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Dragon Fruit Stem Extract Reduces IgG Levels in Pregnant Wistar Rats as an Immunomodulator Candidate

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ABSTRACT

Dragon fruit stem contains many organic acids, proteins, minerals such as potassium, magnesium, calcium and iron, vitamin C, and sources of antioxidants that have properties to lower blood pressure and blood sugar levels and increase immune system activity. Objective: To analyze the extract of dragon fruit stems from Pelaihari in reducing IgG levels in Pregnant Female Wistar Rats as Immunomodulator Candidates. Method: The research design used experimental. This study on test animals, mice were divided into 5 groups, namely the normal control group, negative control (injected with 10% SDMD antigen), pregnant mice were injected with 10% SDMD antigen + dragon fruit stem extract 500mg, 1000mg and 2000 mg-KgBW-day for 20 days, after which IgG were examined. blood samples were examined, TBARS test analysis, and One Way Anova test analysis. Results: There was an effect of dragon fruit extract on reducing IgG levels in pregnant mice ($p<0.05$). Doses of 1000mg and 2000mg dragon fruit extract have the ability to decrease IgG levels in pregnant mice, especially at a dose of 2000 mg-KgBW-day. Conclusion: Dragon Fruit plant stem extract can reduce IgG and improve the immune system in pregnant female mice.

Keywords: Dragon fruit stem, IgG, Immunomodulator

INTRODUCTION

Dragon fruit is an important source of phytochemicals such as polyphenols, flavonoids, and vitamin C, which are related to its antioxidant activity, The red and white dragon fruits especially have recently drawn growing attention worldwide not only because of their economic value but also for their health benefits[1].

Dragon fruit (Hylocereus spp.) is a widely consumed tropical fruit that is considered healthy partly due to its high content of phenolic compounds[2]. The phenolic compounds in pulp possess antioxidant activity and have a range of potential health benefits[3]. The global market value of dragon fruit reached 4.9 billion US dollars worldwide in 2016[4].

Ingredients of natural origin to be used as supplements must be in the dosage form. Natural materials generally have less stable properties in the open air. This allows natural ingredients to be formulated in suitable preparations. Natural ingredients that are formulated in dosage forms will increase the stability of the active ingredients. The purpose of this study was to determine the levels of phytochemicals and secondary metabolites of the Fruit, Stem, and Root of the Dragon Fruit Plant (Hylocereus polyrhizus). Dragon fruit flesh has a higher total phenolic content and antioxidant capacity than the skin, while dragon fruit skin contains higher flavonoids and tannins than the flesh. The results showed that dragon flesh planted in Australia has a total phenolic content and antioxidant capacity that is stronger than the skin, while the

skin of the dragon fruit contains a higher flamed and tannin content compared to the flesh of the fruit[5].
Several studies have shown that fresh fruits and vegetables have excellent antioxidant content and can provide protection against chronic diseases caused by oxidative stress, such as cardiovascular disorders and several types of cancer, phenolic compounds, and flavonoids have been reported to show antioxidant properties[6,7]. The study showed that the extract of the H. Polyrhizus rod had excellent Radical Radical and ABTS antidote activities. Stem extract, skin, and flower in watery ethanol 95% show the effect of protection of excellent DNA damage as well. Stem and flower extract 1000 g/ml in watery ethanol 95% increases the nature of cell migration. These results indicate that the stem, leather, and flower H. Polyrhizus is a source of antioxidant polyphenols and has the potential for application in the pharmaceutical, cosmetics, and food industries[8].
Ethanol extract of red dragon fruit skin from Pelaihari with concentrations of 0.0625; 0.125; 0.25; 0.5; and 1 gram/100 mL gave an average percentage of antioxidant activity of 6.468%; 9.738%; 12.286%; 13.141% and 20.867%, respectively. Ethanol extract of red dragon fruit skin has antioxidant activity with an IC50 value of 3.14 grams/100 ml[9].
Natural ingredients that will be used as medicinal products must meet the aspects of efficacy, safety, and quality. The safety aspect can be assessed, one of which is by the Brine Shrimp Lethality Test (BSLT) method using shrimp larvae. BSLT is an initial method that is often used to observe the toxicity of compounds. BSLT can be applied in the screening of active compounds contained in plant extracts that are shown to be toxic to Artemia Salina Leach larvae[10].
Increasing immunity can be done with herbal medicine therapy to minimize the adverse effects of drugs or target resistance. Herbal medicines can come from fruits, vegetables or plants that contain immunostimulators. Fihiruddin's 2019 study stated that peptides in cabbage (Brassica oleraea) have an immunostimulatory effect, namely by increasing the IgG11 titer[11], so researchers are interested in finding other compounds that have bioactivity as immunostimulators regarding the effect of dragon fruit plant stems on IgG in pregnant female Wistar rats as immunomodulator candidates.

MATERIAL AND METHOD

Design The study used in this study was experimental (experimental), the research location was carried out at the Jamu Pucuk Sirih Factory (Extract Manufacturing) and the Pharmacy Lab of FMIPA ULM Banjarbaru (Pharmacological Activity Test). The samples in this study were pregnant mice weighing 250-300 grams in healthy condition which was characterized by active movement of hair that did not fall out and no visible morphological abnormalities that were injected with 10% SDMD antigen. The number of replications was 5 with the normal control group, the negative control group (pregnant mice induced by 10% SDMD antigen, treatment group 1 (pregnant mice induced by 10% SDMD antigen + dragon fruit stem extract 500 mg-KgBW-day, treatment group 2 + dragon fruit stem extract 1000 mg-KgBW-day), and treatment group 3 + dragon fruit stem extract 2000 mg-KgBW-day. The tools and materials used are Glassware, Maseri Vessel, Rotary Evaporator, Waterbath, Grinder, UV-Vis Spectrophotometer, and Atomic Absorption Spectrocopic (AAS). Dragon Fruit Plant from Pelaihari, Technical Ethanol, Whatman filter paper no.10, Ethanol pro analysis, Aqua sterile, DPPH, Tris Buffer Saline (TBS) solution, Bovine Serum Albumin pro analysis, Pure diclofenac standard. and pH meter, shrimp larvae vessel, aerator, individual mouse cage, IgG level test with Human IgM Elisa kit (ab195215).

Dragon Fruit stem Extract-Making Procedure

The parts of each plant are separated and then washed thoroughly using running water, then drained. The samples were then cut into smaller sizes, then dried in an oven at 60°C for 3 days. The dried samples were then mashed using a grinder until a powder was obtained. The dry sample powder was then weighed as much as 1 kg, then each was immersed in 10 liters of ethanol for 3 days. The solvent was changed every 24 hours. The extract solution was

filtered using filter paper and a hydraulic press to separate the active ingredients from the pulp. The extract solution was then evaporated using a rotary evaporator to become 1/10 part. Then it was evaporated again using a water bath until a thick extract was obtained.

Toxicity Test

The toxicity test used Artemia salina larvae which were tested with the Brine Shrimp Lethality Test (BSLT). The concentration of the test solution for BSL was 1500, 1000, 500, 100, and 0 µg/mL (as a control). After observing for 24 hours, the level of toxicity was determined. determined by counting the number of dead larvae. The LC50 value is determined using Linear Regression Analysis. An extract is declared to have the potential for acute toxicity if it has an LC50 value < 1000 µg/mL

Procedure for Measurement of IgG in Pregnant Mice

The treatment groups given to the test animals were the normal control group, the negative control group (Pregnant mice were induced with 10% SDMD antigen), treatment group 1 (Pregnant mice were induced with 10% SDMD antigen and given 500 mg-KgBW-day of dragon fruit stem extract), Treatment group 2 (Pregnant mice were induced with 10% SDMD antigen and given 1000 mg-KgBW-day of dragon fruit stem extract, and treatment group 3 (Pregnant mice were induced with 10% SDMD antigen and given 2000mg mg-KgBW-day of dragon fruit stem extract) were given for 20 days. Mouse IgG examination Pregnancy was carried out on day 0 before giving dragon fruit stem extract and on the last day 20 after giving dragon fruit stem extract by taking blood samples. Data were analyzed using the One Way ANOVA test.

RESULTS AND DISCUSSION

Results

Toxicity Test Results from Dragon Fruit (Hylocereus polyrhizus) Stems

The results of the toxicity test obtained from the number of deaths of Artemia Salina Leach larvae in each test tube in various concentrations of dragon fruit stem extract treatment are shown in Tables 1 and 2. From this table, it can be seen that various concentrations of dragon fruit stem extract in this experiment showed different effects on the death of Artemia salina Leach larvae

Table 1. Toxicity Test Results on Dragon Fruit Stem Extract

Rate (µg/ml)	Percent Mortality		
	Replication 1	Replication 2	Replication 1
100	20	10	10
500	20	20	20
1000	30	30	30
2000	60	70	60
LC50	1982,14 µg/ml	1867,02 µg/ml	1639,02µg/ml
Average LC50		**1600,05 µg/ml**	
Results (> 1000 µg/ml)		**NOT TOXIC**	

Table 1 shows that the relationship between the dilution concentration of dragon fruit stem extract and the percentage of death of Artemia salina L shrimp larvae is positive linear, this shows that the higher the concentration of dragon fruit stem extract given, the higher the percentage of death of shrimp larvae, which can be seen in terms of magnitude. the regression coefficient value is close. Brine Shrimp Lethality Test (BSLT) is a screening method to

determine the toxicity of a compound extract from natural ingredients that is acutely cytotoxic using Artemia salina Leach larvae as test animals. An extract is declared to have the potential for acute toxicity if it has an LC50 value < 1000 µg/mL. LC50 (Lethal Concentration 50) is the concentration of a substance that can cause death at 50%. Testing of dragon fruit stem extract shows that the LC 50 price is 1600.05 µg/ml > 1000 µg/mL, so it can be said that dragon fruit stem extract is not toxic for consumption because the stem is rich in vitamins and minerals.

Research Results and Data Analysis

Dragon fruit stem extract was tested on mice. Tests were carried out on pregnant female mice. Twenty-five mice were divided into five groups, each group consisting of 5 mice. The group consisted of normal controls, negative controls, a dose of 500 mg-KgBW-day, a dose of 1000 mg-KgBW-day, and a dose of 2000 mg-KgBW-day. All test animals except the normal control group were induced by antigen, namely sheep red blood cells. Administration of antigen will trigger an increase in IgM, except in the normal control group. Next, the group with a dose of 500 mg-KgBW-day, a dose of 1000 mg-KgBW-day, and a dose of 2000 mg-KgBW-day were given the extract every day. The negative control group was not given any exposure. On day 0 and day 20, IgG levels in the blood were analyzed. IgG levels in the blood were analyzed using the Human IgG Elisa Kit (ab195215).

Parametric prerequisite test results

The results of observations/measurements in this study on IgG titers before being analyzed to prove the research hypothesis was tested first with a data normality test carried out using the Kolmogorov-Smirnov test obtained and explained in full as shown in the table below :

Table 2. Normality test results

Observation group	*p-value* IgG levels	Distribution
Normal	0.068	Normal
Negative	0.143	Normal
D500	0.200	Normal
D1000	0.200	Normal
D2000	0.200	Normal

In Table 2, based on the results of the Kolmogorov-Smirnov test, it was found that data on IgG levels in mice for each observation group showed p-values, all of which were greater than the significance level of $\propto = 0.05$. So all data has been proven to be normally distributed.

Table 3. Dragon Fruit stem plant Homogeneity test results

Observation group	*p-value* IgG levels	Distribution
Based on Mean	0.047	Homogen
Based on Median	0.671	Homogen
Based on Median and with adjusted df	0.674	Homogen
Based on trimmed mean	0.055	Homogen

If Sig > 0.05, then the data is homogeneous

Based on table 3, it shows the results of the IgM homogeneity test when administering dragon fruit stem extract (Sig 0.115), P > 0.05, so the data is homogeneously distributed.

Results of measuring IgG levels

Table 4. Test Results of Giving Dragon Fruit Stem Extract on IgG Levels In Pregnant Rats

No	Group	Replication	IgG levels (ng/mL)	
			Average Day 0th	Average Day 20th
1	Normal Control	5	2.39	2.40
2	Negative Control	5	2.26	8.90
3	Dose 1 (500 mg/KgBW/Day)	5	2.39	7.65
4	Dose 2 (1000 mg/KgBW/Day)	5	2.38	6.24
5	Dose 3 (2000 mg/KgBW/Day)	5	2.22	5.48

Table 4 test results show that on day 0 the IgG value was recorded from the test animals. On day 20, the average data for the normal control group showed no significant increase since day 0, due to no antigen exposure to this group. The negative control group showed an increase in IgG in all test animals. This is due to antigen exposure which causes an increase in IgG in this group. On the 20th day, at a dose of 500 mg-KgBW-day, there was still an increase in IgG levels in all mice in this group. On the 20th day, at a dose of 1000 mg-KgBW-day there was a decrease in IgG levels but at a dose of mg-KgBW-day there was a slight increase in IgG levels. This shows that a dose of dragon fruit stem extract of 2000 mg-KgBW-day is able to prevent an increase in IgG due to antigen exposure.

Table 5. Comparison Results of IgG When Giving Dragon Fruit stem Extract to Rats

Observation group	n	Average IgG	*p-value*
Normal Control	5	2.4020[a]	
Negative Control (SDMD)	5	8.9000[b]	0,000
SDMD + Dragon fruit extract 500mg	5	7.6500[c]	
SDMD + Dragon fruit extract 1000mg	5	6.2400[d]	
SDMD + Dragon fruit extract 2000mg	5	5.4840[e]	

Table 5 Statistical results show that normal controls have a significant difference from negative controls with Sig values. 000. These results show that in normal controls that were not given antigen induction compared to negative controls that were induced by antigen without being given dragon fruit stem extract, there were significant differences in IgG levels. Administration of antigen caused a significant increase in IgG levels compared to normal controls. These results validate the data that administration of antigen can cause an increase in IgG levels. Based on statistical results, it is known that the negative control was significantly different from the 500 mg-KgBW-day dose group (Sig. 0.000), the 1000 mg-KgBW-day dose (Sig. 0.000), and the 2000 mg-KgBW-day dose (Sig. 0.000). This shows that administering dragon fruit stem extract is able to suppress and reduce IgG levels in antigen-induced mice, but this reduction does not approach normal values.

DISCUSSION

Test Results on Rats

Dragon Fruit Stem Extract

Testing on animals aims to determine the ability of dragon fruit stem extract to suppress the acute increase in IgG due to antigen induction (Sheep Red Blood Cells).The results of statistical analysis of IgG levels showed that the normal group when compared with the 1000 mg-KgBW-day (Sig. 0,753) or 2000 mg-KgBW-day (Sig. 0,999) dose group had no significant differences. This shows that administering dragon fruit extract at a dose of 1000 mg-KgBW-day or a dose of 2000 mg-KgBW-day is able to suppress the increase in IgG levels to normal conditions. Dragon fruit extract at a dose of 1000 mg-KgBW-day or a dose of 2000 mg-KgBW-day is able to prevent an increase in IgG levels due to antigen induction. When comparing the extract at a dose of 1000 mg-KgBW-day with a dose of 2000 mg-KgBW-day, it has a Sig. 0.876 which shows no significant difference between the two doses. This shows that the administration of dragon fruit extract at a dose of 1000 mg-KgBW-day or a dose of 2000 mg-KgBW-day has equivalent ability.

The stem extract test was carried out on mice using the same method as the test on the fruit extract. The group of mice consisted of five groups. The first group was normal mice without antigen induction and without extract. The second group was a negative control that was induced by antigen but not given extract. In groups three, four, and five, they were induced with antigen, then given extract with consecutive doses of 500 mg-KgBW-day, 1000 mg-KgBW-day, and 2000 mg-KgBW-day. On the 20th day, the IgG levels were determined in all groups. The results of the IgG levels were then analyzed statistically using SPSS. When compared between negative control groups with doses of 500 mg-KgBW-day, doses of 1000 mg-KgBW-day, and doses of 2000 mg-KgBW-day, it is known that the Sig value <0.000 in all groups. This shows that giving extracts at doses of 500 mg/KgBB/Day, doses of 1000 mg-KgBW-day, and doses of 2000 mg-KgBW-day is able to suppress IgG levels even though this ability does not reach the normal value of IgG. Stem extract from dragon fruit plants still has the ability to fight antigens, although not as strong as the ability of dragon fruit extract.

Based on statistical results, it can be seen that administration of antigen can increase IgG levels in mice. Administration of the extract at all doses is known to be able to suppress the antigen-induced increase in IgG in mice. The strongest ability to suppress the increase in IgG occurred in the extract at a dose of 1000 mg-KgBW-day and a dose of 2000 mg-KgBW-day. At a dose of 500 mg-KgBW-day, the extract was unable to suppress antigen as indicated by IgM levels still above normal. This test shows that administering the extract at a dose of 1000 mg-KgBW-day and a dose of 2000 mg-KgBW-day can suppress the increase in IgG due to the presence of antigen. If selected, a dose of 2000 mg-KgBW-day is recommended to be able to suppress the antigen. The smaller the dose given, the more recommended the dose to be chosen.

Immunity It can be defined as the body's ability to identify and fight off a large number of infectious and potentially harmful microorganisms, enabling the body to prevent or fight off disease and inhibit organ and tissue damage. Immunomodulators These are biological or synthetic substances that can stimulate, suppress or modulate any aspect of the immune system. Many medicinal plants exhibit immunomodulatory activity in experimental models at specific doses. Various types of screening methods both in vivo and in vitro have been used to determine their pharmacology. Some medicinal plants may stimulate the immune system, (e.g., Panax ginseng, Ocimum sanctum, Tinospora cordifolia, and Terminalia arjuna), and some may suppress the immune response. Also, various secondary metabolites (e.g., alkaloids, glycosides, saponins, flavonoids, coumarins, and sterols) exhibit a wide range of immunomodulatory activities[12], The doses used were methanol extract of dragon fruit skin (Hylocereus polyrhizus) 500, 100 and 20 µg/ml; soluble fraction 20, 500 and 100 µg/ml; and sediment 500, 100 and 20 µg/ml. The addition of samples with higher concentrations was able to reduce the number of latex phagocytic macrophages. The soluble fraction showed higher macrophage phagocytic activity compared to methanol extract of fruit skin, sediment and

control media [13], Extraction of dragon fruit skin (Hylocereus polyrhizus) with 96% ethanol solvent. The extract doses used were 0.25 mg/g bb, 0.5 mg/g bb, and 1 mg/g bb. There were differences in the distribution of IL-1β levels between groups, where a decrease in IL-1β levels was related to an increase in the dose of red dragon fruit skin extract given as therapy. In vivo, peritoneal fluid and endometriosis lesions of endometriosis model mice[14], The group of mice given 25 g of red dragon fruit powder (Hylocereus polyrhizus) and 5 g of high-fat diet feed decreased the average TNF-α by 6.96 (pg / ml) to 4.67 (pg / ml), likewise mice given 25 g of red dragon fruit powder plus 5 g of high-fat diet feed and swimming exercise experienced a decrease in TNF-α levels from an average of 8.21 (pg / ml) to 5.14 (pg / ml) in In vivo, blood plasma of obese model mice[15] . Dragon fruit skin and flesh extract 2000 µg/ml, 1000 µg/ml, 500 µg/ml and 250 µg/ml. The results of all extracts at a concentration of 2000 mg/ml showed a higher rate of lymphocyte proliferation. Dragon fruit skin methanol extract showed a proliferation rate of 251.16% and dragon fruit methanol and dragon fruit skin ethanol extract showed a proliferation rate of 167.4% and dragon fruit ethanol extract showed a proliferation rate of 148.8% in In vitro sheep blood PBMC culture[16]. IgA and IgG levels increased at DFO (Dragon Fruit Oligosaccharides) doses of 1 g/kg, 2 g/kg and 4 g/kg in vivo in mouse blood plasma[17]. Dragon fruit plants have been proven to inhibit the growth of various types of bacteria such as Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Salmonella pullorum, Staphylococcus epidermidis, Propionibacterium acne, Neisseria meningitidis, Streptococcus pneumoniae, Streptococcus mutans, Proteus mirabilis, Bacillus cereus, Pseudomonas aeruginosa, Listeria monocytogenes, Pseudomonas Sp, Methicillin-Susceptible Staphylococcus aureus (MSSA) and Methicillin-Resistant Staphylococcus aureus (MRSA). The highest antibacterial activity was obtained in the n-hexane fraction of red dragon fruit stems in inhibiting MRSA (19.48 mm). Based on literature studies, dragon fruit plants have a number of phytochemical compounds and show antibacterial activity against various types of bacteria, including Gram-positive and Gram-negative[18]. extract and the dragon fruit stem fraction (Hylocereus polyrhizus) against several groups of compounds, including flavonoids, alkaloids, saponins, tannins and terpenoids. Screening showed that the powder, extract, n-hexane fraction and ethyl acetate fraction contained flavonoids, steroids and saponins. While the water fraction contained flavonoid and saponin compounds[19]. Antioxidant activity test using the DPPH immersion method measured the absorbance at a wavelength of 517 nm and compared with vitamin C, showing that the ethanol extract of red dragon fruit stems (IC50 1020.96 ìg/mL)[20].

Dragon fruit (Hylocereus spp.) contains similar bioactive compounds, including alkaloids, phenols, saponins, steroids, and tannins. However, dragon fruit (Hylocereus spp.) has additional bioactive compounds, namely terpenoids, which give it a comparative advantage over Carica papaya. Dragon fruit exhibits therapeutic effects, including antiplatelet, anesthetic, antifungal, antiviral, anabolic, cholesterol-lowering, antidiabetic, anthelmintic, anticarcinogenic, antimutagenic, styptic, astringent, antiadhesive, antihyperglycemic, antiparasitic, anticancer, antibacterial, anti-inflammatory, antioxidant, and analgesic properties. Overall, both Carica papaya and dragon fruit stems exhibit potential therapeutic effects on human health, for the development of natural medicines.[21]

CONCLUSION

Safe to use Dragon Fruit Plant Extract (Hylocereus polyrhizus) was tested using the Brine Shrimp Lethality (BSL) method. The dose of 2000 mg-KgBW-day is the most significant dose of dragon fruit stem extract (Hylocereus polyrhizus) which was tested pharmacologically to reduce antigen-induced IgG levels in mice, which functions as an immunomodulator in pregnant Wistar mice. It is necessary to test the effectiveness of the dragon fruit stem extract formulation on other variables.

AUTHORS' CONTRIBUTIONS

Conception anddesign: Rubiati Hipni
Analysis and interpretation of the data: Rubiati Hipni, Isnaniah
Drafting of the article: Rubiati Hipni, Hapisah

Critical revision of the article for important intellectual content: Rubiati Hipni, Fitria Jannatul Laili
Final approval of the article: Rubiati Hipni
Collection and assembly of data: Rubiati Hipni, Evi Risa Mariana, Niken Pratiwi

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