The Potential Effects of Reishi Mushroom (Ganoderma lucidum) Consumption on Bone Health Indices and Serum Minerals Profile Disorders Induced by CCl₄ on Rats

Yousif A. Elhassaneen*, Sherif S. Ragab and Mona S. Salman

Department of Nutrition and Food Science, Faculty of Home Economics, Minoufiya University, Shebin El-Kom, Egypt.

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The modern pharmacological therapy is costly and associated with multiple side effects resulting in patient non-compliance. Thus there is a need to explore alternative therapies particularly from herbal sources as these are cost effective and possess minimal side effects. Therefore, the purpose of this study was to elucidate the potential effects of reishi mushroom (Ganoderma lucidum) consumption on bone indices and serum minerals profile disorders induced by CCl₄ on rat. Treatment of animals with CCl₄ caused a significant decreased (p≤0.05) in serum minerals profile (Fe, -31.14%, Ca, -21.36% and P, -25.76%) and bone health indices [bone mineral density (BMD), -19.20%, %), bone G-protein (BG-P), -37.54%and bone mineral content (BMC), -30.56%] compared to normal controls. Supplementation of the rat’s diet with reishi mushroom powder (RMP) (1.0 to 5.0 g/100g) improved their serum minerals content and bone indices. The rate of improving was elevated with the increasing of the RMP concentration. In conclusion, the present work has been undertaken to present scientific information on an medicinal plant, reishi mushroom, which has been documented for its antiosteoporotic activity. So, we recommended RMP by concentrations ranged 1-5% % amount to be included in our daily diets, drinks and food supplementation.

Keywords: Iron, calcium, phosphorus, bone mineral density, bone mineral content, Bone G-protein.

INTRODUCTION

The reishi mushroom (Ganoderma lucidum) is a white rot, wood-decaying fungus that is classified within the family Ganodermaceae of Polyporales which show hard fruiting bodies. The Latin word lucidus means “shiny” or “brilliant” and refers to the varnished appearance of the surface of the mushroom. In China, G. lucidum is called lingzhi, whereas in Japan the name for the Ganodermataceae family is reishi or mannentake (Leskosek-Cukalovic et al., 2010). Nutritional studies indicated that G. lucidum contains mainly protein, fat, carbohydrate and fiber (Stamets, 2000; Hung and Nhi, 2012). Artificially cultivated variety has similar contents of nutritional components compared with wild types, and the extraction significantly increases the amounts of crude protein and carbohydrates and deleted crude fiber. Mizuno (1995) reported the composition of G. lucidum extract (% of dry weight), which consisted of folin-positive material (68.9%), glucose (11.1%), protein (7.3%), and metals (10.2%) (K, Mg, and Ca are the major components with Ge having the 5th highest metal concentration at 489 mg/g). However, there are qualitative and quantitative differences in the chemical composition of G. lucidum products depending on the strain, origin, extracting process, and cultivation conditions (Hobbs, 1995; Stamets, 2000; McKenna et al., 2002; Hung and Nhi, 2012).

Beside the all mentioned nutrients, the fruiting body, mycelia, and spores of G. lucidum contain approximately 400 different bioactive/phytochemical compounds, which mainly include triterpenoids, polysaccharides, nucleotides, sterols, steroids, fatty acids, proteins/peptides, and trace elements (Mizuno,
Amongst all of these compounds, the triterpenoids and polysaccharides are occupied the central position as the most biologically active compounds. Triterpenoids extracted from G. lucidum are reported to be responsible for many of the pharmaceutical properties of the fungus. Thus far, hundreds of triterpenoids have been isolated in G. lucidum and many more are likely to be discovered in the future. Two major types of triterpenoids are ganoderic acids (C_0) and lucidenic acids (C_2), with the total triterpenoid content in G. lucidum ranging from 0.6 to 11 mg/g of dry powder (Kim and Kim, 1999; Min et al., 1999; Boh et al., 2007).

These triterpenoids were reported to mitigate diabetes and regulate inflammatory pathways in cell culture (McKenna et al., 2002). Triterpenoids from G. lucidum also possess significant chemo-therapeutic potential and exhibit cytotoxic effects on colon carcinoma cells (Ruan and Popovich, 2012). It has been reported that triterpenoid fraction of G. lucidum containing ganoderic acid F affect on activities tumor growth and metastasis which were mainly due to inhibition of tumor-induced angiogenesis (Gao et al., 2002). Also, triterpenoids, such as ganoderic acids T–Z isolated from G. lucidum, showed cytotoxic activity in vitro on hepatoma cells (Toth et al., 1993).

Other studies report that different triterpenoids of G. lucidum have strong anti-HIV-1 protease activity and that triterpenes such as ganodermic acids C and D inhibit histamine release (Boh et al., 2007). Moreover, triterpenoids of G. lucidum have been reported to exert various enzyme inhibitory activities. Inhibitors of farnesyl protein transferase (FTP) have been demonstrated to inhibit Ras-dependent cell transformation and thus represent a potential therapeutic strategy for the treatment of human cancers (Lee et al., 1998). For the polysaccharides, more than 100 types have been isolated from the fruiting body, spores, and mycelia, or separated from the broth of a submerged liquid culture of G. lucidum (Wasser, 2005). G. lucidum polysaccharides such as β-D-glucans, heteropolysaccharides, and glycoprotein have been isolated, characterized, quantified at 10–50% in dry weight and are considered the major contributors of bioactivity of the mushroom (Chen et al., 1998; Cheong et al., 1999; Boh et al., 2007). Polysaccharides of G. lucidum also have cancer-fighting properties owing primarily to modulation of the immune system and cellular protection from free radicals (Chen and Miles, 1996; Han et al., 1998). Also, Polysaccharides (β-D-glucans, heteropolysaccharides, and glycoproteins) isolated from G. lucidum demonstrated antitumor activity against Sarcoma 180 in mice (Wasser, 2002).

Extracts from G. lucidum (e.g., polysaccharide fractions, manethonic extracts, and LZ-8) have mitogenic effects on mouse splenocytes and human peripheral blood mononuclear cells (PBMCs) in the presence of various immunostimulating or immunosuppressive agents (e.g., PHA and 12-O-tetradecanoylphorbol 13-acetate) (Mao et al., 1999). Moreover, Animal studies have demonstrated that the polysaccharide fractions of G. lucidum have potential hypoglycemic and hypolipidemic activities. Research with G. lucidum on diabetic mice indicates that free radical scavenging of polysaccharides protects pancreatic islets from oxidative stress. This finding is significant because it suggests that G. lucidum may have therapeutic benefits in the treatment of type 2 diabetes (Gao et al., 2004; Jia et al., 2009). For the unique content of bioactive compounds and their biological roles, G. lucidum has been reported to have a number of pharmacological effects including immunomodulating, antiatherosclerotic, anti-inflammatory, analgesic, chemopreventive, antitumor, radioprotective, sleeppromoting, antibacterial, antiviral (including anti-HIV), hypolipidemic, antifibrotic, hepatoprotective, diabetic, antioxidative and radical-scavenging, anti-aging, hypoglycemic, and anti-ulcer properties (reviewed in Liu, 1999; Wasser, 2005). But there is a dearth of information regarding the relationship between the consumption of G. lucidum and the bone health. Therefore, the purpose of this study was to elucidate the potential effects of reishi mushroom (G.lucidum) consumption on bone health indices and serum minerals profile disorders induced by CCl_4 on rats.

MATERIALS AND METHODS

Materials

Dried fruits of reishi mushroom (Ganoderma lucidum) were obtained as a donation from the Agriculture Research Center (ARC), Ministry of Agriculture, Cairo, Egypt. Casein was obtained from Morgan Chemical Co., Cairo, Egypt. All organic solvents and other chemicals were of analytical grade were purchased from El-Ghomhorya for Drug, Chemical and Medical Instrumental Trading Co. (Cairo, Egypt).

Reishi mushroom powder (RMP)

Dried fruits of reishi mushroom were ground into a fine powder in high mixer speed (Moulinex Egypt, Al-Araby Co., Egypt). The material that passed through an 80 mesh sieve was retained for use.

BIOLOGICAL EXPERIMENTS

Animals

Animals used in this study, adult male albino rats (130-150g per each) were obtained from Helwan Station, Ministry of Health and Population, Helwan, Cairo, Egypt.

Basal Diet

The basic diet prepared according to the following formula as mentioned by (AIN, 1993) as follow: protein (10%), corn oil (10%), vitamin mixture (1%), mineral mixture (4%), choline chloride (0.2%), methionine (0.3%), cellulose (5%), and the remained is corn starch (69.5%). The used vitamin mixture component was that recommended by (Campbell, 1963) while the salt mixture used was formulated according to (Hegsted, 1941).

Experimental design

All biological experiments performed a complied with the rulings of the Institute of Laboratory Animal Resources, Commission on life Sciences, National Research Council (NRC, 1996). Rats (n=35 rats), 130-150g per each, were housed individually in wire cages in a room maintained at 25 ± 2 °C, relative humidity (55±5%), a 12-hr lighting cycle and kept under normal healthy conditions. All rats were fed on basal diet for one-week before starting the experiment for acclimatization. After one week period, the rats were divided into two main groups, the first group (Group 1, 5 rats) still fed on basal diet and the other main group (30 rats) was injected by CCl_4 for two weeks to induce liver impaired rats then classified into sex sub groups as follow: group (2), fed on standard diet only as a positive control; group (3), fed on standard diet containing 1.0
% RMP; group (4), fed on standard diet containing 2.0 % RMP; group (5), fed on standard diet containing 3.0% RMP; group (6): fed on standard diet containing 4.0% RMP and group (6): fed on standard diet containing 5.0% RMP.

**Blood sampling**

At the end of experiment period, 4 weeks, blood samples were collected after 12 hours fasting using the abdominal aorta and rats were scarified under ether anesthetized. Blood samples were received into clean dry centrifuge tubes and left to clot at room temperature, then centrifuged for 10 minutes at 3000 rpm to separate the serum according to Drury and Wallington, (1967). Serum was carefully aspirate, transferred into clean covet tubes and stored frozen at -20°C until analysis.

**Serum mineral content**

Iron (Fe), calcium (Ca) and phosphorous (P) content in plasma samples were determined by the adaptation the method mentioned by Singh et al., (1991). One hundred μl of plasma sample were transferred into a digested glass tube and 2 ml of tri-acids mixture (containing nitric acid: perchloric acid: sulfuric acid in the ratio of 20: 4: 1 v/v respectively) were added to each tube. The tubes content were digested gradually as follow, 30 min at 70 °C; 30 min at 180 °C and 30 min at 220 °C. After digestion, the mixture was cooled, dissolved in MilliQ water, and the volume was increased to 10 ml in volumetric beaker. After filtration in ashless filter paper, aliquots were analyzed for Na and K content using of atomic absorption spectrophotometer, type Perkin - Elmer, Model 2380.

**Bone analysis**

In rats, bone mineral density (BMD) was measured by DEXA scans or abdominal computed tomography (CT) scans via the Siemens method and presented as T score (normal >-1, osteopenia=-1 to 2.5, osteoporosis <2.5) according to Andreas et al., (2014). Bone mineralization, bone mineral content (BMC), of rat femora were determined by μCT scans (μCT40, Scanco Medical AG, Wangen-Brüttisellen, CH) such as mentioned in Andreas et al., (2014). Bone G protein (BG-P) were determined such as mentioned by Linkhart et al., (1996).

**Statistical Analysis**

All measurements were done in triplicate and recorded as mean±SD. Statistical analysis was performed with the Student t-test and MINITAB 12 computer program (Minitab Inc., State College, PA).

**RESULTS AND DISCUSSION**

**Effect of RMP consumption on serum minerals profile of rats treated with CCl4**

Serum minerals profile of rats injected CCl4 and consumed RMP were shown in Table (1). From such data it could be noticed that treatment of animals with CCl4 caused a significant increased (p≤0.05) in Fe (-31.14%), Ca (-21.36%) and P (-25.76%) compared to normal controls. Supplementation of the rat diets with RMP (1.0 to 5.0 g/100g) prevented the rise of mean serum Fe, Ca and P activities. The rate of preventative was increased with the increasing of the RMP concentration. Such as shown in Figure (1), the rate of increasing in the liver enzymatic activities were recorded -25.14, -17.65, -13.38, -7.30 and -4.24 % (For Fe); -19.73, -17.80, -12.46, -7.32 and -4.45% (for Ca) and -23.48, -17.42, -14.39, -9.09 and -6.82% (for P) with the rat diets supplemented by 1.0, 2.0, 3.0, 4.0 and 5.0 g/100g of RMP, respectively. Such as mentioned by Linder (1991) after carbon, hydrogen, oxygen, and nitrogen, those elements required in greatest abundance in the daily diet are calcium, phosphorous, sulfur, potassium, sodium, chlorine, and magnesium. Numerous biochemical and physiological processes require or are modulated by phosphorus. Phosphorus is an essential component of bone mineral, where it occurs in the mass ratio of 1 phosphorus to 2 calcium.

Approximately 85% of the phosphorus in the adult body is found in bone. Phosphorus also plays an important role in many and varied chemical reactions in the body. It is present in soft tissues as soluble phosphate ion; in lipids, proteins, carbohydrates, and nucleic acid in an ester or anhydride linkage; and in enzymes as a modulator of their activities. Energy for metabolic processes drives largely from the phosphate bonds of adenosine triphosphate (ATP), creatine phosphate, and similar (Avioli, 1988). Also, phosphorus is a component of every cell and other important compounds including DNA and RNA which are responsible for cell growth and repair, part of phospholipids present in every cell membrane in the body, a major component of bones and teeth and important for pH regulation (Veot and Veot, 1990). Calcium is concentrated in the organelles and blood, and is also very important in the structure of the skeleton and in the maintenance of the extracellular fluid calcium concentration (reviewed in Eastwood, 1997). Also, iron is a constituent of hemoglobin, myoglobin, and number of enzymes and, therefore, is an essential nutrient of humans (Veot and Veot, 1990). In addition to these functional forms, as much as 30% of the body iron is found in storage forms such as ferritin and hemosiderin (mainly in the spleen, liver and bone marrow), and a small amount is associated with the blood transport protein transferring (reviewed by Wang, 2009).

**Effect of RMP consumption on bone health indices of rats treated with CCl4**

Bone health indices of rats injected CCl4 and consumed RMP were shown in Table (2). From such data it could be noticed that treatment of animals with CCl4 caused a significant decreased (p≤0.05) in Bone mineral density (BMD, -19.20%), Bone g- protein (BG-P, -37.54%) and Bone mineral content (BMC, -30.56%) compared to normal controls. In similar study, Andreas et al., (2014) reported that mice with chronic liver diseases induced by CCl4 frequently exhibit decreased bone mineral densities (BMD), which is defined as hepatic osteodystrophy (HOD). Supplementation of the rat diets with RMP (1.0 to 5.0 g/100g) prevented the lower of mean bone BMD, BG-P and BMC indices. The rate of preventative was increased with the increasing of the RMP concentration. Such as shown in Figure (2), the rate of increasing in the bone indices were recorded -16.00, -11.20, -7.20, -5.60 and -1.60% (For BMD); -32.68, -18.87, -12.40, -7.88 and -6.93% (for BG-P) and -25.00, -22.22, -13.89, -8.33 and -8.33% (for BMC) with the rat diets supplemented by 1.0, 2.0, 3.0, 4.0 and 5.0 g/100g of RMP, respectively.
Table 1: Effects of RMP consumption on CCl₄ induced changes in serum minerals profile

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<thead>
<tr>
<th>Groups</th>
<th>Control (-)</th>
<th>Control (+)</th>
<th>RMP (%)</th>
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<td>Fe (µg/dl)</td>
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<td>Mean*</td>
<td>98.24</td>
<td>67.65</td>
<td>73.54</td>
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<td>SD</td>
<td>4.89</td>
<td>1.98</td>
<td>7.03</td>
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<td>% of Change</td>
<td>0.00</td>
<td>-31.14</td>
<td>-25.14</td>
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<td>Ca (mmol/l)</td>
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<tr>
<td>Mean*</td>
<td>2.022</td>
<td>1.590</td>
<td>1.623</td>
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<tr>
<td>SD</td>
<td>0.120</td>
<td>0.210</td>
<td>0.210</td>
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<td>% of Change</td>
<td>0.00</td>
<td>-21.36</td>
<td>-19.73</td>
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<td>P (mmol/l)</td>
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<tr>
<td>Mean*</td>
<td>1.32</td>
<td>0.98</td>
<td>1.01</td>
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<td>SD</td>
<td>0.12</td>
<td>0.09</td>
<td>0.07</td>
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<tr>
<td>% of Change</td>
<td>0.00</td>
<td>-25.76</td>
<td>-23.48</td>
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* Each value represents the mean of the replicates. Values with the different letters in the same row are significantly different P≥0.05

Figure 1: Effects of RMP consumption on CCl₄ induced changes in serum minerals profile (as a % of control)
### Table 2: Effects of RMP consumption on CCl4 induced changes in bone health indices

<table>
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<th>Groups</th>
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<th>Control (+)</th>
<th>RMP (%)</th>
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<td></td>
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<td>Mean</td>
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<tr>
<td>Bone mineral density (BMD, g/cm²)</td>
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<td>0.125&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
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<td>0.101&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>0.105&lt;sup&gt;c&lt;/sup&gt;</td>
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<td></td>
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<td>0.111&lt;sup&gt;bc&lt;/sup&gt;</td>
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<td>0.116&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>0.118&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>0.123&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>Bone G- protein (B G-P, ng/ml)</td>
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<td>24.11&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>15.06&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>16.23&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>19.56&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
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<td>21.12&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>22.21&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>22.44&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Bone mineral content (BMC,g)</td>
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<td>0.36&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>0.28&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>0.33&lt;sup&gt;a&lt;/sup&gt;</td>
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* Each value represents the mean of the replicates. Values with the different letters in the same raw are significantly different P≥0.05

**Figure 2:** Effects of RMP consumption on CCl₄ induced changes in bone health indices (as a % of control)*

* BMD, bone mineral density; BG-P, bone G- protein; BMC, bone mineral content
In general, the degradation in bone indices (BMD, BG-P and BMC) in animals treated with CCl4 is inherent similarity of the symptoms of osteoporosis. It is one of the most widespread metabolic bone disorders affecting one in three women and one in twelve men at some point in their lives (Liggett and Reid, 2000; Henry, 2001). According to the WHO “Osteoporosis is a disease characterized by low bone mass and microarchitectural deterioration of bone tissues, leading to enhanced fragility and consequent increase in fracture risk that results in fractures with minimal trauma” (Meryl, 1997).

Several factors such as genetic, nutritional and lack of exercise etc., along with aging have been shown to be risk factors in the aetiology of osteoporosis (Malhotra and Mithal, 2008). With aging, however, an erratic absorption of calcium from gut disturbs the calcium homeostasis leading to an imbalance in the calcium regulating hormones (parathyroid hormone and calcitonin) and thereby increase bone turnover (Shoback et al., 1993) Osteoblastic activity and calcium absorption from the gut also suffers with the age (Tanna, 2005). In addition to menopause and aging, hereditary factors, lack of exercise or immobilization, lifestyle, prolonged steroid administration, excessive diet, alcohol intake, smoking, thyroxin therapy and geographical variations are the major causes of osteoporosis, among which lifestyle changes, diet and oestrogen deficiency are modifiable factors, whereas hereditary factors are non-modifiable (Ferguson, 2004). Genetic factors responsible for the onset of osteoporosis can be related to family history, small body frame, skin type, low stature, early grey hair and white women (Kumar and Clark, 2002). In men, osteoporosis can be linked to decreased testosterone levels or loss of long term remodeling efficiency (Gali, 2001).

The earlier treatment regimens for postmenopausal osteoporosis suggested prevention by classical hormone replacement therapy (HRT) which has today become obsolete. Recently, a clinical trial with HRT in healthy postmenopausal women was stopped by the American National Institute of Health as the increase of cardiovascular and mammary cancer risks under HRT far outweighed the benefits, namely a reduction of hip fractures (antioestoporetic effect) and of colon cancer (Rossoouw et al., 2002). Besides HRT, many pharmacological agents, used to manage the osteoporosis act by decreasing the rate of bone resorption, thereby slowing the rate of bone loss or by promoting bone formation. Synthetic agents like calcium carbonate (calcium consumption), vitamin D supplements,Raloxifene and Droloxifene (selective estrogen receptor modulators), calcitonin, bisphos- phonates, sodium fluoride (increases trabecular bone mineralization), along with physical activity to strengthen muscles, stimulate osteoblast formation and prevent resorption (Gali, 2001). They are also however associated with side effects such as hypercalcaemia, hypercalciuria, increased risk of endometrial and breast cancer, breast tenderness, menstruation, thromboem- bolic events, vaginal bleeding, hot flashes, dyspepsia and gastrointestinal ulcers (Canalis et al., 1988; Genant et al., 1989) further, the lack of direct head to head trials of treatments for osteoporosis, with reduction in fractures as an end point, makes it difficult to determine the relative efficacy of the different treatments (Malhotra and Mithal, 2008).

To overcome the wide range of side effects produced by these synthetic drugs, there is an increasing demand for ‘green medicines’ which are thought to be healthier and safer for the treatment of osteoporosis. The phytoestrogens, which are known to bind to the estrogen receptor sites of the cell and trigger the components and processes of estrogenic activity, have a promising role in the treatment of osteoporosis (Adams, 1989). The isoflavonoids are among the most active phytoestrogens in the flavonoid class. Ipri flavone, a synthetic flavonoid derivative (Agnusdei et al., 1989) has been found to be effective in preserving bone mass in several models of experimental osteoporosis (Benvenut et al., 1991). The isoflavones found in soybeans, such as genisten, were found to prevent bone loss in the ovariectomized rat model of osteoporosis (Bahram et al., 1996; Fanti et al., 1998). *Pleurotus eryngii* or king trumpet mushroom or king oyster mushroom, belonging to Family Pleurotaceae, is an edible mushroom, growing in the greater Mediterranean area, in close association with several genera of plants in the Family Apiaceae (Zervakis et al., 2001).

Oral treatment with aqueous extract of *P. eryngii* at the dose of 0.4 ml/day to bilaterally ovariectomized rats for 4 weeks stimulated the activity of bone forming osteoblasts via increasing serum alkaline phosphatase (ALP) activity and osteoprotegerin (OPG) gene expression level, while inhibiting the generation and activity of bone resorbing osteoclasts via decreasing the number of tartrate resistant acid phophatase (TRAP) (+) multinucleated cells and resorption areas. In addition, it was demonstrated that *P. eryngii* attenuated the progress of bone loss in rats with ovariectomy-induced osteoporosis. Although the active substances of *P. eryngii* have not yet been identified, it is suggested that *P. eryngii* contains substances that have the potential to enhance bone metabolism (Kim et al., 2006). An in vitro study also confirmed the proliferating and differentiating effect of *P. eryngii* aqueous extract on osteoblast cells (Kim et al., 2006). Also, Kunyoshi et al., (2006) found that ethanol extract from the fruiting body of *G. lucidum* showed significant effects on the proliferation of MCF-7 cells. This proliferation effect is related to the estrogenic activity of *G. lucidum*, because this proliferation activity was inhibited by the addition of the antiestrogenic compound 4(ICI182,780). The ethanol extract of *G. lucidum* prevented ovariectomy-induced bone loss and decreased the concentration of osteocalcin in the blood serum, similar to the action of 17β-estradiol. These data provide evidence that the ethanol extract of *G. lucidum* protects against bone loss caused by estrogen deficiency, without a substantial effect on the uterus. These results showed that *G. lucidum* might be a useful ingredient for the treatment of sex-hormone- related aging diseases such as osteoporosis.

**CONCLUSION**

The present work has been undertaken to present scientific information on a medicinal plant, reishi mushroom (*G. lucidum*), which has been documented for its antioestoporetic activity. The mechanism of action could be induced by either bind with estrogen receptors which exhibit responses at the cellular and molecular levels, or its act by improving the serum minerals content related to the bone health such Fe, Ca and P. Therefore, we recommended like of that plant part (RMP) by a 5%% amount to be included in our daily diets, drinks and food supplementation process.

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