity was increased in osteoporosis rats, as a result there was decrease in serum osteocalcin level. After treatment with Bengalin, serum osteocalcin level was significantly restored (p < 0.01), as compared with osteoporosis rats. IL1, IL6 and TNF α are potent stimulator of bone resorption may mediate bone loss after estrogen withdrawal (Pacifici, 1998). The levels of TNF α and IL6 increase after menopause (Straub et al., 2000), when estrogen levels decrease, and during the aging processes suggesting a role for these factors in the pathogenesis of postmenopausal osteoporosis as well as other age related diseases. Hormone and local factors such as PTH, calcitriol or 1, 25 dihydroxy D, prosta
glandin E2 and IL1 were acting on osteoclast cells to increase production of RANKL (receptor activator of NF-κβ ligand) and increased the bone resorption through RANK- RANKL interaction. Osteoprotegerin (OPG) is a novel anti--resorptive agent and a soluble secreted protein of the tumor necrosis factor (TNF) receptor family (Lacey et al., 1998) that functions as a decoy receptor for RANKL (Anderson et al., 1997). RANKL-RANK-OPG system provides emphasis on osteoclast differentiation and bone resorbing capacity. RANKL expression is induced by the pro-inflammatory mediator IL1, thereby promoting osteoclastogenesis. IL1 also essential for TNF α induced osteoclast development (Herman et al., 2008). In the present study, it was observed that IL1 and TNF α level was restored by Bengalin, indicating RANKL-RANK-OPG system is likely to be involved.

It was shown that osteoporosis bone lesions were improved by Bengalin treatment observed through DEXA scan studies. Present study correlates well with previously published data (Holdsworth et al., 2000) which indicated that the cross-wedge calibrated DEXA technique provides high-precision measurements of bone mineral content (CV = 0.6%) and bone mineral density (CV = 0.8%) because of the reduced variance and improved object segmentation provided by the CWC-DEXA system.

In subacute toxicity study, Bengalin treatment caused cardio toxicity characterized by increased serum AST, CK level. Myocardial damages have been reported in clinical (Daisley et al., 1999) and experimental envenomation by *Tityus* species found in Trinidad (Corrêa et al., 1997). Tityustoxin, the major neurotoxin was isolated from *Tityus serrulatus* caused cardiac lesions in rats (Corrêa et al., 1997). Here, Bengalin isolated from Indian black scorpion venom also showed cardio toxicity on isolated guinea pig heart and auricle and neurotoxicity on isolated rat phenic nerve diaphragm. However, further work on the detail toxicity study and mechanism of action of Bengalin in bone remodeling process are warranted.
Acknowledgements

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Conflict of interest

I wish that the editor will consider the manuscript. We declare that there is no conflict of interest in this manuscript.

References


