Correlation of Serum ALP Activity with the Healing Process of Femoral Fractures in Rats Used Cissus Quadrangularis Extract as Therapy

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Abstract

The purpose of this study was to find out corellate value of serum ALP activity with the process of fracture healing in rat with therapy Cissus quadrangularis extract. This study used 18 male white rats (Rattus Norwegicus) age of 12 weeks. After 1 week of adaptation, all rats were treated femoral dexter osteotomy under aseptic conditions. Then, reposition of femoral dexter fractures with intramedullary pin fixation. The rats model were evenly randomly divided into three groups at 2nd weeks after surgery: group I treated with normal diet, group II with normal diet + CaCo3 supplement 150 mg/day, group III with normal diet + Cissus quadrangularis extract 500 mg / day per oral. Furthermore, the treatment was carried out for 4 weeks. At the end of the experiment (6th weeks after surgery), the blood samples were taken directly from canthus orbitalis dexter for alkaline phosphatase analysis. The data were analyzed by One Way Anova using SPSS for Windows program. The results indicated that ALP activity was significantly decreased ( p < 0.05). The more complete the fracture healing process, ALP activity the closer to normal value. The conclusion is the serum total ALP activity have a positive correlation with the process of fracture healing and could be used to determine the progress of fracture healing of the surgically treated fractures.

Keywords: ALP activity, fracture healing, cissus quadrangularis.
**Introduction**

Bone fractures healing is a complex biological process. The healing potential of bone, whether in a fracture or fusion model, is influenced by variety of biochemical, biomechanical, cellular, hormonal, and pathological mechanisms (Kalfas, 2001). Thus, the development of agents that possess osteogenic activity is undoubtedly useful in treatment of such conditions (Parisuthiman et al., 2009). The Use of calcium as supplement in clinical trials evaluating calcium and vitamin D are inconsistent with some supplements demonstrating benefits and others not indicating clear utility.

In recent years, herbal medicine has gain increasing interest as an alternative approach and might pose a valuable potential therapeutic agent, because these are considered to be relatively safer than chemically synthesized drugs. One such herbal medicine is *Cissus quadrangularis*, it is an indigenous medical plant of Indians (Cortes et al., 2005). *Cissus quadrangularis* Linn, belongs to the family Vitaceae and is indigenous to India, Sri Lanka, Malaysia, Thailand, Indonesia and Africa. Phytochemical analyses revealed that it consists of a high amount of vitamin C, β-carotene, triterpenoids, β-sitosterols, quadrangularins A, B, and C, and calcium.

The potent fracture-healing property of this plant has been demonstrated in several reports (Parisuthiman et al., 2009). In experimental fracture animal models, local and systemic administration of *Cissus quadrangularis* extract caused less tissue reactions, significantly accelerated complete new bone formation, and reduced healing time (Deka et al., 1994). A study of calcium-45 uptake during fracture-healing process suggested that *Cissus quadrangularis* treatment resulted in early calcification and remodeling phenomenon, as calcium level and the tensile strength of the newly formed bone were rapidly restored to the normal level. In addition, *Cissus quadrangularis* extract could neutralize the anti-anabolic effect of cortisone in the healing of fracture. In view of the new bone-formation process, it is possible that *Cissus quadrangularis* could mediate its osteogenic effect by enhancing osteoblast differentiation and function (Parisuthiman et al., 2009).

Normal fracture healing is generated by increased osteoblastic activity. Osteoblasts secrete large quantities of ALP, which is involved in the process of bone matrix formation and its mineralization (Leung et al., 1993). ALP is believed to either increase the concentration of local inorganic phosphate or neutralize inorganic pyrophosphate, an inhibitor of hydroxyapatite crystal formation (Volpin et al., 1998). Osteoblasts synthesize organic bone matrix (osteoid) and collagen. Osteoblasts also synthesize the enzyme alkaline phosphatase and initiate the process of mineralization. Osteoblasts represent the final stage of differentiation of pluripotential stem cell in the bone marrow.

Experimental studies show that the levels of bone specific alkaline phosphatase (BsALP) in the osteoblastline cells and bones are proportional to the formation of collagen. Clinical studies also show that the level of BsALP in the serum correlates with the bone formation rate (Muljacic et al., 2010).

Komnenou et al., (2005) have studied alkaline phosphatase (ALP) activity useful in evaluating the progress of healing and have concluded that serial determination of serum ALP activity in dogs during fracture healing could be an additional tool in predicting fractures at risk of developing, helping the clinician to choose the appropriate intervention. The purpose of this study was to find out correlate value of serum ALP activity with the process of fracture healing in rat after femoral dexter osteotomy with intramedullary pin fixation used *Cissus quadrangularis* extract as therapy. Furthermore, this research can provide information that serum ALP activity could be used to determine the progress of bone healing of the surgically treated fractures.

While this bone healing is monitored mainly by physical and serial radiologic examinations of the fracture site. However, it is sometimes difficult to distinguish a delayed union from a non union, and advanced imaging techniques may not be available.
Materials and Methods

This study used 18 male white rats (Rattus Norwegicus) age of 12 weeks. After 1 week of adaptation, all rats were treated femoral dexter osteotomy under aseptic conditions. Then, reposition of femoral dexter fractures with intramedullary pin fixation. Surgical procedure performed according to Millet et al., (2001), and anesthetized using a combination of ketamine (25 mg / kg body weight) and Xylazine (8 mg / kg bw) (Yudaniayanti, 2008).

The rats model were evenly randomly divided into three groups at 2nd weeks after surgery: group I treated with normal diet, group II with normal diet + CaCo3 supplement 150 mg/day, group III with normal diet + Cissus qudrangularis extract 500 mg / day per oral. Furthermore, the treatment was carried out for 4 weeks. At the end of the experiment (6th weeks after surgery), the blood samples were taken directly from canthus orbitalis dexter for alkaline phosphatase analysis.

Data analysis for this study using one-way Anova for each treatment, then continued LSD test to determine differences between treatment groups by using SPSS for Windows version 10 (Ghozali, 2006).

Results

The data ALP activity is shown in Table 1. Analysis of ALP activity was performed to determine statistically significant differences between groups ( p < 0.05). The group treated with Cissus qudrangularis extract (group III) had lower amount of ALP activities, but no significant difference with CaCo3 group (group II) (p>0,05). The control Group had the highest ALP activity and statistically significant differences with group II and III (p<0,05).

<table>
<thead>
<tr>
<th>Group</th>
<th>ALP activity (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (control group)</td>
<td>265,32 ± 39,21\textsuperscript{a}</td>
</tr>
<tr>
<td>Group II (CaCo3 group)</td>
<td>176, 17 ± 13,36\textsuperscript{b}</td>
</tr>
<tr>
<td>Group III (Cissus qudrangularis extract group)</td>
<td>145, 83 ± 16,45\textsuperscript{b}</td>
</tr>
</tbody>
</table>

\textsuperscript{a, b} different superscripts in the same row indicate significant differences (p <0.05).

Discussion

The results of alkaline phosphatase activity of rat blood in all groups showed largest increase of the normal value is 57-128 (+ 87 IU/L) (Lumley, 1990). This is due to osteoblast are active mature bone cells when the process of fracture healing. Osteoblasts are synthesize the organic matrix and regulate the mineralization process. Osteogenesis begins with osteoblast formation and secretion of type I collagen, which makes up about 90% of the organic bone matrix, or the osteoid. Once osteoblasts are active, they begin to produce large amounts of alkaline phosphatase, a phosphate-splitting enzyme that is release into the osteoid to initiate the deposition of minerals (Potu et al., 2009). In our study, we did not measure the levels of ALP before treatment and 2nd weeks after the osteosynthesis, because we concern that the animal would be stress that can affect their condition. The ALP levels before osteosynthesis are refers to the normal levels of rats submitted by Lumley (1990).

The group treated with Cissus qudrangularis extract (group III) had lower amount of ALP activities to compared with the others group. In a similar study by Yudaniayanti et al., (2011), base on radiological evaluation that the group treated with Cissus qudrangularis extract showed the fracture healing process is best compared to other treatment groups, with the highest scoring. This is due to the active ingredient in Cissus qudrangularis extract is attributed to the presence of phytogenic steroid that plays a major role in fracture healing and has anabolic properties. The anabolic
phystosteroid has been shown to markedly induce an early regeneration of the connective tissue and rapid mineralization of the callus, so that bone healing is more strength and stable (Cortes et al., 20005). Similar results in the study by Potu et al., (2009), showed that the CQ plant extract enhances the proliferation and differentiation ability of MSCs into osteoblasts. ALP activity, the most widely recognized biomarker for osteoblast activity, was enhanced by a short treatment with CQ. The results are in line with several previous in vivo experiments that have demonstrated that CQ promotes ALP activity and enhances collagen synthesis in the fracture-healing process (Shirwaiker et al., 2003). Based on these results suggested that the petroleum ether fraction may contain active constituents that stimulate osteoblast differentiation and its bioactivity. The phyrogenic steroids found in CQ may be involved in stimulating osteoblastogenesis and may act on estrogen receptors of bone cells (Chopra et al., 1975).

Based on these findings, it can be assumed that ALP activity depends on the stability of the bone fragments after the bone fracture. This has been confirmed by other studies which prove that unstable osteosynthesis results with greater mobility to bone fragments in comparison to the stable osteosynthesis, then it have had the largest increase in ALP activity (Muljacic et al., 2010). The persistent elevated ALP levels above the normal value may be explained by the continuing osteoblastic activity. After the 8th week the serum alkaline phosphatase levels decrease gradually but do not return to normal as a complete turnover cycle in bone remodeling requires an average period of 200 days (Erikson et al., 1994). Nakagawa et al., (2006) have studied the concentration of total ALP rose to a maximum at 3 weeks after surgery and then gradually decreased, returned to the initial values.

In the end of this study, the group which treated with Cissus quadrangularis extract (group III) had lower amount of ALP activities to compared with the others group. This results show that the third treatment group, the process of fracture healing almost complete. Due to a decrease ALP value showed that in osteoblast activity almost complete in bone formation to repair fractures.

Osteogenesis begins with osteoblast formation and secretion of type I collagen, which makes up about 90% of the organic bone matrix, or the osteoid. Once osteoblasts are active, they begin to produce large amounts of alkaline phosphatase, a phosphate-splitting enzyme that is released into the osteoid to initiate the deposition of minerals. Calcium hydroxyapatite, which comprises 70% of the bone mass, crystallizes along the cavities in the three-dimensional collagen network. After mineralization, the complete bone becomes hard and rigid with the mechanical properties necessary to withstand external forces, support the body and protect the internal organs. The proliferation rate and biological activity of the osteoblasts controls the rate of bone formation, and accelerated osteoblast growth is the key factor for efficient bone repair (Stavors et al., 2003).

Conclusion

The serum total ALP activity have a positive correlation with the process of fracture healing and could be used to determine the progress of fracture healing of the surgically treated fractures.

References


