Australian Journal of Basic and Applied Sciences, 3(2): 977-981, 2009
ISSN 1991-8178

Therapeutic Efficacy of Garlic Oil with 1,25 Dihydroxy Vit D_3 and Calcium in Osteoporotic Ovariectomized Rats

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Abstract: Objective: To investigate the therapeutic efficacy of synergestic effect of garlic oil, 1,25 dihydroxy vit-D3 and calcium in osteopenic ovariectomized rats. Materials and Methods: Forty female albino rats were used in the study. They fed a standard chow throughout the study. They were divided into 5groups: Sham operated control group (SH), ovariectomized group (OVX), OVX received garlic oil, OVX received combined drug of Ca and vit. D3 orally, and OVX coadministered with garlic oil, Ca+vit. D3. All the groups were tested for bone turnover parameters (serum osteocalcin and alkaline phosphatase), serum estradiol level and serum calcium & phosphorus. The excised femur bones were subjected for DEXA analysis. Results:In the OVX group mean serum calcium level was significantly decreased but improvement was obtained in the treated animal groups although the change was not statistically significant. Significant decrease in the mean serum estradiol level and significant increase in the mean serum levels of both osteocalcin and alkaline phosphatase were noticed in the OVX group compared to the SH control group. Significant improvement in all these parameters were obtained in the treated groups, but better improvement was obtained when garlic oil was combined with Ca and vit.D.

Key words: Osteoporosis, ovariectomized rats, garlic oil , sham operation, calcium and vit.D.

INTRODUCTION

The vitamin D endocrine system plays pivotal roles in calcium homeostasis and bone mineral metabolism. Vitamin D is a prohormone which is converted into its active hormonal form 1,25 $(oH)_2$ D_3 by 1α hydroxylation in the proximal tubular cells of the kidney. It activates its cellular receptor (VDR) which then activates target genes to engender its biological actions such as intestinal calcium absorption, control of bone remodelling and suppression of parathyroid hormone function (hormone production and cell growth) (Kohupillai, 2008).

A sharp decrease in ovarian estrogen production is the predominant cause of rapid hormone – related imbalance of calcium and subsequent bone loss during the first decade after menopause (Arjmandi *et al*., 1993 and Riis, 1996).

There is evidence that estrogen may be involved directly in determining intestinal calcium absorption.

Also, The available data indicate that the decrease in the basal levels of 1,25 dihydroxyvitamin D_3 could not solely account for the decrease in calcium absorption suggesting that the intestine of elderly or ovariectomized women is resistant to 1,25 $(oH)_2$ D_3 (Mukherjee *et al*., 2006).

Estrogen administration was shown effectively to restore the normal responsiveness of the intestine to 1,25 $(oH)_2$ D_3 in ovariectomized premenopausal women (Gennan *et al*., 1990) and in postmenopausal women (Civilelli *et al*., 1988 and Heaney *et al*., 1998), through up regulating vitamin D receptor (VDR) expression in the duodenal mucosa (Lie *et al*., 1999).

The hydroxylation of vitamin D in the kidney was also found to be modulated by estrogen (Pike *et al*., 1978).

The prospect that phytoestrogens (weak estrogens found in plants or derived from plant precursors by human metabolism) may have similar beneficial effects to mammalian estrogens on bone is an exciting one. The *in vitro* and animal data support the hypothesis that the effects of these compounds are due to their similar structure to estrogen and their binding affinity to both estrogen receptors α and β (Dalais, 2001 and Das *et al*., 2005).

Garlic (Allium Sativum Linn.), a common ingredient of food with many medicinal effects was found to be a substantial contributor of phytoestrogens (Horn- Ross *et al*., 2000).

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Oil extract of garlic was proved to have a therapeutic potential in preventing ovarian hormone insufficiency induced osteoporotic changes in an ovariectomized rat model of osteoporosis (Mukherjee *et al*., 2004). The significant role of garlic in maintaining skeletal health is possibly by promoting intestinal transference of calcium through the partial revival of serum estrogen titer (Mukherjee *et al*., 2006).

The aim of our work is to investigate the therapeutic efficacy of garlic oil combined with 1,25 dihydroxy vitamin D3 and calcium in osteoporotic ovariectomized rats.

MATERIALS AND METHODS

I-Animals and Diet:

This study was carried out with 40 female white albino rats weighing 150-200g. They were reared and maintained individually in an environmentally controlled animal laboratory (12h light / dark schedule at 25±2°C).

They were fed a standard chow throughout the study. The animals were either subjected to bilateral ovariectomy (OVX) or to sham operation (SH) under ether anesthesia (Goseki *et al*., 1996).

The beginning of therapy started 3 months after OVX and sham operations.

The guidelines of the ethical care and treatment of the animals followed the regulations of the ethical committee of NRC.

The animals were divided into 5 groups 8 rats in each group .

Group I: Sham operated rats (SH) as control group received corn oil 100mg/kg body weight /day orally.

Group II: Bilaterally ovariectomized rats (OVX) received corn oil 100 mg/kg body weight/day orally.

Group III: Bilaterally ovariectomized rats (OVX) received garlic oil 100mg/kg body weight /day orally. (Mukherjee *et al*., 2006).

Group IV: Bilaterally ovariectomized rats (OVX) received combined drug of Ca and vit D3 of a dose 250 ug/kg body weight / day orally.

Group V: Bilaterally ovariectomized rats received garlic oil (100 mg/ kg body weight) + combined drug vit. D3 and Ca (250 ug/kg body weight) / day orally.

Ii-sample Collection:

Blood Collection:

After 6 months post-operative i.e 3 months after starting the treatment animals were anaesthetized by diethyl ether and the blood was withdrawn from the optical vein Cocchetto, & Bjornsson 1983). The samples were collected in clean polypropylene tubes, left to clot at 37°C for 10 minutes, then centrifuged and sera were separated. All samples were frozen at -20°C until used.

Biochemical Parameters Measured:

Serum osteocalcin: was measured using ELISA technique (kits derived from immuno-diagnostic systems limited UK (Price *et al*., 1981).

Serum estradiol hormone was measured using ELISA technique a kit derived from (Adaltis Italia, Italy), according to the method of Ratcliff & Carter, 1988.

Serum calcium and phosphorus were measured colorimetrically (kits derived from centronic Gmbh-Germany) according to Biggs& Moorehead, 1974 and Henry, 1974.

Serum alkaline phosphatase was measured using spectrophotometric procedure, Biodiagnostic comp. (Belfield& Golderg, 1971).

III-DEXA Analysis for Bones:

The rats were dissected and the excised femurs were cleaned from the surrounding musculature. These bones were weighed and then stored in saline solution to enhance scanning resolution using Norland XP-46 (3.9.6 / 2.3.1).

Statistical Analysis:

Statistical analysis of the results was carried out using the standard computer program SPSS (V.904, Echo soft corporation USA 1998). Normally distributed results were compared using student "t" test. Differences among groups were evaluated using one way ANOVA. Results were expressed as mean ±SD. "P" values less than 0.05 were considered to be significant.

RESULTS ANS DISCUSSION

Biochemical Parameters:

Results of the serum biochemical parameters of the different studied animal groups are presented in table (1). Mean serum calcium level was significantly decreased in the ovariectomized group compared to the sham control group. This decrease was improved in the treated animal groups although the improvement was not statistically significant.

There was no significant changes in the mean serum phosphorus level between the different studied groups.

On the contrary compared to the sham control group, ovariectomized rats showed significant increase ($P<0.01$) in the mean serum levels of both ostecoalcin and alkaline phosphatase which indicates increased bone turnover rate. This increase was significantly corrected in all treated animal groups but best results were obtained in the group supplied with a combination of calcium, vitamin D_3 and garlic oil.

Ovariectomy also caused significant marked reduction is the mean serum estradiol level, this reduction was significantly improved after treatment with garlic oil, but better improvement was obtained when garlic oil co-administered with vitamin D_3 and calcium.

Bone Parameters:

Results of bone parameters are presented in table (2). Compared with the sham-operated control group, the ovariectomized group of animals showed significant decrease in the total bone mineral density (BMD) of the right femur. Results further proved that only the treatment of the ovariectomized rats with a combination of calcium, vitamin D and garlic oil could significantly elevate the BMD. No change was obsderved as regard BMD in the proximal region in all groups.

As regard BMD in the distal region of the right femur there was significant decrease in the ovariectomized group compared to the sham control group. Best results were obtained in case of supplementation of garlic oil in combination with calcium and vitamin D_3, where there was effective increase in the distal BMD.

Bone mineral content (BMC) of the proximal, distal and total regions showed a significant decrease in the ovariectomized group compared to the sham control group, this decrease was significantly improved after co- administration with calcium, vitamin D and garlic oil.

Table. 1: Serum biochemical parameters in the different studied groups.

	SH	OVX	OVX + garlic	OVX+Ca +vit.D	OVX+ garlic +ca with vit. D
Calcium mg/dl	8.97±0.41	7.50[a] ± 0.95	8.39±0.29	8.33±0.31	8.73±0.32
Phosphorus mg/dl	5.15±0.08	5.22±0.14	5.38±0.18	5.23±0.15	5.26±0.22
Osteocalcin ng/L	15.8±0.35	20.13[a]±1.25	17.46[b]±0.31	16.133[b]±0.36	15.7[b]±0.63
Alkaline Phosphatase U/L	138.66±4.82	171.24[a]±10.70	142.5[b]±8.21	145.41[b]±7.80	132.0[b]±8.10
Estradiol pg/ml	80.6± 7.30	15.30[a]±4.20	37.80[a b]±9.30	19.42[a b]±6.50	40.2[a b]±8.80

Values are given as mean SD for 8 rats in each group.
a: Significant difference at $P<0.01$ compared to sham group.
b: Significant difference at $P<0.01$ compared to OVX group.

Table. 2: Bone mineral content (g), mineral density (g/cm^2) in proximal, distal and total regions of the femur in the different studied groups.

	SH	OVX	OVX + garlic	OVX+Ca.+ vit. D	OVX+ galic + Ca with vit.D
BMC (proximal)	0.063± 0.004	0.048 [a]± 0.005	0.052±0.002	0.053±0.005	0.060 [b]±0.006
BMC (distal)	0.068± 0.003	0.050 [a]± 0.052	0.057± 0.070	0.055± 0.090	0.066 [b]± 0.087
BMC (total)	0.190± 0.006	0.120 [a]± 0.102	0.163± 0.004	0.150± 0.400	0.188 [b] ± 0.007
BMD (proximal)	0.160± 0.004	0.113± 0.009	0.128± 0.011	0.126± 0.005	0.138± 0.006
BMD (distal)	0.168± 0.003	0.100 [a]± 0.009	0.132±0.032	0.130± 0.098	0.164 [b]± 0.041
BMD (total)	0.141±0.031	0.103 [a]± 0.011	0.109± 0.160	0.109± 0.010	0.144 [b]± 0.033

Values are given as mean SD for 8 rats in each group.
a: Significant difference at $P<0.01$ compared to SH group.
b: Significant difference at $P<0.01$ compared to OVX group.

Discussion:

Because of its incidence and the health-related problems it gives rise to , postmenopausal osteoporosis has become a social problem requiring appropriate management strategies. Replacement therapy is effective for both prevention and therapy, but recent findings have shown that its long term administration is not safe as was previously thought (Chiechi & Micheli, 2005).

Also the efficacy and safety of several vitamin D metabolites and analogs, including the active hormonal form of vitamin D_3 (1, 25 – (oH) $_2$ D_3) have been tested in women with postmenopausal osteoporosis. Each of these metabolites and analogs has a narrow therapeutic window for the prevention and treatment of osteoporosis (Sairanen *et al*., 2000).

Therefore our work aimed to study the efficacy of supplementation of garlic oil together with vitamin D_3 and calcium in suppressing ovariectomy induced bone resorption.

Our experimental situation of bilateral ovariectomy demonstrated marked reduction in the mean serum estradiol level in the OVX group compared to the SH control group. Significant recovery of serum estradiol level was observed in the groups treated with garlic oil or with the combination of vitamin D_3, calcium and garlic oil. This is in accordance to a study done by Mukherjee *et al*., 2006 who demonstrated that supplementation of an oil extract of garlic in an ovariectomized rat model of osteoporosis is effective in restoring significantly the reduced serum estradiol level. They explained that the phytoestrogenic active principle in the oil extract of garlic possibly stimulated estrogen synthesis at extragonadal sites. Such extragonadal estrogen synthesis finds support from earlier observations that circulating estrogen concentration elevates gradually with time after ovariectomy in rats (Zhao *et al*., 2005).

Furthermore even in postmenopausal women, estradiol is produced in a number of extragonadal sites and acts locally at these sites. These sites include the mesenchymal cells of adipose tissue, osteoblasts, chondrocytes of bone, numerous sites in the brain and breast vasculature (Simpson & Davis, 2001 and Simpson *et al*., 2001).

Our results also proved reduced BMD resulting from increased bone turnover rate as indicated by the higher serum osteocalcin and serum alkaline phosphatase level in the OVX group compared to the SH control group. These results are in agreement with those of Goseki *et al*., 1996 and Mukherjee *et al*., 2004, who demonstrated that bone remodelling in rats is accelerated after ovariectomy. In our experiment such high rate of boneturnover was well corrected in all supplemented groups but best results were obtained in the group coadministered with calcium, vitamin D_3 and garlic oil. Similarly Mukherjee *et al*., 2004 proved that the high rate of bone turnover was well corrected by supplementation with oil extract of garlic suggesting that garlic oil may have protective action against ovarian hormone insufficiency – related bone resorption. In another study by Choi *et al*., 2001 they proved that phytoestrogens perform their antiosteoporotic effect by stimulating osteoblastic activity through an estrogen receptor mediated action , or by increasing the production of insulin like growth factor-1 (IG-F_1) which is known to enhance osteoblastic activity (Sugimoto *et al*., 1997). and positively affect bone mass in postmenopausal women (Boonen *et al*., 1996 and Chiechi & Micheli, 2005).

In the current study significant decrease in the mean serum calcium level was noticed in the OVX compared to the SH control group. This decrease was improved in all the supplemented groups although the change is not statistically significant. Also the BMC in the proximal , distal and total regions of the femur showed significant decrease after ovariectomy and improvement was significantly apparent after treatment with a combination of calcium, vitamin D_3 and garlic oil.

These results found support from an earlier proposed hypothesis that menopause and estrogen deficiency are associated with increased renal excretion of calcium and intestinal resistance to 1,25 dihydroxy vitamin D_3, and thereby reduced calcium absorption, this will result in a rapid decrease in calcium content within the bones leading to demineralization and eventually development of postmenopausal osteoporosis (Francis *et al*., 1984 and Gennari *et al*., 1990).

Also, Mukherjee *et al*., 2006 proved the effect of garlic oil supplementation in the improvement of serum calcium level in the OVX animals and they suggested that the phytoestrogen present in garlic possibly could restore hydroxylation of vitamin D_3 and thereby an increase in the intestinal transference of calcium.

Conclusion:

Treatment our experimental animal groups with vitamin D_3, calcium and oil extract of garlic could effectively restore the reduced calcium level, correct the high rate of bone turnover, elevate the reduced serum estradiol level and improve both BMC and BMD after ovariectomy. This effect was more better in the group coadminstered with the three components.

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