Bone mineralization by OST-6 (OsteoCare), a herbomineral preparation, in experimentally induced rickets in rats

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SUMMARY

In the present study the efficacy of OST-6 (OsteoCare), a herbomineral preparation, on bone mineralization in experimental rickets has been evaluated. This was accomplished by feeding pregnant rats and subsequently their pups with vitamin D and calcium deficient (VDCD) with low phosphorus diet. The parameters such as serum and bone mineral contents (calcium and inorganic phosphorus), serum alkaline phosphatase, sex hormones and histology of bone were considered. VDCD resulted in a significant reduction in bone and serum calcium and inorganic phosphorus, increased serum alkaline phosphatase and decreased sex hormones (testosterone in males, progesterone and oestrogen in females). Histologically the bone showed osteodystrophic changes and disproportionate cartilaginous proliferations in the epiphyseal region. Incorporation of OST-6 into feed at 5% concentration resulted in a complete reversal of rickets, which was substantiated by biochemical and histological observations. It has been concluded that OST-6 is useful in the management of rickets in a natural way through herbal resources.

Key words: experimental rickets; OST-6 (OsteoCare); herbomineral preparation; bone mineralization; histology.

INTRODUCTION

Rickets is a metabolic disorder of the growing skeleton caused by a disturbance in calcium and phosphorus metabolism, characterized by an inadequate mineralization of bone and a disproportionate growth of the epiphyseal cartilage (McDade, 1977). Several etiological factors such as nutritional deficiencies of calcium, phosphorus, vitamin D as well as inadequate sunlight have been reported to play a role in the causation of rickets with the formation of a less compact and weak bony matrix with diminished rigidity (Boyd, 1961).

Vitamin D sufficiency is particularly important in infancy and its deficiency can be caused by inadequate dietary intake, intestinal malabsorption or a diminished synthesis of active metabolite as a consequence of an inadequate exposure to sunlight. While in advanced countries dietary deficiencies are very uncommon because of the wide spread use of fortified milk, bread and vitamin supplements, the situation in developing countries is highly variable (Jannie and Donaid, 1989).

Ayurveda, an ancient system of Indian medicine, mentions several plants that are useful in the correction of fracture and bone metabolic disorders. OST-6 (OsteoCare) is an herbomineral preparation formulated with such plants and bhasmas which are well known for their

*Terminalia arjuna* is extensively used in the treatment of osteodystrophic conditions (Nadkarni, 1996a). *Withania somnifera* is considered a rejuvenator in Ayurveda. It helps in relieving the pain associated with osteodystrophic conditions and is also useful in cases of general debility, nervous exhaustion and muscle pain (Nadkarni, 1996b). *Commiphora mukul* helps in mineralization of the bones (Nadkarni, 1996c). Praval bhasma is a rich and natural source of calcium and, due to appropriate Ayurvedic processing has the advantage of easy absorption from the intestine (Asundi and Dixit, 1978).

In the present study, OST-6 was evaluated for its anti-ricketic potential using an experimental rat model.

**MATERIALS AND METHODS**

**Animals**

Twenty pregnant rats (16 weeks old, inbred), weighing between 250-275g were selected and randomized into 4 groups of 5 animals each. They served as the base groups for the reproduction of pups to provide models for evaluation. The animals were housed in standard laboratory conditions provided with a temperature of 22±3°C, relative humidity of 50-55% and a 12 hr light and dark cycle. Drinking water and a synthetic pelleted diet (Lipton India Ltd., Mumbai) were supplied *ad-lib* throughout the study period. Experimental protocol was approved from The Ethical Committee of the Institution. All the animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by The National Academy of Sciences and published by The National Institutes of Health.

**Preparation of OST-6**

Each gram of OST-6 contains *Terminalia arjuna* (bark 250 mg), *Withania somnifera* (root 250 mg), *Commiphora mukul* (gum resin 280 mg) and Praval bhasma (220 mg). The plant constituents were powdered and weighed individually and then mixed in appropriate proportion with Praval bhasma. The constituents of plant material were procured from local supplier and identified by Dr. R. Kannan, Botanist, R&D Centre, The Himalaya Drug Company and voucher specimens were preserved at R&D Centre. Such two or more batches of preparations from raw materials of different origin were standardised by finger print analysis for characterisation using high performance thin layer chromatography (HPTLC).

**HPTLC analysis**

One gram of OST-6 was weighed and extracted by refluxing on a water bath with 15 ml of dichloromethane. Extract was filtered and concentrated to 2 ml. 10µl of concentrate was spotted on pre-coated silica gel plate. Plates was developed using dichloromethane: Methanol
Plate was scanned using densitometer at 254 nm. Finger print of OST-6 is shown in Figure 1.

**In vivo study**

The design for the development of the groups was done in two phases as shown below:

**Phase I**

**Group 1:** This group received the normal pelleted diet from day 1 of pregnancy through 21 to 60 days postpartum.

**Group 2:** This group received calcium and vitamin D free synthetic diet with low phosphorus (0.4%) from day 1 of pregnancy to 60 days postpartum.

**Group 3:** This group received 5% OST-6 in the calcium and vitamin D free synthetic diet with low phosphorus (0.4%) from day 1 of pregnancy to 60 days postpartum.

**Group 4:** This group received 5% of a calcium mixture (which is equivalent to 10g elemental calcium and 2500 IU of Vitamin D/kg of diet) in the calcium and vitamin D free synthetic diet with low phosphorus (0.4%) from day 1 of pregnancy to 60 days postpartum.

The pups from the above mentioned respective groups were maintained with their mothers until they were weaned. Twenty-six pups from each group comprising equal number of males and females formed the materials for the study and received their respective diets for another 39 days. The body weights of all the rats were recorded at weekly intervals. These groups are designated as derived groups (DG) as shown below:

**Phase II**

**DG 1:** This group received the normal pelleted diet from the day of weaning to the 39th day.

**DG 2:** This group received calcium and vitamin D free synthetic diet with low phosphorus (0.4%) from the day of weaning to the 39th day.

**DG 3:** This group received 5% OST-6 in the calcium and vitamin D free synthetic diet with low phosphorus (0.4%) from the day of weaning to the 39th day.

**DG 4:** This group received 5% of a calcium mixture (which is equivalent to 10g elemental calcium and 2500 IU of Vitamin D/kg of diet) in the calcium and vitamin D free synthetic diet with low phosphorus (0.4%) from the day of weaning to the 39th day.

**Necropsy and processing of the tissues**

On the 40th day the rats of all the four groups were anesthetized by using diethyl ether for collection of blood samples and then euthanised. Serum samples were used for the estimation of calcium (Sarkar and Chauhan, 1967), inorganic phosphorus (Varley, 1980), alkaline phosphatase (Horn, 1972), progesterone, estrogen and testosterone (the estimations were carried out by using the Coat-A-Count Kit, Diagnostic Products Corporation, Los Angeles).

The animals of the respective groups were then systematically necropsied and both femur bones from each rat of the respective groups were removed and divided into two sets. One set was taken for bone mineral estimation after ashing in an electric furnace at 700°C for 8 hrs. The other set was again divided by longitudinal division of each bone into two halves and fixed in 10% neutral buffered formalin.
The fixed soft tissues as well as bone material after decalcification, were processed by the paraffin technique (Drury, 1980). Sections of 5µ thickness were cut and stained by the routine H&E method (Bancroft and Cook, 1988). Sections of femur bone were taken from the same position in all the groups.

**Statistical analysis**

Data obtained are expressed as mean ± SEM and statistical significance was ascertained using the ANOVA followed by unpaired student’s ‘*t*’ test.

**RESULTS**

**Effect on body weight**

The body weight of the animals under different groups is shown in Figure 2. Animals belonging to DG2 showed a significant (*p*<0.001) decrease in body weight both in male and females as compared to DG1. The rats of DG3 and DG4 showed a significant (*p*<0.001) improvement in body weight gain as compared to DG2 rats.

**Biochemical profile**

The biochemical profile in relation to the serum levels of calcium, inorganic phosphorus, alkaline phosphatase and sex hormones as well as levels of bone calcium and inorganic phosphorus have been summarized in Table 1. Animals of DG2 showed significant (*p*<0.001) decrease of serum and bone calcium and inorganic phosphorus as compared to DG1. The serum and bone calcium and inorganic phosphorus levels in DG3 and DG4 were comparable to DG1.

![Figure 2](image)

**Fig. 2.** The effect of OST-6 (OsteoCare) on body weight (M ± S.E.)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum Calcium (mg/dl)</th>
<th>Bone Calcium (mg/gm)</th>
<th>Serum Phosphorus (mg/dl)</th>
<th>Bone Phosphorus (mg/gm)</th>
<th>ALP (IU/l)</th>
<th>Estrogen (pg/ml)</th>
<th>Progesterone (ng/ml)</th>
<th>Testosterone (ng/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DG1</td>
<td>10.47± 0.142</td>
<td>120.00± 5.42</td>
<td>16.99± 0.50</td>
<td>17.99± 0.93</td>
<td>100.37± 4.28</td>
<td>9.67± 2.30</td>
<td>36.32± 2.65</td>
<td>168.00± 41.14</td>
</tr>
<tr>
<td>DG2</td>
<td>4.94± 0.171</td>
<td>38.85± 7.93</td>
<td>5.28± 0.31</td>
<td>10.81± 1.82</td>
<td>265.25± 12.47</td>
<td>3.72± 0.46</td>
<td>28.07± 1.50</td>
<td>8.11± 5.64</td>
</tr>
<tr>
<td>DG3</td>
<td>8.96± 0.24</td>
<td>100.62± 6.34</td>
<td>14.64± 0.49</td>
<td>16.39± 1.12</td>
<td>101.56± 4.32</td>
<td>9.97± 1.98</td>
<td>38.80± 3.93</td>
<td>134.03± 38.97</td>
</tr>
<tr>
<td>DG4</td>
<td>10.10± 0.346</td>
<td>94.86± 8.42</td>
<td>14.58± 0.55</td>
<td>15.86± 0.85</td>
<td>108.55± 3.85</td>
<td>10.18± 1.01</td>
<td>35.92± 2.92</td>
<td>137.50± 37.45</td>
</tr>
</tbody>
</table>

*p*<0.05 DG2 vs DG1, *p*<0.05 DG2 vs DG3, *p*<0.05 DG2 vs DG4 and *Not significant* DG3 and DG4 vs DG1.
There was significant ($p<0.001$) decrease in the levels of testosterone in males, progesterone and oestrogen in females of DG2 as compared to DG1. These levels were significantly increased in DG3 and DG4 and were comparable with that of DG1.

In DG2 there was significant elevation in serum alkaline phosphatase activity (total as well bone specific) as compared to DG1. In DG3 the level of this enzyme was comparable to DG4.

**Histopathology**

Bone sections from all the experimental groups were reviewed for the histological changes. The untreated animals exhibited normal compactness of the bone and narrow band of cartilage in DG1 is shown in Figures 3 and 4 respectively. In DG2, the cortical bone sections showed thinning of bone cortex and trabeculae with disproportionate proliferation of the epiphyseal cartilage and fibrous tissue replacements (Figs. 5 and 6). In DG3 and DG4, the histological findings of the bone were near normal and are comparable to DG1 (Figs. 7-10).

**DISCUSSION**

The objective of this study was to evaluate the efficacy of OST-6 using experimentally induced rickets and comparing the responses with suitable controls. Calcium and vitamin D deficiency with low phosphorus is known to result in defective bone
calcification, which in turn affects body weight gain as well as development of the gonads. Growth retardation nearly invariably accompanies hypophosphatemic rickets (Chan and Bartter, 1979). In the present study the reduction in body weight was noticed in DG2 animals throughout the experiment and the same was near normal in DG3 and DG4 compared to DG1.

Rickets was originally described as a disease of long bones with a widened epiphyseal plate and wide osteoid seams (Weinstein et al., 1984). It has been found that the production of rickets involved dietary deficiency of calcium and vitamin D with low phosphorus diet (Clarke et al., 1989). Our studies showed that rickets can be induced by feeding the pregnant mother and subsequently their pups with vitamin D and calcium deficient with low phosphorus diet. Widening or proliferation of epiphyseal cartilage is a characteristic feature of rickets (Clarke et al., 1987). In the present experiment, there was an increased proliferation and widening of epiphyseal cartilage of the bone in DG2 animals. The animals of the groups DG3 and DG4 showed a near normal band of cartilage width and thus presented the absence of lesions of rickets.

It is well known that rickets is a metabolic disorder and is phosphorus dependent, where there will be reduction in serum inorganic phosphorus levels (Miura et al., 1996). In the present study, the serum inorganic phosphorus levels in control group was 16.00 mg/dl and was reduced to approximately 50% in DG2 i.e., 5.2 mg/dl, in DG3 it was 14.64 mg/dl and 14.58 mg/dl in DG4. From the above observation, it is evident that there will be significant reduction in inorganic phosphorus levels in rachitic rats, which is in concurrence with earlier report (Miura et al., 1996). The incorporation of OST-6 in DG3 significantly improved the concentration of inorganic phosphorus in serum and the same was observed in DG4. It is well known that vitamin D elevates the serum inorganic phosphorus levels in rachitic rats by stimulating phosphate reabsorption in kidneys and intestine (Tanaka and DeLuca, 1974). The pharmacological mechanism for the increase in the serum concentration of inorganic phosphorus induced by OST-6 has not yet been clarified; however, it clearly improved hypophosphatemia in rachitic rats.
It is well known that sex hormones participates actively in the process of bone mineralization (Correa et al., 1992; Wimalawansa and Wimalawansa, 1999; Lane et al., 1999). In the present study we observed significant reduction of all sex hormones (estrogen, progesterone and testosterone) in DG2 and its near normalization with OST-6 helping in resisting the bone from the damage caused by calcium and vitamin D deficient and low phosphorus diet. Also this observation correlates with the increased body weight in DG3 and DG4 compared to DG2, thus establishes the fact of improved bone mineralization in DG3 and DG4 contributing to the improved body weight gain.

It has been established that due to an increased bone turnover the serum alkaline phosphatase gets increased in rickets and other bone metabolic disorders (Fitzpatrick, 1996). In DG2, with the experimental induction of rickets, the serum alkaline phosphatase levels were elevated substantially and the findings were similar to those of earlier published report (Fitzpatrick, 1996). In the DG4 that received the standard corrective therapy provided the picture of diminished alkaline phosphatase activity compared to DG2, which is an indication of positive response to the therapy. DG3, which received OST-6, also showed a reduction in alkaline phosphatase activity indicating value of OST-6 as a corrective comparable to DG4. These findings lend credence to the fact that OST-6 is rich in calcium and vitamin D like activity, which can be used in the management of rickets naturally through the herbal resources. Further studies are in progress to establish the efficacy of OST-6 in clinical situations of rickets.

REFERENCES


