INTRODUCTION

28-Homocastasterone (28-HC) structurally a keto-isoform is an active plant growth regulator among the brassinosteroid (Bs) family members [1]. 28-homocastasterone and 28-homobrasslinolide (aldo-isoform) are actively synthesised in plants by CYP72B1 enzyme and contribute to a wide range of physiological processes during the plant life cycle from seed development to modulation of flowering, senescence, stress response and photomorphogenesis [2].

Humans are exposed to 28-homocastasterone hormones through consumption of plant materials as food and herbal based folk medicine. Assimilation of 28-homocastasterone hormone into tissues induces metabolic changes in animal cells [3]. However, an earlier study in our lab employed with 28-homobrasslinolide and 28-homocastasterone for the production of cellular blood elements and it can be influenced by nutritional, hormonal, physiological, pathological factors and drugs intake [5]. Humans are consuming phyto-oestrogen more or less on a regular basis and we assume that it may be influenced haematologically for the production of cellular blood elements and it can be influenced by nutritional, hormonal, physiological, pathological factors and drugs intake [5].

Bone marrow cells are the principal hematopoietic organ and responsible for the production of cellular blood elements and it can be influenced by nutritional, hormonal, physiological, pathological factors and drugs intake [5]. Haematopoiesis and bone histomorphology in the treated group was noted. A significant reduction in electrolyte level (p<0.05) and increased in alkaline phosphatase activity (p<0.05) was noted in treated groups. A significant reduction in electrolyte level (p<0.05) and increased in alkaline phosphatase activity (p<0.05) was noted in treated groups.

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Keywords: Phytohormone, 28-homocastasterone, Diabetic, Bone, Osteoblast

OBJECTIVE: To study the effect of brassinolide keto isoform 28-homocastasterone in diabetic male rat bone marrow cells, bone histological changes, electrolytes and alkaline phosphatase activity in rat blood.

METHODS: Diabetes was induced in the group (n=6) of rats with a single peritoneal injection of streptozotocin (60 mg/kg bwt). With a treatment schedule of 15 consecutive days, control (n=6) and diabetic rats received 666 µg/kg bwt of 28-homocastasterone. Circulating blood cell count, cell indices, bone marrow cells, bone histology, electrolytes Na⁺, K⁺, Cl⁻, P, Ca²⁺ and alkaline phosphatase activity was assessed.

RESULTS: Cytological examination showed an increased erythrocyte progenitor and megalakaryocyte cell lineage along with improved osteoblastic and bone histomorphology in the treated group was noted. A significant reduction in electrolyte level (p<0.05) and increased in alkaline phosphatase activity (p<0.05) was noted in treated groups.

CONCLUSION: It is suggested that brassinosteroid keto isoform 28-homocastasterone exhibits a hematopoietic effect in diabetic rat and improves bone histology while reducing hyperglycemic damage in bone.

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Bone marrow aspiration cytology

Following euthanasia, the femur bone was removed, and excess muscle and fat were trimmed. The bone is split longitudinally, and marrow was opened. Using a fine brush, a small plug of marrow was gently extracted and smeared onto glass slides and immediately fixed in methanol for H/E staining [5]. The stained slide films were examined under camera fitted binocular microscope (Olympus 100) at 20x magnifications.

Histomorphology analysis of bone

The dissected femur bone was fixed in 10% formaldehyde for 24 h after removal of the surrounding soft tissues. Decalcification was performed with a 10% ethylenediamine tetraacetate (EDTA) solution for 5 d. The fixed and decalcified femur bone was embedded in paraffin and sectioned at 5 µm using digitalized microtome. Paraffin bone sections were stained with hematoxylin/eosin (H/E stain) and masson trichrome stain [13]. The histomorphology of bone was examined under the binocular microscope (Olympus 100) at 20x and 40x magnification.

Statistical analysis

Results of the investigations were expressed as mean±SD and the data was analysed by one-way ANOVA employing SPSS software 16.0 version. The value of p<0.05 was considered significant.

RESULTS

Rats administered 28-HC (100 µg/150 gm bwt) through oral gavage for 15 d (table 1) showed increased RBCs and Hb level in treated diabetic group compared to diabetic control group significantly (p<0.05). RBCs count increased 5.6% in the treated diabetic group and 2% in normal treated group. Hb level increased 5.66% in the treated diabetic group compared to the untreated diabetic group. On the other hand total WBCs count noted decreased by 33.33% in normal 28-HC treated rat compared to normal control. In addition, normal 28-HC treated rat platelets count decreased significantly compared to control (P<0.05).

Table 1: Effect of 28-homocastasterone on blood cells count

<table>
<thead>
<tr>
<th>Groups</th>
<th>RBC 10 x 6/µl</th>
<th>Hb g/l</th>
<th>TWBC 10x3/µl</th>
<th>Platelets 10x3/µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.8±1.02</td>
<td>15.9±0.08</td>
<td>0.94±0.42</td>
<td>266±14</td>
</tr>
<tr>
<td>Control+28-HC</td>
<td>7.86±0.95</td>
<td>16.80±0.62*</td>
<td>11.50±0.86*</td>
<td>179±9*</td>
</tr>
<tr>
<td>Diabetic</td>
<td>6.42±0.84</td>
<td>12.60±0.73</td>
<td>03.50±0.26</td>
<td>444±12</td>
</tr>
<tr>
<td>Diabetic+28-HC</td>
<td>6.78±0.72*</td>
<td>14.90±0.85*</td>
<td>04.90±0.37*</td>
<td>334±16*</td>
</tr>
</tbody>
</table>

Values are expressed±SD. Group n=6. †Group Indicates statistical significance against normal control (p<0.05). *Indicates statistical significance against diabetic control (p<0.05).

Change in mean corpuscular volume (MCV) (table 2) decreased by 9% on diabetic treated rat and only by 0.8% in treated control rat. The diabetic control rat MCV, however, remained elevated 13% above that of the normal control. In a similar manner, the mean corpuscular hemoglobin (MCH) showed a downward trend in 28-HC treated control and in the treated diabetic rat, the reduction was 8%. In contrast, MCH level showed an increase of 1.9% on diabetic control rat. The mean corpuscular hemoglobin concentration (MCHC) was determined as a percentage of the cell content. Similar to the observation noted for MCH, MCHC in treated control rat decreased 4.8% in the 15 d treatment. However, in the diabetic control, the change was 2.5% above the normal control. In the treated diabetic rat there was 6% below diabetic control.

Table 2: Effect of 28-homocastasterone on blood cells indices

<table>
<thead>
<tr>
<th>Groups</th>
<th>MCV fL</th>
<th>MCH %</th>
<th>MCHC %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.0±0.53</td>
<td>21.4±0.62</td>
<td>35.7±0.35</td>
</tr>
<tr>
<td>Control+28-HC</td>
<td>5.96±0.38</td>
<td>20.2±0.35</td>
<td>34.0±0.48</td>
</tr>
<tr>
<td>Diabetic</td>
<td>6.81±0.64</td>
<td>21.8±0.5</td>
<td>36.6±0.38</td>
</tr>
<tr>
<td>Diabetic+28-HC</td>
<td>6.21±0.52*</td>
<td>20.0±0.45</td>
<td>32.3±0.42</td>
</tr>
</tbody>
</table>

Values are expressed±SD. Group n=6. †Group Indicates statistical significance against normal control (p<0.05). *Indicates statistical significance against diabetic control (p<0.05).

Table 3: Effect of 28-homocastasterone on platelets indices

<table>
<thead>
<tr>
<th>Groups</th>
<th>MPV fL</th>
<th>PDW fL</th>
<th>PCT fL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.1±1.20</td>
<td>8.4±1.21</td>
<td>0.23±0.05</td>
</tr>
<tr>
<td>Control+28-HC</td>
<td>6.9±1.58*</td>
<td>7.9±1.20*</td>
<td>0.15±0.08†</td>
</tr>
<tr>
<td>Diabetic</td>
<td>9.7±1.25</td>
<td>10.0±1.39</td>
<td>0.12±0.06</td>
</tr>
<tr>
<td>Diabetic+28-HC</td>
<td>7.0±0.93*</td>
<td>8.7±1.24*</td>
<td>0.12±0.08</td>
</tr>
</tbody>
</table>

Values are expressed±SD. Group n=6. †Group Indicates statistical significance against normal control (p<0.05). *Indicates statistical significance against diabetic control (p<0.05).

Table 4: Effect of 28-homocastasterone on blood electrolytes level

<table>
<thead>
<tr>
<th>Groups</th>
<th>Na mEq/l</th>
<th>K mEq/l</th>
<th>Cl mEq/l</th>
<th>P mEq/l</th>
<th>Ca++ mEq/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.3±6</td>
<td>9.8±1.20</td>
<td>96±4</td>
<td>6.3±0.24</td>
<td>12.2±1.25</td>
</tr>
<tr>
<td>Control+28-HC</td>
<td>12.0±4*</td>
<td>7.9±0.4</td>
<td>90.2±*</td>
<td>6.0±0.45†</td>
<td>11.1±0.94*</td>
</tr>
<tr>
<td>Diabetic</td>
<td>148±5</td>
<td>12±0.6</td>
<td>106±4</td>
<td>7.2±0.15</td>
<td>14.6±1.23</td>
</tr>
<tr>
<td>Diabetic+28-HC</td>
<td>130±6*</td>
<td>6.0±0.8*</td>
<td>85±3*</td>
<td>6.3±0.18*</td>
<td>10.6±0.83*</td>
</tr>
</tbody>
</table>

Values are expressed±SD. Group n=6. †Group Indicates statistical significance against normal control (p<0.05). *Indicates statistical significance against diabetic control (p<0.05).
In diabetic control rat, there was a significant increase in the serum electrolytes sodium, potassium, and calcium (table 4). Oral administration of 28-HC reduced the levels of serum electrolytes Na⁺, K⁺, Cl⁻, P and Ca²⁺ when compared to normal rats. In contrast (table 5) serum ALP activity increased in the 28-HC treated group compared to respective controls.

Bone marrow aspiration examination (fig. 1) normal control bone marrow aspirate showed the normal cellularity and active trilineage hematopoiesis. In 28-HC treated normal rat, moderate cellularity with myeloid suppression and increased immature megakaryocytes and myeloid cells in the bone marrow aspirate were observed. Diabetic control rat, marrow aspirate showed hypocellularity with relative suppression of erythrocytes and myeloid cells. Diabetic 28-HC treated rat, cellular with relative myeloid suppression and increase in immature megakaryocytes were observed.

Fig. 1: Bone marrow aspiration cytology following 15 d oral administration of 28-HC

Fig. 2: Bone histology following 15 d oral administration of 28-HC

Histologic features were studied in H/E and masson trichrome stained bone tissues of normal and 28-homocastasterone treated rat femur bone were examined. Normal control rat bone marrow biopsy showed normal bone trabeculae with normal bone elements. 28-HC treated normal (fig. 2 and fig. 3) rat bone tissue biopsy showed moderate cellularity with myeloid suppression, increased immature megakaryocytes, immature myeloid cells and trabeculae showed osteoblasts. Diabetic control rat bone tissues showed patchy hypocellularity with relative suppression of megakaryocytes cellular with osteoclasts in the bony trabeculae. Diabetic 28-HC treated rat (fig. 2 and fig. 3) bone tissue showed myeloid suppression, increased immature megakaryocytes and bony trabeculae is rimmed by osteoblasts.
Blood circulation was analyzed, confirming the effect of 28-HC [15]. Perhaps, remains premature, which are morphologically and functionally abnormal [14, 15]. Increased glucose oxidation in the presence of transition metals has been shown to produce membrane damage by membrane lipid peroxidation and protein glycation [13]. This could be the reason for the altered electrolyte balance in diabetic rat, which resulted in the elevated serum sodium, potassium and calcium in STZ-induced diabetic rats. However, administration of 28-HC reduced the glucose level and restored tissue electrolytes and this can be the possible cause for the reduction of the serum electrolyte levels [16].

CONCLUSION

The present study confirms that the plant ketosteroid 28-homocastasterone exhibits the hematopoietic effect. When administered, this compound improved platelet indices in diabetic rat blood and increased RBC, Hb and WBC levels significantly. Histology study in bone tissues showed that increased osteoblastic activity and improved bone histomorphology in 28-HC treated rat. The molecular mechanism underlying alteration in marrow cells and bone histology changes need further study.

ACKNOWLEDGEMENT

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CONFLICT OF INTERESTS

Authors declare no conflict of interest.

REFERENCES


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