Clinical and Radiographic Assessment of Single or Combined Treatment with *Lepidium sativum* and Alendronate of Non-Surgically Treated Chronic Periodontitis in Postmenopausal Osteoporotic Women

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Abstract

Aim of the study: The aim of the study was to compare the clinical outcomes, radiometric indices (antigonial index and mental index) before and after non- surgical treatment of chronic periodontitis in postmenopausal osteoporotic women receiving alendronate (ALN) and Lepidium sativum either singly or combined.

Material and methods: The study was conducted on 45 postmenopausal osteoporotic women with chronic periodontitis. The study population was divided randomly and equally into three groups: A, B and C (15 patients each). Group A: local debridement was performed for these patients. They received ALN at a dose of (70 mg/ once/ week) for six months as regular treatment of osteoporosis. Group B: patients were given finely powdered dried seeds of Lepidium sativum (LS; 1 gm tid) for six weeks plus local debridement. Group C: patients received combined treatment of LS at a dose of 1 gm/tid (for six weeks) and ALN at a dose of 70 mg once weekly for six months plus local debridement. Gingival index (GI), pocket depth (PI) and clinical attachment level (CAL) were measured together with ADAM8 level in the GSF of the study group at baseline, three and six months.

Results: By comparing the means of GI at baseline, three months and six months within the three groups, Group C showed the highest significant decrease in the mean GI (p = 0.01). Comparing the pocket depth and the clinical attachment level, Group B and C showed comparable pocket depth reduction and Group C reported the highest clinical attachment gain at p=0.001. By comparing the mean of radiometric indices of the three groups through the whole study period, Group C showed the greatest increase in the MI index from baseline to six months at p=0.0001.

By comparing the mean percentage of change (%) in GI among the three groups, Group C showed the statistically significant highest mean percent decrease in GI. By comparing the mean percentage of change of pocket depth before and after treatment, both Groups B and C showed significantly higher percentage of PD reduction than Group A after three months and six months at p = 0.002 and p = 0.001, respectively, while no significant difference was detected between groups B and C through the whole study period. By comparing mean percentage of changes of CAL, Group C showed the highest percentage of CAL change.

Conclusion: Within the limits of the study, it is concluded that Lepidium sativum can be used in the treatment of osteoporotic postmenopausal women with chronic periodontitis who refuse treatment with ALN. The combined use of LS and ALN may have a synergistic effect resulting in a more favorable clinical response, increased bone mass than using ALN alone when combined with conventional therapy in treatment of chronic periodontitis in postmenopausal osteoporotic women.

Key words: host modulation, Lepidium sativum, periodontitis, osteoporosis, radiomorphometric indices

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Introduction

Both periodontal diseases and osteoporosis are bone resorptive diseases that are multifactorial in etiology (Basha, 2014). There may be a bidirectional relationship between osteoporosis and periodontitis. A positive association between these common diseases has been demonstrated (Wactawski-Wende *et al.,* 2005; Karayianni *et al*, 2009; Pepelassi, 2012). Insufficient estrogen is closely related to periodontitis and osteoporosis. Estrogen deficiency leads to increased expression of inflammatory cytokines alveolar bone loss and high bone turnover rates (kholsa et *al.*2011; Luo *et al.,* 2014).

The use of radiomorphometric indices may provide the opportunity of early identification of osteoporotic patients, who actually need treatment (**Uysal** *et al.,* 2007). Quantitative and qualitative indices have been used to assess bone and osteoporosis using panoramic radiography. **(**Lazic *et al.*, 2002) Quantitative indices include gonial angle, antegonial angle, antegonial index (AI), mental index (MI) and panoramic mandibular index (PMI), while qualitative indices include the mandibular cortical index (MCI) (Zlataric and Celebic, 2003).

Konstantinos, *et al.* (2007) found a significant correlation between vertebral, femoral bone mineral density (BMD) and the mandibular cortical width (MI). They demonstrated that a decrease in mandibular cortical width of 1 mm increases the likelihood of osteopenia or osteoporosis by 43%.

With the current understanding of the host response and periodontal disease pathogenesis, it is likely that pharmaceutical inhibition of host response pathways may be an adjunctive or alternative strategy for treating periodontal disease (Van Dyke and Serhan, 2003; Rangarajan *et al.*, 2014). A variety of different drug classes have been evaluated as host modulation agents, including; non-steroidal anti-inflammatory drugs (NSAIDs), tetracycline and non-antimicrobial formulations of tetracycline; sub antimicrobial doxycycline dosing (SDD) (Lee *et al.*, 2004; Golub, *et al.,* 2008; Preshaw, 2008), chemically modified tetracyclines (Agnihotri and Gaur, 2012) and bisphosphonates, (BPs) (Alencar *et al.*, 2002).

BPs produce a sustained reduction in the levels of biochemical markers of bone remodeling, returning them to the premenopausal range. They also increase bone mineral density and decrease the risk of osteoporotic fracture in postmenopausal women (Badran et al,2009). Long-term intervention studies have shown that continuous alendronate (ALN) therapy is associated with a sustained therapeutic effect on bone density and remodeling and improved bone mass (Veena and Prasad, 2010; Kazuhiro, 2013).

Despite the benefits of these drugs, bisphosphonate related osteonecrosis of the jaw (BRONJ) which was first reported in 2003 is a severe side effect of bisphosphonate therapy. Since this initial identification, many cases have been reported (Bagan *et al.,* 2006). BRONJ has been reported to be 0.7/100,000 person/years of exposure (Ruggiero *et al.,* 2009).

Medical plants constitute a promising source of phytotherapy drugs due to their minor side effects, affordable cost and efficiency when compared to synthetic drugs. Based on numerous chemical and pharmacological studies, many plants and their compounds have been shown to possess anti-osteoporotic activity (Krishnarajua *et al.,* 2005).

Lepidium sativum (LS) has been considered as an important medical plant since the Vedic era (Sharma and Agarwal, 2011). LS has immunomodulatory properties and good wound healing activities. It decreases nuclear factor (NF)-kB, nitric oxide (NO), COX-2 and leukotriene B4 (LB4) which are thought to play roles in the pathogenesis of inflammatory diseases (Diwakar *et al*, 2011). Numerous bioactive components with anti-osteoporotic potential have been isolated from the plant. These components modulate bone metabolism through estrogen receptors by inhibit bone resorption and stimulation of bone formation **(**Picherit *et al*, 2000).

We hypothesized that the combined use of LS and ALN may have a synergistic effect resulting in more favorable clinical response and increase in bone mass than using either of them singly combined with conventional therapy in treatment of chronic periodontitis in postmenopausal osteoporotic women.

Thus, this study was performed to compare the clinical outcomes, radiometric indices (mental index and antigonial index) in postmenopausal osteoporotic women with chronic periodontitis and receiving alendronate and *Lepidium sativum* either singly or combined before and after non- surgical treatment.

Material and methods

Study population

This longitudinal randomized clinical study was conducted on 45 postmenopausal osteoporotic women with chronic periodontitis selected from Al Hussein Hospital and the Outpatient Clinic of Oral Medicine, Periodontology, Diagnosis and Radiology Department, Faculty of Dental Medicine, Al-Azhar University (Girls Branch), Cairo Egypt. The study population consisted of age-matched patients. All patients were informed with the nature of the study. A written informed consent was obtained from each included participant. The research design was approved by the Ethical Committee, Faculty of Dental medicine, Al-Azhar University. The participants of this study were collected during the period from September 2014 to May 2015.

Patient selection

Bone Mineral Density (BMD) in (gm /cm^2) was measured using a DXA scanner (DXA scanner LUNAR Madison, WI prodigy/DPX, model 7681 USA). Osteoporotic patients were selected based on site and gender specific cut-off values of the standard deviations (T-scores) compared to a reference group of young normal adults. Patients with T-score below -2.5 were considered as osteoporotic.

Once the DXA images had been recorded, the investigator positioned a customized rectangular region of interest (ROI) on the lumbar spine, left femur and right forearm images on-screen and the computer software calculated the BMD of these regions (Tonguc *et al.*, 2012).

Criteria for patient selection

Inclusion criteria

1. Female subjects of age range between 45-65 years and free from any systemic disease except for natural menopause, according to modified Cornell Medical Index.
2. Patients diagnosed as osteoporotic based on (DXA) scores of hip or spine (T scores less -2.5).
3. Patients with probing depths (PD) ≥ 5 to 7 mm or clinical attachment levels (CAL) ≥ 4 to 6 mm and vertical bone loss ≥ 3 mm on standardized intraoral periapical radiographs.

Exclusion criteria

1. Women with a history of surgically induced menopause, tobacco abuse, bone destructive lesions of jaw/metabolic bone diseases, diabetes mellitus, thyroid diseases and connective tissue disease. Those on hormone replacement therapy (HRT)/corticosteroids or chemotherapy and / or radiotherapy.
2. Patients with history of periodontal therapy or use of antibiotics in the past 6 months prior to examination.

Primary outcome and sample size estimation

Comparisons between the three groups of pocket depth and clinical attachment level were tested using ANOVA test. Sample size of 15 patients was estimated using G power program (University of Düsseldorf, Düsseldorf, Germany) at 80% power of the study and 0.05 level of confidence.

Randomization

A computer-generated table was used to provide a random distribution of patients. Only one person randomly enrolled the patients to groups using a single allocation ratio (1:1) and a computer program (Excel 2013 v15.0, Microsoft Windows, RAND function).

Grouping

The study population was divided randomly and equally into three groups; A, B and C (15 patients each).

Group A: Fifteen osteoporotic postmenopausal women suffering from chronic periodontitis. They received ALN as part of regular treatment of osteoporosis at a dose of (70 mg/once/week) for six months. Local debridement was done for these patients.

Group B: Fifteen osteoporotic postmenopausal women suffering from chronic periodontitis. They refused ALN treatment. They were given finely powdered dried seeds of *Lepidium sativum* (1 gm/tid) for six months added to local debridement (Paranjape and Mehta, 2006).

Group C: Fifteen osteoporotic patients suffering from chronic periodontitis. Subjects of this group received combined treatment of *Lepidium sativum* at a dose of 1 gm/tid (for six months) and ALN at a dose of 70 mg once weekly for six months added to local debridement.

Periodontal treatment protocol

All septic foci in the patients' mouth were removed prior to the start of treatment. Patients were treated periodontally by non- surgical approach as follows:

1. Each patient received an initial phase of detailed instruction in self-performed plaque control measures.
2. Scaling and root planning (SRP) using hand instrument and/or ultrasonic device were performed for each patient in (2-4) sessions
3. Recall visits were done for every patient every 2 weeks to ensure plaque control.

Periodontal assessment

Clinical monitoring was performed by a calibrated investigator who remained masked to the treatments provided. Patients were evaluated clinically as well as by assessing ADAM8 biomarker levels in GCF. Periodontal examination and GCF collection were performed at baseline, three months and six months.

A periodontal examination was done for each patient by the same examiner as follows: Gingival index (GI): the gingival status was evaluated using the (GI) of Loe and Silness (1963). Probing pocket depth (PPD): pocket depth was measured using Williams's periodontal probe. PPD measurements were recorded at six location points around the circumference of each tooth. Clinical attachment level (CAL): the readings were recorded at the same location of PD.

Panoramic radiographic measurements

Digital panoramic radiographic examination was performed at base line, three months and six months for all groups, by the same examiner using panoramic machine (OP-100 G.E, Finland, 2004) with exposure parameter (Current of 16 mA and voltages of 75 kV) and exposure time of 17.6 seconds. A photo stimulable phosphor storage plate was utilized. The plate was then scanned using the Vista Scan image laser scanner and digitalized at resolution of 300 dpi and 265 (8 bits/pixel) gray shade formats. The scan resolution pitch settings were adjusted for panoramic radiograph. Image processing and analysis was performed using Vista Scan system (Durr Dental Germany).

Linear radiomorphometric assessment

DBSwin software was used to perform vertical linear measurements in panoramic radiographic images, and subsequently panoramic radiographic indices was calculated. Mental index (MI) was measured which is the mandibular cortical thickness at the mental foramen region. Antegonial index (AI) is a measurement of cortical width in the region anterior to the gonion at a point identified by extending a line of `best fit' on the anterior border of the ascending ramus down to the lower border of the mandible. Where the anterior border of the ramus was markedly curved, the line was drawn to fit as closely as possible to the straighter, inferior, part of the bone margin above the third molar region (Ledgerton *et al.*, 1999).

Statistical Analyses

Numerical data were presented as mean and standard deviation (SD) values. Data were explored for normality using Kolmogorov-Smirnov test of normality. When variables where found to be normally distributed, ANOVA test was used to compare means of percent change in clinical parameters, radiomorphometric index among the three groups. Tukey post hoc test was used to detect which group is responsible for the significance. Kruskal-Wallis test was used for comparisons between groups when variables where not normally distributed. The significance level was set at $p \leq 0.05$. Statistical analysis was performed with SPSS 18.0, Chicago, IL, USA.

Results

Comparison of the change of mean clinical parameters and radiometric indices (Antigonial index and Mental index) within the group

By comparing the means of GI at baseline, three months and six months in three groups, Group C showed the highest significant decrease in gingival index (p = 0.01). In comparing pocket depth and the clinical attachment level, Groups B and C showed comparable pocket depth reduction and Group C reported the highest clinical attachment (p = 0.001). (*Table 1*).

By comparing the mean of radiometric indices of the three groups through the whole study period, Group C showed the greatest increase in the MI index from baseline to six months (p = 0.0001) (*Table 2*).

Table 1. Comparisons of means of different clinical parameters within the group at different time intervals.

Variable		Group A		Group B		Group C	
		Mean± SD (mm)	*P* value	Mean± SD (mm)	*P* value	Mean± SD (mm)	*P* value*
Gingival index	Baseline	1.5±0.9	0.513	1.55±0.3	0.015*	1.10±0.45	0.010*
	3 months	1.5±0.53		0.57±0.58		0.03±0.07	
	6 months	1.35±0.49		0.50±0.50		0.10±0.15	
PD Reduction	Base line	3.53±0.79		3.64±1.09	0.000*	3.47±0.23	0.000*
	3 months	3.07±1.03	0.070*	2.64±0.60		2.67±0.22	
	6 months	2.86±0.89		2.38±0.48		2.27±0.29	
CAL Gain	Base line	3.90±0.63		2.87±0.94	0.032*	2.91±0.70	0.001*
	3 months	3.31±0.45	0.011*	2.16±0.70		2.37±0.60	
	6 months	3.14±0.42		2. ±0.69		2.03±0.45	

SD: standard deviation, GI: gingival index, PD: pocket depth, CAL: clinical attachment level,
*statistically significant p < 0.05.

Table 2. Comparing means of radiometric indices (MI & AI) within the group at different time intervals.

Variable		Group A		Group B		Group C	
		Mean± SD (mm)	*P* value	Mean± SD (mm)	*P* value	Mean± SD (mm)	*P* value
AL	Base line	3.23±0.43	0.000*	3.05±0.50	0.000*	3.04±0.51	0.000*
	3 months	4.22 ±0.52		4.24±0.51		4.12±0.79	
	6 months	4.62±0.65		4.50±0.36		4.32±0.70	
M I	Base line	4.00±0.76	0.000*	3.96±0.79	0.001*	3.57±0.81	0.000*
	3 months	5.44±0.67		4.77±0.72		5.40±0.68	
	6 months	6.32±0.94		5.24±0.94		6.62±0.67	

AI: antigonial index, MI: mental index.
*statistically significant p < 0.05.

Comparison of the mean percentage of change of clinical parameters and radiometric indices (Antigonial index and Mental index)

By comparing the mean percentage of change in GI among the three groups, Group A showed statistically significant lowest mean percentage decrease in GI. Group B showed statistically significant lower mean percentage decrease than Group C. Group C showed the greatest statistically significant mean percentage decrease in GI through the whole study period (*Table 3*).

By comparing the mean percentage of change of pocket depth among the three groups over the period from baseline to three months, Groups B and C showed a higher percentage of change than Group A with statistical significance (p = 0.002). From baseline to six months, Groups B and C showed a higher percentage of change than Group A with statistical significance (p = 0.001). No significant difference was detected between Groups B and C (*Table 3; Figure 1*).

By comparing the mean percentage of change of the clinical attachment level among the three groups over the period from baseline to three months, Group B showed a statistically significant higher percentage of change than Group A (p = 0.037). Group C was not significantly different from Group A nor from Group B. From baseline to 6 months, Group B and C showed a statistically significant higher percentage of change than Group A (p = 0.002) (*Table 3; Figure 2*).

Table 3. Comparing means of percentage of decrease of different clinical parameters and radiometric indices (AI&MI) among the groups at different time intervals.

Variable	Time change	Group A	Group B	Group C	*p* - value
		Mean ± SD%	Mean ± SD%	Mean ± SD%	
GI Index	Baseline – 3 months	8.0% ± 6.2	66.8% ± 32.4	97.8% ± 5	0.003*
	Baseline – 6 months	18.2% ± 9.5	67.1% ± 31.5	93.6% ± 9.8	0.006*
PD reduction	Baseline – 3 months	14.64% ± 9.76	26.09% ± 9.65	23.0% ± 5.6	0.002*
	Baseline – 6 months	19.95% ± 9.67	31.77% ±11.57	34.59% ± 8.68	0.001*
CAL gain	Baseline – 3 months	14.55% ± 4.83	23.65% ±12.06	18.33% ± 9.57	0.037*
	Baseline – 6 months	19.07% ± 3.85	27.15% ±11.20	29.69% ± 7.46	0.002*
AI	Baseline – 3 months	24.94% ± 12.33	23.26% ± 8.18	26.59%± 16.86	0.781
	Baseline – 6 months	31.77% ± 13.05	29.38% ± 10.34	42.73%± 7.13	0.002*
MI	Baseline – 3 months	26.01% ± 13.08	17.31% ± 6.96	33.32% ± 15.07	0.0001*
	Baseline – 6 months	35.59% ± 15.06	24.19% ± 8.99	45.72% ± 11.86	0.0001*

SD: standard deviation, GI: gingival index, PD: pocket depth, CAL: clinical attachment level, AI: antigonial index, MI: mental index.
*statistically significant p < 0.05.

Figure 1. Line chart showing the mean percent of change of PD of the three groups throughout the whole study period

After comparing the antegonial angle mean percentage of change between the three groups over the period from baseline to three months, no statistical significance was found between the three groups, and from baseline to six months, Group D showed a statistically significant higher percentage of change from Groups B and C (p-value = 0.002) (*Table 3; Figure 3).*

After comparing the mental index mean percentage of change among the three groups over the period from baseline to three months, Group C showed a statistically significant higher percentage of change than Group B (p = 0.0001), while Group A was not statistically significantly different from Group B nor from Group C. From baseline to six months, Group A and C showed astatistically higher percentage of change than Group B (p = 0.0001) *(Table 3; Figure 4).*

Discussion

Upon reviewing the literature, LS or combined use of LS and ALN in postmenopausal osteoporotic women suffering from chronic periodontitis has not been previously investigated. Thus, the current study was performed to compare the clinical outcomes, panoramic radiomorphometric indices (AI and MI) of postmenopausal osteoporotic women with chronic periodontitis receiving alendonerate and *Lepidium sativum* either singly or combined. This was done before and after non-surgical periodontal treatment.

In the current study, alendronate was used at a dose of 70 mg weekly because it is six to 10 times more potent than pamidronate and up to 1000 times more potent than etidronate in inhibition of bone resorption. Moreover, it is capable of inhibiting periodontitis associated osteoclastic activity (Veena and Prasad, 2010; Gupta et al, 2011). A weekly dose of ALN of 70 mg has similar efficacy to a daily dose of ALN of 10 mg with reduced esophageal irritation (Kazuhiro, 2013). *Lepidium sativum* was given as an oral dose of LS seed powder of 1 gm thrice daily according to previous research by Paranjape and Mehta (2006).

The improvement in the clinical parameters in Groups B and C may be explained through the resolution of periodontal inflammation via the anti-inflammatory properties of *Lepidium sativum* which resemble those of non-steroidal anti-inflammatory drugs (Radwan *et al.,* 2007). LS seeds contain antioxidants substance such as β-carotene (Gopalan *et al.*, 2000) ascorbic acid and α- tocopherol (Elshal *et al.*, 2013).

Chapple *et al.* (2007) found an inverse relationship between plasma vitamin C and total antioxidant capacity (TAOC). Moreover, a previous study found that *P. gingivalis* infection was associated with low concentrations of vitamin C in plasma, which may increase colonization of *P. gingivalis* or disturb the healing of the infected periodontium (Pussinen *et al.*, 2003). A study by Linden *et al.* (2009) observed significantly lower levels of α- and β-carotene, in moderate and generalized severe periodontitis as compared to the remaining population. Carotene was effective in suppressing the activation of proinflammatory pathways through the quenching of free radical molecules (Opara and Rockway, 2006).

The anti-inflammatory property of LS has been demonstrated in many *in vitro* studies (Kumar *et al,* 2013*;* Nita *et al.,* 2013; Raval et al, 2013) and in clinical trials in management of inflammatory disease (Paranjape and Mehta, 2006; Raval and Pandya, 2009)

The improvement of the clinical parameters of LS treated groups is in agreement with a previous study that demonstrated a noticeable improvement in the cellular content of the periodontal ligament of diabetic rats fed with LS seeds at 30 days although the orientation of fibers was not achieved. At 45 days, the periodontal ligament exhibited superior orientation and cellularity (El Kilani and Hakam, 2013).

Moreover, α-tocopherol present in LS seeds shares similarities with the structure of estrogen (Elshal *et al.*, 2013). This estrogen-like and antioxidative activity of LS produce bone protective effects, and may directly regulate the proliferation and activity of OB and OC (Puel *et al.*, 2004), thus improving periodontal parameters

In the present study, radiographic analysis revealed that all intervention groups showed a statistically significant increase of AI at six months and MI after three months and six months respectively. Group C showed the highest percentage of change. These finding are in agreement with a study by Selimović *et al.* (2011) who found ALN 70 mg weekly increased hip BMD and decreased cortical porosity. It improved parameters of hip structure geometry including cortical thickness and produced more uniform mineralization. Moreover, ALN therapy increased BMD by 29.6% (Lucisano *et al.*, 2013). Previous studies have demonstrated significant effects of ALN on the increased cortical thickness (Watkins *et al.*, 2012; Zebaze, 2014).

These results are in accordance with previously reported benefits of LS seeds. LS induced a marked influence on fracture healing in rabbits (Juma, 2007). LS seeds contain considerable amount of fatty acids (Bafee and Ali, 2009) which inhibit OC formation and bone resorption in the co-culture system (Lee *et al.*, 2010). Quercetin present in LS reversed the decreased biomechanical quality and the impaired microarchitecture of the femurs in diabetic rats (Liang *et al.*, 2011).

In the present study the highest increase in radiometric indices was recorded in the group receiving combined treatment (LS and ALN) and is agreement in agreement with a previous study by Elshal *et al.* (2013) who found that although treatment with LS significantly raised serum Ca, P and ALP levels in glucocorticoid-induced osteoporosis (GIO) in rats, the tibia bone weights were heavier in LS and LS+ALD groups compared to those of animals treated with ALD alone. They demonstrated that co-treatment of ALN and LS, through a short duration (four weeks), has significantly improved the biochemical bone indices and restored microarchitecture of femurs and vertebral bones of the GC rats compared to ALD and LS single treatment group.

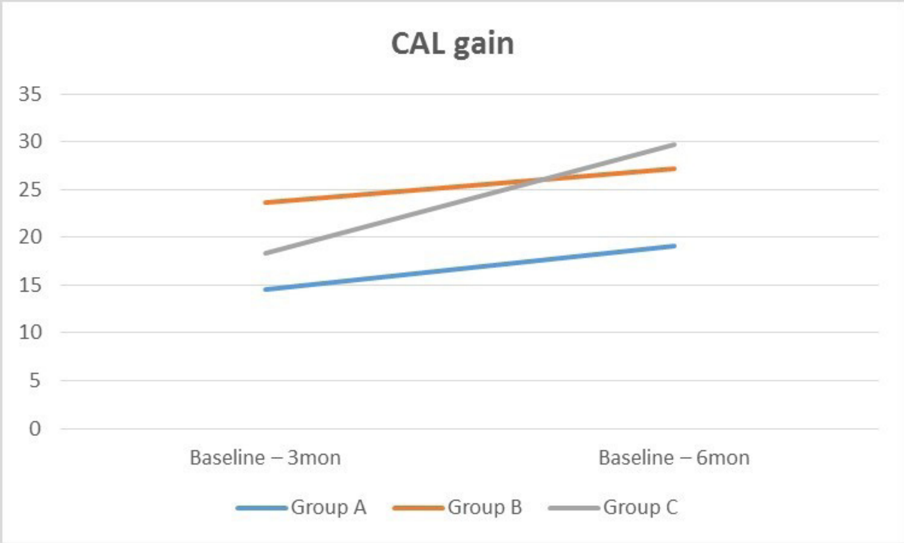

Figure 2. Line chart showing the mean percent of change of CAL of the three groups throughout the whole study period

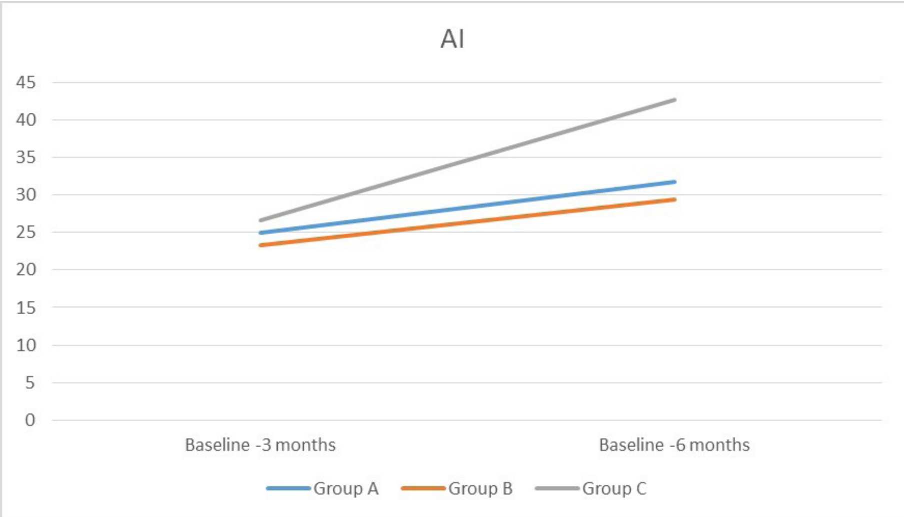

Figure 3. Line chart showing the mean percent of change of AI of the three groups throughout the whole study period

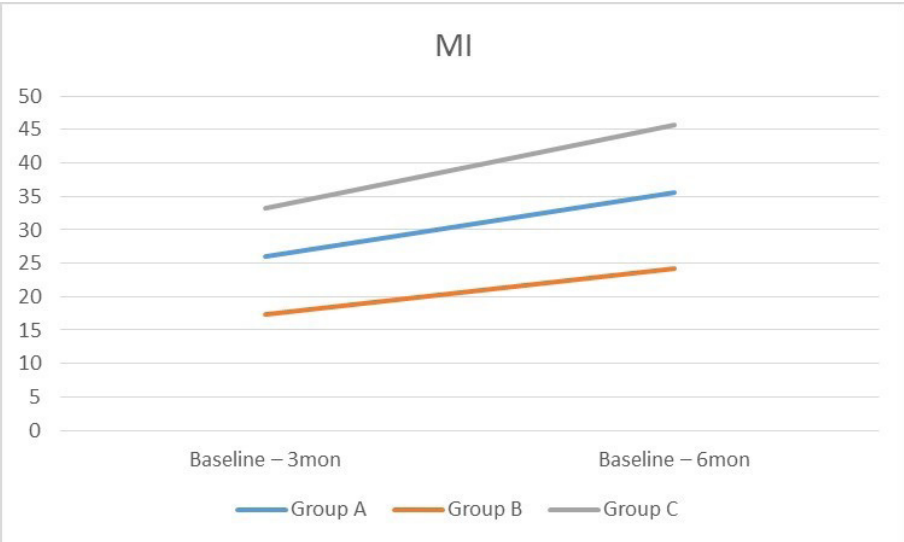

Figure 4. Line chart showing the mean percent of change of MI of the three groups throughout the whole study period

Conclusion

Within the limits of the study, it can be concluded that *Lepidium sativum* can be used in the treatment of osteoporotic postmenopausal women with chronic periodontitis who refuse treatment with ALN. The combined use of LS and ALN may have a synergistic effect resulting in a more favorable clinical response, increased bone mass than using ALN alone when combined with conventional therapy in treatment of chronic periodontitis in postmenopausal osteoporotic women.

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