

REPORT

The effects of *Cosmos caudatus* (Ulam Raja) supplementation on bone biochemical parameters in ovariectomized rats

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Abstract: *Cosmos caudatus* (ulam raja) contains high mineral content and possesses high antioxidant activity which may be beneficial in bone disorder such as postmenopausal osteoporosis. The effects of *C. caudatus* on bone metabolism biomarkers in ovariectomized rats were studied. 48 Sprague-Dawley rats aged three months were divided into 6 groups. One group of rats was sham-operated while the remaining rats were ovariectomized. The ovariectomized rats were further divided into 5 groups: the control, three groups force-fed with *C. caudatus* at the doses of 100mg/kg, 200mg/kg or 300mg/kg and another group supplemented with calcium 1% *ad libitum*. Treatments were given 6 days per week for a period of eight weeks. Blood samples were collected twice; before and after treatment. Parameters measured were bone resorbing cytokine; interleukin-1 and the bone biomarkers; osteocalcin and pyridinoline. Serum IL-1 and pyridinoline levels were significantly increased in ovariectomized rats. Supplementation of *C. caudatus* was able to prevent the increase of IL-1 and pyridinoline in ovariectomized rats. Besides that, *C. caudatus* showed the same effect as calcium 1% on biochemical parameters of bone metabolism in ovariectomized rats. In conclusion, *Cosmos caudatus* was as effective as calcium in preventing the increase in bone resorption in ovariectomized rats.

Keywords: *Cosmos caudatus*, osteoporosis, ovariectomy, calcium.

INTRODUCTION

Osteoporosis is characterized by reduction in bone strength and increased risk of bone fractures (Kung *et al.* 2006). This is due to the imbalance in bone metabolism between bone formation and bone resorption. There are several factors which regulate bone metabolism which include cytokines (Datta *et al.*, 2008). Changes in bone metabolism may be assessed by measuring bone metabolism biomarkers such as osteocalcin (bone formation marker) and pyridinoline (bone resorption marker) (Delmas *et al.*, 2000).

In postmenopausal women, the deficiency of estrogen increases the risk of developing osteoporosis. Estrogen was found to have antioxidant properties (Badeau *et al.*, 2005). It was also shown to increase the expression of glutathione peroxidase in osteoclasts (Lean *et al.*, 2005), an enzyme which is responsible for the degradation of hydrogen peroxide. Estrogen deficiency will reduce the expression of the enzyme and renders the bone susceptible to hydrogen peroxide attacks.

In osteoporosis, lipid peroxidation is increased due to the reduction in antioxidants (Maggio *et al.*, 2003) and reactive oxygen species are found to play a role in bone

metabolism (Sontakke and Tare 2002). Free radicals have also been shown to be cytotoxic to osteoblastic cells (Moreau *et al.*, 1998). Loss of estrogens accelerates the effects of aging on bone by decreasing defense against oxidative stress which leads to bone loss (Almeida *et al.*, 2007).

Since free radicals and lipid peroxidation are involved in bone metabolism and may be the culprit in causing bone loss, substances having antioxidative activities can overcome the detrimental effects. Our previous studies have shown the beneficial effects of palm-oil derived tocotrienols in several experimental osteoporosis; ovariectomized (Norazlina *et al.*, 2000), steroid-induced (Ima-Nirwana and Fakhurazi 2002) and nicotine-induced (Norazlina *et al.*, 2007). The effects of palm-oil derived tocotrienols may be attributed to its antioxidative activities.

Thus, in finding alternatives in the treatment of osteoporosis, we turn to a local plant, *Cosmos caudatus* or locally known as 'ulam raja' (King's salad). Previous study has shown that this plant has antioxidative activities (Huda-Fauzan *et al.*, 2007). It contains phenolic compounds that contribute to the color, antioxidant and anticarcinogenic properties of the plants. For every 100 g of *Cosmos caudatus*, the total phenolic compound is

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21.41 mg. It is also found that *Cosmos caudatus* had extremely high antioxidant capacity of about 2,400 mg l-ascorbic acid equivalent antioxidant capacity (AEAC) per 100 g of fresh sample (Shui *et al.*, 2005). It is also believed that *Cosmos caudatus* promote the formation of healthy bones (Ismail 2000). This may be due to the rich mineral content of *Cosmos caudatus* such as calcium, phosphorus, magnesium, iron and potassium (Nutriweb Malaysia). Thus, we hypothesized that *Cosmos caudatus* may exert protective effects on bone of ovariectomized rats which is a suitable animal model for studying postmenopausal osteoporosis. In this study, the effects of *Cosmos caudatus* on bone biomarkers were determined.

MATERIAL AND METHODS

Animals and treatment

Fourty-eight 3-month old Sprague-Dawley female rats were obtained from the Laboratory Animal Resource Unit, Universiti Kebangsaan Malaysia. The rats were then randomly assigned to six groups of eight rats each. One group was sham-operated while the remaining five groups were ovariectomized. Treatment was carried out for six weeks according to the following treatment groups: Group 1; Sham-operated group which received vehicle deionized water (C), group 2; ovariectomized group which received vehicle deionized water (OVX), groups 3-5; ovariectomized and supplemented with *C. caudatus* extract at the doses of either 100 (CC100), 200 (CC200) or 300 (CC300) mg/kg body weight and group 6; ovariectomized and supplemented with 1% calcium (Ca) *ad libitum*. The rats were housed three per cage under 12-hour natural light/dark cycles and given tap water *ad libitum*.

Diets

All rats received normal rat chow obtained from Gold Coin (Port Klang, Selangor, Malaysia). Fresh *Cosmos caudatus* was obtained from a local vendor and its water extract with the concentration of 100g/300ml was obtained from School of Chemical Sciences & Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia using water extraction method which was previously described (Huda-Faujan *et al.* 2007). To prepare the 100 mg/kg dose of *C. caudatus*, 24 ml of the extract (100g/300ml) was dissolved in 56 ml deionized water. For the 200 and 300 mg/kg doses, 48 ml and 72 ml of the extract were dissolved in 32 ml and 8 ml of deionized water respectively. A total of 0.1 ml/100 g rat weight of the prepared solutions was given orally to the respective groups via oral gavage, 6 days a week, up to 8 weeks. Calcium was prepared by mixing 1 g of lactic acid-hemicalcium salt (Sigma, USA) with 100 ml of deionized water.

Ovariectomy

The rats were anaesthetized with Ketamil and Ilium Xylazil-20, 1:1 (Troy Laboratories, Australia). The lower

abdomen of the rats were shaved and incised. Fallopian tubes and ovaries were identified and absorbable catgut suture was used to tie the fallopian tubes below the ovaries. The ovaries were then removed. The rats were given normal diet for 2 weeks for the wound to recover before dietary manipulation was started.

Bone biochemical markers

Blood samples were collected twice, at the initiation of the treatment and upon completion of treatment. Serum was obtained for the measurement of bone biochemical markers. Serum interleukin-1 α (IL-1 α) was measured by using rat ELISA kits (Bender MedSystems, Vienna, Austria). Serum osteocalcin was determined by using ELISA kit purchased from Nordic Bioscience Diagnostic A/S (Herlev, Denmark). Serum pyridinoline was measured by using METRA EIA kit from Quidel Co. (San Diego, USA).

Analyses of data

The statistical test applied was the One-Way analysis of variance (ANOVA) test using the Statistical Package for Social Sciences software. The Tukey's honestly significant difference test was selected as the post-hoc test.

This study was approved by the University's Animal Ethics Committee, approval code: PP/FAR/2008/Norazlina/12-August/225-Sept-2008-Aug-2009.

RESULTS

Serum interleukin-1

After six weeks of treatment, the serum interleukin-1 levels were increased in OVX group (fig. 1). No differences were observed in the other groups between the pre- and post-treatment values. Similarly, no significant differences were seen between the different groups.

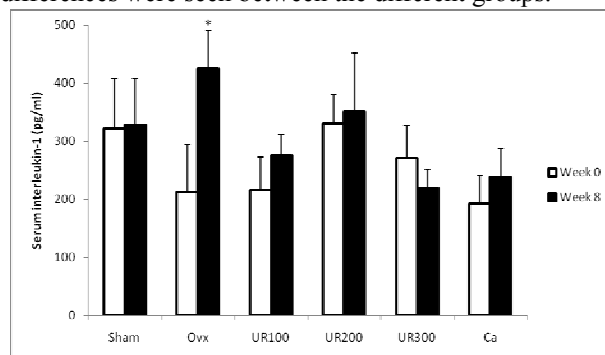


Fig. 1: Serum interleukin-1 levels in ovariectomized rats treated with *Cosmos caudatus* or calcium.

Data presented as mean $\pm$ SEM ($p < 0.05$). '*' indicates significant difference compared to week 0. Sham: sham-operated, Ovx: ovariectomized control, UR100: ovariectomized and supplemented with *C. caudatus* (100 mg/kg), UR200: ovariectomized and supplemented with *C. caudatus* (200 mg/kg), UR300: ovariectomized and supplemented with *C. caudatus* (300 mg/kg), and Ca: ovariectomized and given 1% calcium.

Serum osteocalcin

Osteocalcin levels showed a trend of reduction in all groups and significant differences were observed between before and after treatment values for the C, OVX, CC200 and Ca groups. No significant changes were seen between the different groups (fig. 2).

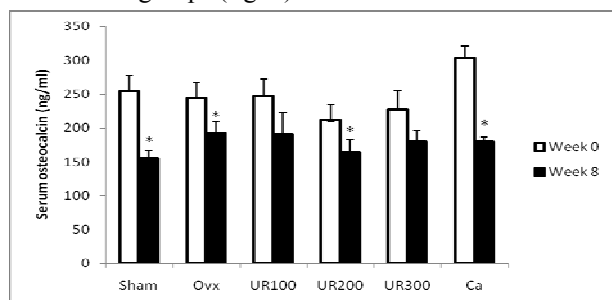


Fig. 2: Serum osteocalcin levels in ovariectomized rats treated with *Cosmos caudatus* or calcium.

Data presented as mean $\pm$ SEM ($p < 0.05$). ‘*’ indicates significant difference compared to week 0. Sham: sham-operated, Ovx: ovariectomized control, UR100: ovariectomized and supplemented with *C. caudatus* (100 mg/kg), UR200: ovariectomized and supplemented with *C. caudatus* (200 mg/kg), UR300: ovariectomized and supplemented with *C. caudatus* (300 mg/kg), and Ca: ovariectomized and given 1% calcium.

Serum pyridinoline

The only significant change that was observed was in group OVX in which the rats had higher pyridinoline values after treatment as compared to before treatment (fig. 3).

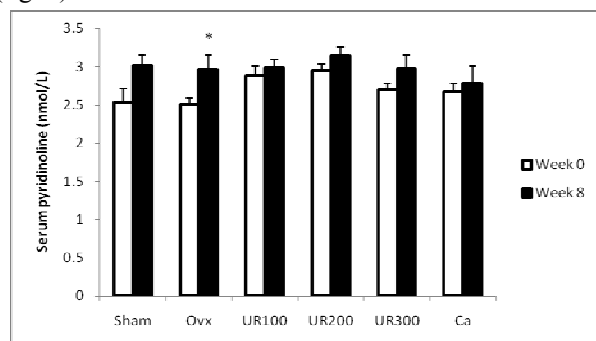


Fig. 3: Serum pyridinoline levels in ovariectomized rats treated with *Cosmos caudatus* or calcium.

Data presented as mean $\pm$ SEM ($p < 0.05$). ‘*’ indicates significant difference compared to week 0. Sham: sham-operated, Ovx: ovariectomized control, UR100: ovariectomized and supplemented with *C. caudatus* (100 mg/kg), UR200: ovariectomized and supplemented with *C. caudatus* (200 mg/kg), UR300: ovariectomized and supplemented with *C. caudatus* (300 mg/kg), and Ca: ovariectomized and given 1% calcium.

DISCUSSION

Ovariectomy is shown to increase interleukin-1 beta and interleukin-10 within 10 days after ovariectomy in rats (Percegoni et al., 2009). Another study reported that

ovariectomy caused an increase in production of pro-inflammatory cytokines such as interleukin-6 and interleukin-8 in adipose tissue (Bruun et al., 2003).

The increase in these pro-inflammatory cytokines is due to estrogen deficiency. Estrogen has been found to be able to modulate the production of the pro-inflammatory cytokines such as interleukin-1, by decreasing its production (Rogers et al., 2007). Thus, in estrogen deficiency, the modulation of pro-inflammatory cytokines production will be lacking and the cytokines levels will be increased. Furthermore, in estrogen deficiency condition, the ability to defend against oxidative stress is reduced (Manolagas 2010). It has been shown that oxidation-derived free radicals intervene in bone resorption, promoting osteoclastic differentiation and eventually increase in bone resorption (Sontakke and Tare 2002).

The role of the cytokines in bone metabolism has been documented by previous study. Pro-inflammatory cytokines which also include interleukin-1, interleukin-6 and tumor necrosis factor- α , are regulators of bone resorption and has been implicated in bone disorder (McLean 2009). In another recent study, it was shown that postmenopausal osteoporosis is an inflammatory disorder, due to the association between estrogen deficiency and cytokines such as receptor activator of nuclear factor kappa-B ligand (RANKL) and interleukin-17 (De Selm et al., 2012).

In the present study, we observed an increase in interleukin-1 levels in the ovariectomized rats after 8 weeks. This is in agreement with other findings previously mentioned. This finding is suggestive of an increase in bone resorption. However, in the groups supplemented with *Cosmos caudatus* and calcium, no increment of IL-1 was observed. This further suggests that the supplements were able to prevent the increase in IL-1 thus prevent bone resorption.

C. caudatus is rich with phenolic compound and has a strong antioxidant activity which is proposed to have protective effects against lipid peroxidation and able to scavenge free radicals (Abas et al., 2003). Besides having high phenolic compound, *C. caudatus* is found to have high flavonoid content such as catechin, epicatechin and quercetin (Mustafa et al., 2010). These properties of *C. caudatus* may be responsible in protecting bone from oxidative stress and subsequently prevent bone loss. In addition, *C. caudatus* is found to have anti-inflammatory effects where the methanolic extract showed the highest anti-inflammatory activity followed by aqueous extract (Ajaykumar et al., 2012). The reduction in interleukin-1 seen in the *C. caudatus* treated groups in this study may be due to the anti-inflammatory property of *C. caudatus*.

Cosmos caudatus has been shown to improve structural parameters of bone histomorphometry in ovariectomized

rats (Mohamed *et al.*, 2012). However, to date, no study has been done on *Cosmos caudatus* in relation to bone metabolism or bone cytokines. The effects of *C. caudatus* on bone observed in this study may be attributed to its phenolic and flavonoid compounds. One study has shown that polyphenolic fraction of berry fruits suppressed the production of inflammatory mediators such as interleukin-1 in gingival fibroblasts (Zdarilová *et al.*, 2010). Another study using black tea extract showed a suppression of interleukin-1 secretion from peripheral blood mononuclear cells (Neyestani *et al.*, 2009). These effects were attributed to the antioxidant and anti-inflammatory properties of the substances.

Previous studies have shown that other phenolic substances, such as p-coumaric (Folwarczna *et al.*, 2009), tyrosol and hydroxytyrosol from olive oil (Puel *et al.*, 2008), also protect bone in ovariectomised animals. Thus *Cosmos caudatus* which also possessed phenolic compounds may have similar effects on bone.

Upon ovariectomy, bone formation and bone resorption markers such as osteocalcin and deoxypyridinoline were found to be increased in a previous study (Kawakita *et al.*, 2009). Similar observations were found in another study, in which osteocalcin and pyridinoline were increased post-ovariectomy (Hertrampf *et al.*, 2009). These findings were suggestive of increased bone turnover in ovariectomized rats. In the present study, we observed a post-ovariectomy increase in pyridinoline levels but not osteocalcin levels. Different treatment period may be responsible for this difference, 8 weeks in our study whereas 12 weeks in the previous studies. However, the increase in pyridinoline level seen in our study indicates an increase in bone resorption.

Cosmos caudatus, at all doses, was found to prevent the increase in pyridinoline levels in this study. Its effect is comparable to the group supplemented with calcium. This is suggestive of a possible protective effect of *C. caudatus* against the adverse effects of ovariectomy on bone metabolism. In one study, a Chinese herbal medicine, Du-Zhong, which has a high content of polyphenols, was able to decrease levels of bone turnover markers in ovariectomized rats (Zhang *et al.*, 2009).

The phenolic compounds in *C. caudatus* need to be identified in order to better understand its effects. Different phenolic acids may exert different effects; either beneficial or harmful, on bone metabolism as shown in a previous study (Folwarczna *et al.*, 2009). Thus, further studies are required to confirm whether these compounds were responsible for the effects on bone and which type of phenolic is involved.

In addition to the phenolic compound arguments above, the beneficial effects of *Cosmos caudatus* on bone may also be attributed to its mineral content since it possesses

high calcium content (Nutriweb Malaysia), hence the comparable effects seen between *Cosmos caudatus* and calcium supplementation in this study. However, further studies are needed to determine the calcium content in *Cosmos caudatus* to ascertain these findings.

In conclusion, *Cosmos caudatus* may play a role in preventing bone loss in ovariectomised rats by reducing bone resorption. To establish the mechanism by which *C. caudatus* act, further studies on serum mineral levels, estrogen levels, antioxidant activities and parathyroid hormone levels must be carried out. In addition, the effects of *C. caudatus* on other bone parameters should also be determined.

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