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Anti-osteoporotic effect of medical herbs and calcium supplementation on ovariectomized rats

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Abstract Estrogen deficiency and insufficient calcium in diet in postmenopause create serious problems with resultant osteoporosis and fractures. In the present study, forty adult albino rats weighting 200–220 g were used and divided into five groups. Group 1, control group included non-ovariectomized untreated rats. Group 2, ovariectomized)OVX) rats. Group 3, OVX rats daily received $CaCO_3$ (27 mg/kg b.w.). Group 4, OVX rats daily received a mixture of herbs (sage, rosemary and thyme) (30 g/kg b.w.). Group 5, OVX rats daily received both herb mixture and $CaCO_3$. The results showed significant decrease in serum calcium (Ca), inorganic phosphorus (P), total proteins (TP), calcitonin, bone mass density (BMD), reduced glutathione (GSH) and catalase (CAT) in OVX rats compared to the control group. While a significant increase in serum parathyroid hormone (PTH), osteocalcin, alkaline phosphatase (ALP) and acid phosphatase (ACP) were recorded. Also the recorded data showed increases in femur, tibia and vertebral lipid peroxidation (MAD) content and the activities of acid phosphatase and alkaline phosphatase in the ovariectomized rats. On the other hand, when OVX rats were fed on sage, rosemary and thyme supplemented diets or $CaCO_3$ in a single or in combination, the data recorded a significant improvement in all the above mentioned parameters. Finally, the results of this study indicated that the combination of common herbs (sage, rosemary and thyme) with calcium carbonate, was more effective in reduction and prevention of osteoporosis in ovariectomized rats. So, it can be concluded that consumption of mixed herbs supplemented diets might be considered as a functional food for retarding risks of osteoporosis associated with estrogen deficiency in OVX states.

Abbreviations: OVX, ovariectomized rats; Ca, calcium; P, phosphorus; ACP, acid phosphatase; ALP, alkaline phosphatase; BMD, body mass density; CAT, catalase; GSH, reduced glutathione; MAD, malondialdehyde; PTH, parathyroid hormone; OCN, osteocalcin; TP, total protein

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Introduction

Osteoporosis that is associated with ovarian hormone deficiency following menopause (postmenopausal osteoporosis) is by far the most common cause of age-related bone loss. A sharp decrease in ovarian estrogen production is the predominant cause of rapid hormone related bone loss during the first decade after menopause (Gruber et al., 1984). Traditional therapies for postmenopausal Osteoporosis have emphasized agents that inhibit bone resorption such as estrogen (Centrella and Canalis, 1985), calcitonin (Canalis et al., 1988; Rudman et al., 1981) and bisphosphonates (Bennet et al., 1984). Although the most effective method to reduce the rate of postmenopausal bone loss is estrogen replacement therapy, it may be accompanied by side effects (Genant et al., 1989) and thus recommended only for women who are at high risk of osteoporosis and who have no contraindications. Therefore, it would be most helpful to study a naturally occurring substance that minimizes bone loss in postmenopausal women. Also, recent moves away from hormone replacement therapy suggested calcium as the simplest and cheapest strategies to treat and prevent osteoporosis. (Wallace et al., 2004).

Calcium is an essential element in bone mineralization and formation being the key component of hydroxyapatite and its use as a monotherapy for osteoporosis are reviewed (Blanch and Pros, 1999; Flynn, 2003). Optimizing the dietary intake of calcium is the nutritional goal to prevent osteoporotic fractures in postmenopausal women (Deprez and Fardellone, 2003). Adding three servings of yoghurt to the daily diet of older women resulted in a significant reduction in bone resorption (Shaker et al., 2005).

There is an increasing interest in phytochemicals as new sources of natural antioxidant and antimicrobial agents. The use of synthetic antioxidants in the food industry is severely restricted as to both application and level (Tawaha et al., 2007). Currently, there is a strong debate about the safety aspects of chemical preservatives, since they are considered responsible for many carcinogenic and teratogenic attributes, as well as residual toxicity. Plant-derived polyphenols receive considerable attention because of their potential antioxidant and antimicrobial properties (Moreira et al., 2005). Phenolic compounds exhibit a considerable free-radical scavenging (antioxidant) activity, which is determined by their reactivity as hydrogen or electron donating agents, the stability of the resulting antioxidant derived radical, their reactivity with other antioxidants and, finally, their metal chelating properties (Genena et al., 2008).

Salvia officinalis L. (sage, common sage, garden sage or Dalmatian sage) *Rosmarinus officinalis* L. (Rosemary) and *Thymus vulgaris* L., (thyme) are medicinal and aromatic plants of the Lamiaceae (Labiatae) family, native to Mediterranean countries which today are cultivated all over the world (Galimuhtasib et al., 2000). Sage is a popular herb commonly used as a culinary spice for flavoring and seasoning that has also been used for centuries in folk medicine for the treatment of a variety of ailments; also it is found that sage strongly inhibits bone resorption. Sage is a herbrich ‘‘essential oils are a class of volatile oils obtained from plants, possessing the odor and other characteristic properties of the plant’’. Monoterpenes, the major components of essential oils, belong to the group of isoprenoids containing 10 C-atoms. Monoterpenes are widely distributed in the plant kingdom and are present in some herbs commonly used in human nutrition. As essential oils and monoterpenes have a pleasant odor and taste when used at low concentration, they have been extracted since ancient times also from many plants, both edible and inedible, and are used today as food additives. As essential oils and monoterpenes are lipophilic compounds, they readily cross cell membranes and are therefore absorbed through the skin and lung there; therefore, there is a long history of use of essential oils and monoterpenes for many medical applications in ointments, and bath additives to be used in the relief of head and chest colds as well as muscle pain (Abdallah et al., 2010).

Rosemary (*R. officinalis* L.) and *T. vulgaris* L., (thyme) are also spice and medicinal herbs widely used around the world. The main compounds are α-pinene, bornyl acetate, camphor and 1,8-cineole (Pintore et al., 2002).

Thyme is employed to season and suppress offensive odors, such as trimethylamine odor, in foods. The essential oil is well recognized for its medicinal properties in the treatment of bronchitis, whooping cough and tooth-ache. It is possible that the flavonoids present may be important. It was found that the main components of the essential oil were thymol and carvacrol (Porte and Godoy, 2008).

In the light of these findings, the present study was undertaken to evaluate weather intake of phytoesterogenic herbs (sage, rosemary and thyme) could play a positive role in reducing development of osteoporosis and bone loss associated with estrogen deficiency in OVX rats.

Materials and methods

Experimental animals

Forty adult female albino rats (*Rattus rattus*) weighing 200–220 g were used for experimentation. Animals were provided with a balanced standard diet and water ad-libitum. The animals were randomly divided into 5 groups each of 8 rats: