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THE EFFECT OF AVOCADO (*PERSEA AMERICANA* MILL) SEED POWDER ON THE LYMPHOCYTES AMOUNT IN *ESCHERICHIA COLI* INDUCED MICE (*SPRAGUE DAWLEY*)

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ABSTRACT : Avocado (*Persea americana* Mill) is one of the plants that can be used as herbal medicine, while Indonesia is one of the three major avocado producers in the world. All parts of avocado plant can be used as herbal medicine, but until now, it's still lack of information. In fact, parts of avocado were thrown away as waste, including its seed. On the other hand, the substance contained in avocado seed has pharmacological effect, namely as an analgesic and anti-inflammatory. The aim of this study was to find out the effect of avocado seed powder on the lymphocytes number in *Escherichia coli* induced Mice (*Sprague dawley*). A total of twenty-five healthy male mice were divided into five groups respectively to be treated differently for 7 days. Group I was administered sterile distilled water orally as the negative control (without *E. coli* induction). Group II was administered sterile distilled water orally as the positive control. Group III was administered 5% of avocado seed powder orally. Group IV was administered 10% of avocado seed powder orally. Group V was administered 15% of avocado seed powder orally by means of oral gavage. The blood samples were taken on day 1, 3 and 7. The lymphocytes amount was counted and analysed. Comparing all groups to the one with the negative control, there were significant differences between them, except group IV on day 1. During day 3 and 7 all groups, excluding group IV had significant differences ($p<0.05$) with the increase of the lymphocytes average amount. In conclusion, 10% of avocado seed powder can effectively suppress the lymphocytes amount in *E. coli* induced mice.

Key words : Avocado, lymphocytes, *Escherichia coli*, communicable disease.

INTRODUCTION

Avocado is one of the plants which has medicinal efficacy, while Indonesia is one of the three major avocado producers in the world. All parts of avocado plant, namely its leaf, root, and seed, can be used as medicine, but this has not been known (Permadi, 2005). In its use, however, there were many parts of this plant thrown away as waste, including its seed.The substances contained in avocado seed are polyphenols, flavonoids, triterpenoids, quinones, tannins, monoterpenoids, and sesquiterpenoids compounds (Zuhrotun, 2007). Those substances are the ones which make avocado seed has pharmacological effect, namely as an analgesic and anti-inflammatory (Permadi, 2005). Inflammation is a normal protective response towards tissue injury caused by physical trauma, damaging chemicals, or microbiological substances. Inflammation is triggered by the release of chemical mediators from injured tissue and cell migration. Lymphocytes are white blood cells involved in vertebrates' immune system. There are two major categories of lymphocytes: large granular lymphocytes and small lymphocytes (Mycek, 2001).

Escherichia coli is an opportunistic microbe found inside humans' colon as a normal flora. This bacterium has a unique characteristic for it maycause primary infection in the intestine, as well as its capability in generating infection in other body tissues outside the intestine (Ganiswarna, 2005). *E. coli* has the same characteristics as other Gram-Negative Bacteria (GNB), namely it has Lipopolysaccharides (LPS) as the endotoxin in its cell walls. This LPS can be used as strong inflammatory agents (Hossain-Ibrahim *et al,* 2006). In every laboratory research, experimental animal models such as mice (*Sprague dawley*) can be used. Mice can represent mammals, including humans, since they have the same neuroendocrine systems as human beings (Lindsey and Russell, 2006). The aim of this study was to examine the effect of avocado seed powder on the lymphocytes number in *Escherichia coli* induced Mice (*Sprague dawley*).

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METHODOLOGY

This study is an experimental laboratory research with post only control group design as both the sample and the treatment are more controlled, measurable, and the effect of the treatment is more reliable (Notoatmodjo, 2002). This study was conducted at Biomedical Laboratory of Physiology of Dentistry Faculty, University of Jember. Male mice that had been induced by *E. coli* bacteria and avocado seed powder with the concentration of 5%, 10% and 15% was administered. The way this research worked was by preparing experimental animal models by adapting them to the cage environment at the Physiology Laboratory of Dentistry Faculty, University of Jember for 7 days. The distribution of standard food (using *Turbo* brand as the chicken feed) and drink was carried out ad libitum every day. The mice were then weighed and grouped randomly into 5 groups.

The avocado seed powder was obtained by chopping 250 grams of avocado seeds using the varieties of long green avocado. The seeds were then dried utilizing an oven at 40ºC during 24 hours. After that, the dried seeds were grinded using a grinding machine and sifted using a 100-mass sieve executed at the Food Processing Laboratory of Agriculture Faculty, University of Jember. This resulted in the gain of 100 mass avocado seed powder as much as 100 mg with a concentration of 100%. For a concentration of 5%, 5 mg of avocado seed powder was mixed with 95 ml of sterile distilled water. For a concentration of 10%, 10 mg of avocado seed powder was mixed with 90 ml of sterile distilled water. For a concentration of 15%, 15 mg of avocado seed powder was mixed with 85 ml of sterile distilled water.

The preparation of *Escherichia coli* bacteria suspension was done by taking 1 ose of *E. coli* bacteria in 1 ampoule of *E. coli* bacteria, then diluting it with 1 cc sterile distilled water (serial dilution) in a laminar flow cabinet, producing a 10^{-1} dilution. After that, *E. coli* bacteria with a concentration of 10^{-5} were covered with sterile cottons and incubated for ±24 hours in an incubator. The suspension of *E. coli* bacteria was injected subcutaneously into the back of the mice that had been previously shaved with a concentration of 10^{-5} as much as 0,01 cc[12]. Each group was given different treatments for 7 days as follows. Group I: 5 mice were administered sterile distilled water orally as much as 0, 5 ml twice a day as the negative control (without *E. coli* bacteria induction). Group II: 5 mice were administered sterile distilled water orally as much as 0, 5 ml twice a day as the positive control. Group III: 5 mice were administered avocado seed powder with a concentration of 5% as much as 0,5 ml twice a day orally. Group IV: 5 mice were administered avocado seed powder with a concentration of 10% as much as 0, 5 ml twice a day orally. Group V: 5 mice were administered avocado seed powder with a concentration of 15% as much as 0.5 ml twice a day orally.

Blood samples were taken on day 1, 3 and 7 by piercing the tip of the mice's tails with a scalpel. Those blood samples were used as the peripheral bloodsmears utilizing glass objects and then stained with 1 ml of wright's stain for 1-3 minutes. To obtain the counting area, the peripheral blood smears were observed using a binocular microscope with 400 times magnification. After that, one drop of emersion oil was placed on the preparation that would be examined. The lymphocytes amount per 100 leukocytes can be observed and counted with 1000 times magnification.

RESULTS

Based on the observations, the data on the average number of the lymphocytes can be seen in Table 1. Prior to the statistical analysis test, the P-P Plot normality test had to be carried out to determine whether the data was normally distributed. The data was normally distributed, the variant homogeneity test was then performed using the Levene's test ($p>0.05$).

Based on the analysis result, it was known that the data carried out using ANOVA was homogeneous. Therefore, two-way ANOVA test could be executed hereafter. It could be seen from the analysis result done using two-way ANOVA test on the observation parameters (between treatments and controls) that the F

Table 1 : The average of lymphocyte amount in the *E. coli* induce mice's peripheral blood smears.

Samples	The lymphocytes amount in respectively day					
	1		3		7	
	Mean	SD	Mean	SD	Mean	SD
Negative Control	39.4	3.5071	38.0	3.8079	47.8	1.9235
Positive Control	31.4	2.4083	23.6	3.2863	32.0	1.5811
5% Avocado Seed Powder	32.2	2.1679	32.4	3.7815	37.6	1.6733
10% Avocado Seed Powder	40.8	4.3243	42.0	3.1623	42.4	2.8810
15% Avocado Seed Powder	26.8	2.1679	13.0	1.0	26.2	2.1679

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count's value was greater than the F table's. Likewise, the significance value was 0.000. H_0 was rejected and H_1 was approved or, in other words, the inflammation amount was determined between settings and controls. Once, it was known that the data was significant ($p<0.05$), a further examinationusing the Tukey's Least Significant Difference (LSD) test was then necessaryto be conducted to find out which lymphocytes that were significantly different.

From the Tukey's LSD test, all groups that were compared to the one with the negative control and observed every day had significant differences, except the group with 10% of avocado seed powder on day 1. Compared to the avocado seed concentration that had been given, the group with 15% of avocado seed powder had a significant difference ($p<0.05$) on all days, be it on day 1, 3 and 7. Compared to the length of the treatments, both the group with the positive control and the one with 15% of avocado seed powder had significant differences ($p<0.05$) with a decrease in the lymphocytes average number. On day 3 to day 7 all groups, except the one with 10% of avocado seed powder had significant differences ($p<0.05$) with an increase in the lymphocytes average number.

DISCUSSION

The lymphocytes amount of the group with the negative control decreased on day 1 to day 3, but did not differ significantly. On day 3 to day 7 the lymphocytes amount of the group with the negative control significantly increased. The increase of the lymphocytes amount was caused by a chronic inflammation occurring on day 7. On day 3 to day 7 the lymphocytes amount of the group with the negative control should not increase since it was not given triggers of inflammation. Even though the group with the negative control was not injected with *E. coli* bacteria to trigger the inflammation, the lymphocytes amount still increased. This might occur due to the treatments given to the mice's tail since day 1. Those treatments triggered the inflammation and might cause the entry of particular bacteria leading to inflammation. Not long after the piercing, local arteriolar dilatation happened which were preceded by brief vasoconstriction. The increase of vascular permeability occurred while the plasma proteins and white blood cells released into a specific tissue called exudation and it was the major picture of acute inflammatory reactions (Robbins *et al*, 1995).

Compared to other given treatments, the group with the negative control, namely the one that was not induced by *E. coli* bacteria, had a significant difference, except the group with 10% of avocado seed powder on day 1. On day 3 to day 7 the group with the negative control differed significantly to all groups with treatments, including the one with 10% of avocado seed powder. This could be happened due to the one with the negative control experienced an inflammatory process and was only administered with sterile distilled water which did not really help in suppressing the inflammatory process. Compared to all treatments, the group, which was given 10% of avocado seed powder was the one whose value was almost the same asthe group with the negative control. Avocado seed powder with a concentration of 10% could suppress the change in the lymphocyte amount. This might be due to the existence of tannins and flavonoids in avocado seeds.

Tannins have antioxidant and anti-inflammation properties. Besides, this organic substance also contains metal ion chelators such as Fe. When infections occur, tannins are able to hamper the reproduction of microorganisms by binding the Fe. When microorganisms invade, they can take advantage of the Fe inside one's body in order to help them multiply. Whereas tannins, which are metal ion chelators can bind Fe, thus bacteria may not multiply (Brock, 1986). Moreover, they are able to affect the inflammatory responses by their activities which remove free radicals. There is a proof supporting the correlation between inflammations and free radical reactions. For example, nitric oxide (NO), a free radical produced by nitric oxide synthase (NOS), acts as the second messenger during the inflammatory process. Inducible nitric oxide synthase (iNOS) is produced in response to any inflammatory cytokines and lipopolysaccharide (LPS). iNOS stimulates NO production which then arouses further inflammation. It is estimated that the mechanism of tannins is likely to hamper the inflammatory marker by the oxidation of tannins and the reduction of radical oxidation species, including free radicals. In addition to that, Pentagalloyl Glucose (PGG), one of gallotannins (hydrolyzable tannin), can also hamper Prostaglandine^2 (PGE^2), which acts as an inflammation mediator (Jeffers, 2006). If this mediator is hampered, the inflammation will be hampered as well. The resistance occurring in this inflammatory process also obstructs the increase of leukocytes, so that lymphocytes, which include leukocytes, also experience obstacles in escalating their amount in chronic inflammation process, which are supposed to increase in such a chronic inflammation.

Flavonoids play an important role in maintaining permeability as well as increasing capillary resistance. Flavonoids mainly work on the microvascular endothelium to reduce the occurrence of hyperpermeability and

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edema. The anti-inflammatory properties of flavonoids are originated from their mechanism that hampers the release of arachidonic acid and the secretion of lysozyme enzymes from neutrophil and endothelial cells as well as hampers the proliferation and exudation phases of the inflammatory process. The obstruction in the release of arachidonic acid from inflammatory cells will cause the lack of arachidonic substrate for the cyclooxygenase and lipoxygenase pathways, which later will suppress the amount of prostaglandin, prostacyclin, endoperoxide, hydroxyeicosatetraenoic acid, and leukotriene in other parts. That suppresion will likely affect the inflammatory process as well as the leukocytes migration, which will influence the suppression of lymphocytes amount enhancement (Belanti, 1993).

The lymphocytes amount of the group with the positive control decreased significantly from day 1 to day 3. In this situation, chronic inflammation occurred with an increase in the amount of PMN and monocytes, while the new lymphocytes amount increased. There was a significant increase of the lymphocytes amount from day 3 to day 7. It was due to the fact that the inflammation had started to become chronic on day 7. The groups with 10% of avocado seed powder treatment did not differ significantly on day 1 and 3, nor did on day 3 and 7.It can be clearly seen that 10% of avocado seed powder was able to affect the lymphocytes amount and suppress the change, thus the groups did not differ significantly from day 1 to day 7. Compared to the groups with 5% and 15% of avocado seed powder treatments, the one which most effectively suppressed the lymphocytes amount was the group with 10% of avocado seed powder, whose lymphocytes amount did not significantly differ during all days of observation. Therefore, avocado seed powder with a concentration of 10% is more effective both in the short and long term.

Drug absorption is influenced by several factors such as solubility, diffusion ability across the cell membrane, drug concentration, circulation, contact surface area, drug dosage form, and method of use. As the concentration of 10% is higher than 5%, the absorption is even higher, so the effect is also greater. However, any drugs in high doses may inflict toxic effects (Nindia, 2001). In the group with 15% of avocado seed powder, the lymphocytes amount decreased significantly on day 1 and 3. This also happened to the group with the positive control. On day 3 and 7 a significant increase occurred. Avocado seeds did not reduce the increase of lymphocytes amount in chronic inflammation. This might be because the concentration of the avocado seed was too large, sothe lymphocytes amount remained the same. This large concentration was what caused toxicity effects inside the mice's bodies, where it is known that large amounts of tannins can cause irritation to the tissue. Besides, it also can cause Fe deficiency resulting in anemia. Anemia caused by iron deficiency might affect humoral, specific, and nonspecific immunity as well as cytokine activity that plays a vital role in the immune mechanism (Ekiz *et al*, 2005).

CONCLUSION

Based on the result of the observation that has been done, the conclusions can be drawn as follows Avocado seed powder can affect the lymphocytes amount in mice induced by *E. coli* bacteria. The concentration of 10% of avocado seed powder can suppress the increase of lymphocytes amount, specifically in chronic inflammation, which in this study happened on day 7.

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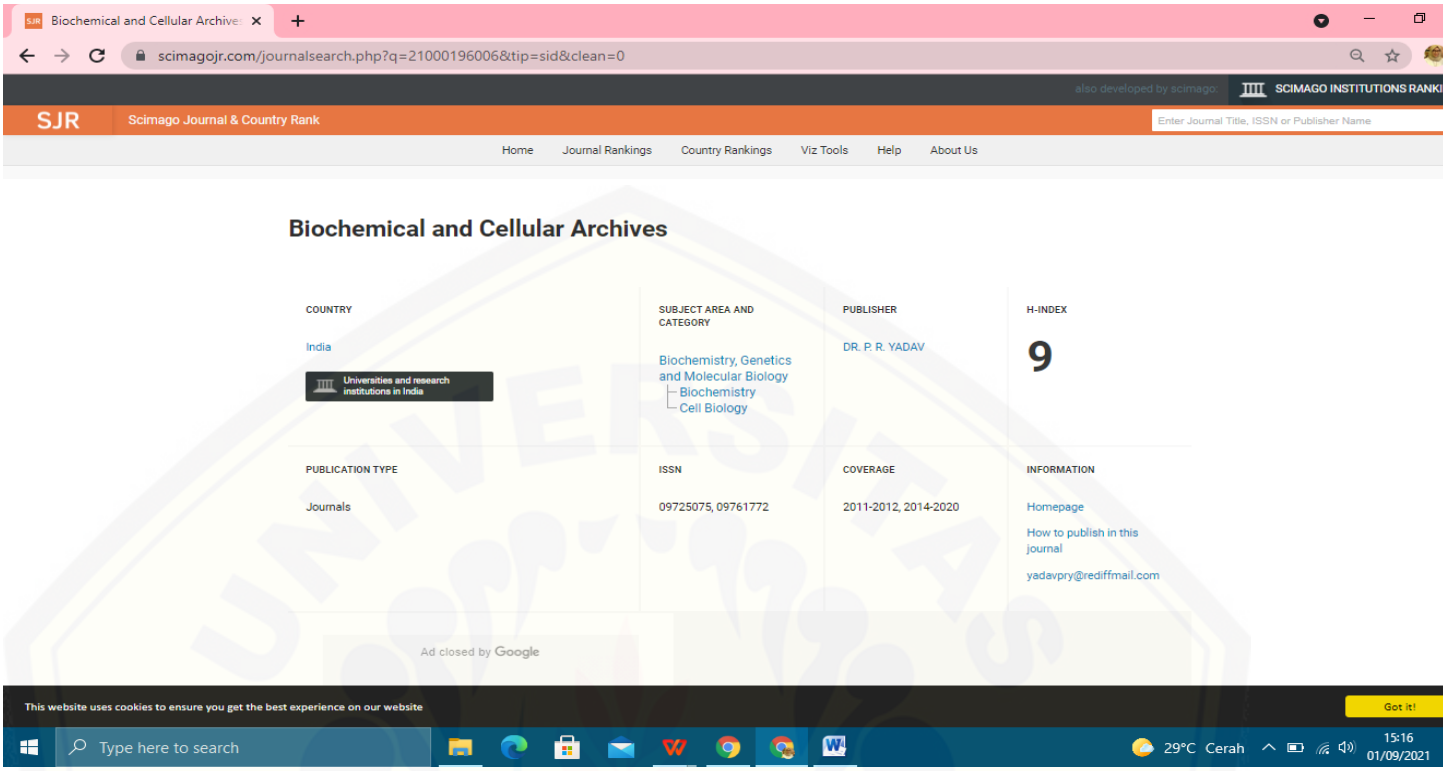

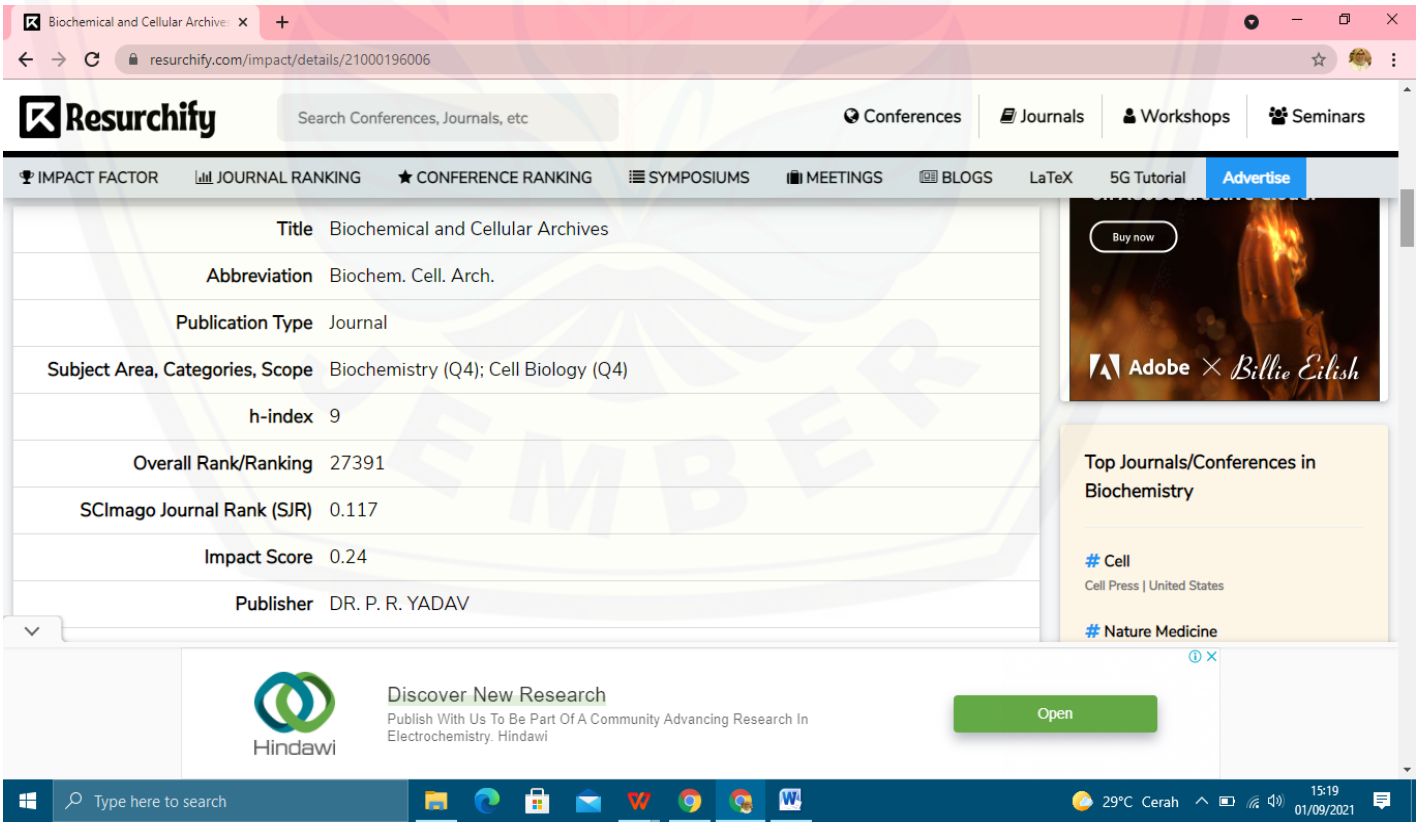

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