One of the serious systemic skeletal diseases is osteoporosis, which emerges owing to many etiologies including hypogonadism, hypersteroidism, and menopause (1, 2). In osteoporosis, mineral density of bone tissue becomes significantly low, which results in deteriorated and fragile bones. Such bones are extremely prone to breakage (3).

Bone physiology depends on the delicate balance between bone resorption and regeneration. Bone resorption depends on deterioration of osteoblasts and osteoclasts through the process of apoptosis. Reversibly, bone regeneration involves the uninterrupted supply of new osteoblasts and osteoclasts (4). The functions of osteoblasts and osteoclasts are principally related with increased consumption of glucocorticoids, decreased level of sex steroids and senescence. Additionally, the prolonged lifespan of osteoclasts and shortened lifespan of osteoblasts leads to disturbance in equilibrium between bone resorption and regeneration.

An upregulation in osteoclastogenesis and osteoblastogenesis in the marrow has been observed in people with decreased level of sex steroids under the effect of upregulated levels and influence of cytokines, such as IL-1 and IL-6 (5). In addition, there is diminished bone formation in both aged male and female during each remodeling cycle. Furthermore, there is significant increase in the rate of bone remodeling in menopausal women (6).

On excessive usage of glucocorticoids, there is reduction in the intestinal absorption of calcium. Moreover, glucocorticoid excess also induces defects in the metabolism of vitamin D. Both of these changes lead to hypercalciuria and various pathological changes, including increased resorption...
of bones as well as decreased proliferation of osteoblasts. As a result, reduction in the biosynthesis of various macromolecules occurs resulting in deficiency of sex steroids, this situation escorts to hyperparathyroidism (7). Additionally, there is increased apoptosis as well as reduced synthesis of osteoblasts and osteocytes, on excessive usage of glucocorticoids (8).

The factors responsible for osteoporosis development include modifiable and non-modifiable factors. The former category involves medication use, calcium and vitamin D usage, sex hormones, anorexia nervosa, alcohol consumption, and smoking, while age, gender, body size, family history, and ethnicity are named as non-modifiable factors (2). The osteoporosis can be prevented or treated through daily exercise, balance diet, and various drugs. Estrogen, bisphosphonates, calcitonin, and sodium fluoride are the drugs which are used to treat osteoporosis (9).

The usual therapeutic approaches for managing osteoporosis focus on the remodeling of bones through various modes involving the provision of estrogens, calcium and phosphorus in bones; the stimulation of parathyroid hormone (PTH) synthesis; the induction of OPG (osteoprotegerin) expression, osteoblast proliferation, and the osteoclast apoptosis; and reduction in the level of IL-1, 4, and 6 (10).

Therapies that play important role in reducing bone resorption include vitamin D, calcium, hormone, and bisphosphonate. Little therapeutic outcome has been noted in terms of improved bone mineral density when vitamin D or calcium alone is supplemented in deficient persons (10). Hormone therapy is another approach for managing the osteoporosis in the postmenopausal women, however it should be dealt as a short-term therapy since its long-term use may lead to development of breast and/or uterus cancer (11). Along with the preventive effect against osteoporosis, bisphosphonate intake produces some undesired effects in upper gastrointestinal that may persist for many years after their discontinuation (12). For example, potential undesired effect of estrogen is an increased risk of venous thrombosis (13). Additionally, therapies that play important role in stimulating the bone regeneration include PTH, which is involved in bone anabolism. At a dose of 20 µg, PTH (1-34) effectively inhibited fracture risk in postmenopausal women. However, the discontinuity of PTH is followed by decline in bone mineral density (14). This drawback of PTH has been managed by using an antiosteoporotic compound, strontium ranelate after discontinuing PTH (15, 16).

The above given summary of synthesized drugs used for preventing and treating the osteoporosis elaborates their specific drawbacks. In comparison to these drugs, some natural therapeutic agents from various medicinal plants are found to possess promising antiosteoporotic activity with lesser side effects, even after long-term use. It is noteworthy that the plant medicines possess plenti-
ful active compounds. It is useful in treating osteoporosis, which is a disease with multipathways and multitargets of pathogenesis (16).

*Drynaria fortunei* is a well known medicinal plant whose dried rhizome is extensively used for treatment of bone diseases, inflammation, and hyperlipemia (17). It is especially used in Traditional Chinese Medicines (TCM). *Drynaria fortunei* (Kunze) J. Sm. is a perennial pteridophyte that belongs to family, Polypodiaceae (18). Due to use of dried rhizome of *Drynaria fortunei* in disease therapy, the term “Rhizoma Drynariae” is generally used for this herbal drug. In TCM, Rhizoma *Drynariae* is also called as “Yang-tonifying” herb; it means herb used to treat bone diseases (18, 19). Moreover, various flavonoids and triterpenoids are found in Rhizoma *Drynariae* extract, and these compounds are considered to be responsible for improving the bone cell viability (20). Naringin and neoeriocitrin are leading examples of flavonoids isolated from Rhizoma *Drynariae* extract (18). These compounds have antiosteoporotic activity, possibly due to its capability of activating the estrogen receptors as well as replacing estrogen (21). The important examples of triterpenoids isolated from Rhizoma *Drynariae* extract include 24-ethyl-9,19-cyclolanost-25-en-3; 3-ol, hop-22(29)-ene and fern-9(11)-ene, respectively (18). Naringin has been demonstrated to possess the capability of stimulating new bone formation (20). Figure 1 reveals the structure of Rhizoma *Drynariae*. In addition, Figure 2 describes the chemical structure of naringin and neoeriocitrin, isolated from *Drynaria fortunei* (20).

The pharmacological data of the extracts, serum, and Rhizoma *Drynariae* total flavonoids has been found from literature search about various modes of action of Rhizoma *Drynariae* and its compounds against osteoporotic models of osteoblasts, osteoclasts and animals (22-27). For example, significant increase in alkaline phosphatase activity in the cell lines, enhancement in proteoglycan synthesis, and improvement in calcification of the cultivated chick embryo bone primordium is observed after Rhizoma *Drynariae* injection (28). After treatment of rat osteoblasts with Rhizoma *Drynariae*, considerable bone recovery via its antioxidant action has been reported (29). A biochemical study has reported significant effect of Rhizoma *Drynariae* on osteoclastic cell lines (23, 30).

This review article reports the summary of current studies about antiosteoporotic potential of the dried rhizome of *Drynaria fortunei* with special focus on its mode of action. **ANTIosteoporotic potential of the dried rhizome of *Drynaria fortunei***

The Rhizoma *Drynariae* has effectively been used for many years in the eastern Asia (especially in China and Korea) as an anti-inflammatory, hypolipemic, anti-atherosclerotic, and anti-osteoporotic agent (31-33). Numerous current studies elaborate therapeutic effectiveness of *Drynariae* Rhizoma in osteoporosis and bone fracture in various models such as the ovariectomized rat model. The modes of action of Rhizoma *Drynariae* include stimulation of BMP-2 and ALP (alkaline phosphatase), aggregation of bone matrix proteins such as type I collagen, and increased expression of up-regulated Runx2 and osteocalcin (34). Beside Rhizoma *Drynariae* total flavonoids, Rhizoma *Drynariae* has been used for bone treatment in different forms including Rhizoma *Drynariae* medicated-extracts and serum.

**Extracts**

The mouse bone cells culture was exposed to various dilutions of *Drynariae* Rhizoma extract to investigate its anti-resorption potential. From colorimetric MTT assay conducted for determination of mitochondrial activity of these cells, significant anti-resorptive effect of *Drynariae* Rhizoma extract was observed. It is noteworthy that no cytotoxicity was observed in this experiment. This outcome is in agreement with another report (35). A dilution of 100 µg/mL of *Drynariae* Rhizoma extract produced maximum bone protective effect. Alternatively, osteoclasts contain cathepsin k, an endoproteinase that is found largely in the lysosomes, which is considered to be responsible for bone resorption via matrix degradation. Cathepsin k is present only in the osteoclasts. Cathepsin k expression can be inhibited by gene silencing, leading to inhibition of bone resorption as well as the collagen decomposition (36), while cancellous bone turnover is observed when cathepsin k overexpression. Thus, cathepsin k may be considered as a specific biomarker in bone resorption. These information suggest that cathepsin k may be a crucial target (37, 38).

Another experiment involving the treatment of mouse long bone cells such as osteoclasts and osteoblast with wortmannin (the PI3-kinase inhibitor) and calphostin C (a specific inhibitor of protein kinase C), was conducted. The results revealed the inhibitory effect on the osteoclast-recruited intracellular dispersion of cathepsin k. Similar findings were obtained when *Drynariae* Rhizoma extract was used instead of wortmannin. On the other hand, mannose-6-phosphate receptor
is involved in the re-entrance of the secreted proenzymes into cells. Thus, one more experiment was carried out to study the inhibitory effect of wortmannin and Drynariae Rhizoma extract on the possibility of this re-entrance in the absence or presence of mannose-6-phosphate. The results revealed the dose-dependent inhibitory effect on the osteoclast-reconciled intracellular dispersion of cathepsin k. Additionally, an elevated level of wortmannin and Drynariae Rhizoma potency was observed in the presence of mannose-6-phosphate. Conclusively, dose-dependent inhibition of in vitro bone resorption and cathepsin k processing by Drynariae Rhizoma was noted. In this way, Drynariae Rhizoma extract can be taken as a prodrug with bone resorption inhibiting feature, since it possesses capability of ceasing the maturing process of cathepsin k in osteoclasts-containing long bone cells (39). Later on, Jeong et al. (34) studied the effect of Drynariae Rhizoma extract on bone tissue formation, ossification, using MC3T3-E1 (non-transformed osteoblasts) and rat bone marrow cells. As a result, Drynariae Rhizoma was found to be involved in stimulation of the ALP activity and mineralization in a dose-dependent in a concentration range of 50-150 µg/mL. Moreover, significantly elevated levels of bone morphogenetic protein-2 and ALP mRNA were noted at a concentration of 100 µg/mL of Drynariae Rhizoma. Additionally, non-significant elevated levels of type I collagen mRNA were noted at a concentration of 60 µg/mL of Drynariae Rhizoma, leading to the gene expression inhibition of collagenase-1 during 15-20 days of culture. Decisively, these anabolic effects of Drynariae Rhizoma results in an increase in proliferation and differentiation of osteoblasts in vitro, revealing the bone protective activity of Drynariae Rhizoma (40).

In order to verify the in vitro results that Rhizoma Drynariae extract promotes in vitro bone cell viability through an increase in intracellular total protein, alkaline phosphatase and acid phosphatase (23), the systemic effect of Rhizoma Drynariae extract on bone structure in normal mice was examined using micro-CT scanning. Wong and Rabie used 8 week old male BALB/c mice to compare the bone structures of mice treated with and without Rhizoma Drynariae extract. The results of this in vivo study show that there is 0.25 mm enhancement in the proximal end of the left tibia of each mouse. The quantitative morphometric analysis of the bone structures revealed that there was an increase in the bone density as evident from augmented bone volume/tissue volume ratio and bone trabeculae by 6.45% and 10.00%, respectively, in Rhizoma Drynariae extract treated mice. It could be concluded from these results that the bone density can be improved by oral intake of Rhizoma Drynariae extract. This in vivo study supported the previous in vitro findings about the anabolic effect of Rhizoma Drynariae extract, i.e., this extract enhanced the bone cell activity (23, 41). In another study to test bone strengthening activity of Rhizoma Drynariae, Wong et al. investigated the systemic effect of Rhizoma Drynariae extract on bone formation in eight week old male BALB/c mice. The results showed that there is enhancement in the trabecular number and bone density by 10% and 6.45%, respectively, which reveals the change in bone histomorphology in Rhizoma Drynariae extract treated mice as compared to control group of these animals. As evident from the identification of osteoblasts and osteocytes in the newly formed bone, it was also noted that there was induction of new bone formation on the margins of the defects in Rhizoma Drynariae treated mice. It indicates the systemic effect of this extract on bone formation and healing. In addition, this activity of Rhizoma Drynariae could be due to its active constituent, naringin. Naringin has been demonstrated to be involved in the up-regulation of osteogenic factor expression, which results in angiogenesis and/or osteogenesis. As concluding remarks, the osteogenic effect of naringin and other active ingredients of Rhizoma Drynariae on fracture-prone bones including femur neck and lumbar spine may also be investigated (42).

Serum

The influence of serum medicated with different doses of RDTF (Rhizoma Drynaria total flavonoids) on the osteoblasts of newborn SD rats cultured by collagenase method, was investigated. These cells were then tested for MTT, PNPP, PI and Annexin V/PI analysis and it was observed the significantly increased proliferation and alkaline phosphatase activity in RDTF treated osteoblasts than that of the untreated osteoblasts, demonstrating the anti-osteoporosis activity in a time dependent manner (43).

Rhzoma Drynaria total flavonoid extract

RDTF are obtained through extraction of dry rhizomes of Drynaria fortunei, followed by isolation and purification (44). Naringin is the main active constituent of RDTF. The RDTF play an important role in treatment of the bones with lesions, low density and strength, increased blood viscosity, low
bone mineralization, and resorption especially post-
menopausal osteoporosis (39, 45-49).

Kang et al. prepared water and ethanol extract of
Drynariae Rhizoma to compare the extraction
efficacy of total flavonoids. Moreover, both extracts
were tested in vitro for comparing their antioxidant
and anti-osteoporosis features. The ethanol extract
showed better extraction efficacy and antioxidant
activity in comparison to that of water extract.
Additionally, the ethanol extract showed better pro-
liferation and differentiation of cultured mouse
(KP100 CD-1) osteoblastic cells in vitro. Based on
these results, it can be concluded that the ethanol
extract of Drynariae Rhizoma might be more effect-
tive for osteoporosis treatment (50).

In postmenopausal osteoporosis, Rhizoma
Drynariae is capable of sustaining the normal tra-
becular structure as well as inhibiting rate of bone
turnover through mimicking the estrogen. In addi-
tion, RDTF possess potent anti-oxidative and osteo-
protective activity, possibly through its potential of
restoring OVX-induced osteoporosis in rats.
Moreover, the increased osteoblasts proliferation as
well as the reduced osteoclasts activity in rat bones-
treated with RDTF, was observed in vitro (51).

Smad is a protein that plays a role in intracel-
lar signaling for silk threonine kinase receptors
type I and II, which are involved in the generation of
bones. In order to examine the influence of RDTF
on expression of the Smad1 and Smad5 mRNA, Zhu
et al. (52) administered RDTF to the ovariectomized
rats (except normal group consisting of non-
ovariectomized rats) in high, moderate, and low
doses. The femur bone assays of the sacrificed rats
showed that Smad1 and Smad5 were more
expressed in RDTF treated rats, as compared to nor-
mal group rats. This effect was dose independent
(52).

Pang et al. (53) conducted a study to explore
the bone protection in young ovariectomized
(C57/BL6J) mice by using RDTF based on its estro-
gen-like activity. The maximum increase in trabecu-
lar-rich bone mineral densities at distal femur and
lumbar spine in the ovariectomized mice treated
with 0.173 mg of RDTF per gram mice weight per
day, was observed. The peripheral quantitative com-
puted tomography approach was employed to study
bone mineral densities. The co-incubation of RDTF
with 17β-estradiol antagonist ICI 182, 780 in rat
osteoblast-like UMR-106 cells did not show stimu-
lation of osteoblasts by RDTF; it showed ER-
dependent osteoblastic functions of RDTF.
Moreover, transient transfection in UMR-106 cells
was also studied. It revealed that ERE-dependent
luciferase activity depended on dose of RDTF
through ER-α and ER-β. Conclusively, RDTF not
only activates ER leading to growth regulation of
the osteoblasts, but also protects mice from ovar-
ietomy-induced osteoporosis. Additionally, the post-
menopausal women with osteoporosis should also
be clinically treated to assess therapeutic efficacy of
RDTF. Such results were already published by Liu
et al., demonstrating that RDTF-3H-TdR mixture
promotes the alkaline phosphatase activity in UMR-
106 cells in a dose- and time-dependent manner in
osteoblast culture in vitro (54).

In order to provide experimental confirmation
about the mode of Rhizoma Drynariae for osteo-
porosis treatment, an interventional study was con-
ducted to study the effect of RDTF on the osteo-
clasts through detection of bone mineral density,
bio mechanics, serum cathepsin k concentration, and
cathepsin k mRNA expression in the proximal meta-
physis of theibia in 72 female Sprague-Dawley
ovariectomized rat models of osteoporosis.
Additionally, a three-point bending approach was
used for measuring the maximum load of theibia to
assess the influence of RDTF on bone strength. In
comparison to that in the estrogen and normal
groups, there were significant differences in the
bending load, serum cathepsin k concentrations, and
cathepsin k mRNA expression in the RDTF rats.
Moreover, RDTF showed a concentration-depend-
ents effect on bone mineral density (55). In a study
about interventional effect of RDTF on cathepsin k,
bone density of the ovariec tomized rat model with
osteoporosis under treatment using RDTF was
determined and found RDTF mediated-increase in
bone density, in comparison to the control group
(56-60).

Isolated compound - naringin

The leading example of flavonoid isolated
from Rhizoma Drynariae extract is naringin that
plays an important role in bone metabolism and
osteogenesis (39, 61, 62). It is capable of suppress-
ing the retinoic acid-provoked osteoporosis in rats;
enhancing expression of BMP-2 resulting in bone
formation; increasing the proliferation and
osteogenic differentiation of bone mesenchymal
stem cells in osteoporosis diseases in human (63-
65). Due to double directional adjusting effect (i.e.,
estrogenic and anti-estrogenic functions), naringin is
found to manage osteoporosis via selectively bind-
ing with estrogenic receptor (66, 67). In addition,
current studies have proved the usefulness of
naringin in ovariec tomized-induced bone loss in
mice/rats (68-71).
Clinical trials using Rhizoma Drynariae for treating osteoporosis

The fundamental remedies for osteoporosis include bisphosphonates and estrogen, but these agents have significant side effects. Therefore, natural remedies are currently being investigated, particularly from plants, since possibility of finding of natural ingredients is significantly higher in plants than indiscriminate prospect in conventional approaches, as evident from their folk use in osteoporosis. In contrast to the admired view “herbals are natural and harmless”, herbal safety is of prime importance, since some herbals have been reported to have serious side effects including hepatotoxicity. Thus, clinical usage of herbal drugs should be recommended after thorough quality control testing and standardization. Table 1 reflects the clinical trials using Rhizoma Drynariae for treating osteoporosis. It is clearly evident from most of the studies that the anti-osteoporotic activity of Rhizoma Drynariae is comparable to various standards including tibolone, estradiol valerate, medroxyprogesterone, and nilestriol, in terms of bone mass density change during study for 180 days. Moreover, these studies reported no adverse effects of Rhizoma Drynariae, except for tibolone that resulted in uterine bleeding (8.6%) (72).

CONCLUSION

The literature study proposes various modes of anti-osteoporotic activity of RDTF as given here:

Firstly, RDTF promoted the proliferation of MCF-7 (human breast cancer cell line) and ROS17/2.8 (osteoblast-like cell line), even more efficiently than estradiol and genistein showing the estrogen-like effect of RDTF. Secondly, RDTF inhibited the lacunae production in osteoclasts leading to inhibition of cellular expression of cathepsin k. Finally, RDTF inhibited transport and expression of the cathepsin k precursor in cells, with the same effectiveness as that of wortmannin. These studies verify that RDTF treats osteoporosis through inhibition of cathepsin k.

Acknowledgment

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REFERENCES


Table 1. Clinical trials using Rhizoma Drynariae for treating osteoporosis.

<table>
<thead>
<tr>
<th>No.</th>
<th>Treatment of subjects</th>
<th>Treatment group</th>
<th>Treatment duration (days)</th>
<th>Bone mass density change (g/cm²)</th>
<th>Adverse effects (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rhizoma Drynariae</td>
<td>PT</td>
<td>180</td>
<td>+0.005 (lumbar); +0.025 (femur)</td>
<td>Not observed</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>Tibolone</td>
<td>HT</td>
<td></td>
<td>+0.043 (lumbar); +0.051 (femur)</td>
<td>Uterine bleeding (8.6%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Rhizoma Drynariae</td>
<td>PT</td>
<td>180</td>
<td>+0.024 (femur)</td>
<td>Not observed</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>Estradiol valerate, medroxyprogesterone</td>
<td>HT</td>
<td></td>
<td>+0.015 (femur)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Rhizoma Drynariae</td>
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<td>180</td>
<td>+0.038 (femur)</td>
<td>Not observed</td>
<td>75</td>
</tr>
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<td></td>
<td>Nilestriol</td>
<td>HT</td>
<td></td>
<td>+0.039 (femur)</td>
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</tr>
<tr>
<td>4</td>
<td>Rhizoma Drynariae</td>
<td>PT</td>
<td>90</td>
<td>+0.103 (forearm)</td>
<td>Not observed</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>Nilestriol</td>
<td>HT</td>
<td></td>
<td>+0.056 (forearm)</td>
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Antioosteoporotic effect of the rhizome of Drynaria fortunei (Kunze)...

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