Original Article

Evidence for a prospective anti-osteoporosis effect of black tea (*Camellia Sinensis*) extract in a bilaterally ovariectomized rat model

Asankur Sekhar Das MSc, Maitrayee Mukherjee MSc and Chandan Mitra PhD

Department of Physiology, Presidency College, College Street, Calcutta, India.

The purpose of this study was to examine whether whole aqueous black tea extract (BTE) prevents bone loss induced by ovarian hormone deficiency. Eighteen 95 –100 days old female albino rats were randomly assigned to three treatment groups [sham -operated control (sham); bilaterally ovariectomized (ovx) and ovx + aqueous black tea extract (BTE)] and sacrificed after 28 days. All animals were fed a standard laboratory diet with free access to deionized water except on days of urinary parameter studies when animals were given only calcium free deionized water during the entire 24 h period of urine collection. Body weight study revealed that rats in the ovx group had significantly higher final body weight than rats in the sham group. This higher final body weight was not observed in animals receiving BTE. The ovx group also had significantly higher abdominal fat mass and liver weight and significantly lower uterus, right kidney and left kidney weights than in other two groups. All these organ weight changes in ovx group also were not observed in animals receiving BTE. Results of urinary studies revealed that rats in the ovx group had significantly higher urinary excretion of calcium (Ca), phosphate, creatinine (Cr), calcium to creatinine (Ca : Cr) ratio (P<0.001) and hydroxyproline (HPr) (P<0.01) than rats in the sham group. Significant recovery of all these parameters were observed in animals receiving BTE. The ovx group also had significantly higher (P<0.001) serum alkaline phosphatase (AP) and tartrate-resistant acid phosphatase (TRAP) activity than rats in the other two groups. These changes could not be seen in animals receiving BTE. Also, identical changes were seen in bone density experiments. Rats in the ovx group had significantly lower densities of the right femur (P<0.001), eighth thoracic rib (P<0.001), eighth thoracic vertebra (P<0.05), and fourth lumbar vertebra (P<0.01) than rats in the sham group; and significant improvement in densities of these bones were seen in animals supplemented with BTE. Animals of ovx group also showed significant decrease in calcium and phosphate level in all these bones which could be regained significantly when these animals were supplemented with BTE. Our findings suggest that aqueous BTE may be effective in preventing bone loss due to ovarian hormone deficiency. Because serum activity of AP, TRAP and urinary loss of bone minerals (Ca and Phosphate) and also the organic components of bone (Cr and HPr) were significantly greater in the ovx group, compared to sham animals and ovx + BTE group. This confirms that ovariectomy enhances and BTE suppresses the rate of bone turnover. The density results of ovx + BTE group are significantly greater than rats in the ovx group, suggesting further that formation exceeded resorption. Detailed studies are underway to clarify the mechanism of this protective effect of BTE on hypogonadal bone loss.

Key Words: ovariectomy, black tea extract, bone turnover, bone density, osteoporosis

Introduction

Ovarian hormone deficiency associated osteoporosis following menopause, postmenopausal osteoporosis, is by far the most common cause of age-related bone loss.[1] This disorder is characterized by reduced amount of bone leading to diminished physical strength of the skeleton and an increased susceptibility to fracture.[2] All over the world it is a major public health issue. There is no evidence that postmenopausal bone loss itself causes any symptoms and it usually becomes clinically apparent when a fracture occurs, by which time the disease is well set-in and possibly irreversible. Progressive bone loss has therefore been called the " silent epidemic" or "silent thief".[2]

Traditional therapies for postmenopausal osteoporosis have emphasized on agents that inhibit bone resorption such as synthetic oestrogens, calcitonin and biphosphonates.[3] Among these alternatives, although oestrogen replacement therapy is by far the most effective method to reduce the rate of postmenopausal bone loss, it may be accompanied by fatal side effects like breast cancer[4] and is thus recommended only for women who are at high risk

Correspondence address: Professor Chandon Mitra, 14/17A, Golf Club Road, Calcutta-700 033, India
Tel: + 033 2413 1383/2417-8645®; Fax: + 033 22198149 (o)
Email: chandon_mitrapresi@yahoo.com
Accepted 15 December 2003

of osteoporosis and who have no contraindications.[1]

A review article was recently published on the evidence for current therapies for postmenopausal osteoporosis and establishment of practical guidelines for the management of osteoporosis by family physicians.[5] Khan proposes that, in addition to calcium and vitamin D, approved pharmacological therapies should include majority of the selective oestrogen receptor modulators, bisphosphonates, calcitonin and hormone replacement therapy (HRT). Although parathormone (PTH) may offer another treatment alternative, therapeutically it is yet to be readily available and is less well studied.[6,7] Furthermore, the potential bone forming agents currently available may either have serious side effects, may not improve skeletal health, or may not decrease susceptibility to fracture.[1] Therefore, it would be a need-based study to find a naturally occurring substance that minimizes bone loss in postmenopausal women, thus de-creasing the necessity for drug therapy.

Tea is the most widely used beverage worldwide and occupies a prime position as a favourite beverage in oriental countries like Japan, India and China. Its therapeutic value came to the forefront with studies showing that tea has antioxidant properties, is anti-hypertensive, anti-lipidemic, anti-diabetic, anti-neoplastic and hypocholesterolemic.[8] The role of tea drinking, a daily habit in Asia, has been identified as a protective factor against osteoporosis.[9,10] A recent epidemiological report from Cambridge, Great Britain suggested that the skeletal health is better preserved in older women who drink tea.[11] Flavonoids and polyphenols of phyto origin have received attention for their beneficial health effects. The oestrogenic activity of naturally occurring isoflavones, by virtue of their ability to bind nuclear oestrogen receptor, was reported over a decade ago[12] and only recently has the role of soybean isoflavones and their glycosides in preventing menopausal symptoms, osteoporosis, high cholesterol and cancer been reported.[13] Studies have also confirmed that soybean isoflavone glycosides, which are pharmacologically and structurally similar to the synthetic phytoooestrogens,[1] are capable of preventing bone loss in ovariectomized rats fed a calcium-deficient diet.[13] Several in vitro studies have confirmed identical results with phytooestrogens.[14,15] These promising reports of beneficial effects of soy flavonoids on post-menopausal bone loss have led us to hypothesize that natural beverage tea, which is also rich in polyphenol esters (theaflavine gallate and digallate) and complex polyphenols (thearubigens)[10] and being so easily available and widely consumed all over the world, might be equally effective in modulating bone loss due to ovarian hormone deficiency. To test this hypothesis, we used a standard bilaterally ovariectomized rat model for osteoporosis and supplementation with aqueous BTE. The promising effect of flavonoids on bone health has immense implications should tea be shown to be effective in the prevention or management of osteoporosis as a natural therapeutic agent.

Materials and methods

Animals and diets

Eighteen 95-100 days female albino rats, weighing 90-100g were used for this study. Upon arrival at our institute, the rats were housed in an environmentally controlled animal laboratory and maintained on a 12h light/dark schedule at 25 ± 2°C throughout the experimental period. They were acclimated for seven days in laboratory environment and fed with a standard laboratory diet containing 67.36% carbohydrate, 22.7% protein, 5.7% fat, 0.4% calcium, 0.3% phosphorus and 0.195 nmol vitamin D_3 per g of diet.[1] They were also given free access to deionized drinking water, except on days of urinary parameter studies when animals were given only calcium free deionized water during the entire 24 h period of urine collection. After acclimation, rats were regularly checked for two consecutive normal reproductive cycles. They were then randomly divided by initial body weight into three groups consisting of six animals in each group: (A) sham-operated control; (B) bilaterally ovariectomized; (C) bilaterally ovariectomized + BTE.

Under light ether anaesthesia, bilateral (dorsolateral) ovariectomies were performed in the groups B and C, and animals of group A were subjected to sham-operation. They were fed with that same diet as described above. After seven days of recovery from surgical convalescence, animals of group C were treated orally with 2.5% aqueous black tea extract (BTE) at a single dose of 1 mL/100g body weight daily for 28 days. Animals in the other two groups received only deionized water as placebo. During the period of BTE treatment, group A was pair-fed with experimental groups B and C so as to overcome the impact of any altered food intake in the experimental groups.

Preparation of 2.5% aqueous BTE

The black tea extract was prepared from CTC (Curl, Tear and Crush) BOP (Broken Orange Pickoe) grade black clonal tea. It was processed and supplied by Tocklai Experimental Station, Jorhat, Assam, India to the Drug Development Division, Indian Institute of Chemical Biology, Jadavpur, Calcutta. We received a generous gift from that institute and a fresh 2.5% aqueous BTE was prepared everyday following the method of Wei *et al.* [16]

Body weights and organ weights

On completion of the experimental period, the final body weight of animals of all three groups were recorded. They were sacrificed on the scheduled date and fresh weight of organs, viz, whole liver, right and left kidney, uteri and total abdominal fat were recorded. Guidelines for the ethical care and treatment of animals from the Animal Care & Ethics Committee, Presidency College, Calcutta, India were strictly followed which has been following the recommendations and guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India, New Delhi, India.

Estimation of urinary calcium, phosphate, creatinine and hydroxyproline

Fasting urine was collected for 24 h (9am to 9am) according to the standard laboratory procedure[17], as described elsewhere by Chanda *et al*.[18] Urinary calcium, phosphate, creatinine and hydroxyproline content were estimated according to the methods described, respectively, by Adeniyi *et al*,[19] Lowry and Lopez,[20] Nath,[21] and Bergman and Loxley.[22]

Estimation of serum alkaline phosphatase and tartrate-resistant acid phosphatase activity

Blood was collected directly from the heart under urethane anaesthesia (1.7 mg/g body weight). Serum was obtained by using standard laboratory protocol. Serum alkaline phosphatase (AP) and serum tartrate-resistant acid phosphatase (TRAP) activity were estimated spectrophotometrically (Double Beam Spectrophotometer, Shimadzu 160A; Shimadzu Corporation, Kyoto, Japan) by using the method of Mitchell *et al*.,[23] and reagent kit (LABKIT, Spain) respectively.

Measurement of bone density

The right femur, eighth thoracic rib and eighth thoracic vertebra and fourth lumbar vertebra were freed of soft tissue and cleaned. Fresh weight of each bone was recorded. Bone density (g/cm^3 bone volume) was measured according to the method as described by Arjmandi *et al.,*[1] by using Archimedes' principle.

Estimation of bone calcium and bone phosphate level

Right femur, eighth thoracic rib, eighth thoracic vertebra, and fourth lumbar vertebra were removed and cleaned of adhering tissue. The whole bone was extracted two times with a 1:1 mixture of ethanol and diethyl ether for 48 h and one time with diethyl ether for 24 h. The dehydrated and defatted bones were ashen for 48 h at 600˚C and hydrolyzed in 6 N HCl for determination of calcium and phosphate.[24] Calcium and phosphate were estimated according to the method as described respectively by Adeniyi *et al*.,[19] and Lowry and Lopez.[20]

Data

Data were expressed as mean ± SE. Significance was determined by Student's *t* - test using PSI-PLOT Version 2.0 (Poly Software International; 1992, 1993). Differences were considered significant if $P < 0.05$.

Results

Body weights and organ weights

Initially, animals of all the three groups were more or less of similar mean body weight. At the end of the study (28 days), the ovx group (Group B) had a significantly higher body weight ($P<0.01$) compared to sham-control (Group A). The significantly higher body weight could not be seen in the animals of group C (ovx + BTE) which returned almost to control values (Table 1). The lower mean final body weight of the animals of group C were not a result of significantly less food intake, because all animal groups were pair-fed and thus their food intake was similar.

The organs that were examined for any change in their weight are also listed in Table 1 and are presented relative to body weight. Results showed that ovariectomy caused atrophy of the uterus which could be prevented by BTE supplementation (Group C). Similar observations were made with both kidneys. BTE supplementation could revive significantly the ovariectomy-induced reduction in kidney weight. On the contrary, the abdominal fat mass and liver weight were significantly higher in ovx group (Group B) when compared with rats in the sham-control group (Group A). However, BTE supplementation could significantly reduce both abdominal fat mass and liver weight in Group C animals (ovx + BTE).

Urinary calcium, phosphate, creatinine, hydroxyproline excretion profiles and calcium to creatinine ratio

The urinary calcium, phosphate, creatinine and hydroxyproline excretion profiles together with Ca:Cr ratio of sham group (Group A), bilaterally ovariectomized (Group B) and ovariectomized rats supplemented with aqueous BTE (Group C) are shown in Table 2. Compared with the sham-operated group, ovx animals showed a significant increase in all the urinary parameters studied, viz, calcium, phosphate, creatinine and Ca : Cr ratio at $P < 0.001$ level and hydroxyproline at $P<0.01$ level. An elevated response of all these parameters were significantly counter regulated in rats receiving aqueous BTE.

Table 1. Body weight and relative organ weights of sham group (Group A), ovariectomized group (Group B), ovareictomized + BTE treated group (Group C) of rats.

Parameters	Experimental Groups			*P* value	
	Sham (Gr. A)	Ovx (Gr. B)	Ovx +BTE (Gr. C)	Gr. A Vs Gr. B	Gr. B Vs Gr. C
Initial body weight (g)	94.4 ± 1.78	97.4 ± 3.4	96.08 ± 1.8	$P>0.05$	$P> 0.05$
Final body weight (g)	96.3 ± 1.82	117.8 ± 4.21	97.9 ± 1.23	$P<0.01$	$P< 0.01$
Organ weight (g/100g body weight)					
Uterus	0.1572 ± 0.003	0.0767 ± 0.006	0.1482 ± 0.008	$P<0.001$	$P<0.001$
Abdominal fat	1.1624 ± 0.074	2.2097 ± 0.296	1.1051 ± 0.112	$P<0.01$	$P<0.01$
Liver	3.27 ± 0.116	4.14 ± 0.282	3.35 ± 0.196	$P<0.05$	$P<0.05$
Right kidney	0.3474 ± 0.004	0.3149 ± 0.006	0.3403 ± 0.007	$P<0.01$	$P<0.05$
Left kidney	0.3436 ± 0.005	0.2926 ± 0.005	0.3479 ± 0.02	$P<0.001$	$P<0.01$

Values expressed as mean ± SE ($N = 5$).

Serum alkaline phosphatase and tartrate-resistant acid phosphatase activity profiles

The serum alkaline phosphatase (AP) activity profiles of rats of sham, ovx and ovx + BTE groups are shown in Figure 1. Rats of ovx group (Group B) showed a significant increase in serum alkaline phosphatase activity when compared to animals of sham group (P <0.001) (Group A). This increase in AP activity was significantly lowered (P <0.001) in rats on receiving BTE (Group C). Likewise, the significant (P <0.001) increase in TRAP in ovariectomized animals (Group B), compared to control (Group A), could be effectively reduced by aqueous BTE treatment (Group C) (Fig 2).

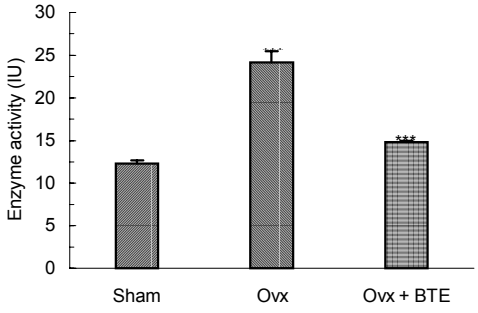

Figure 1. Effect of ovariectomy and ovariectomy + BTE on serum alkaline phosphatase (AP) activity in different groups of rat. Error bars represent means ± SE. (N = 6). In statistical analysis Group B (ovx) has been compared with Group A (sham-control) and Group C (ovx + BTE) with Group B (ovx). *** Denotes significant difference P<0.001.

Bone density profiles

Animals in the ovx group (Group B) had significantly lower densities of the right femur (P<0.001), eighth thoracic rib (P<0.001), eighth thoracic vertebra (P<0.05) and fourth lumbar vertebra (P<0.01), compared with the sham group (Group A). BTE supplementation in these animals (Group C) were seen to recover the density of all these bones significantly: right femur (P<0.05), eighth thoracic rib (P<0.001), eighth thoracic vertebra (P<0.05) and fourth lumbar vertebra (P <0.05) (Fig 3).

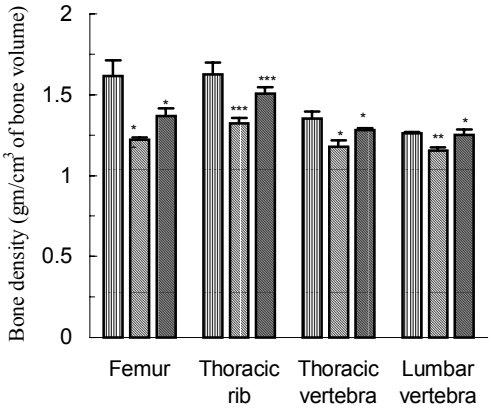

Figure 2. Effect of ovariectomy and ovariectomy + BTE on serum tartrate-resistant acid phosphatase (TRAP) activity in different groups of rat. Error bars represent means ± SE. (N=6). In statistical analysis Group B (ovx) has been compared with Group A (sham-control) and Group C (ovx + BTE) with Group B (ovx). *Denotes significant difference P<0.01 and ** denotes P<0.001.

Bone calcium and bone phosphate levels

Bone calcium and phosphate levels are shown in Table 3. Animals of ovx group (Group B), compared to sham group (Group A), showed a marked decrease in calcium and phosphate level of right femur (Calcium: P<0.001; Phosphate: P<0.05)), eighth thoracic rib (Calcium: P< 0.001; Phosphate: P<0.01), eighth thoracic vertebra (Calcium: P<0.001; Phosphate: P<0.05) and fourth lumbar vertebra (Calcium: P< 0.05; Phosphate: P<0.01). Significant recovery of both mineral content of these bones were seen when ovx animals were supplemented with BTE (Group C).

Discussion

The main purpose of this study was to evaluate whether whole aqueous BTE is effective in preventing bone loss due to ovarian hormone deficiency caused by bilateral ovariectomy. We report here for the first time the experimental data in support of an anti-osteoporosis effect of aqueous BTE. Oestrogenic activity of naturally occurring isoflavones by virtue of their ability to bind nuclear oestrogen receptor was reported earlier[12] and only recently the role of soybean isoflavones and their glycosides in preventing menopausal symptoms, osteoporosis, high cholesterol and cancer have been reported.[13] To test whether similar promising responses, especially pro-oestrogenic and anti-osteoporosis, could be obtained in ovariectomized animals by polyphenol esters and complex polyphenols present in BTE, we initially tested body weight and organ weight parameters (Table 1). This was followed by more direct primary and secondary osteoporosis marker parameters (Table 2 and 3;

Table 2. Urinary excretion of calcium, phosphate, creatinine, hydroxyproline and calcium : creatinine ratio in sham group (Group A), ovariectomized group (Group B) and ovariectomized + BTE treated group (Group C) of rats.

Parameters	Experimental groups			*P* value	
	Sham (Gr. A)	Ovx (Gr. B)	Ovx + BTE (Gr. C)	Gr. A vs Gr. B	Gr. B vs Gr. C
Calcium (mg/24 h)	0.61 ± 0.029	2.36 ± 0.12	0.81 ± 0.029	P<0.001	P<0.001
Phosphate (mg/24 h)	2.26 ± 0.093	3.32 ± 0.093	2.75 ± 0.070	P<0.001	P<0.01
Creatinine (mg/24 h)	0.79 ± 0.017	1.57 ± 0.12	0.84 ± 0.018	P<0.001	P<0.001
Hydroxyproline (mg/24 h)	0.312 ± 0.05	0.716 ± 0.08	0.332 ± 0.08	P<0.01	P<0.01
Calcium : Creatinine	0.603 ± 0.025	1.644 ± 0.13	1.06 ± 0.089	P<0.001	P<0.01

Values are expressed as mean ± SE (N = 5).

Table 3. Bone calcium and bone phosphate levels of the sham group (Group A), ovariectomized (Group B), and ovariectomized + BTE treated group (Group C) of rats. Values expressed as mean ± SE (*N*=6).

	Experimental groups			*P* value	
	Sham (Gr. A)	Ovx (Gr. B)	Ovx +BTE (Gr. C)	Gr. A Vs Gr. B	Gr. B Vs Gr. C
Bone calcium level *(percent of ash weight)*					
Right Femur	23.16 ± 0.64	18.61 ± 0.73	22.94 ± 0.83	*P*<0.001	*P*<0.01
Eighth Thoracic rib	35.49 ± 1.57	24.56 ± 0.31	32.87 ± 1.85	*P*<0.001	*P*<0.01
Eighth Thoracic vertebra	21.48 ± 0.80	11.30 ± 0.91	21.08 ± 0.34	*P*<0.001	*P*<0.001
Fourth Lumbar vertebra	21.00 ± 1.24	17.15 ± 0.40	20.23 ± 1.03	*P*<0.05	*P*<0.05
Bone phosphate level *(percent of ash weight)*					
Right Femur	20.08 ± 0.58	17.72 ± 0.84	21.15 ± 0.69	*P*<0.05	*P*<0.01
Eighth Thoracic rib	22.85 ± 0.37	20.78 ± 0.51	22.31 ± 0.34	*P*<0.01	*P*<0.05
Eighth Thoracic vertebra	20.31 ± 0.40	17.53 ± 0.82	20.73 ± 0.32	*P*<0.05	*P*<0.01
Fourth Lumbar vertebra	20.29 ± 0.59	16.84 ± 0.47	20.95 ± 0.18	*P*<0.01	*P*<0.001

Fig. 1-3) to assess the extent of bone turnover and bone loss.

With respect to controlled food intake and body weight in pair-fed conditions, animals in the ovariectomized group had significantly greater final body weights than rats in the sham group. Despite similar food consumption, ovariectomy-induced greater body weight gain - this has also been reported earlier.[25] Ovariectomy-induced body weight gain could not be seen in animals when treated with BTE (Table 1). To our knowledge, this is the first time such an observation has been made for BTE treatment. Also, compared to sham-operated rats, greater gain in weight of abdominal fat and liver in the ovariectomized group could not be seen in animals receiving BTE (Table 1). It has been suggested that isoflavone glycosides present in soybean protein,[1,13] the polyphenol esters and complex polyphenols present in BTE might serve as non-steroidal pro-oestrogenic compounds and thus, like oestrogen, expectedly prevented ovariectomy-induced body weight and organ weight gain.[1]

The role of tea in decreasing the growth and developmental aspects of transformed cells[26] may deserve mention in this context. In a pair-fed experimental condition, significant reduction in abdominal fat on receiving BTE, compared to sham animals (Table 1), may be of particular interest. This is because it raises questions whether or not polyphenol esters and complex polyphenols of BTE, similar to that of soybean protein isolate,[1] stimulates the synthesis of growth hormone known to decrease adipose tissue mass[27] and increase bone mass.[28,29] Interestingly, this particular observation may have other indirect significance because studies have been in progress for some years to develop or identify an anabolic or bone-forming agent[3,30] which can be useful therapeutically in elderly patients with osteoporosis. Our notion of BTE acting as a pro-oestrogenic compound possibly has received further acceptance by our results with uterus weight. The uterus atrophy, as expected in the animals of ovariectomized group, could be regained by BTE-treatment (Table 1) suggesting an uterotrophic activity of the compounds present in BTE. This finding also corroborates well with earlier observations that naturally occurring isoflavonoids have oestrogenic activity.[12,13,31] Similarly to the uterus, weight regaining response was observed in both kidneys of rats receiving BTE (Table 1), thus suggesting a possible tissue-specific effect of BTE.

The rate of bone loss in a post-menopausal situation may be indirectly assessed with the use of a number of biochemical markers.[2] It is established that the biochemical estimates of bone formation and bone resorption increase sharply at the menopause,[32] and that the higher the bone turnover, the higher the rate of bone loss.[33] Fast bone losers have elevated concentrations of these markers, compared with slow bone losers.[34,35] Bilaterally ovariectomized rats in our present study had an increase loss of 24 h urinary calcium and phosphate, compared to the sham group. But these responses were significantly lowered in bilaterally ovariectomized rats on receiving BTE (Table 2). This suggests that this significant decrease in urinary excretion of calcium and phosphate could be attributed to decreased bone resorption and/ or increased bone formation or both.

To ascertain whether or not such bone loss was an inevitable consequence due to marked changes in bone turnover (as expected under ovariectomized conditions), we evaluated two specific biochemical markers of bone turnover, namely serum alkaline phosphatase activity and urinary calcium to creatinine ratio. As expected, both the parameters were seen to be significantly higher in animals of the ovariectomized group, compared to sham-operated animals. On receiving BTE, these values were lowered significantly indicating that BTE was effective in preventing bone loss due to ovarian hormone deficiency (Table 2) since a rise in serum alkaline phosphatase and the urinary calcium to creatinine ratio have been linked with collagen degradation, bone resorption and osteoporosis.[36, 37-39]

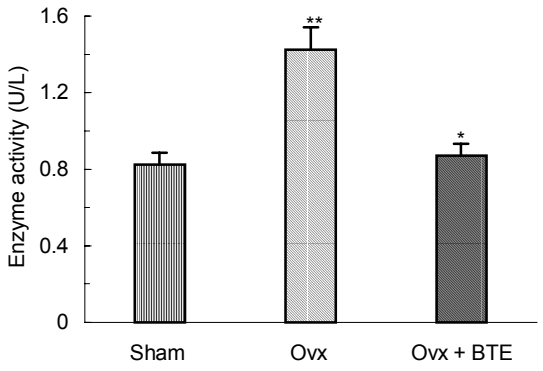

Figure 3. Effect of ovariectomy and ovariectomy + BTE on densities of right femur, 8th thoracic rib, 8th thoracic vertebra, and 4th lumbar vertebra in ( ) Group A (sham-control), ( ) Group B (ovx) and ( ) Group C (ovx + BTE) of rats. Error bars represent means ± S.E. (n=6). In statistical analysis Group B has been compared with Group A and Group C with Group B. *Denotes significant difference $P<0.05$, ** denotes $P<0.01$, and *** denotes $P<0.001$.

The markers of bone resorption and osteoclastic activity measure circulating or urinary concentrations of fragments of bone matrix that are released during bone resorption, or enzymatic activities associated with osteoclasts. The close association of increased serum concentrations of TRAP and urinary hydroxyproline respectively as a potential index for osteoclastic activity and degradation of Type I collagen are well established.[40] In our studies both TRAP and hydroxyproline were seen significantly greater in the ovx group, compared to sham and ovx + BTE-treated group (Fig. 2 and Table 2). These data further emphasize that BTE seems to have positive influence in counteracting the increased osteoclastic activity as well as bone resorption due to ovarian hormone deficiency. This was cross-examined in our studies with bone minerals. Ash content of calcium and phosphate from different bones were significantly lower in the ovx group than in the sham and ovx + BTE – treated group (Table 3), thus supporting our speculation that BTE possibly has been effective in preventing bone loss under the conditions of our study. Moreover, our data on bone density measurements (Fig. 3) suggest that bone loss due to ovarian hormone deficiency is prevented by BTE – administration. Since trabecular bone is readily lost due to ovariectomy in this animal model,[1] it may be expected that this type of bone may be more responsive to BTE – treatment than is cortical bone. However, this was not supported by our data. As bone density is the primary determinant of bone breaking strength,[37] these results further suggest that BTE may also have an effective role in restoring the bone breaking strength in a situation when high bone turnover prevails because of ovarian hormone deficiency. Bone density results in our study possibly provide another important clue towards the ongoing search to develop or identify an anabolic or bone forming agent which like soybean protein isolate[1] might stimulate the synthesis of growth hormone.

In summary, this study for the first time provides experimental evidence to suggest that BTE has positive anti-osteoporosis and bone mass preserving effects. It further supports the suggestion of earlier epidemiological reports that drinking tea might be beneficial in preserving skeletal health.[9-11]

Acknowledgement

The financial assistance by the National Tea Research Foundation, Kolkata, India is gratefully acknowledged.

References

1. Arjmandi BH, Alekel L, Hollis BW, Amin D, Stacewicz-Sapuntzakis M, Guo P, Kukreja SC. Dietary soybean protein prevents bone loss in an ovariectomized Rat model of Osteoporosis. J Nutr 1996; 126: 161-167.
2. Riis BJ. The role of bone turnover in the pathophysiology of osteoporosis. Br J Obstetrics Gynaecology May 1996; 103 (Suppl. 13): 9-15 .
3. Mundy GR. Osteoporosis into the year 2010. Br J Obstetrics Gynaecology May 1996; 103 (Suppl. 13): 32-38.
4. Armstrong K, Popik S, Guerra C, Ubel PA. Beliefs about breast cancer risk and use of postmenopausal hormone replacement therapy. Med Decis Making July-Sept 2000; 20 (3): 308-313.
5. Khan A. Advances in osteoporosis therapy. 2003 update of practical guidelines. Can Fam Physician 2003; 49: 441-447.
6. Follin SL, Hansen LB. Current approaches to the prevention and treatment of postmenopausal osteoporosis. Am J Health Syst Pharm May 2003; 60 (9): 883-901.
7. Nieman LK. Management of surgically hypogonadal patients unable to take sex hormone replacement therapy. Endocrinol Metab Clin North Am Jun 2003; 32 (2): 325-36.
8. Krishnamoorthy KK. The nutritional and therapeutic value of tea. Proc International Symposium on Tea Science, Japan 1991: 6-11.
9. Fujita T. Osteoporosis in Japan: factors contributing to the low incidence of hip fracture. Adv Nutr Res 1994; 9: 89-99.
10. Kao PC, Peng FK. How to reduce the risk factors of osteoporosis in Asia. Chung Hua I Hsueh Tsa Chih (Taipei) March 1995; 55 (3): 209-213.
11. Hegarty VM, May HM, Khaw KT. Tea drinking and bone mineral density in older women. Am J Clin Nutr Apr 2000; 71 (4): 1003-1007.
12. Miksicek RJ. Commonly occurring plant flavonoids have ooestrogenic activity. Mol Pharmacol 1993; 44 (1): 37-43.
13. Toshiya T, Takehiko U, Kuniaki H, Haruo N, Kuniro T, Hitoshi I. New 6-0-Acylisoflavone glycosides from soybeans fermented with Bacillus *subitilis (natto)*. I. 6-0-succinylated isoflavone glycosides and their preventive effects on bone loss in ovariectomized rats fed a calcium-deficient diet. Biol Pharma Bull 1999; 22 (11): 1193-1201.
14. Wattel A, Mentaverri R, Prouillet C, Mullie C, Dupont C, Kamel S, Brazier M. Effects of two flavonols, quercetin and kaempferol, on in vitro bone resorption and osteoclast apoptosis. Ann Nutr Metab 2001; 45: 217 – 234.
15. Lorenzetti S, Paterno A, Germani D, Cianfarani S, Branca F. Phytoooestrogens and IGF-1 in vitro regulation of bone resorption by osteoclasts. Ann Nutr Metab 2001; 45: 217 – 234.
16. Wei H, Zhang X, Zhao Ji-Fu, Wang ZY, Bickers D, Lebwohl M. Scavenging of hydrogen peroxide and inhibition of ultraviolet light-induced oxidative DNA damage by aqueous extracts from green and black teas. Free Radical Biology and Medicine 1999; 26: (11/12) 1427-1435.

17. Nath RL, Nath RK. Practical Biochemistry in Clinical Medicine, 2nd edition. Calcutta: Academic Publishers, 1990; 8.
18. Chanda S, Islam MN, Parmanik P, Mitra C. High-lipid diet intake is a possible predisposing factor in development of hypogonadal osteoporosis. Jpn J Physiol 1996; 46 (5): 383-388.
19. Adeniyi KO, Ogunkeye OO, Isichei CO. Thyroidectomy and thyroxine administration alter serum calcium levels in rat. Acta Physiologica Hungarica 1993; 81 (1): 95-99.
20. Lowry HO, Lopez AJ. The determination of inorganic phosphate in the presence of labile phosphate esters. J Biol Chem 1946; 162: 421-428.
21. Nath RL, Nath RK. Practical Biochemistry in Clinical Medicine, 2nd edition. Calcutta: Academic Publishers, 1990: 94
22. Bergman I, Loxley R. The determination of hydroxyproline in urine hydrolysates. Clin Chim Acta1 970; 27: 347-349.
23. Mitchell RH, Karnovsky MJ, Karnovsky MF. The distribution of some granule associated enzyme in guinea pig polymorphonuclear leucocyte. Biochem J 1970; 116: 207-216.
24. Yeh JK, Liu CC, Aloia JF. Effects of exercise and immobilization on bone formation and resorption in young rats. Am J Physiol (Endocrinol Metab. 27) 1993; 264: E 182-189.
25. Kalu DN, Liu CC, Salerno E, Hollis BW, Echon R, Ray M. Skeletal response of ovariectomized rats to low and high doses of 17 β-estradiol. Bone Miner 1991; 14: 175-187.
26. Weisburger JH. Tea antioxidants and health. In: Cadenas E, Packer L, eds. Handbook of Antioxidants. New York: Marcel Dekker Inc, 1996; 480.
27. Rudman D, Feller AG, Nagraj HS, Gergans GA, Lalitha PY, Goldberg AF, Schlenker RA, Cohn L, Rudman IW, Mattson DE. Effects of human growth hormone in men over 60 years old. N Eng J Med 1990; 323: 1-6.
28. Arjmandi BH, Liu CC, Yu S, Kalu DN. Effect of growth hormone on trabecular bone loss, Osteoclasts, Ostoblasts and their marrow progenitors. J Bone Miner Res 1994; 9 (1): S 382.
29. Kalu DN, Liu CC Arjmandi BH, Salerno E, Salih MA, Hollis BW. Growth hormone but not rhIGF-I reversed bone loss due to ovariectomy in rats. J Bone Min Res 1993; 8 (Suppl. 1): S 271.
30. Turner CH. Toward a cure for Osteoporosis: reversal of excessive bone fragility. Osteoporosis Int 1991; 2: 12-19.
31. Lerner L, Turkhimer AR, Borman A. Phoretin, a weak oestrogen and oestrogen antagonist. Proc Soc Exp Boil Med 1963; 111: 115-117.
32. Christiansen C, Riss BJ, Rodbro P. Screening procedure for women at risk of developing post menopausal osteoporosis. Osteoporosis Int 1990; 1: 35-40.
33. Hansen MA, Overgaard K, Riis BJ, Christiansen C. Role of peak bone mass and bone loss in postmenopausal osteoporosis.12 year study. Br Med J 1991; 303: 961-964.
34. Hui SL, Slemenda CW, Johnston CC. The contribution of bone loss to postmenopausal osteoporosis. Osteoporosis Int 1990; 1: 30-34.
35. Christiansen C, Riis BJ, Rodbro P. Prediction of rapid bone loss in postmenopausal women. Lancet 1987; 1: 1105-1108.
36. Lindsay R, Coutts JRT, Hart DM. The effect of endogenous oestrogen on plasma and urinary calcium and phosphate in oophorectomized women. Clin Endocrinol 1977; 6: 87-93.
37. Myburgh KH, Noakes TD, Roodt M, Hough FS. Effect of exercise on the development of osteoporosis in adult rats. J Appl Physiol 1989; 66: 14-19.
38. Delmas PU. Biochemical markers of bone turnover. J Bone Miner Res 1993; 8: 5549-5555.
39. Gert BJ, Shao P, Hanson UA *et al*. Monitoring bone resorption in early postmenopausal women by an immunoassay for cross-linked collagen peptides in urine. J Bone Miner Res 1994; 9: 135-142.
40. Stepan JJ. Enzyme tests in bone disease. In: Moss DW, Rosalki SB, eds. Enzyme tests in diagnosis. New York: Oxford University Press Inc, 1996; 155-188.