Evaluation of the Efficacy and Safety of Reosto in Postmenopausal Osteoporosis: A Prospective, Randomized, Placebo-Controlled, Double Blind, Phase III Clinical Trial

Dr. Deepti Dongaonkar, MD, DGO
Professor, Department of Obstetrics & Gynaecology,
Dr. Rajeev Mehta, MD (Radiology)
Hon. Professor, Department of Radiology
Grant Medical College and Sir JJ Group of Hospital, Mumbai, India
Dr. Kolhapure, S.A.* MD
Senior Medical Advisor, R&D Center, The Himalaya Drug Company, Bangalore, India.

[*Corresponding author]

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ABSTRACT
The current management of postmenopausal osteoporosis (PMO) includes the use of estrogentic compounds, SERMs, bisphosphonates, calcitonin, and PTH; however, the associated adverse effects limit their long-term usage. Recently, phytoestrogens have been shown to be beneficial in the management of PMO. Reosto is a polyherbal formulation and is a rich source of phytoestrogens and calcium, and this study was planned to evaluate the efficacy and safety of Reosto in the management of PMO.

The study was a prospective, randomized, double blind, placebo-controlled, phase III clinical trial, conducted as per the ethical guidelines of GCP. One hundred and five healthy women, with natural or surgical menopause, who had amenorrhea in the preceding 6 months were included in the study. All women who were consuming any drug, which is known to affect bone metabolism, those women suffering from endometrial polyps or hyperplasia or other estrogen-dependent tumors were excluded from the study. At the randomization visit, a detailed medical history was obtained from all the enrolled women. Subsequently, all women underwent a thorough systemic and gynecological examination, routine and bone-specific biochemical investigations. All women underwent a baseline BMD examination using DEXA. All women were randomized in Reosto and placebo groups, and both the groups were advised to consume the respective medications in dose of two tablets twice daily, orally, for 6 months. All the enrolled women were monitored at monthly intervals for 6 months, for any reported or observed adverse effects. The predefined primary efficacy end points were improvement in the BMD score and bone-specific biochemical parameters. The predefined secondary safety end points were incidence of short- and long-term adverse events, and overall patient compliance to the drug treatment. Statistical analysis was done according to intent-to-treat principles.
Out of the 105 enrolled women, 11 women had normal BMD, and 16 women were lost to follow-up and the data of 78 women (39 from each group) was considered for statistical analysis.

This study observed a significant increase in the T-score and BMD of lumbar spine, femur neck, and hip, in the Reosto group (which indicate the desirable remineralization of osteoporotic bones). There was also a significant increase in serum calcium and serum phosphorus, and a significant decrease in serum alkaline phosphatase levels, in the Reosto group (which indicates the beneficial changes in the biochemical bone markers). Also, there were no clinically significant changes in any of the hematological parameters, nor was there any clinically significant adverse reaction, and the overall compliance to the treatment was excellent (which reflects the short- and long-term safety of Reosto). Therefore it may be concluded that the treatment with Reosto is effective and safe in the management of PMO.

INTRODUCTION
Low BMD, microarchitectural deterioration, increased bone fragility, and an increased susceptibility to fracture/s are the cardinal features of OP. “T-score” (as measured by DEXA), which is the number of SDs of BMD above or below the BMD of young-normal mean, is used to define OP. As per WHO, a reduction in the T-score by 2.5 times of SD of the mean peak value of young adults is classified as OP. Osteoporosis may be either primary (subdivided into type I/PMO and type II/senile OP), or secondary OP (also referred as type III OP).

Postmenopausal osteoporosis is responsible for a major chunk of women’s morbidity and mortality, and the mortality rates from PMO-related fractures are greater than that of combined mortality rates from breast and ovarian cancers. The management of PMO according to modern medicine includes the use of estrogenic compounds, SERMs, bisphosphonates, calcitonin, and PTH. However, the associated adverse effects with these drugs limit their long-term usage. Recently, phytoestrogens (plant derived osteogens) have been shown to be beneficial in the management of PMO.

Reosto is a polyherbal formulation, and is a rich source of phytoestrogens and calcium. Reosto is recommended for the management of PMO, and contains the powders of Commiphora wightii, Vanda roxburghii, Terminalia arjuna, Withania somnifera, Sida cordifolia, Godanti bhasma and Kukutandatvak bhasma, and is processed in Coriandrum sativum, Aloe vera, and Bambusa arundinacea. This study was planned to evaluate the efficacy and safety of Reosto in the management of PMO.

AIM OF THE STUDY
This study was planned to evaluate the clinical efficacy and safety (short- and long-term) of Reosto in the management of PMO.

MATERIALS AND METHODS
Study design
The study was a prospective, randomized, double blind, placebo-controlled, phase-III clinical trial, conducted at the Department of Obstetrics and Gynecology, Grant Medical College, Mumbai, India, as per the ethical guidelines of Declarations of Helsinki, in accordance to the principles of GCP. The study protocols, case record forms, regulatory clearance documents, product-related information and informed consent forms were submitted to the “Institutional Ethics Committee”, and were approved by the same.
Inclusion criteria
One hundred and five healthy women, with natural or surgical menopause (ovaries removed) and who had amenorrhea in the preceding 6 months, and who were willing to give written informed consent were included in the study.

Exclusion criteria
All women who were consuming any drug, which is known to affect bone metabolism (e.g. SERMs, bisphosphonates, calcitonin, vitamin D analogs, corticosteroids, etc.), and those women who were suffering from endometrial polyps or hyperplasia or other estrogen-dependent tumors were excluded from the study. Similarly, all women who had moderate or severe hypertension, and those who were not willing to give informed written consent were excluded from the study.

Study procedure
At the randomization visit, a detailed medical history was obtained from all the enrolled women. Subsequently, all women underwent a thorough systemic and gynecological examination, routine biochemical investigation (Hb, TLC, DLC, ESR, LFTs and RFTs), bone-specific biochemical investigations (serum calcium, serum phosphorus and serum alkaline phosphatase). All women underwent a baseline BMD examination (of the lumber spine, femur neck, hip) using DEXA (Machine make: HOLOGIC QDR 1000 (S/N2417P). All women were randomized into Reosto and placebo groups, and both the groups were advised to consume the respective medications in dose of two tablets twice daily, orally, for 6 months.

Monitoring and follow-up
All the enrolled women were monitored at monthly intervals for 6 months, for any reported or observed adverse effects. At each follow-up visit, the investigator recorded any information about intercurrent illness, therapeutic interventions and concomitant medication/s. Medications considered necessary for the patient’s welfare, and which will not interfere with the study medication (e.g. NSAIDs) were allowed at the discretion of the investigators. A detailed evaluation of BMD, hematological, and bone-specific biochemical investigations were repeated at the end of 6 months.

Adverse events
All the adverse events reported or observed by women were recorded with information about severity, date of onset, duration and action taken regarding the study drug. Relation of adverse events to the study medication were predefined as “Unrelated” (a reaction that does not follow a reasonable temporal sequence from the time of administration of the drug), “Possible” (follows a known response pattern to the suspected drug, but could have been produced by the patient’s clinical state or other modes of therapy administered to the patient), and “Probable” (follows a known response pattern to the suspected drug that could not be reasonably explained by the known characteristics of the patient’s clinical state).

Women were allowed to voluntarily withdraw from the study, if they experienced serious discomfort during the study or sustained serious clinical events requiring specific treatment. For women withdrawing from the study, efforts were made to ascertain the reason for dropout. Non-compliance (defined as failure to take <80% of the medication) was not regarded as treatment failure, and reasons for non-compliance were noted.
Primary and secondary end points
The predefined primary efficacy end points were improvements in the BMD score and bone-specific biochemical parameters. The predefined secondary safety end points were incidence of short- and long-term adverse events, and overall patient compliance to the drug treatment.

Statistical analysis
Statistical analysis was done according to intent-to-treat principles. The reduction in symptom- and sign-scores was evaluated by “Repeated Measure Two-Way ANOVA Test”, which was followed by “Bonferroni’s post-tests”, for evaluating the changes in various parameters from baseline values to the values after six months. The minimum level of significance was fixed at 95% confidence limit and a 2-sided p value of <0.005 was considered as significant.

RESULTS
Out of the 105 enrolled women, 11 women had normal BMD, and 16 women were lost to follow-up and the data of 78 women (39 from each group) was considered for statistical analysis. There was no significant difference in the body mass index of enrolled women, in both the groups (F=1.314, p=0.4096, NS).
There was a highly significant increase in the BMD of lumbar spine (t=8.566, p<0.001; HS), femur neck (t=7.2050, p<0.001; HS), and hip (t=13.78, p<0.001; HS) in the Reosto group, as compared to the placebo group, at the end of the study (Table 1). There was a significant increase in the T score of lumbar spine (t=5.10, p<0.001; S) (Table 2 and Figure 1) and femur neck (t=2.608, p<0.05, S) in the Reosto group, as compared to the placebo group, at the end of the study (Table 2 and Figure 2).

| Table 1: Comparative changes in BMD scores before and after treatment in women from Reosto and placebo groups |
| --- | --- | --- | --- |
|  | Reosto | Placebo |
|  | Before | After | Before | After |
| Lumbar spine | Mean | 0.79 | 0.89 | 0.75 | 0.75 |
|  | SEM | 0.02 | 0.01 | 0.02 | 0.02 |
|  | Summary* | Drug: t=8.566, p<0.001, HS; Placebo: t=0.3822, p>0.05, NS |
| Femur neck | Mean | 0.60 | 0.71 | 0.68 | 0.67 |
|  | SEM | 0.01 | 0.01 | 0.01 | 0.01 |
|  | Summary* | Drug: t=7.2050, p<0.001, HS; Placebo: t=0.8406, p>0.05, NS |
| Hip | Mean | 0.73 | 0.85 | 0.81 | 0.81 |
|  | SEM | 0.01 | 0.02 | 0.02 | 0.01 |
|  | Summary* | Drug: t=13.78, p<0.001, HS; Placebo: t=0.4202, p>0.05, NS |

*Two-way ANOVA Test followed by “Bonferroni’s post-test”
Table 2: Comparative changes in T-score before and after treatment in women from Reosto and placebo groups

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<tr>
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<th>Reosto</th>
<th>Placebo</th>
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<tr>
<td></td>
<td>Before</td>
<td>After</td>
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<tr>
<td>Lumbar spine</td>
<td>Mean</td>
<td>-2.36</td>
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<tr>
<td></td>
<td>SEM</td>
<td>0.15</td>
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<tr>
<td>Summary*</td>
<td>Drug: $t=5.10$, $p&lt;0.001$, HS; Placebo: $t=0.57$, $p&gt;0.05$, NS</td>
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<tr>
<td>Femur neck</td>
<td>Mean</td>
<td>-2.25</td>
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<tr>
<td></td>
<td>SEM</td>
<td>0.18</td>
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<tr>
<td>Summary*</td>
<td>Drug: $t=2.608$, $p&lt;0.05$, S; Placebo: $t=0.8728$, $p&gt;0.05$, NS</td>
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*Two-way ANOVA Test followed by “Bonferroni’s post-test”

There was a highly significant increase in serum calcium ($t=4.48$, $p<0.001$; HS), and serum phosphorus ($t=6.30$, $p<0.001$, HS) and a highly significant decrease in serum alkaline phosphatase levels ($t=3.11$, $p<0.001$; HS) in the Reosto group as compared to the placebo group, at the end of the study (Table 3).

There were no clinically significant changes in any of the hematological parameters. There were no clinically significant adverse reactions (either reported by women, or observed by the investigators), and the overall compliance to the treatment was excellent, in both the groups.
Table 3: Comparative changes in biochemical parameters before and after treatment in women from Reosto and placebo groups

<table>
<thead>
<tr>
<th></th>
<th>Reosto</th>
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<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
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<tr>
<td>Serum calcium</td>
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<tr>
<td>Mean</td>
<td>9.38</td>
<td>10.06</td>
<td>9.47</td>
<td>9.64</td>
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<tr>
<td>SEM</td>
<td>0.13</td>
<td>0.12</td>
<td>0.12</td>
<td>0.15</td>
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<tr>
<td>Summary*</td>
<td>Drug: ( t = 4.48, p &lt; 0.001 ), HS; Placebo: ( t = 0.61, p &gt; 0.05 ), NS</td>
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<td>Serum phosphorus</td>
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<tr>
<td>Mean</td>
<td>3.37</td>
<td>4.09</td>
<td>3.71</td>
<td>3.71</td>
</tr>
<tr>
<td>SEM</td>
<td>0.07</td>
<td>0.11</td>
<td>0.08</td>
<td>0.04</td>
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<tr>
<td>Summary*</td>
<td>Drug: ( t = 6.30, p &lt; 0.001 ), HS; Placebo: ( t = 0.04, p &gt; 0.05 ), NS</td>
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<tr>
<td>Serum alkaline</td>
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<tr>
<td>phosphatase</td>
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<tr>
<td>Mean</td>
<td>191.90</td>
<td>146.40</td>
<td>191.10</td>
<td>159.40</td>
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<tr>
<td>SEM</td>
<td>8.67</td>
<td>10.06</td>
<td>9.58</td>
<td>12.13</td>
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<tr>
<td>Summary*</td>
<td>Drug: ( t = 3.11, p &lt; 0.001 ), HS; Placebo: ( t = 2.17, p &gt; 0.05 ), NS</td>
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*Two-way ANOVA Test followed by “Bonferroni’s post-test”

DISCUSSION

Bone remodeling is the primary function of osteoblasts and osteoclasts, and the other factors (such as hormones (esp. estrogen), growth factors and cytokines) play a regulatory role in bone homeostasis.\(^7,8\) Diet, daily milk intake, food habits and exercises do influence the bone regulatory mechanism.

Postmenopausal estrogen deficiency is held responsible for increase in the osteoclast activity, which leads to the depletion of calcium, collagen, and proteins from bone, with the resultant increase in bone porosity and risk for fracture/s.\(^9\) Bone loss is most rapid during the first few
years after menopause, and the rate declines there after in advanced old age.\textsuperscript{10} Within the Asian populations, numerous observational studies have shown that postmenopausal women consuming the foods containing phytoestrogens had the highest BMD.\textsuperscript{11,12}

Phytoestrogens suppress bone resorption, negate the bone resorption effects of PTH, vitamin D, and prostaglandins, increase Ca\textsuperscript{2+} retention in bone, and augment the action of estrogen on bone, and it appears that phytoestrogens are a rational alternative to HRT/ERT in PMO.\textsuperscript{13,14}

Of the two estrogen receptors (ER\textsubscript{α} and ER\textsubscript{β}) in osteoblasts, there is an increased expression of ER\textsubscript{β} during bone mineralization, which is particularly pertinent to the effects of phytoestrogens.\textsuperscript{15}

Nitric oxide in addition to its atheroprotective effect, plays an important role in osteoblast function and bone turnover.\textsuperscript{16} Reactive oxygen species and superoxide production contributes to osteoclastic bone resorption.\textsuperscript{17} Also, the generation of ROS activates Tumor necrosis factor-\textsubscript{αB} (TNF-\textsubscript{αB}), which mediates the actions of osteoclasts.\textsuperscript{18} Nicotine amide dinucleotide phosphate (NADPH) oxidase enzyme system generates the superoxide generation in osteoclasts\textsuperscript{19} and there is decreased superoxide dismutase and glutathione peroxidase activity in women with PMO.\textsuperscript{20} It appears that higher antioxidant intake has a protective role on bone health.\textsuperscript{21,22} In addition to menopause and advanced age, dyslipidemia, hyperhomocystinemia, hypertension, and diabetes have also been associated with increased risk of low BMD.\textsuperscript{23}

This study observed a significant increase in the BMD and T-score of lumbar spine, femur neck, and hip, in the Reosto group (which indicate the desirable remineralization of osteoporotic bones). There was also a significant increase in serum calcium, serum phosphorus, and a significant decrease in serum alkaline phosphatase levels, in the Reosto group as (which indicates the beneficial changes in the biochemical bone markers). Also, there were no clinically significant changes in any of the hematological parameters, nor were there clinically significant adverse reactions, and the overall compliance to the treatment was excellent (which reflects the short- and long-term safety of Reosto). These beneficial effects might have been due to the synergistic actions of the ingredients, which are well documented.

The main ingredients of Commiphora wightii are flavonoids.\textsuperscript{24} Vanda roxburghii contains alkaloids, glycosides, tannins, saponins, sterols, and resins.\textsuperscript{25} The active ingredients of Terminalia arjuna are tannins, triterpenoid saponins (arjunic acid, arjunolic acid, arjungenin, and arjunglycosides), flavonoids (arjunone, arjunolone, and luteolin), gallic acid, ellagic acid, oligomeric proanthocyanidins, phytosterols, calcium, and minerals.\textsuperscript{26,27} The principle constituents of Withania somnifera are withanolides,\textsuperscript{28} withaferin A, withanosides, coumarins, scopoletin, aesculetin, triterpene, and phytosterols.\textsuperscript{29,30} The active ingredients of Sida cordifolia are quinazoline alkaloids (ephedrine, and y-ephedrine), phytosterols, mucins, potassium nitrate, resins, resin acids, 6-phenyl ethylamine, carboxylated tryptamines, hypaphorine, vasicinone, vasicine and vasicinol.\textsuperscript{31} The active ingredients of Coriandrum sativum are petroselinic acid, linoleic acid, palmitic acid, stearic acid, vaccenic acid, and myristic acid. The active constituent of Aloe vera is acetylated beta-D-mannan substituted with D-galactose and D-glucose.\textsuperscript{32}

Sida cordifolia and Withania somnifera are rich sources of phytoestrogens inhibit bone resorption and negate the bone resorption effects.\textsuperscript{33} Two experimental studies documented that Reosto renormalized the increased levels of alkaline phosphatase (indicating its beneficial effect in bone formation), and decreased the urinary excretion of pyridinoline,
deoxypyridinoline and hydroxyproline (indicating inhibition of bone resorption) and also inhibited the formation of osteoclast like cells in the presence of 1, 25-(OH)$_2$ Vitamin D$_3$.

Guggulsterones from *Commiphora wightii* inhibit the formation of NO, and inhibit NF-κB activation and thereby control TNF-α stimulation in osteoclasts. Arjunaphthanoloside from *Terminalia arjuna* has potent antioxidant, antiradical and antiliperoxidative activities, and inhibits NO production. Vanda roxburghii has potent anti-inflammatory activity. *Withania somnifera* has powerful adaptogenic and antistress potential. Kukkutandatvak bhasma (hen eggshell powder) and Godanti bhasma (seashell powder) are rich sources of elemental organic calcium carbonate, which has higher bioavailability.

Guggulsterones from *Commiphora wightii* inhibit LDL oxidation mediated by either catalytic ions or free radicals (generated by azo compounds, lipoxygenase, or macrophages), as evidenced by the decrease in the LDL oxidation. *Terminalia arjuna* also has potent antihypercholesteremic activity.

Therefore, it can be summarized that the observed beneficial effects of Reosto in PMO might be due to the inhibition of bone resorption along with the negation of the bone resorption effects of endogenous factors (by the phytoestrogens from *Sida cordifolia* and *Withania somnifera*), antioxidant action (of guggulsterones from *Commiphora wightii* and arjunaphthanoloside from *Terminalia arjuna*), anti-inflammatory activity (of *Vanda roxburghii*), adaptogenic and antistress activity (of *Withania somnifera*), supplementation of elemental organic calcium carbonate (by Kukkutandatvak bhasma and Godanti bhasma), and desirable cardioprotective actions (of guggulsterones from *Commiphora wightii* and *Terminalia arjuna*). Thus, Reosto appears to inhibit bone resorption and renormalizes bone formation through its estrogenic, antioxidant, anti-inflammatory, adaptogenic and calcium supplementation properties, along with the desired control of associated risk factors such as CVDs.

**CONCLUSION**

Postmenopausal osteoporosis is responsible for a major chunk of women’s morbidity and mortality in the west. The management of PMO in modern medicine includes the use of estrogenic compounds, SERMs, bisphosphonates, calcitonin, and PTH. However, the adverse effects associated with the use of these drugs, limit their long-term usage. Recently, phytoestrogens (plant derived osteogens) have been shown to be beneficial in the management of PMO. Reosto is a polyherbal formulation, which is a rich source of phytoestrogens and calcium; and this study was planned to evaluate the efficacy and safety of Reosto in the management of PMO.

This study observed a significant increase in the T-score and BMD of lumbar spine, femur neck and hip in the Reosto group (which indicate the desirable remineralization of osteoporotic bones). There was also a significant increase in serum calcium and serum phosphorus, and a significant decrease in serum alkaline phosphatase levels, in the Reosto group (which indicates the beneficial changes in the biochemical bone markers). Also, there were no clinically significant changes in any of the hematological parameters, nor were there clinically significant adverse reactions, and the overall compliance to the treatment was excellent (which reflects the short- and long-term safety of Reosto).

Thus, Reosto appears to inhibit the bone resorption and renormalizes bone formation through its estrogenic, antioxidant, anti-inflammatory, adaptogenic and calcium supplementation
properties, along with the desired control of associated risk factors such as CVDs. Therefore, it may be concluded that the treatment with Reosto is effective and safe in the management of PMO.

REFERENCES