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DRAGON FRUIT (*HYLOCEREUS COSTARIOENSIS'S*) EXTRACT AS ANTIINFLAMATION AND ANTI STRESS OXIDATIVE IN OBESE WISTAR RATS WHICH GIVEN HIGH-FAT DIET

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ABSTRACT

A high-fat diet can increase body fat until obesity occurs. Obesity could raise the production of *reactive oxygen species*(ROS). The production of ROS continuously can caused of oxidative stress which is characterized by increased levels of malondialdehyde (MDA). When oxidative stress occurs continuously it can trigger atherosclerosis and cell damage. The aims of study to determine the potential anti-oxidative and anti-inflammatory activities of dragon fruit extract. Twenty sevens wistar rats which have 150-300 g body weight were separated in 3 groups where each group was given standard feed (control), high fat (HF) and high fat with dosage 100 mg per BW per day of red dragon fruit extract (DFF). Four weeks later blood plasma taken and analyzed contain of MDA, IL-6 and TNF-α.The average values of the parameters show a significant differences were computed by One Way Anova then continued by Post Hoc test. The results of MDA, TNF-α and IL-6 content form blood plasma of wistar rats on a high fat diet showed a significant difference ($p<0.05$) compared with control rats. Otherwise the decrease of the MDA, TNF-α and IL-6 levels in the rat blood plasma, which fed with dragon's fruit flesh has a significant difference compared with the high fat group. In his case, it can be concluded that the Dragon fruit extract has potential to prevent the possibility of oxidative stress and inflammation to avoid atherosclerosis.

KEYWORD: Antiinflammation, stress oxidative, dragon fruit flesh, Obese wistar rats.

INTRODUCTION

Obesity is an emerging problem of health and is important to know the early of cardiovascular disease.[1] In the condition of obesity there will be an increase in fat deposits, which represents one the most common chronic conditions and dysfunction and increased oxidative stress. In addition, obesity is also associated with the risk of cardiovascular disease, decreased neurological disease, and increased risk of death.[2] The consequence of obesity is an impact on increased energy consumption and reduced physical exercise. Many research also indicate chronic low grade inflammation is associated with obesity and metabolic complication. This symptom occurs because many chronic disorders including were currently associated with inflammation.[3]

Inflammation is a biological symptom than arises from the immune system where triggered by various factors such as the presence of pathogen, cell damaged and toxic compounds. These factors could result acute and/or chronic inflammation of the organs such as the heart, pancreas, liver, kidneys, lungs, brain, digestive tract, reproductive system which can lead to tissue damage or disease.[4] Inflammation and oxidative stress are most importance factor in cardiovascular disease that contribute to the onset of atherosclerosis symptoms. Vascular -related proinflammatory and proatherogenis disorder in adult and also couldoccur in obese children. Increased inflammatory was characterized such as by leptin, interlekuin 6 (IL-6), c-reactive protein CRP, tumor necrosis-α (TNF-α) and increase oxidative stress marker by malondialdehyde (MDA).[5] The occurrence of chronic systemic inflammation does not only occur in certain tissue, but can also occur in the vascular system, many organs and internal system. Inflammation which often associated with free radical damage and oxidative stress may not cause pain, because some internal organs do not respond to pain. Pathological symtoms of chronic inflammation are indicated by continuous active inflammation and tissue damage. Macrophages, neutrophils and eosinophils are immune cells that play a direct role in a production of chronic inflammatory.[6]

Prevention of oxidative stress, inflammation and atherosclerosis can be done by reducing fat level in blood the way of decreasing food intake, gastrointestinal, absorption, reducing endogenous synthesis, and

increasing bile and excreta expenditure.[7] This prevention can be done by consuming natural medicine.

One of natural medicine that can be used to prevent obesity and increase cholesterol in the blood is extract of red dragon fruit (*Hylocereus costarioensis*). Red dragon fruit skin contains lots of vitamin C and red fruit flesh were rich anthocyanin, polyphenol, phytoalbumin, and contains minerals, fiber, phosphorus and calcium. Red dragon fruit seeds also contain vitamin E and polyunsaturated fatty acids.[8] The powder of dragon fruit contains many bioactive compounds for example phenolic acid, vitamin C, anthocyanin and alkaloid.[1] Koba (2007) said that high concentration of flavonoid sindicates high antioxidative activity against the free radical.[9] Flavonoids and the other phenolic compounds are generally the result of secondary metabolites plant having aromatic ring that contains at least one hydroxyl groups. It has been reported that many phenolic compounds were produced by plants. It is very importance toknow that half of these phenolic compounds are flavonoids as aglycones, glycosides and methylated derivatives. It has been reported that phytochemical compounds found in herbal medicines, both in the form of flavonoids and other phenolic compounds, can function as effective antioxidants, anticancer, antibacterial, cardioprotective agents, anti-inflammatory, immune enhancing, skin protection from UV radiation, and potential applied in the pharmaceutical and medical field.[10]

Anthocyanin is a polyphenol which rich pigment which is responsible for the red, violet and blue color if various fruit and vegetables. Anthocyanin is one of the flavonoids in dragon fruit.[11] Anthocyanin also can fix blood lipid profile and has vasoprotective effect.[12]

From the above information we like to study the effect of red dragon fruit extract as an antistress oxidative and antiinflammation as well this done by giving dragon fruit extract on obese wistar rat

MATERIALS AND METHODS

Materials

The sample used is extract of red dragon fruit flesh (*Hylocereus costaricensis*) which grows in Badung regency, Bali. MDA (Malondialdehyde) ELISA Kit Elabscience Rat IL-6 Elisa Kit Multi Science and using Rat TNF-α Elissa Kit Multi Science. The animals used were wistar rats which were two month old.

Methods

Making the Extract of Red Dragon Fruit (*Hylocereus costaricensis*)

One kilogram of red dragon fruit flesh is cut into small pieces then blended and extracted by macerating with 95% methanol at normal conditionsduring 24 hours. The filtrate was collected and concentrated with a rotary evaporator, so that the concentrated extract of methanol was obtained. The dose of red dragon fruit flesh extract given to mice is 100 mg/WB kg/day.

High Fat Diet

A high-fat diet was prepared by mixing a standard diet of cp 550 (60%) and 20% lard and 20% duck egg yolk then madepellet form and given for 30 days.[13]

Experimental Animals

Twenty sevens female rat wistar aged 2 month and weighted 150-300 g used and obtained from Center Study of Animal Diseases (CSAD) Faculty Animalof Veterinary, Udayana University.Eighteen rats were made obese by being given high fat and nine rats were given standard feed as a control group. All rats were reared individually in sized plastic cages 36x28x12 cm. After 30 days fed high fat diet, 9 rats given additional feed by extract dragon fruit with dosage 100 mg/kg wb/day for 4 weeks.[14]

Malondialdehyde (MDA) content analysis

Malondialdehyde(MDA) content was analyzed using the MDA ELISA Kit method from Elabscience;Catalog No: E-EL-0060.The procedure to analyze the MDA as follow. Firstly, all reagents were paced at room temperature before used. Preheating is done for 15 minutes on the microplate reader before measuring the OD. Second, a washing buffer solution was madeby diluting 30 mL of concentrated wash buffer to 750 mL with deionized or distilled water.

The standard working solution was prepared by centrifuging the standard at 10,000 x g for 1 minute, then added of reference standard and sample diluents 1,0 mL then leaving it for 10 minutes and reversing for several times. After completely dissolved, mixed it evenly using pipette and produced a stock solution of 2000 ng/mL.The stock solution series was prepared by preparing 7 tube filed 500µLof reference sample dilution. Into the second tube added with the solution from the first tube, so continue the work until the 7^{th} tube so that the stock standard working solution concentration is obtained 1000; 500; 250; 125; 62.5; 31.25 and 15.625 ng/mL respectively.

Solution of biotinylated detection Ab prepared by diluted 100x of concentrated biotinylated detection Ab to 1x working solution with biotinylated detection Ab diluents. The other solution is concentrated HRP Conjugate working prepared by diluted 100x concentrated HRP Conjugate working solution with concentrated HRP Conjugate diluents.

MDA analysis procedure as follow. Firstly, each of 7 standards with different concentrations added 50 µL into the well. The plate closed using sealer that is already in the kit then incubated at 37^{o}C for 45 minutes. The next step is aspirated or decanted the solution from each well then added 350 µL of buffer solution and soaked for 1 -2 minutes. The next stages solution in the well aspirated or

decantated from each well and dried using clean absorbent paper. This treatment was repeated 3 times. Into each well, 100 µl of HRP conjugate working solution was added to it then covered with plate sealer and incubated at 37°c for 30 minutes then the solution aspirated or decanted from each well and this treatment repeated 5 times. The next 90 µL of substrate reagent added to each well and covered with new plate sealer then incubated at 37°C for 15 minutes. 50 µL of stop solution was added to each well then determine the optical density at 450 nm.

The IL-6 and TNF-α content analysis

The content of IL-6 uses the Rat IL-6 Elisa Multi Science Kit; Catalog no: EK 306/2 and TNF-α content analyzed using Rat TNF-α Elissa Multi Science Kit; Catalog no: EK 382/2.

The standard of IL-6 mixed with a solution of 250 µL standard diluents which created a standard solution (2000 pg/mL). The same way to create with concentration (1000 pg/mL). Seven tubes which contain 250 µL of standard diluents were prepared for each of the IL-6 and TNF-α standard. The standard IL-6 (2000 pg/mL) and TNF-α (1000 pg/mL) used to produce a 1:1 dilution series. Each tube was mixed evenly before being transferred to the next tube.

The reagents of IL-6 and TNF-α prepared among as follows. Washing Buffer reagent was prepared by pouring the entire contents (50 mL) into graduated cylinder then added with deionized water to 1000 mL.The Washing Buffer solution was placed in a clean bottle and save at 2 -25°C and this solution stable for 30 days. Assay buffer made by pouring the entire contents (5 mL) of the Assay Buffer into 100 mL graduated cylinder and make the volume 50 mL with deionized water. This solution stored at 2-8°C and stable for 30 days. Detect Antibody was prepared by diluting 1:100 concentrated Detect antibody with Assay Buffer in clean plastic tube as needed. Detect Antibody is confirmed to be used 30 minutes after being diluted.The reagent of Streptavidin-HRP was prepared by dissolving 1:100 concentrated Streptavidin-HRP with Assay Buffer in aplastic tube. The Streptavidin-HRP solution is confirmed 30 minutes after preparation.

The procedure to analyze the TNF-α and IL-6 as follow. Firstly, all reagents and standards were prepared as directed. Second, 100µl 2-fold diluted standard added to standard well in duplicate, 100 µl standard diluents added to blank well in duplicate. Third, 80µl assay buffer and 20 µl sample added to the sample well. Fourth, 50µl diluted detect antibody added to each well. The second, third, and fourth stages must be completed within 15 minutes, then incubated for 2 hours at room temperature. After that, aspirated and washed 6 times. The 100µl Streptavidin-HRP added to each well, then incubated for 45 minutes at room temperature. Aspirated and washed 6 times. The 100 µl substrate solution added to each well, then incubated for 5 – 30 minutes at room temperature. Protected from light. The 100 µl stop solution added to each well. The last stagewas determined at 450 nm within 30 minutes. Correction was done at570 or 630 nm.

RESULTS AND DISCUSSION

Results

The MDA content, TNF-α and Blood plasma IL-6

MDA content, TNF-α and IL-6 blood plasma of rats presented in Table 1

Table 1: Mean MDA, TNF-α and IL-6 levels of rat blood plasma.

Parameters	control	P	High fat (HF)	p	Dragon fruit flash (DFF)
MDA (ng/mL)	98.80±0.96^{bc}	0.000 0.000 0.000 0.000	474,71±3.88^{ac}	0.000 0,000 0,000 0,000	179,10±0,14^{ab}
TNF-α (pg/mL)	74.96±2.71^{bc}	0.000 0.000 0.000 0.000	530.88±6.51^{ac}	0.000 0.000 0.000 0.000	92,16±3,32^{a,b}
IL-6 (pg/mL)	81.72±1.48^{bc}	0.000 0.000 0.000 0.000	377.11±1.48^{ac}	0.000 0.000 0.000 0.000	57,41±3,44^{ab}

Average ± SD (n = 5) followed by a superscript in the same row shows a significant difference.MDA: Malondialdehyde; TNF-α: Tumor necrosis factor-α; IL-6: Interleukin-6

a. Shows a significant difference from the control p<0.05
b. Shows a significant difference from HF p<0.05
c. Shows a significant difference from DFF p<0.05

DISCUSSION

From Table 1, it presented that after 4 weeks of MDA levels there was a significant difference (p<0.05) in high fat treatment (HF) where the mean MDA level was 474.71 ± 3.88 ng/mL compared to the control treatment

MDA levels averaged 98.80 ± 0.98 ng/mL. The significant differences ($p<0.05$) also occurred for treatment of dragon fruit flesh extract (DFF) where the mean level was 179.10 ± 0.14 ng/mL compared to the control. From Table 1 it can also be seen that there was a significant difference ($p <0.05$) for TNF-α levels in high fat treatment (HF) where the average TNF-α level was 530.88 ± 6.51pg/mL compared to the control treatment with a mean TNF-α level of 74.96 ± 2.71 pg/mL. Significant differences ($p <0.05$) also occurred for TNF-α levels in the treatment of dragon fruit flesh extract (DFF) with an average of 92.16 ± 3.32 pg/mL compared to controls.From Table 1 it can also be seen that there was a significant difference ($p <0.05$) for IL-6 levels in high fat treatment (HF) where mean IL-6 levels were 377.11 ± 1.48 pg/mL compared to the treatment control with a mean IL-6 level of 81.72 ± 1.48 pg/mL. Significant differences ($p <0.05$) also occurred for IL-6 levels in the treatment of dragon fruit meat extract (DFF) with an average value of 57.41 ± 3.44 pg/mL compared to controls 81.72 ± 1.48 pg/mL.

From the data in Table 1 the comparison of mean values of MDA, TNF-α and IL-6 shows an increase in high-fat treatment. Compared with controls, high-fat treatment experienced an elevate in the value of MDA, TNF-α and IL-6 by 78.33%; 79.19% and 85.88% respectively. Compared to the high-fat treatment, the mean value of MDA decreased in the treatment of the administration of dragon fruit extract by 62.27%.The mean value of IL-6 for the treatment of the administration of dragon fruit meat extract decreased by 84.78% compared to high-fat treatment. The mean value of TNF-α for treatment of dragon fruit meat extract decreased by 82.64% compared to high-fat treatment.

Increased content of MDA, TNF-α and IL-6 in high-fat treatment is a sign that at high fat treatment there is an increase in oxidative stress. Atherosclerosis is characterized by an increasing of levels of cholesterol and lipoprotein, especially low-density lipoprotein (LDL) and triglycerides (TG), which are the main causes of atherosclerosis and coronary heart disease.In general, oxidative damage occurs in plasma LDL because of the hydroxyl (OH) radical produced by metal ions present in the serum, which undergo changes due to the oxidation process.[15] Oxidative stress occurs when an imbalance between free radicals and antioxidant defense mechanisms, which results in the development of endothelial dysfunction, inflammation and atherogenesis.[16] Increased oxidative stress results increased production of inflammatory cytokines which can trigger the production of free radical.[17,18]

The anthocyanin content of dragon fruit extract is 35.88 ± 1.42 mg/kg.The anthocyanin content of dragon fruit extracts could improve conditions for oxidative damage. In giving dragon fruit flesh extract, there was a decrease in MDA levels during the treatment. These decrease in MDA levels indicates that there is a decrease in oxidative damage. This condition is caused by compounds such as anthocyanin, which is contained in dragon fruit flesh and dragon fruit skin, able to repair damage caused by oxidative stress. Anthocyanin is a polyphenol compound that is rich in pigments, play importance role for the formation of red, purple and blue colors from various fruits and vegetables. Anthocyanin is one type of flavonoids that is widely found in dragon fruit.[11]

CONCLUSION

The yield of the research showthat there was significant differences at $p <0.05$ of MDA, TNF-α and IL-6 levels in blood plasma treatedwith high-fat were 474.71 ± 3.88 n /mL; 530.88 ± 6.51 pg/mL and 377.11 ± 1.48 pg/mL respectively compared to the controls of 98.80 ± 0.98 ng/mL; 74.96 ± 2.71 pg/mLand 72 ± 1.48 pg/mLrespectively. Significant decreases in $p<0.05$ for plasma content of MDA, TNF-α and IL-6 blood in obese rats fed dragon fruit fleshextract were compared with high fat treatment, which were 62.2%; 82.64% and 84.78% respectively

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Conflicts of interest

The author declares no financial conflicts of interest

Support fund

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Animal Ethic

All the expemental works with the animal were carried out after obtaining approval from the organization of Animal ethics committee (ethical clearance) Nomor: 32/UN14.2.9/PT.01.04/2020.

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