The Influence of *Aia Tempayang* on Interleukin-2 (IL2) Levels on Female *Rattus Norvegicus Sprague Dawley* Strains in Breast Cancer Prevention

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ABSTRACT

Background: Breast cancer is a condition in which the cells in the breast tissue grow rapidly and uncontrollably. Risk factors for cancer are those caused by carcinogenic factors, behavior, and diet. Breast cancer causes a decrease in immunity. It can be prevented by consuming high-antioxidant foods such as Aia Tempayang. Aia Tempayang is made from caesalpinia sappan l., scaphium scaphigerum/seeds and ocimum basilicum seeds, each of which contains an antioxidant compound that prevents cancer.

Objectives: To analyze the effect of Aia Tempayang on the decline of interleukin-2 (il-2) as a deterrent to breast cancer.

Materials and Methods: Female Sprague Dawley was 35 days old n= 30, and divided into five groups: normal control group (K1); control groups induced by DMBA without intervention (K2); treatment group induced by DMBA + caesalpinia sappan l. 0.072 g + scaphium scaphigerum 0.012 g + basilicum seeds 0.045 g (X1); treatment group induced by DMBA + caesalpinia sappan l 0.144 g + scaphium scaphigerum 0.024 g + basilicum seeds 0.09 g (X2); and treatment group induced by DMBA + caesalpinia sappan l 0.288 g + scaphium scaphigerum 0.048 g + basilicum seeds 0.18 g (X3). After 35 days of intervention, serum IL-2 was analyzed using ELISA method. Data analysis used Paired-T Test, One Way ANOVA, and Post-Hoc Bonferroni follow-up test.

Results: There was a significant difference in serum IL-2 (p=0,000) between supplementation groups found after intervention.The X1, X2, and X3 groups showed decreased of IL-2 to the K2 group without intervention.

Conclusion: Aia tempayang was effective in reducing interleukin-2 levels in the group of mice induced by DMBA for 35 days with doses of X1, X2 and X3. The dose closest to the normal group is the intervention group X3 with a dose of Caesalpinia sappan l 0.288 g + Scaphium scaphigerum 0.048 g + Basilicum seed 0.18 g.

Keywords : breast cancer; interleukin-2; caesalpinia sappan l; scaphium scaphigerum; basilicum seeds.

BACKGROUND

Breast cancer is the most common cancer in women and is the most common cause of death by cancer after lung cancer, with a percentage of 12%. There are 3.5 million women with breast cancer.[1] It is a condition in which cells in breast tissue grow rapidly and do not grow controlled in breast tissue.[2] The emergence of new cells is caused by uncontrolled cell division and is followed by invasion and metastasis of cells into other tissues and organs of the body. Many risk factors cause breast cancer, that are carcinogens, behavior, and food. Carcinogens are cancer-inducing substances that interfere with metabolic processes.[3] Cancer decrease the patient's immunity. Immunity is a reaction in the body against foreign substances that enter the body molecularly or cellularly.[4] Cells involved in the immune system in the body are T cells produced by the thymus and B cells produced in the bone marrow. Cancer cells in the body are responded to

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by the innate and adaptive immune systems, which involve macrophages and T helper (Th) cells. Helper T cells can produce IL-2 cytokines, which are regulators of the growth and differentiation of lymphocyte cells. Macrophages and IL-2 are two components of the immune system.[5] Interleukin-2 (IL-2) produced by T helper cells is a growth factor for all subpopulations of T lymphocyte cells and is responsible for the clonal expansion of T lymphocytes after T lymphocytes recognize antigens.[6]

There have been various types of treatment for breast cancer, but some of these treatments have certain side effects. Meanwhile, several studies have shown that some cancer sufferers use herbal medicine as an alternative treatment. Herbal medicine is used as an immunomodulator and is recognized as the most frequently used alternative treatment for cancer. Immunomodulators are substances that can help improve immune system function.[7]

Herbal medicinal plants have antioxidant active substances that function to boost the immune system and ward off radicals.[8] One of the uses of herbal drinks as an alternative treatment is *Aia Tempayang*, which is a traditional drink typical of West Sumatra that is brewed. The ingredients used are derived from several ingredients, Caesalpinna Sappan, *Scaphium Scaphigerum*, and *Basillicum Seeds*. Each ingredient contains compounds that prevent cancer. Caesalpinia Sappan contains bioactive flavonoids and phenols that play a role as antioxidants, and the main substance contained in sappan wood is brazilin, which acts as an anti-inflammatory, anti-bacterial, anti-diarrhea, and anti-cancer agent.[9] Brazilin substances that can modulate the immune system, especially T cell activity. Thus, it can increase cell activity and suppress the decrease in interleukin-2.[10]

Schapium Affine contains alkaloids, flavonoids, glycosides, tannins, and saponins that are useful for treating diseases such as intestinal infections, fevers, inflammation, asthma, and pharyngitis.[11] The content of methanol and ethanol extracts from *Scaphium Affine* is known to have antioxidant activity that can effectively reduce free radical activity. *Basil Seeds* are one of the ingredients used in steeping. The content contained is antioxidants and contains essential oils as the main component and other components such as tannins, cardiac glycosides, flavonoids, and other phenolic compounds and saponins. Basil essential oil has been shown to have immunomodulatory, hyperglycemic, hypolipidemic, anti-inflammatory, and anti-microbial effects.[9] This research on *Aia Tempayang* has never been done on experimental animals or humans, so the researchers wanted to prove the effect of giving Aia tampayang to decrease interleukin-2 levels.

MATERIALS AND METHODS

The research was carried out from April to May 2021. The ingredients for Aia Tempayang were obtained from the traditional market of the city of Solok, West Sumatra. Intervention research on experimental animals at the Nutrition Laboratory of the Inter – University Center of Food and Nutrition Studies (PSPGPAU), Gajah Mada University,Yogyakarta. Animal Laboratory for 42 days, from acclimatizasation to blood collection for the post test. Research on experimental animals has been approved by the Medical/Health Bioethics Commission, Faculty of Medicine, Sultan Agung Islamic University, Semarang, with the Ethical Clearance number No.28/II/2021/Commission on Bioethics.

Process of Making an *Aia Tempayang*

Aia Tempayang consists of *Caesalpinia Sappan*, *Scaphium Scaphigerum*, and *Basilicum Seeds*. All these items were stored with aluminum foil to prevent physical damage. A brewing process involves mixing all the ingredients and then brewing them at 70^0C temperatures for 20 minutes, then filtering the entire material.

Experimental Animals

In this experimental study, randomized post-test only design with a control group (n=30) on female white wistar rats was used. The subjects of this study were female *Rattus norvegicus* Sprague Dawley (SD) rats that were induced by DMBA at the same time as the intervention. DMBA induction is induced through a subcutaneous areola mouse area twice a week for 5 weeks with dose 20 mg/kgBB. The rats used were female rats aged 35 days with a body weight 150-300 grams, healthy, and active.

The rats are divided randomly into five groups (six female rats per group): normal control group (K1); control groups induced by DMBA without treatment (K2); treatment group induced by DMBA + caesalpinia sappan 1 0.072 g + scaphium scaphigerum 0.012 g + basilicum seeds 0.045 g (X1); treatment group induced by DMBA + caesalpinia sappan 1 0.144 g + scaphium scaphigerum 0.024 g + basilicum seeds 0.09 g (X2); and treatment group induced by DMBA + caesalpinia sappan 1 0.288 g + scaphium scaphigerum 0.048 g + basilicum seeds 0.18 g (X3). The rats are groubed with 27^0C thermometers, 70% higrometers, and AC 17^0c, and the condition of the mouse is 12 dark hours and 12 bright hours. All rats are grouped in the mammary glands to detect abnormal mass development after five weeks of intervention.

The standard feed used is AD II standard feed, composed of 15% crude protein, 3–7% crude fat, 12% water content, 6% crude fiber, 7% ash, 0.9–1.1% calcium, and 0.6-0 phosphorus. 9%). Rat body weight measurements were carried out once every 7 days using a digital animal scale. Examination of interleukin-2 levels was carried out using the ELISA method at the end of the study.

Statistical Analysis

The data used was the primary data of weight measurement and interleukin-2 level examinations, which is a comparison between the healthy control group and intervention group. Weight measurement data was recorded at the beginning of the study and then recorded every week. *SPSS* was used to analysis data.

The data were tested for normality using the Shapiro-Wilk test. The first statistical analysis determined the differences in weight data pre-test and post-test. The average weight of the experimental animals was normally distributed, with the Paired t-test and *One - Way* Anova used to examine changes and differences in the group, and the Kruskal-Wallis test to differences in body weight changes between experimental groups of animals. The second statistical analysis is the post-test carried out on interleukin-2 levels data, which proved the IL-2 Level was normally distributed. Furthemore, the *One-Way* Anova and Bonferroni Post-Hoc tests were used to examine the difference in the intervention effects in the group.

RESULTS

The body weight characteristics of the experimental animals during the acclimatization period ranged from 160–188 g. No experimental animals dropped out during the study. The intervention of Aia Tempayang for 35 days resulted in an increase in the body weight of rats. The results of statistical tests on changes in body weight of experimental animals before and after administration of Aia Tempayang (Table 1) showed an increase in body weight of experimental animals before and after DMBA induction and Aia Tempayang intervention were significantly different in groups (p = 0.000).

Table 1. Test Animal Body Weight Value (g) Before and After Intervention Aia Tempayang

Group	n	Before (Mean±SD)	After (Mean±SD)	p	Δ Median (min-Max)
K_1	6	175,83±4,579[a]	216,00±4,858[a]	0,000	40,50(39-41)[b]
K_2	6	178,50±3,728[a]	169,67±3,266[a]	0,000	-9,00(-10- -8)[b]
X_1	6	181,00±4,775[a]	210,33±4,546[a]	0,000	29,50(28-30)[b]
X_2	6	182,67±3,559[a]	222,50±3,450[a]	0,000	40,00(38-41)[b]
X_3	6	181,17±5,037[a]	221,33±4,412[a]	0,000	40,50(39-41)[b] P = 0.000[b]

p= Paired T-Test; [a]= One Way ANOVA; [b]= Kruskal-Wallis

The results of the paired t-test in the table above show that there is a significant difference in the average body weight between groups before and after the 35-day aia tempayang intervention in each group (p = 0.000). Based on the Kruskal-Wallis test, it showed that there was a significant difference in weight change between groups ($p<0.05$).The results of the One-Way ANOVA test showed that there was a significant difference in body weight at the end of the intervention and the DMBA induction on weight changes between groups (p = 0.000). evidenced by p-value < 0.05. Descriptively, the lowest percentage increase in body weight of experimental animals was shown by group K_2, which were DMBA-induced rats without Aia tempayang intervention. The provision of the Aia Tempayang intervention was higher in weight gain in the treatment group and was equivalent to the increase in body weight in the healthy control group who received standard feed.

Table 2. Interleukin-2 Levels in Experimental Animals (pg/dl)

Group	n	Interleukin-2 Levels	p
K_1	6	0,226 ± 0,035[a]	0,000
K_2	6	1,317 ± 0,213[a]	
X_1	6	0,799 ± 0,032[a]	
X_2	6	0,425 ± 0,033[a]	
X_3	6	0,290 ± 0,023[a]	

p=One Way Anova, [a]=Post-Hoc Bonferroni

One-way ANOVA statistical test results (Table 2) showed interleukin-2 levels were significantly different in the five groups (p = 0.000). The results of Bonferroni's Post-Hoc statistical test showed an increase in interleukin-2 levels in the K2 (1,317 ± 0,213) group compared to the X1, X2, and X3 intervention groups, whose results were close to those of the normal group (0,226 ± 0,035). This proves that the intervention dose of aia tempayang in groups X1, X2, and X3 has the ability to reduce levels of interleukin-2 in rats with breast cancer. There was no difference in group X3 (p = 0.032) compared to K1. This indicates that steeping Aia tempayang with a dose of *caesalpina sappan* = 0.288 g, *scaphium scaphigerum* = 0.048 g, and *basillicum seeds* = 0.18 g is the best dose to reduce interleukin-2 levels in breast cancer rats. The larger of the dose, the lower the interleukin-2.

DISCUSSION

The weight gain of experimental animals in the intervention group of rats was significantly different compared to the sick rat group. The weight loss that occurred in the K2 group was due to the carcinogenic DMBA, which was given continuously in the absence of received antioxidants.[12] Compared to mice that were not given DMBA, group K1. Weight loss is the result of a nutritional disorder known as cachexia syndrome. In cancer patients, cachexia ranges from 40–80% and causes death in 30–50% of cancer patients.[13] Cachexia occurs due to various factors, that is inadequate food intake, metabolic disorders, and specific humoral and inflammatory responses.[14]

Inteleukin-2 is a cytokine that plays a role in regulating the immune response. It functions as a mitogen for T cells, which potentially increases the proliferation and function of T cells, B cells, and NK cells, improves antigen formation, and increases the production and release of other cytokines. In a stable or normal body state, the amount of IL-2 is reduced by Treg cells, so the amount is quite low. The reduction in the amount of IL-2 by Tregs is in line with the function of Tregs, namely suppressing the immune response, so that there is no excessive immune response, such as autoimmune.[15] The group of rats induced by DMBA and the intervention of Aia tempayang steeped decreased the amount of IL-2 due to a decrease in the number of cancer cells due to apoptosis, so that the signals for the formation of IL-2 were reduced and relatively close to the amount in normal mice. The development and activity of T cells can be stimulated by the addition of an immunomodulator.

Aia Tempayang is a traditional drink made from several food ingredients, it is made from *caesalpina sappan*, *basillicum seeds*, and *scaphium scaphigerum*. Where the secondary metabolite content in each ingredient contains antioxidants and is in the form of chemical compounds, that is flavonoid compounds.[16] This compound can suppress the decrease in the number of leukocytes and has the potential as an anti-inflammatory. One of the community's efforts to suppress the high prevalence of breast cancer is to increase the consumption of antioxidant-rich foods. The flavonoids in the wrought Aia Tempayang can used on interferon γ produced by T cells, which will stimulate phagocytic cells and increase the secretion of IL-2.

In the study of Swarnalatha (2014), it was also stated that flavonoids can affect inflammation and lymphocyte proliferation. In relation to breast cancer, some tumor cells are presented by dendritic cells to induce T cells to proliferate and secrete large amounts of IL-2 and IFN-. Interleukin-2 is found in high amounts in the body tissues of breast cancer patients, especially at the tumor site.[17] This causes an increase in the number of Th2 and Treg cells, which inhibit the immune system's anti-tumor response to cancer. Administration of IL-2 as immunotherapy in cancer patients has also been shown to increase the number of circulating Treg cells, thereby causing a very strong suppression of the immune system.[18] As evidenced by the absence of nodules in the breasts of rats, flavonoids showed selective ability in terms of killing cancer cells and inhibiting cancer angogenesis and cancer metastasis.[19]

Antioxidants have an important role in protecting the body from free radical attack. Antioxidants such as flavanoids, vitamin C, which are found in brewed aia tempayang can boost the immune system to fight and protect the body against cancer and infections.[20] Through this mechanism, the effectiveness of all bioactive compounds and nutrients that act as antioxidants, anti-inflammatory, and anti-cancer agents in Aia Tempayang is enhanced. After 35 days of treatment, the mean decrease in interleukin-2 levels was higher at dose III and was equivalent to that of the healthy control group.

This study had limitations, that the long-term effect of steeping aia tempayang on the prevention of breast cancer is unknown, and it is not known what ingredients are the most effective in the active components of aia tempayang steeping.

CONCLUSIONS

The aia tempayang intervention was able to reduce interleukin-2 levels in the intervention group at a dose of 0.288 g of *caesalpina sappan*, 0.048 g of schapium scaphigerum, and 0.18 g of basillicum seeds and was significantly different compared to the group of rats induced by DMBA without intervention.

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