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Accelerative Effect of Fenugreek Seeds on the Healing of Mandibular Fracture in Male Dromedary Camels and Monitoring of the Healing by Bone Biomarkers

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Abstract: The present study was designed to determine the possible effect of fenugreek (*Trigonella foenum graecum*) seeds on acceleration of healing of mandibular fracture in male dromedary camels. The mandibular fracture healing was monitored by determining the concentrations of serum bone resorption {pyridinoline (PYD) cross-links} and formation {Bone Alkaline Phosphatase (BAP) and osteocalcin} biomarkers. Twenty adult male camels with recent bilateral mandibular fractures were used in this study. Interdental wiring technique using stainless steel wire was adopted to immobilize the fractured horizontal rami. The camels were randomly divided into two groups: Treatment group and control group. The results showed that feeding camels in the treatment group with fenugreek (100 g/camel/day for 2 weeks) accelerated mandibular fracture healing. It was concluded that serial determination of the concentrations of serum PYD, BAP and osteocalcin during mandibular fracture could be a useful tool in predicting fracture healing in male dromedary camels.

Key words: Pyridinoline (PYD) cross-links, bone alkaline phosphatase (BAP), osteocalcin, mandibular fracture, fenugreek, camel

INTRODUCTION

Fenugreek (*Trigonella foenum graecum*) is an annual herb that has a long history as both a culinary and traditional medicinal plant. The seeds of fenugreek have strong flavor and are commonly used as a spice in food preparation. Literature reviews have revealed several benefits of fenugreek seeds under various experimental studies. Fenugreek has been shown to possess significant antidiabetic antiatherosclerotic (Sharma *et al*., 1996), antiulcerogenic (Pandian *et al*., 2002), anticholestrol (Thompson Coon and Ernst, 2003), antioxidant properties (Dixit *et al*., 2005) and antineoplastic effects (Sur *et al*., 2001) particularly colon tumor (Devasena and Menon, 2003, 2007) and breast cancer (Sebastian and Thampan, 2007). It has also been used to protect liver from ethanol-induced hepatotoxicity (Thirunavukkarasu *et al*., 2003; Kaviarasan and Anuradha, 2007) and regulate hyperthyroidism (Tahiliani and Kar, 2003).

Mandibular fracture is a common condition that occur in male camels particularly during the breeding or rutting season when a savage male camel fights with other camels (Gahlot, 2000). Mandibular fractures can also occur in females but in small percentage (Gahlot, 1990). Most mandibular fractures appear as bilateral horizontal fractures for the mandibular fractures. Both external and internal fixation techniques have been reported to be used as a successful treatments (Gahlot, 2000; Ramadan, 1994). The techniques of external fixation include interdental wiring and percutaneous transfixation (Gahlot, 2000). Internal fixation techniques include bone plating and intramedullary pinning (Ramadan, 1994).

The concentrations of bone resorption biomarkers such as pyridinoline crosslinks (PYD) and bone formation biomarkers such as Bone Alkaline Phosphatase (BAP) and osteocalcin can be determined

in blood. They provide useful instruments for studying remodeling process in bone diseases, bone fractures and physical exercise. The pyridinoline (PYD) cross-links have received considerable attention as one of the most promising biomarkers of bone and cartilage resorption. PYD cross-links are derived from hydroxylysine and lysine residues within the collagen molecule and are found in mature type 1, 2 and 3 collagens (Robins, 1983). The cross-links stabilize collagen fibrils by covalently linking collagen molecules between two telopeptides and a triple-helical sequence at two intermolecular sites.

Total Alkaline Phosphatase (TAP) is a membrane-bound enzyme that hydrolyzes phosphate esters. It is found in most organs of all mammalian species. Its mechanism of release and specific function are still unknown. Bone-specific Alkaline Phosphatase (BAP) is the major component of TAP that its level can be determined in blood.

Osteocalcin (gamma carboxy-glutamic acid-Gla) also referred to as Bone Gla Protein (BGP), is a small vitamin K-dependent and calcium binding protein that contains 49 amino acids with three Gla residues. It is the most abundant noncollagenous protein in bone tissue. Osteocalcin is biosynthesized and secreted by osteoblasts (Beresford *et al*., 1984) and is therefore considered a specific osteoblastic marker produced during bone synthesis. During incorporation into the bone matrix, a small fraction is secreted into the blood where it can be detected.

The current study was carried out to determine the effect of fenugreek (*Trigonella foenum graecum*) seed powder on healing of mandibular fracture in dromedary camels and also to monitor the concentrations of PYD, BAP and osteocalcin in serum of dromedary camels that had mandibular fractures and surgically treated with interdental wiring technique.

MATERIALS AND METHODS

Camels

Twenty adult male camels, 7-10 years old, which had been presented to the Veterinary Teaching Hospital at Qassim University during the rut season (January-April, 2007) for treatment of mandibular fractures, were used in the present study. The camels had relatively recent bilateral mandibular fractures (1-2 days before the presentation). All the camels were in good health as assessed by physical and clinical examinations including body temperature, respiratory rate, heart rate, renal and hepatic functions. Each camel was controlled in the sitting position and sedated with xylazine (0.2 mg kg^{-1} IV Bomazine BOMAC Laboratories Ltd., New Zealand) and the mandibular fracture was surgically immobilized with interdental wiring technique using 1.4 mm thick monofilament stainless steel wire. The steps of interdental wiring technique have been described in details by Gahlot (2000). Penicillin (10,000 IU kg^{-1} b.wt. IM) was injected postoperatively once a day for five days. The camels were randomly divided into two groups: Treatment group (N = 10) and control group (N = 10). In both groups, the camels were fed with cow milk for a period of three weeks, followed by soft food including green alfalfa for 2 weeks. Starting from the 6th week, the camels were fed with dry alfalfa gradually mixed with green alfalfa. In addition, camels in the treatment group were fed with fenugreek (*Trigonella foenum graecum*) seed powder (100 g/camel/day) mixed either with their milk or water.

Sample Collection

Blood samples were collected in plain vacutainer tubes from the jugular vein of each camel immediately before the fixation of the fracture and then once a week for 7 weeks. Serum samples were stored at -20°C till assayed.

Biomarker Assays

Commercial immunoassay kits were used to assess the concentrations of PYD, BAP and osteocalcin (Quidel Cor., California, USA). Briefly, PYD assay was performed by adding 50 μL of

assay reagent and 25 µL of standards, controls, or serum samples to each well of the coated 96-well plate. After adding 75 µL of PYD antibody to each well, the plate was incubated overnight in the dark refrigerator. After washing with washing buffer, 150 µL of enzyme conjugate were added to each well. The plate was incubated for one hour at room temperature and then washed with washing buffer. The plate was incubated for 40 min at room temperature. Stop solution (100 µL) were added to each well and the automated plate reader was used to read the optical density at 405.

BAP assay was performed by adding 125 µL of assay buffer and 20 µL of standards, controls, or serum samples to each well of the coated 96 well plate. After incubation for three h at room temperature, the plate was washed with washing buffer. After adding 150 µL of working substrate solution, the plate was incubated for 30 min at room temperature. Finally, 100 µL of stop solution were added to each well and the optical density was read at 405 nm using an automated plate reader. Osteocalcin was performed by adding 25 µL of standards, samples to each well of a 96 well plate that previously coated with human osteocalcin. The plate was incubated for two h at room temperature after adding 125 µL of antiosteocalcin antibody to each well. After washing with buffer, 150 µL of enzyme conjugate were added to each well. The plate was incubated for one h at room temperature and then washed with buffer. After that, 150 µL of working substrate solution were added to each well and the plate incubated for 40 min at room temperature. Finally, 50 µL of stop solution were added to each well and the optical density was read at 405 nm using an automated plate reader.

Statistical Analysis

A repeated measures design was used as the statistical model and one-way Analysis of Variance (ANOVA.) was used to determine the differences between groups and within a group. Tukey's Honset Significant Difference was used to determine the multiple comparisons of means. The significance level was set at $p<0.05$ and SAS software (Statistical Analysis System) was used to perform all statistical calculations.

RESULTS AND DISCUSSION

Seven weeks were sufficient for complete healing of mandibular fractures in fenugreek-treated camels compared to 10-11 weeks for control group. In both groups the wires were left for extra two weeks and then removed. Submandibular abscesses and oral wounds due to the embedding of wire in the mouth were the major postoperative complications seen in the present study. Only one camel in the treatment group and three camels in the control group developed submandibular abscesses postsurgically. Four camels in the treatment group and five camels in the control groups had oral wounds as a result to the embedding of wires in the mouth.

Table 1 shows the weekly concentrations of serum PYD in fenugreek-treated camels compared with the control along the experimental period. The levels of PYD in both groups were relatively high at day zero and gradually decreased. In the treatment group, a significant reduction in levels of PYD was observed at week two and continued until end of the study, whereas in the control group, a significant decrease was detected starting from week four and continued until end of the study.

Table 1: Mean levels of serum PYD (nmol L^{-1}), serum BAP (U L^{-1}) and serum osteocalcin (ng mL^{-1}) in the treatment group compared with the control group along the experimental period

		Weeks							
Parameters		0	1	2	3	4	5	6	7
Serum PYD	Treatment	15.6	12.3	7.5	6.5	7.1	6.4	7.2	7.3
(nmol L^{-1})	Control	16.8	15.7	13.4	12.2	9.5	8.5	6.4	6.3
Serum BAP	Treatment	45.4	84.2	111.0	123.8	117.3	113.9	94.8	84.3
(U L^{-1})	Control	50.6	63.4	78.5	84.9	92.5	91.5	94.5	92.5
Serum osteocalcin	Treatment	20.4	22.3	25.6	34.9	40.6	52.7	56.6	55.8
(ng mL^{-1})	Control	21.4	23.8	26.4	27.1	28.5	32.4	35.6	40.4

Starting from week one, the levels of BAP significantly increased in both groups and continued high until the end of the study (Table 1). However, the concentrations of BAP in the treatment group were significantly higher than those in the control group.

Starting from week 3, levels of osteocalcin significantly increased in camels of the treatment group and continued high until the end of the study (Table 1).

In the present study, stainless steel wire (Thick 1.4 mm) was surgically used for the immobilization of bilateral mandibular fracture in dromedary camels. The major two postoperative complications seen in the present study were the formation of submandibular abscesses and oral wounds due to the burying of wire in the mouth. Gahlot (2000) has reported similar complications when using 1-2 mm thick copper wire. In the present study, only four camels (one from the treatment group and three from the control group) had developed post surgical submandibular abscesses whereas in Gahlot report (2000) submandibular abscesses are usual postoperative complications seen in camels with bilateral fractures. One advantage of using stainless steel wire over the other types of wire is its stability in contaminated wound and this might be the reason for the relatively lower incidence of the formation of postoperative submandibular abscesses in the present study. Another advantage of stainless steel wire includes its consideration as the standard for judging knot security and tissue reaction to suture materials (Fossum *et al*., 1997). Only one camel from the treatment group had postoperative submandibular abscess compared to three camels in the control group. The reason for that is unknown however, Bin-Hafeez *et al*. (2003) have found that aqueous extract of fenugreek enhanced immune functions in mice. The submandibular abscess was surgically opened and the pus was drained out and the wound was dressed with 2.5% tincture of iodine (Gahlot, 2000). One disadvantage of metallic sutures including stainless steel is there tendency to cut tissue. In the present study, four camels from the treatment group and five camels from the control group had oral wounds as a result of the embedding of wires in the mouth. The wounds were treated successfully by flushing with physiological saline and 0.5% of iodine solution. To decrease the tissue cutting effect, the wire can be threaded into an appropriate diameter of polyethylene tube.

The present study has shown that adding fenugreek (*Trigonella foenum graecum*) seed powder (100 g/camel/day) to the feeding milk or water of male dromedary camels significantly accelerated the mandibular fracture healing. The exact mechanism by which the fenugreek seed powder affects bone healing is not clear. However, Bone Morphogenetic Protein (BMP) effects for fenugreek could be suggested here. In addition, the seeds of fenugreek are very rich with amino acids. Shang *et al*. (1998) isolated 17 amino acids from fenugreek seeds seven of them were essential amino acids.

Physical and serial radiological examinations are a routine methods used to monitor bone healing of the fracture site. However sometimes, it is difficult to distinguish a delayed union from a nonunion and advanced imaging techniques may not be available. Serum biomarkers of bone turnover may be clinically useful in evaluating the progress of fracture healing. There is no any report that has dealt with changes in bone turnover biomarkers during the healing process of bilateral mandibular fracture in dromedary camels. The present study has shown that the levels of bone resorption biomarkers (PYD) were high at the beginning and gradually decreased with time. An early significant increase was seen in the concentration of BAP with a maximum level at week three (in the treatment group) and later significant increase was detected in the levels of osteocalcin. Ingle *et al*. (1999) have studied the changes in bone turnover following ankle fractures in women. They have found that bone formation biomarkers significantly increased between 1 and 4 weeks and BAP returned to baseline at 52 weeks, but osteocalcin remained elevated, whereas bone resorption biomarkers did not increase by the time and NTx was decreased at 52 weeks (Ingle *et al*., 1999). Veitch *et al*. (2006) have found that there was an earlier increase in bone resorption biomarkers and later rise in bone formation biomarkers. Recently, It has been reported that changes in bone turnover biomarkers in normal bone healing primarily dependent on the fracture size (Stoffel *et al*., 2007). In the present study, the locations of bilateral mandibular fractures in all the camels were just before the first premolars in both sides. Therefore, all the fractures were expected to have relatively the same size.

The beginning increase of the levels of PYD was an expected result because bone fracture leads to increase bone turnover (Akesson *et al*., 1993; Garnero *et al*., 1996; Riis *et al*., 1996). Van Daele *et al*. (1996a, b) have reported that increased levels of collagen crosslink biomarkers can be used to predict hip fracture risk in elderly women. It has been thought that collagen crosslinks predict future fracture with the same magnitude that blood pressure predicts stroke or serum cholesterol predicts risk of coronary artery disease (Marshall *et al*., 1996). Thus, the increase of PYD concentration is associated with increase in bone resorption and that usually occurred in conditions of bone fractures at least in the beginning of fracture occurrence. Akesson *et al*. (1993) have found increased urinary cross-link excretion within hours after hip fracture in human patients. The present study showed that the reduction level of PYD in treatment group was significantly lower than those in the control group. This might indicate that fenugreek negatively affects bone resorption in camels with bilateral mandibular fractures.

PYD are not found only in mature type 1 collagen which is the major type of collagen in bone tissues (Von Der Mark, 1999), but also found in types 2 and 3 collagens (Robins, 1983. Type 2 collagen is the major collagen found in hyaline cartilage (Miller, 1971), whereas type 1 and 3 collagens are the major collagens found in various soft tissue structures including ligaments and tendons (Palmer and Bertone, 1996; Todhunter, 1996). Therefore, the source of PYD elevation observed in the present study was probably due to the changes in all of these structures together. However, because bone is by far the most abundant source of collagen matrix and because its rate of turnover is markedly higher than some other connective tissues such as cartilage, PYD concentrations in biological fluids are likely to be predominantly derived from bone. Deoxypyridinoline (DPD) is another cross-link found in high amounts in mineralized tissues and therefore considered to be specific for bone collagen degradation (Eyre *et al*., 1984). In the present study, DPD levels were not assessed due to the unavailability of the assay when doing the study. In addition, many studies have reported no change between PYD and DPD in reflecting bone resorption (Akesson *et al*., 1993).

The present study showed that the levels of BAP in both groups significantly increased one week post the fracture immobilization and continued until the end of the study. In the treatment group, BAP reached its maximum level by week three, whereas the increase of BAP was gradual with less skewed curve in the control group. It has been reported that levels of TAP significantly increased of about 30% two weeks after tibial shaft fractures (Bowles *et al*., 1996). In another study, Nakagawa *et al*. (2006) have shown that the levels of TAP rose to a maximum at 3 weeks after surgery and then gradually decreased for femoral neck and trochanter fractures. A preliminary report has indicated that BAP predicts vertebral and nonvertebral fracture (Ross *et al*., 1997) and it was a useful tool to predict osteoporotic fractures in postmenopausal women (Ross *et al*., 2000). In the present study, the amount of increase in the levels of BAP in the treatment group was significantly higher than those in the control group. This might indicate that fenugreek seed powder has a positive effect on the synthesis and/or secretion of BAP.

The present study has shown that there was gradual increase in both groups in the levels of osteocalcin from week to the following week. The concentration of osteocalcin significantly increased starting from weeks 3 and 5 in the treatment and the control groups, respectively. Starting from week 3, the levels of osteocalcin in the treatment group were significantly higher than those in the control group. It has been reported that levels of osteocalcin increased in the first four days post fracture, then decreased until five weeks, after which there was an increase of around 35% by 20 weeks, in comparison to day one of the fracture (Bowles *et al*., 1996). Joerring *et al*. (1992) have reported that following Colles' fracture, the concentrations of osteocalcin significantly increased by 44% within one week compared to baseline (fracture sustained less than 24 h earlier) and had returned to baseline at 9 months. In the present study, the increase in the levels of osteocalcin reached an average two-fold after 49 days. The average increase of osteocalcin in camels of the treatment group was approximately three-folds. This might indicate that fenugreek seed powder positively enhanced osteocalcin formation during bone fracture healing in camels.

In the present study, levels of BAP increased at least two weeks earlier than that of osteocalcin. Although BAP and osteocalcin are considered bone formation biomarkers, their correlation in serum has been reported to be weak (Alsobayil, 2005). It has been attributed that the lack of having strong correlation between the two biomarkers was because each one of them reflects different stages of osteoblast function (Delmas *et al*., 1990). In fact, BAP represents an early osteoblast biomarker because it presents in preosteoblasts and osteoblasts, whereas osteocalcin is considered a later biomarker of osteoblast differentiation and bone mineralization (Hauschka and Gallop, 1977; Naylor and Eastell, 1999). This might explain the earlier elevation of serum BAP and not osteocalcin in the present study. In addition there were changes in the osteoblastic activity during bone fracture healing which might affect the fluctuation in the concentration of bone formation biomarkers (Taniguchi *et al*., 2003).

In conclusion, the serial determination of the concentrations of serum PYD, BAP and osteocalcin during mandibular fracture could be a useful tool in predicting fracture healing in male dromedary camels. This study also has shown that adding fenugreek seed powder to the ration of camels that had bilateral mandibular fractures and surgically immobilized by interdental wiring technique significantly stimulated bone formation and inhibited bone resorption. The resultant was the enhancement of bilateral mandibular fracture healing in dromedary camels.

REFERENCES

Akesson, K., P. Vergnaud, E. Gineyts, P.D. Delmas and K.J. Obrant, 1993. Impairment of bone turnover in elderly women with hip fracture. Calcif Tissue Int., 53 (3): 162-169.

Alsobayil, F.A., 2005. Circadian rhythm of bone formation biomarkers in serum of dromedary camels. Vet. Med. J. Giza, 53 (3): 873-882.

Beresford, J.N., J.A. Gallagher, J.W. Poser and R.G. Russell, 1984. Production of osteocalcin by human bone cells *in vitro*: Effects of $1,25(OH)_2$ D_3, $24,25(OH)_2$ D_3, parathyroid hormone and glucocorticoids. Metab. Bone Dis. Rel. Res., 5 (5): 229-234.

Bin-Hafeez, B., R. Haque, S. Parvez, S. Pandey, I. Sayeed and S. Raisuddin, 2003. Immunomodulatory effects of fenugreek (*Trigonella foenum graecum* L.) extract in mice. Int. Immunopharmacol., 3 (2): 257-265.

Bowles, S.A., N. Kurdy, A.M. Davis, M.W. France and D.R. Marsh, 1996. Serum osteocalcin, total and bone specific alkaline phosphatase following isolated tibial shaft fracture. Ann. Clin. Biochem., 33 (pt 3): 196-200.

Delmas, P.D., P.A. Price and K.G. Mann, 1990. Validation of the bone gla protein (osteocalcin) assay. J. Bone Miner. Res., 5 (1): 3-4.

Devasena, T. and V.P. Menon, 2003. Fenugreek affects the activity of beta-glucuronidase and mucinase in the colon. Phytother. Res., 17 (9): 1088-1091.

Devasena, T. and V.P. Menon, 2007. Fenugreek seeds modulate 1,2-dimethylhydrazine-induced hepatic oxidative stress during colon carcinogenesis. Ital. J. Biochem., 56 (1): 28-34.

Dixit, P., S. Ghaskadbi, H. Mohan and T.P. Devasagayam, 2005. Antioxidant properties of germinated fenugreek seeds. Phytother. Res., 19 (11): 977-983.

Eyre, D.R., M.A. Paz and P.M. Gallop, 1984. Cross-linking in collagen and elastin. Annu. Rev. Biochem. 53: 717-748.

Fossum, T.W., C.S. Hedlund, D.A. Hulse, A.L. Johnson, H.B. Seim, M.D. Willard and G. Carroll, 1997. Biomaterials, Suturing and Hemostasis. In: TW Fossum Small Animal Surgery. Mosby, St. Louis, pp: 42-56.

Gahlot, T.K., 1990. Repair of mandibular fractures in camels. Int. Conf. Camel Prod. Improv. Tobruk (Libya).

Gahlot, T.K., 2000. Fractures. In: TK Gahlot Selected Topics on Camelids. The Camelid Publishers, pp: 382-407.

Garnero, P., E. Hausherr, M.C. Chapuy, C. Marcelli, H. Grandjean, C. Muller, C. Cormier, G. Bréart, P.J. Meunier and P.D. Delmas, 1996. Markers of bone resorption predict hip fracture in elderly women: The EPIDOS prospective study. J. Bone Miner. Res., 11 (10): 1531-1538.

Hauschka, P.V. and P.M. Gallop, 1977. Purification and Calcium Binding Properties of ksteocalcin, the Gamma-carboxyglutamate Kontaining Protein of Bone. In: Calcium Binding Proteins and Calcium Function. Wasserman, R. (Ed.). Elsevier/North-Holland, Amsterdam, pp: 338-347.

Ingle, B.M., S.M. Hay, H.M. Bottjer and R. Eastell, 1999. Changes in bone mass and bone turnover following ankle fracture. Osteoporos Int., 10 (5): 408-415.

Joerring, S., L.T. Jensen, G.R. Anderson and J.S. Johansen, 1992. Types 1 and 3 procollagen extension peptides in serum respond to fracture in humans. Arch. Orthop. Trauma. Surg., 111 (5): 265-267.

Kaviarasan, S. and S. Anuradha, 2007. Fenugreek (*Trigonella foenum graecum*) seed polyphenols protect liver alcohol toxicity: A role on hepatic detoxification system and apoptosis. Pharmazi, 62 (4): 299-304.

Marshall, D., O. Johnell and H. Wedel, 1996. Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. BMJ., 312 (7041): 1254-1259.

Miller, E.J., 1971. Isolation and characterization of a collagen from chick cartilage containing three identical alpha chains. Biochemistry, 10 (9): 1652-1659.

Nakagawa, H., M. Kamimura, K. Takahara, H. Hashidate, A. Kawaguchi, S. Uchiyama and T. Miyasaka, 2006. Changes in total alkaline phosphatase level after hip fracture: Comparison between femoral neck and trochanter fractures. J. Orthop. Sci., 11(2): 135-139.

Naylor, K.E. and R. Eastell, 1999. Measurement of Biochemical Markers of Bone Formation. In: Dynamics of Bone and Cartilage Metabolism, Seibel, M.J., S.P. Robins and J.P. Bilezikian (Eds.). SanDiego, Academic Press, pp: 401-410.

Palmer, J.L. and A.L. Bertone, 1996. Joint biomechanics in the pathogenesis of traumatic arthritis. In: Joint Disease in the Horse. McIlwraith, C.W. and G.W. Trotter. W.B. Saunderss Company, Philadelphia. pp: 112-114.

Pandian, R.S., C.V. Anuradha and P. Viswanathan, 2002. Gastroprotective effect of fenugreek seeds (*Trigonella foenum graecum*) on experimental gastric ulcer in rats. J. Ethnopharma, 81 (3): 393-397.

Ramadan, R.O., 1994. Fractures. In: RO Ramadan Surgery and Radiology of The Dromedary Camel. Al-Jawad Printing Press. Saudi Arabia, pp: 226-248.

Riis, B.J., M.A. Hansen, A.M. Jensen, K. Overgaard and C. Christiansen, 1996. Low bone mass and fast rate of bone loss at menopause: Equal risk factors for future fracture: A 15-year follow-up study. Bone, 19 (1): 9-12.

Robins, S.P., 1983. Cross-linking of collagen. Isolation, structural characterization and glycosylation of pyridinoline. Biochem. J. 215 (1): 167-173.

Ross, P.D., R.D. Wasnich and W.K. Knowlton, 1997. Skeletal alkaline phosphatase (Tandem®-R Ostase®) measurements predict vertebral fractures: A prospective study. J. Bone Miner. Res., 12 (Suppl. 1): S150.

Ross, P.D., B.C. Kress, R.E. Parson, R.D. Wasnich, K.A. Armour and I.A. Mizrahi, 2000. Serum bone alkaline phosphatase and calcaneus bone density predict fractures: A prospective study. Osteoporos Int., 11 (1): 76-82.

Sebastian, K.S. and R.V. Thampan, 2007. Differential effects of soybean and fenugreek extracts on the growth of MCF-7 cells. Chem. Biol. Interact, 170 (2): 135-143.

Shang, M., S. Cai and X. Wang, 1998. Analysis of amino acids in *Trigonella foenumgraecum* seeds. Zhong Yao Cai, 21 (4): 188-190.

Sharma, R.D., A. Sarkar, D.K. Hazra, B. Misra, J.B. Singh, B.B. Maheshwari and S.K. Sharma, 1996. Hypolipidaemic effect of fenugreek seeds: A chronic study in non-insulin dependent diabetic patients. Phytother. Res., 10 (4): 332-334.

Stoffel, K., H. Engler, M. Kustera and W. Riesen, 2007. Changes in biochemical markers after lower limb fractures. Clin. Chem., 53 (1): 131-134.

Sur, P., M. Das, A. Gomes, J.R. Vedasiromoni, N.P. Sahu, S. Banerjee, R.M. Sharma and D.K. Ganguly, 2001. *Trigonella foenum graecum* (Fenugreek) seed extract as an antineoplastic agent. Phytother. Res., 15 (3): 257-259.

Tahiliani, P. and A. Kar, 2003. The combined effects of Trigonella and Allium extracts in the regulation of hyperthyroidism in rats. Phytomedicine, 10 (8): 665-668.

Taniguchi T., T. Matsumoto and H. Shindo, 2003. Changes of serum levels of osteocalcin, alkaline phosphatase, IGF-I and IGF-binding protein-3 during fracture healing. Injury, 34 (7): 477-479.

Thirunavukkarasu, V., C.V. Anuradha and P. Viswanathan, 2003. Protective effect of fenugreek (*Trigonella foenum graecum*) seeds in experimental ethanol toxicity. Phytother. Res., 17 (7): 737-743.

Thompson Coon, J.S. and E. Ernst, 2003. Herbs for serum cholesterol reduction: A systemic view. J. Fam Pract, 52 (6): 468-478.

Todhunter, R.J., 1996. Anatomy and Physiology of Synovial Joints. In: Trotter Joint Disease in the Horse, McIlwraith, C.W. and G.W. (Eds.). W.B. Saunders Company, Philadelphia, pp: 1-28.

Van Daele, P.L., M.J. Seibel, H. Burger, A. Hofman, D.E. Grobbee, J.P. Van Leeuwen, J.C. Birkenhäger and H.A. Pols, 1996a. Case-control analysis of bone resorption markers, disability and hip fracture risk: The Rotterdam study. BMJ., 312 (7029): 482-483.

Van Daele, P.L., M.J. Seibel, H. Burger, A. Hofman, D.E. Grobbee, J.P. Van Leeuwen, J.C. Birkenhäger and H.A. Pols, 1996b. Evidence for uncoupling of bone formation and bone resorption in women with hip fractures: A prospective study. Osteoporous Int., 6 (Suppl. 1): S199.

Veitch, S.W., S.C. Findlay, A.J. Hamer, A. Blumsohn, R. Eastell and B.M. Ingle, 2006. Changes in bone mass and bone turnover following tibial shaft fracture. Osteporos Int., 17 (3): 364-372.

Von Der Mark, K., 1999. Structure, Biosynthesis and Gene Regulation of Collagens in Cartilage and Bone. In: Dynamics of Bone and Cartilage Metabolism, Seibel, M.J., S.P. Robins and J.P. Bilezikian (Eds.). Academic Press, San Diego, pp: 3-29.