

The amount of macrophages and activated plasma cells on wound healing process affected by spirulina

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ABSTRACT

Background: *Spirulina* which grows abundantly in tropical seas have been investigated to enhance immune system. The administration of spirulina in tooth extraction sockets was expected to optimise the function of immunocompetent cells. Therefore, wound healing process would be improved. **Purpose:** The aim of this study was to prove that administration of spirulina could influence immune system in tooth extraction sockets. **Method:** There were 28 *Cavia cobayas* used in this study and were put in group of four. Mandibular left incisors were extracted from each of them. The basis made from mixture of polyethylene glycol (PEG) 400 and PEG 4000 was administrated into each socket in control group (TG0). In addition, spirulina 12% was administrated into group TG1, spirulina 24% was administrated into group TG2, and spirulina 48% was administrated into group TG3. All of the *Cavia cobaya* were decapitated and the jaws were removed in day 5 after tooth extraction. The jaws were decalcified in EDTA solution, formed into paraffin block, processed for hematoxylin and eosin (H & E) and immunohistochemistry staining afterwards. Datas were analysed statistically using Anova method. **Result:** There was an augmentation in the number of macrophages and activated plasma cells after spirulina application. The administration of higher concentrations of *Spirulina* leads to greater amount of macrophages and activated plasma cells in each groups. **Conclusion:** In conclusion, spirulina is able to increase the amount of macrophages and activated plasma cells which play important role in healing process.

Keywords: spirulina; macrophages; plasma cells

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INTRODUCTION

Tooth extraction is traumatic to oral mucosa, especially gingiva. The wound would heal after days. There are possible complications interfering healing process, such as excessive bleeding, pain, infection, swelling, and dry socket in certain individuals might occur due to impaired immune response which leads to improper formation of various components that involved in healing process.¹ Anti-inflammatory agents are commonly given following tooth extraction to support eliminating inflammation. However, synthetic drugs that are widely used are relatively unreachable and unaffordable by community so exploring traditional herbs are worth considering.

The use of herbal drug has reached greater development in the past twenty years.² Algae are abundantly grown in tropical sea, including Indonesia. One of the benefits of spirulina is to substitute synthetic anti-inflammatory drug.³ Spirulinas contain protein, iron, gamma-linoleic acid, carotenoid, vitamins, and have been widely used as health supplement.^{4,5}

According to its structure, spirulina is digestible and absorbable due to minerals, fibers, and vitamins, nucleic acid, fatty acid, and carbohydrate contained in spirulina.⁶ Moreover, spirulina is allegedly as an anti-allergic agents due to its ability to decrease the level of specific immunoglobulin E (IgE),⁷ and histamine.⁸ Spirulina is classified as class 1 food, which is safe for consumption

in accordance with The United States of Pharmacopeial Convention (USP).⁹

Macrophage is one of chronic inflammatory cells which is responsible for phagocytosis infectious microbes following tooth extraction. Spirulina works by targeting macrophages, but the molecular mechanism about the role of Spirulina in wound healing process has not been clearly noted.¹⁰ Another component involved in healing process is plasma cells which produces antibodies as response to antigen exposure. Secretory immunoglobulin A (sIgA) is typically antibodies found in oral mucosa. Previous studies reported that spirulina increases the amount of sIgA.¹¹ The variables of this study are the amount of macrophages and activated plasma cells due to their vital roles in healing process. The purpose of this study is to demonstrate the ability of spirulina gel to affect the amount of macrophages and activated plasma cells in wound following tooth extraction.

MATERIALS AND METHODS

This research was an experimental laboratory with samples of 28 males *Cavia cobaya* weighing 300-350 grams, aged 2-3 months which have been adapted to the environment for 1 week. The mandibular left incisives were extracted and spirulina was administrated into each sockets. The spirulina have been dried previously and formed into gel using mixture of polyethylene glycol (PEG) 400 and PEG 4000 (3:1) as basis. The concentrations of spirulina gels used in this study were 12%, 24%, and 48%. There were 4 groups in this study: control group (TG0) which was only administrated with basis, spirulina 12% was administrated into treatment group 2 (TG2), spirulina 24% was administrated into treatment group 3 (TG3), spirulina 48% was administrated into treatment group 4 (TG4). Spirulina was administrated to the animals right after the tooth extraction. The experimental animals were eliminated in order to remove the lower jaws in day 5. The samples were processed for hematoxyline and

eosin (H & E) to observe the number of macrophages and immunohistochemistry staining using monoclonal antibody anti-IgG to observe the amount of activated plasma cells which contain immunoglobulin (Ig) in the cytoplasm. Quantities of macrophages and activated plasma cells were counted using light microscope (400x). Data obtained were analysed using Anova and HSD test afterwards.

RESULTS

The results obtained from control group and treatment groups are described in Table 1 and 2. The results demonstrate that there were more macrophages and activated plasma cells observed in treatment groups than in control group. Figure 1 indicate that higher concentration of Spirulina leads to greater amount of macrophages and activated plasma cells.

Table 1. Mean and standard deviation of macrophages

Groups	N	Mean	Standard Deviaton
Control	7	19	3.19
Spirulina 12%	7	37	6.80
Spirulina 24%	7	52	4.58
Spirulina 48%	7	58	7.42

Table 2. Mean and standard deviation of activated plasma cells

Groups	N	Mean	Standard Deviaton
Control	7	5	1.58
Spirulina 12%	7	7	2.56
Spirulina 24%	7	13	4.58
Spirulina 48%	7	26	7.42

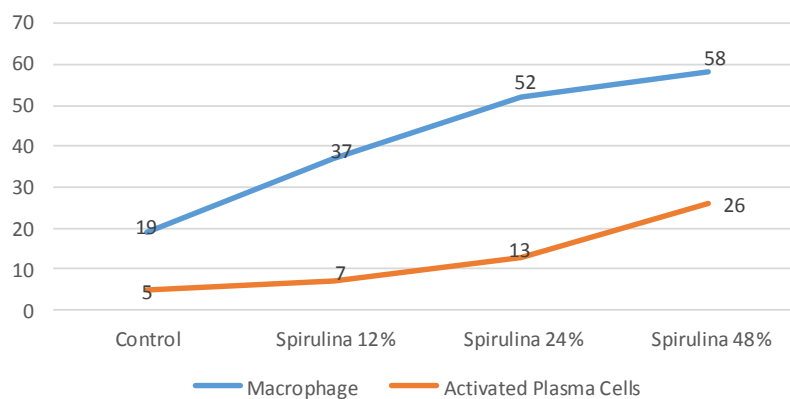


Figure 1. The amount of macrophages and activated plasma cells.

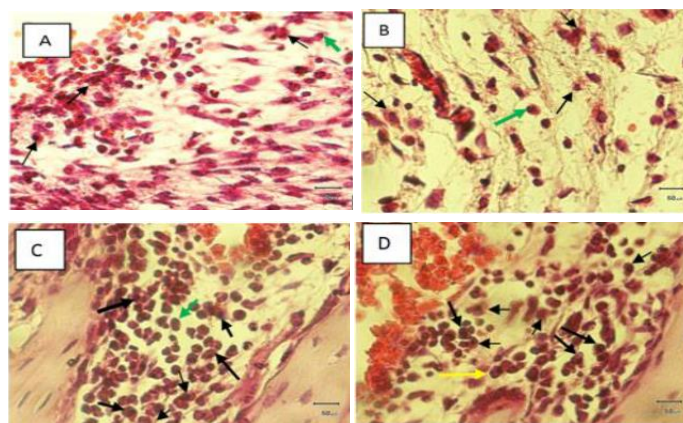


Figure 2. Macrophages in H & E staining. black arrows indicates macrophages (A) control group; (B) spirulina 12%; (C) spirulina 24%; (D) spirulina 48%.

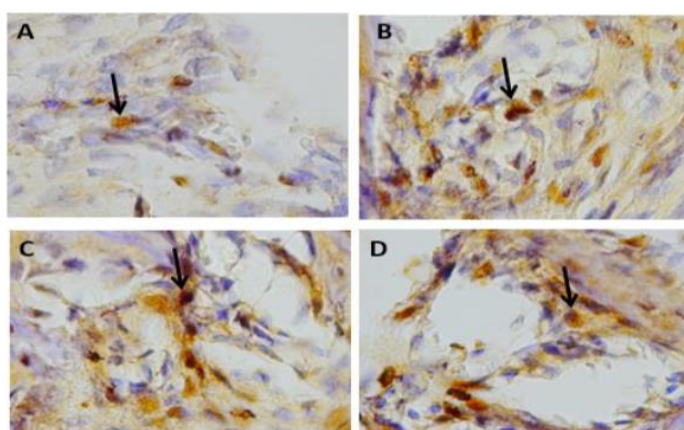


Figure 3. Activated plasma cells in immunohistochemistry staining with monoclonal antibody anti-IgG. The black arrows indicate activated plasma cells (A) control group; (B) spirulina 12%; (C) spirulina 24%; (D) spirulina 48%.

TG4 (spirulina 48%) has the greatest number of macrophages. The Anova test showed significant differences of the amount of macrophages and activated plasma cells with Sig. value 0.000 ($p < 0.05$). Thus, the administration of spirulina could enhance immune response through increasing the number of macrophages and activated plasma cells.

Figure 2 illustrates macrophages after the administration of spirulina in post-extracted tooth sockets of *Cavia cobaya* using H & E staining, and were observed under light microscope (400x). The black arrows indicate macrophages, whereas the yellow and green ones indicate plasma cells. However plasma cells appeared in HE staining were not counted.

Figure 3 illustrates activated plasma cells after the administration of Spirulina in post-extracted tooth sockets of *Cavia cobaya* using immunohistochemistry staining, and were observed under light microscope (400x). The black arrows indicate activated plasma cells which contain antibodies in the cytoplasm. The blue shadow formations which are numerous in Figure 3A are inactivated plasma cells due to absence of antibodies in their cytoplasm.

DISCUSSION

The increasing numbers of macrophages and activated plasma cells verify that bioactive components contained in spirulina could enhance immune system through affecting macrophages and activated plasma cells,¹³ and stimulates VEGF to form collagen which is beneficial for healing process.¹⁴

The ability of spirulina in increasing the function of innate immunity is due to polysaccharide called Immulina which is 100 times more effective in maximising the activity of macrophages mediated by TLR-2 and CD14, as well as increasing the production of TNF- α and IL-1 β . The elevation of the cytokines activates fibroblasts to generate FGF2.¹³

The modulation of immune response by spirulina is initiated by increased proliferation of macrophages which is the target cell of spirulina.¹⁶ Macrophages act as antigen presenting cell (APC) mainly in epithelial tissue, responsible for phagocytosis of immunogenes and presents them in order to be identified by T effector cells. Extracellular immunogenes are identified by macrophages

and B lymphocytes, and presented by MHC class II afterwards. CD4⁺ which functions as T helper would then recognise the peptides and help B lymphocytes to produce antibodies. The interaction between APC and T cell is called immunological synapse.¹⁷

There are two possible ways in activating T effector cells by macrophages. The macrophages express costimulator to bind T naive cells, activate T cells, secrete IL-12 to stimulate the differentiation of T naive cells into effector cells and initiate cell mediated immunity. The responses of T cells occur around 12-18 hours after antigen exposure.¹²

Another variable observed in this study was the amount of activated plasma cells which produce antibodies and are generated from differentiated and matured B cells. The life span of plasma cells ranges up to several years.¹⁸ The increasing number of plasma cells in this study are proportional to the production of antibodies involved in humoral immunity needed in healing process. Antibodies work as effector in humoral immune response that bind to antigens and outgrow antigens through neutralising process that enhances phagocytic cells. The result of this study corresponds to previous study reported that spirulina could enhance cellular and humoral immune response.^{4,11}

The enhancement of spirulina-induced proliferation and activity of macrophage leads to secretion of IL-1 β and IL-6. The cytokine IL-1 β stimulates proliferation of T cell, enhance the function of T cytotoxic and NK cells, and to induce B cells to differentiate into plasma cells. Whereas IL-6 stimulates the production of antibodies by plasma cells.¹⁹ The mechanism of increased antibodies production following administration of spirulina is due to increasing number of CD11b⁺ or through augmenting level of IL-6 which is vital in the development of B cells.¹¹

Complements are essential in humoral immune response. Extracellular immunogens activate complement system through alternative pathway. One of the generated protein is C3d which is able to bind immunogenes. When B lymphocyte recognises antigens through its receptors, B cells also recognises the binding of C3d with immunogenes through such specific receptors for C3d. The binding of C3d and antigens provide signal for B cells to differentiate into plasma cells and produce antibodies subsequently. It shows that complement play a role as signals in humoral immune response.²⁰

The stimulation by antigens alters B lymphocytes to have more interaction with T helper cells. The increasing expression of B7 costimulator induced by B cell activation provides signal to activate T cell and its receptors. The antibodies response to antigen requires assistance by T helper cells.²¹ T helper cells recognise antigens presented in B cells by expressing CD40 ligand (CD40L) and produce cytokines. CD40L subsequently bind with CD40 expressed by B lymphocytes. That binding delivers signal to B cell to stimulate proliferation, synthesis, and secretion of antibodies. The cytokines produced by T helper cells bind with receptors in the surface of B lymphocyte to induce proliferation and differentiation of B cells, and to

induce antibody production.¹² Hence, there is a synergistic relationship between macrophage and activated plasma cells through the mechanism of immunological synapse.¹⁷ In conclusion, spirulina is able to increase the amount of macrophages and activated plasma cells which play important role in healing process.

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