



Energy Dispersive Spectroscopy (EDS) Analysis of Calcium and Phosphorus Minerals Elements on Bone After Insertion Immediate Implant on Socket Filled with Extract Aloe vera

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Abstract

Objective: To examine the effect of aloe vera that containing bioactive materials on the levels of calcium (Ca) and phosphorus (P) minerals and their ratios around the immediate implanted in alveolar bone. **Material and Methods:** Research method by conducting experimental test on experimental animals: 9 male mongrel dogs are divided into 3 groups each 3 tails. In each animal was pulled the second premolar teeth on right side and left side, and immediately inserted titanium implant (3mm x 10mm diameter) after the socket filled with 10% aloevera extract on the right side and control on the left side. Analysis of calcium and phosphorus minerals content formed by examination of Energy Dispersive Spectroscopy (EDS) on Scanning Electron Microscope on implant and around bone tissue was done on days 14, 28 and 56. The result of statistical analysis using repeated ANOVA with independent t test. The level of significance was set at 5%. **Results:** There was a significant difference on calcium level between control and treatment groups on days 14, 28 and 56 ($p < 0.05$). There was no significant difference on phosphorus level between control and treatment groups ($p > 0.05$). The ratio of Ca / P in both control and treatment groups was also significantly different in every observation day ($p < 0.05$). **Conclusion:** The addition of aloe vera extract that containing bioactive materials has an effect on increasing levels of mineral elements calcium and calcium-phosphorus ratio after immediate implant insertion.

Keywords: Aloe; Immediate Dental Implant Loading; Calcium; Phosphorus.

Introduction

Placement of implants in post-extraction syringes or instant implants is a way to simplify clinical procedures and surgery and maintain alveolar dimensions [1]. In the implant position post dental extrantion, the marginal distance occurring in the buccal and palatal or lingual aspects will form new bone on the defect side of the alveolar ridge [2]. Some human studies suggest implant survival rates on sockets that do not affect, but are not limited to, small amounts of research and patient cases [3].

Healing of sockets in tooth extraction is characterized by three overlapping phases of the hemostasis phase or formation of blood clots, bone formation, and bone remodeling [4]. In the hemostasis phase platelet cells produce growth factors and mediators (cytokines) involved in angiogenesis ie Platelet-Derived-Growth-Factor (PDGF) and Transforming-Growth-Factor-beta (TGF-b) [5]. In the bone formation phase, osteoprogenitor migrates to the wound site, then develops and differentiates into osteoblasts. Osteoblasts secrete local growth factors, extracellular matrix, and induce mineralization [6]. The bone remodeling phase begins when the osteoclast absorbs minerals and bone matrix. The mononuclear cells prepare the surface for the osteoblasts, then differentiate to produce the synthesis of the matrix. The mineralization of matrix and osteoblast differentiation into osteocytes completes the remodeling cycle [7].

Calcium (Ca) and Phosphorus (P) are elements that affect the activity of bone-forming cells and bone resorption. P plays a role in osteocyte maturation that plays a role in bone mineralization and systemic P homeostasis. The first step of Ca-P crystal nucleation takes place within the vesicle matrix that is rooted from the osteogenic cell plasma membrane, then migrates into the extracellular skeletal compartment. This process leads to the formation of hydroxyapatite crystals and subsequent relation to collagen organic matrix fibrils. Extracellular matrix on bone formation will be mineralized calcium hydroxyapatite, a compound composed of calcium (Ca) and phosphorus (P) [8].

In the osseointegration process of implantation, osteoinductive and osteoconductive properties are important as resorbable biomaterials in increasing bone tissue growth formation [9]. Osteoinductive induces the process of undifferentiated cells so that it could be differentiated [10]. Osteoconductive is the physical properties of the active ingredient added to the implant in order to support the osseointegration process [9]. The osteoconductive properties are found in the demineralization of matrix bone and calcium phosphate [11].

Several surface implant modifications have been used to improve the quantity and quality of bone-to-implant interfaces in order to accelerate the healing and implantation osseointegration stage with bone [12]. In the last two decades the focus on chemical anisotropic substrates combined with growth (mitogenic) and inductive (morphogenic) substances. The bioactive coatings of most classes of biomaterials continue to evolve to be acceptable in human clinical trials, and the focus of research has shifted to a combination of synthetic implants and biologically active materials [13].

In dentistry, several types of biomaterials as active ingredients have been used for oral rehabilitation in implantology (titanium implants) and regeneration of periodontal tissues [14]. The

use of medicinal plants is believed to contain only slightly toxicity properties compared to other synthetic chemical components [15].

Some plants that have biological activity and potential for the healing application of a disease, one of which is aloe vera [15]. The potential chemical content of aloe vera is derived from the gel, with pH 4.5 being polysaccharide glucomannan and acemannan [16]. Acemannan as polysaccharide in aloe vera accelerates bone formation, promotes enlargement of Bone Marrow Stromal Cell (BMSC), differentiation and expression of growth hormone vascular endothelial growth factors (VEGF), bone morphogenic protein-2 (BMP-2), Osteopontin (OPN), Bone Sialo Protein (BSP), and mineralization [17]. Acemannan stimulates BMD and healing in tooth socket at 4 weeks after extraction [17]. Glucomannan in aloe vera contains mannose and gibberellin, a growth hormone that interacts with fibroblast growth receptors that will stimulate activity and proliferation and significantly influence the synthesis of collagen [18]. Aloe vera gel also helps stimulate bone growth around the endosseous implants. The production of prostaglandins from arachidonic acid and cyclooxygenase is inhibited by aloe vera. Increased wound healing by removing bacteria was observed in in vivo study. The antimicrobial and anti-inflammatory properties of aloe vera reduce inflammation when placed around dental implants and also prevent periimplantitis [19].

Aloe vera also contains an anthraquinone glycoside called emodin, which promotes mineralization of the matrix by increasing bone formation [20]. Aloe vera has a calcium content of 458mg / ppm, and phosphorus 20.10 / ppm [21]. Among micronutrients, calcium (Ca) and phosphorus (P) are the two major constituents of calcium hydroxyapatite, bone mineral that strengthens the mechanical resistance of the organic matrix, 80% of bone mass contains Ca and P.

From the results of several studies show the efficacy of aloe vera in helping the process of acceleration of osseointegrasi and remodeling alveolar bone in the socket after tooth extraction. The calcium and phosphor minerals contained in aloe vera are expected to act as osteoconductive substances for the growth of neovascularization and infiltration of osteogenic precursor cells.

Energy Dispersion X-ray Spectroscopy (EDS or EDX) is an analytical technique used to analyze the element or characterization of the material element composition of a sample, EDS is part of an Electron Microscope (Scanning Electron Microscopy/SEM), which has a microscope imaging capability in identifying specimens. The data generated by the EDS analysis consists of spectra showing the peaks corresponding to the elements that make up the composition, analyzing the actual element mapping of a sample. The calcium and phosphor minerals contained in aloe vera can be an osteoconductive substance for the growth of neovascularization and the infiltration of osteogenic precursor cells into the bone implant interface [22].

The purpose of this study is generally as a scientific study for the development of aloe vera as a supplementary ingredient in the process of implantation process, and specifically aims to know the influence of g aloe vera extract addition to the mineral content of calcium and phosphorus of alveolar bone after the installation of immediate implants in mongrel.

Material and Methods

This research is a laboratory experimental study (True-Experimental Design). Includes the science of Pharmacy, Physics, Microbiology and Dentistry in a manner of Randomized Post Test Control Group Design, with animals as object of research 9 male mongrel dogs. Sample inclusion criteria were 1 year old mongrel dog, 10-12 kilogram weight and healthy (no dull, fallen, bald, and active) hair. Exclusion criteria when there is weight loss of more than 10% after adaptation period in the laboratory.

The animals were divided into 3 groups of observations, each group consisting of 3 samples. Each sample will receive an anesthetic procedure with intravenous sodium pentothal (30mg / ml) and a second right lower tooth extraction premolar is performed. In the previous extraction socket filled with 10% aloevera aloe gel as much as 0.5ml, then inserted titanium implant on the distal socket (Treatment Group) and second left premolar tooth extraction and then immediately inserted titanium implant (Implant SA Osstem size 3.5 x 10, 4 x 10, Osstem Implant Co., Dong Yeonje-Gu Busan, Republic of Korea) without aloe vera extract gel (Control Group) on the distal socket.

Immediate implants were inserted in accordance with the surgical sequence procedure of Osstem. In each group of samples, Group I analyzed the 14th day post insertion immediate implant, Group II for the 28th day of analysis and Group III for 56 day analysis. Prior to treatment, all experimental animals of mongrel dogs were adapted and maintained in animal experimental cages made of iron. Animal adaptation was performed for 7 days before treatment to condition animals in good health. Foods in the form of dog food diet. The temperature and humidity of the room are left in the natural range. In the manufacture of aloe vera extract, the material used is *Aloe barbadensis* Aloe vera leaves Miller (has been tested plant determination and approved officially part of BioFarmasi Post Graduate University of Hasanuddin Makassar). Making aloe vera extract using Maseration technique. Aloe vera (*Aloe barbadensis* miller) washed and weighed and then peeled and removed the meat, cut into 2x2x2cm in a form of dice, then weighed for clean weight. This washing process is done with the aim to remove dirt and bacteria found on the surface of aloe vera.

Aloe is then immersed in a 96% ethanol solvent for 72 hours, every 24 hours filtered and taken that has been dissolved, the remaining aloe vera in the bottle is replaced with a new ethanol solution. After getting dissolved aloevera then put into the rotary evaporator to remove the remaining ethanol solution, for 6 hours, after that obtained aloe vera thick extract. To obtain 10% aloe vera gel 10ml used in this research is first aquades 8ml material is heated at 70-90°C, then insert methyl paraben 0,2% until dissolve, then added hydroxyethylcellulose and carboxymethyl cellulose 15% then homogenized at a rate of 1000rpm until a clear gel mass (base gel) is formed. At the base of the gel was then added another 15% glycerin material and homogenized again. After homogeneous base gel is allowed to be put at 30-40°C, then added 1 gram of aloe vera viscous extract while homogenized to evenly in gel base.

After 14 days in Group I sample then perform surgical procedures with pentothal sodium anesthesia (30mg / ml), surgical sutures were opened and tissue cuts were taken to remove the

implants and bone tissue around, after which the wound was sewn back. On day 28, surgical procedures were performed with sodium pentothal anesthesia (30mg / ml) in Group II sample and, as well as the 56th day, the same procedure was performed in Group III sample. The implant and the surrounding tissue of both groups were each control group and the treatment group (n = 3) removed from the sample periodontal tissue, cleansed in 4% neutral formaldehyde solution for 10 min, dehydrated by ethanol, then dried or vacuum. The implant samples taken were viewed using SEM with various magnifications (100x and 200x).

Scanning electron microscopy (SEM) examination (Tescan VEGA 3, Tescan Orsay Holding, Kohoutovice, Czech Republic) was used as an Energy Dispersive Spectroscopy (EDS) analysis to see the elemental graphs contained. The following two-sided parameters were selected: mesial and distal and surface ratios. Three implants were chosen randomly for analysis.

Data Analysis

To test the normality of data then used Shapiro Wilk test. The ANOVA test and t-independent test were used. Calculation of the average value of Energy Disperse Spectrum Examination namely calcium, phosphorus and calcium phosphorus ratio is also done to see the effect of adding aloe vera active ingredient on bone around implant. All examination results were recorded and data analysis using SPSS program version 24.0 (IBM Corp., Armonk, NY, USA).

Ethical Aspects

All procedures performed in this study were ethical and ethical clearance issued by the Ethics Commission Faculty of Dentistry University of Hasanuddin Makassar.

Results

On day 14 of observation the average calcium content was 19.00, phosphorus was 18.04 and Ca / P ratio 1.05. On day 28 the average calcium content of 20.11, phosphorus 17.49 and the ratio of Ca / P 1.15. On day 56 the average calcium content was 23.63, phosphorus 15.81 and the ratio of Ca / P 1.57. There was an increase in the average of calcium levels, showed a significant difference ($p < 0.05$). Calcium to Phosphorus ratio also increased showed significant difference. The average phosphorus level decreased in the series of day observation but with no significant difference (Table 1 and Figure 1).

Table 1. Comparison of calcium, phosphorus and calcium to phosphorus based on treatment days.

Days of Observation	Calcium	Phosphorus	Ca/P Ratio
	Mean \pm SD	Mean \pm SD	Mean \pm SD
14	19.00 \pm 1.78 ^a	18.04 \pm 0.41 ^a	1.05 \pm 0.10 ^a
28	20.11 \pm 1.92 ^{ab}	17.49 \pm 0.75 ^a	1.15 \pm 0.15 ^{ab}
56	23.63 \pm 5.01 ^{ac}	15.81 \pm 2.71 ^a	1.57 \pm 0.59 ^{ac}
p-value	0.034	0.120	0.018

Repeated Anova; Value $p \leq 0.05$: Significant.

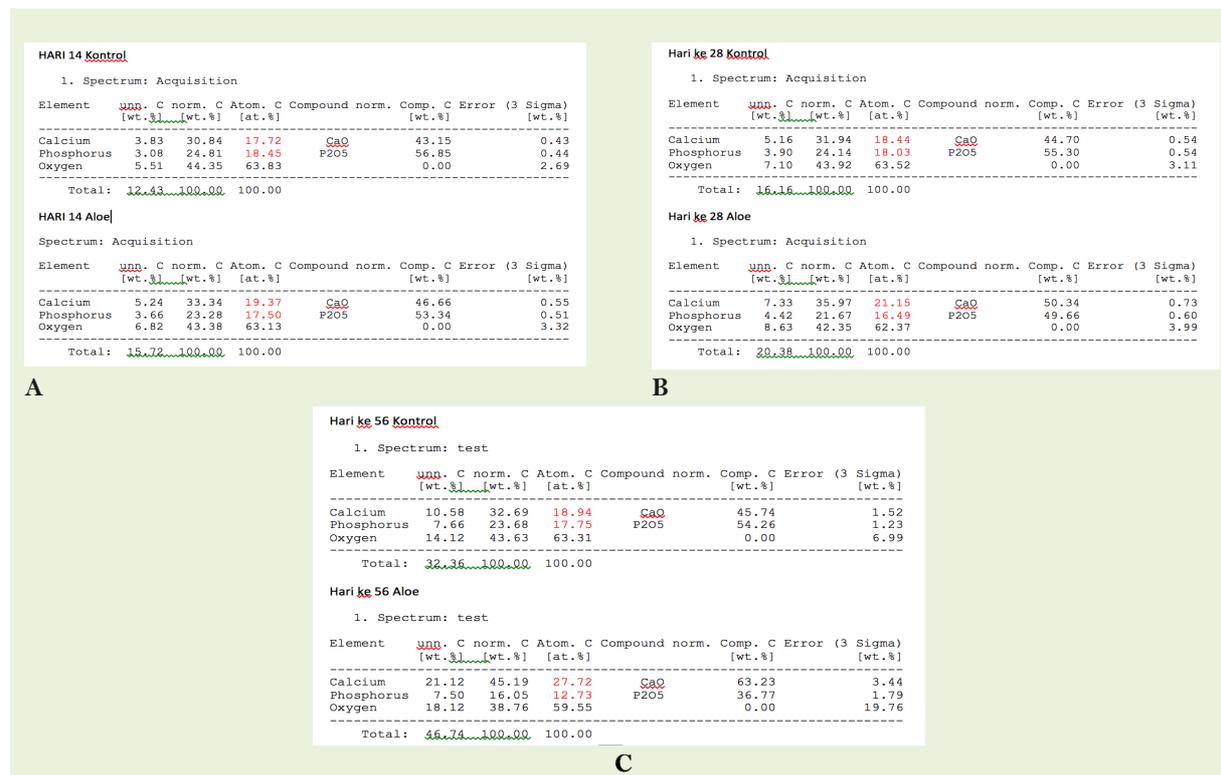


Figure 1. A: Levels of calcium and phosphorus in the control group EDS and aloe vera group day 14. B: Levels of calcium and phosphorus on the control group EDS and aloe vera group day 28. C: Levels of calcium and phosphorus on the control group EDS and aloe vera group day 56.

The mean of calcium levels in the control implant group was 18.47, phosphorus 18.11 and Ca / P ratio 1.02 (Table 2). In the group of implants in the socket filled with aloe vera extract the average calcium was 23.35, phosphorus 16.11 and the ratio of Ca / P 1.50. The calcium levels show a significant difference between the control implant group and the treatment implant group, as well as in the Ca / P ratio show significant differences between the two groups (p<0.05). While the phosphorus level although there are differences but there was no significant difference in phosphorus levels between the Control implant group with the treatment implant group (Figure 2).

Table.2 Comparison of calcium, phosphorus and Ca / P ratio between control group and treatment group.

Groups	Calcium Mean ± SD	Phosphorus Mean ± SD	Ca/P Ratio Mean ± SD
Control	18.47±0.78 ^a	18.11±0.27 ^a	1.02±0.45 ^a
Aloe vera	23.35±3.70 ^{ab}	16.11±2.13 ^a	1.50±0.45 ^{ab}
p-value	0.022	0.069	0.049

Repeated Anova; Value p ≤ 0.05; Significant.

Table 3 shows the comparison of calcium, phosphorus and Ca / P ratio by observation day in the control group is seen on the day 14, the mean calcium concentration was 17.63, phosphorus 18.20 and Ca / P 0.97 ratio. On day 28 the average calcium was 18.50, phosphorus 18.07 and Ca / P ratio 1.02. On day 56 the average calcium was 19.30, phosphorus 18.08 and Ca / P ratio 1.07. Calcium levels increase with increasing days of observation, while phosphorus levels decrease, Ca / P ratio

also increases. The calcium level and phosphorus levels of the control group show no significant differences in each series of observation days. However, Ca / P ratio shows significant difference ($p < 0.05$).

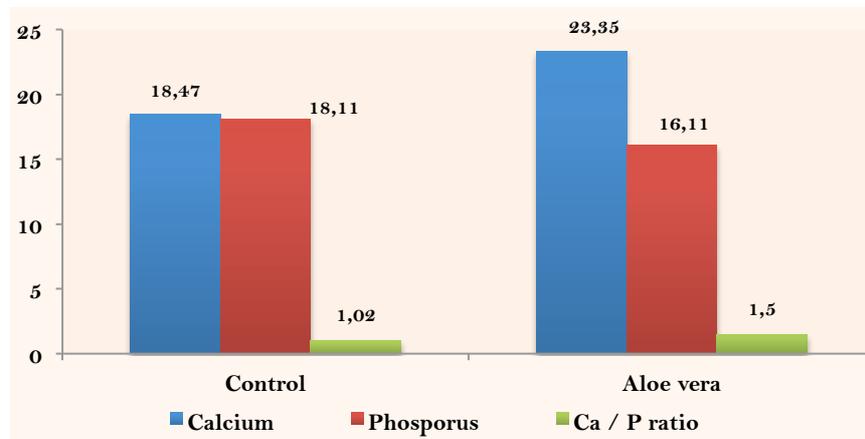


Figure 2. Comparison of calcium, phosphorus and calcium-phosphorus ratio between control implants group and treatment implant group.

Table 3. Comparison of calcium, phosphorus and Ca / P ratio by day of observation in the control implant group.

Days of Observation	Calcium Mean \pm SD	Phosphorus Mean \pm SD	Ca/P Ratio Mean \pm SD
14	17.63 \pm 0.13 ^a	18.20 \pm 0.35 ^a	0.97 \pm 0.11 ^a
28	18.50 \pm 0.78 ^a	18.07 \pm 0.50 ^a	1.02 \pm 0.00 ^{ab}
56	19.30 \pm 0.50 ^a	18.08 \pm 0.50 ^a	1.07 \pm 0.00 ^{ac}
p-value	0.07	0.936	0.007

Repeated Anova; Value $p \leq 0.05$: Significant.

Table 4 shows the increasing levels of calcium in the observed series of days, while the phosphorus level decreases, the Ca / P ratio increases. Comparison of calcium, phosphorus and Ca / P ratio according to observation day on treatment implantation group is seen on day 14 mean calcium level was 20.37, phosphorus level 17.88 and ratio of Ca / P 1.14. On the day 28 the average calcium content was 21.73, the phosphorus was 16.91 and the ratio of Ca / P was 1.29. On day 56 the average rate of calcium was 27.96, phosphorus was 13.54 and the ratio of Ca / P was 2.07 (Figure 3). The values of calcium, phosphorus and Ca / P ratio of the treatment implants group show significant differences on each observation day ($p < 0.05$).

Table 4. Comparison of calcium, phosphorus and Ca / P ratio by observation day in the aloevera-injected implants group

Days of Observation	Calcium Mean \pm SD	Phosphorus Mean \pm SD	Ca/P Ratio Mean \pm SD
14	20.37 \pm 1.41 ^a	17.88 \pm 0.53 ^a	1.14 \pm 0.05 ^a
28	21.73 \pm 0.81 ^{ab}	16.91 \pm 0.59 ^{ab}	1.29 \pm 0.00 ^{ab}
56	27.96 \pm 0.34 ^{ac}	13.54 \pm 1.15 ^{ac}	2.07 \pm 0.15 ^{ac}
p-value	0.009	0.011	0.021

Repeated Anova; Value $p \leq 0.05$: Significant.

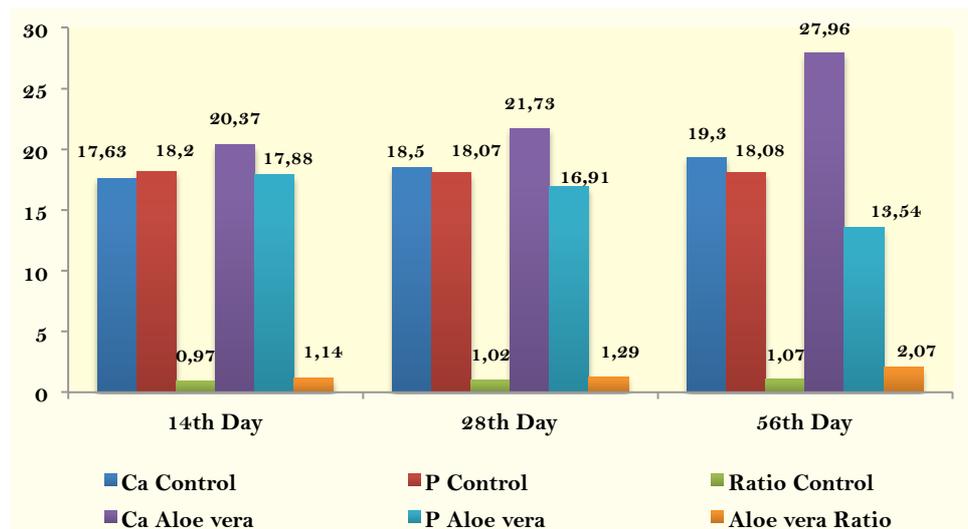


Figure 3. Comparison of calcium, phosphorus and calcium-phosphorus ratio between controls implants group and treatment implant group on daily observation.

Discussion

This study was done to analyze the average value of calcium and phosphorus levels and their ration after the installation of immediate implant on fresh sockets that were not filled with 10% aloe vera gel extract and immediate implant in fresh socket filled with 10% aloe vera gel extract. Analysis of calcium, phosphorus and ration levels with EDS using Scanning Electron Microscopy (SEM). This histomorphometric SEM evaluation can also analyze Bone to Implant Contact (BIC) and is defined as the most common method in most studies as a standard procedure for evaluation of bone formation on the surface of the implant. Specifically, differences in BIC values and calcium mineral content, bone phosphorus between treatment and control surfaces were statistically analyzed to compare the osteogenic potential of the implant surface [23].

The success of dental implants to last a long time depends very much on the osseointegration process that occurs. Among the several parameters that influence the success of the implant, the surface between the implant and bone plays an important role in prolonging the implant's endurance and in enhancing the function of the prosthesis [24]. The interaction mechanisms that occur in the area between the implant and the surrounding tissues show that the composition, surface energy, and surface impurity of the implant material greatly determine the implant performance in the tissues of the osseointegration, and that the surface of the implant material must be bioactive [25].

Through various studies known aloe vera has 11 useful contents. Some of them are amino acids, anthraquinones, enzymes, hormones, minerals, salicylic acid, sterols, monosaccharides, polysaccharides and vitamin [26]. Mineral content of aloe vera, including calcium, phosphorus, iron, sodium, magnesium, manganese, copper, and zinc. Based on the research, enzymes owned by aloe vera include amylase, catalase, cellulose, carboxypeptidase, carboxylolase, phosphatase, lipase, nucleotidase, alkaline, and proteolitease [26]. The concentration of Calcium (Ca) in aloe vera (ppm) is 458.00 and Phosphorus (P) 20.10. References used in this study is aloevera extract gel with a content

of 10%, based on previous research that application of 10% aloe vera substrate for 1 day can decrease inflammatory rate [27].

After tooth extraction will occur trauma and inflammation, glucomannan and gibberelin in aloe vera will stimulate increased fibroblast proliferation and accelerate wound healing with epithelial cell proliferation and prevent infection [28]. Then when the implant is placed inside a tooth extraction socket by using a drilling device occurs the process of osteoclastogenesis. The osteoclastogenesis of osteoclast differentiation and the activation of a combination of costimulatory molecules, M-CSF (Macrophage Colony stimulating factor) and RANKL (Receptor Activator of Nuclear factor κ B Ligand), ie osteoclast precursors replicate and induce RANK in inflammatory conditions produced by cells B and T lymphocytes [29]. In this condition the role of aloe emodin and anthraquinones in Aloe vera is to prevent inflammation, thereby decreasing osteoclastogenesis, thus will not occur bone resorption of bone cells.

Another role of aloe vera in preventing inflammation and decreasing osteoclastogenesis is in the occurrence of bleeding, homeostasis, and the formation of fibrin blood clots on the surface of bone tissue during the implant placement process [30]. The first cells that appear on the surface of the biomaterial during contact with blood (adsorption), coagulation, and fibrinolytic phases are platelets, monocytes, and polymorphonuclear granulocytes and erythrocytes. The level of calcium in the blood plasma will be maintained by the mechanism of homeostasis at this stage [31].

The process of osseointegration or bone formation of interface between the surfaces of the implant is called osteogenesis or ossification. In osteogenesis the development of bone precursor cells is divided into developmental stages: Mesenchymal stem cells, osteoprogenitor cells, pre-osteoblasts, osteoblasts and mature osteocytes. After the osteoprogenitor cells form the osteoblastic line, then proceed with three stages of development of cell differentiation ie proliferation, maturation matrix, and mineralization. At the time of osteoblast differentiation this growth hormone VEGF induces new capillary formation [32-34]. Alkaline Phosphatase (ALP) is considered an early marker of osteoblast differentiation and induces mineralization [35]. At the time of maturation the OPN matrix works by connecting the hydroxyapatite crystals with the bone matrix, at the same time the blood vessels of the havers system transport phosphorus and calcium to the matrix so that the bone matrix becomes hard. OPN and BSP are the major non-collagen proteins that play an important role in bone. Acemannan as polysaccharide in aloe vera significantly stimulates BMSC proliferation, by inducing BMSC differentiation and improving VEGF, BMP-2 regulation in extracellular matrix synthesis, and mineral deposition [17]. The process of remodeling and functioning of mineral homeostasis, especially calcium and phosphorus in the bones, among others, is influenced by hormones. Steady plasma calcium levels will help to deposit calcium in bone [36]. Calcium minerals in aloe vera play a role in the implant osseointegration process by inducing active bone apposition on the surface of the implant in the medullary space so that the bone component becomes denser.

In the case of osseointegrated implants, high-resolution microscopic results show the interfacial zone of afibrillar at the bony-implant interface, where it appears that mineralization in tissues

generally does not directly touch biomaterials. The intercellular layer is rich in non-collagen proteins as well as certain plasma proteins. The new bone-titanium interface presents a thin layer of proteoglycans and glycoprotein [24]. In the tissue around the implant the content of bioactive materials in aloe vera added to the socket after tooth extraction causes certain biological reactions, thus promoting the acceleration of the unification process between the implant and the bone. The minerals of calcium and phosphorus in aloe vera form strong bonds with adjacent tissues, and as biomaterials support the association with bone tissue through bridge calcium and phosphorus.

In this study calcium levels increased significantly with everyday observations, both in the control group and in the treatment group. Similarly, the comparison of elevated levels of calcium between the control group and the treatment group also increased significantly. Phosphorus levels in the statistical analysis were not significantly different, with levels declining as the day of observation increased, which could be explained by a positive Ca / P ratio as a sign of bone ossification. In the control group, the immediate implant group in which the sockets were not added aloe vera extract gel, increased levels of calcium and decreased phosphorus levels were statistically insignificant. This is different from the treatment group i.e the immediate implant group in the sockets added aloe vera extract gel that the calcium level increases and the phosphorus level decreases and is statistically significant. The ratio of Ca / P in all groups also experienced a significant increase. In the control group the value of Ca / P ratio at day 56 of observation was 1.07, in treatment group value of Ca / P ratio 2.07. In the treatment group the ratio of calcium to phosphorus has a statistically significant difference ($p < 0.05$). This calcium to phosphorus (Ca / P) ratio data yields a Ca = 2.07 P ratio. The assumption of calcium levels increased 2 times from the levels of phosphorus. This study is in accordance with the report of the European Food Safety Authority, which reported the ratio of calcium and phosphorus plasma to bone formation in the range of 1.5: 1 [34]. While in the control group the ratio of Ca / P ratio of only 1.07 on day 56 indicated that there is a significant bone formation [37].

Conclusion

The addition of 10% aloe vera gel in post-extraction syringes installed in immediate implants can increase calcium levels in bone and significantly increase the Calcium / Phosphorus ratio on the surface of the implant as a marker of bone formation and faster osseointegration occurrence.

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