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Activity of Hylocereus Costarioensis's Extract as Antiobesity and Hypolipidemic of Obese Rats

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ABSTRACT

The objective of this experiment was to study the antiobesity activity and hypolipidemic of red dragon fruit flesh (Hylocereus costariensis) on obese female wistar rats with high fat diet. Twenty-seven head of female wistar rats were used in this experiment. They were divided into 3 groups with 9 rats as control group and 18 obese rats as groups 2 and 3. Group 1 (control group) was given standard feed CP 551, Group 2 was given high fat diet and group 3 was given high fat diet plus extract of red dragon fruit flesh in the amount of 100 mg per kg body weight. Parameters measured were : body weight change, and physical of characteristic of rats which were recorded every week. After 30 days of feeding, blood samples were collected for profile lipid analysis and also performed analysis of fat and cholesterol in feces and weighing of visceral fat and organs. The results showed, there were significant decrease ($P<0.05$) in body weight, Index Obesity Lee, organs weight, visceral fat weight, total cholesterol, Low Density Lipoprotein (LDL), triglycerides, VLDL, and and ratio of total cholesterol/HDL of rats in Group 3 compared with rats in group 1 and group 2, while in contrast the concentration of rats HDL cholesterol, fat and cholesterol of fecal in group 3 significantly increased compared to rats in group 1 and group 2. It can be concluded that the extract of Red Dragon Fruit flesh had activity as antiobesity and hypolipidemic and could prevent oxidative stress and inflammation.

Key words: *Antiobesity, Extract of Red Dragon Fruit Flesh, Hypolipidemic, Fat, Female Wistar Rat.*

INTRODUCTION

The prevalence of obesity and its complication such as coronary heart disease, diabetic, hypertension, and hyperlipidemic is increasing nowadays. Obesity is caused by the imbalance between food intake and its excretion in the body. Obesity and hyperlipidemia is a risk factor in elevating oxidative stress which accelerate atherosclerosis.[1-5] High fat diet could increase the fat concentration in blood plasma and also can cause obesity which can be a risk factor of coronary heart disease.[4, 6, 7] Prevention of obesity is done by medicinal treatment directly from nature and it is better than chemical and surgical treatment.[5] One of nature herbs that can be used to prevent obesity and the elevation of blood cholesterol is red dragon fruit (Hylocercus costaricencis). This fruit is very popular in Bali because of their color and are mostly used for offerings in temple festivals ; besides, it is cheaper than imported fruit. This fruit is well-known because of its high antioxidant and fiber. The skin of this fruit is also rich in vitamin C, and its meat is also rich in antocyanin, polyphenol, phytoalbumin, minerals, phosphor and calcium. The seed of this fruit also contains vitamin E and polyunsaturated fatty acids. [8, 9] This fruit is also given to patients for its high fiber and it can elevate the excretion of hypercholesterolemia toxin. Research also showed that dragon fruit has antioxidant and anti-proliferative effect and the potency to inhibit the growth of cell B16F16 melanoma tumor. In Malaysia, red dragon fruit is used as a natural food coloring in food and cosmetic industry. The fruit also has the potency as detox, prevents constipation and assists fat absorption in blood. The fruit also can be consumed for people having coronary heart disease. [10-13]

Anthocyanin is a polyphenol which is rich in pigment, which is responsible in the formation of colors such as red, violet and blue in fruit and vegetables. Antocyanin is also a kind of flavonoid which is found in dragon fruit. [14] A study was done by Wybraniec et al., 2001, and it was found that flavonoid in dragon fruit is betacyanine (5-O-[6'-O(3"-hydroxy-3"-methyl-glutaryl) -β-D-glucopyranoside]. Anthocyanin also can fix the profile of blood lipid, has a protective effect and has the ability to inhibit CETP (cholesterol ester transfer protein) as well. [15, 16] By inhibiting CETP, the concentration of HDL will increase and LDL will decrease. [17] Antocyanin also has anti-inflammatory effect by inhibiting citocyn like tumor necrosis factor (TNF-α). Decreasing of TNF-α will elevate oxidation of fatty acid in liver, and cholesterol synthesis will be inhibited by liver cell. [18]

In the present study, we would like to study the effect of red dragon fruit (Hylocereus costaricensis) as antiobesity and hypolipidemic on wistar rats using dragon fruit flesh extract with the amount of 100 mg/kg body weight to know the decrease of rat body weight, and blood fat, and increase of fecal fat and cholesterol.

MATERIAL AND METHODS

Diet

There were 2 diets in this study

1. Diet one (standard diet/CP 551). The compositions of this diet were : water 13% ; protein 18.5 – 20.50% ; fats 4% ; fiber 6% ; ash 8%, ça 0.90% and phosphor 0.70%. This diet was used for feeding rats in group 1.
2. Diet two was consisted of 60% standard diet ; 20% egg yolk of duck and 20% lard (high fat diet). This diet was to group 2 and 3.

The extract of dragon fruit flesh is made by extracting meat of red dragon fruit using methanol 70% and then evaporated using vacuum rotary evaporator. The dosage of this diet was 100 mg/kg body weight/day

Experiment Animals

The animals used in this experiment were 27 female wistar rats aging 11 – 12 weeks and weighing 150 – 200 gram originated from the Center Study of Animals (CSAD) of veterinary Faculty, Udayana University. These rats were divided into 3 groups consisted of 9 rats. Group 1 (control) was given standard diet CP 551, group 2 were given high fat diet, and group 3 were given high fat and extract dragon fruit flesh. Group 2 and 3 were obese rats (18 rats). Ddetermination of obesity was done using an index of obesity Lee. [19] Rats were obese if Lee index > 0.3 with a 4-week duration of induction. All the experimental works with the animal, were carried out after obtaining approval from Organization of Animal Ethics Committee (ethical clearance) No : 0142/KE-PH/VIII/2016.

All groups of rats were kept separately at room temperature with 12 hours dark and 12 hours light every day for 30 days and water was given ad libytum. After 30 days, all the rats were fasted for 12 hours. Blood samples were taken via sinus orbital, and put in blood tubes. The samples then were centrifuged at 5000 g for 15 minute at 4^{o}C and then stored in the freezer for further analysis.

Serum Lipid Analysis

Determination of serum total cholesterol was done using Chop-PAP according to E. Merck. Total cholesterol then was calculated by dividing absorbance of sample with absorbance standards solution (0.240 mg/dL) multiplied by cholesterol standard (200 mg/dL). [20, 21]

The principle of HDL determination was done by the addition of phosphotungstic acid and magnesium ion into the sample until kilomicron VLDL and VDL precipitated. The HDL content was calculated by multiplying the absorbance of sample by 318 (mg/dL). [20, 21]

Determination of LDL cholesterol was done by subtracting the total cholesterol with VLDL and HDL, while determination of VLDL was done by using triglyceride, where VLDL equaled to one overtime (1/5) of triglyceride. [20, 21]

Determination of triglyceride was done by GPO – PAP method. Triglyceride was determined after enzymatic hydrolysis with lipase. Concentration of triglyceride was then calculated by dividing the absorbance of sample by triglyceride standard (0,145) multiplied by triglyceride (TG) constant (200 mg/dL). [20, 21]

Analysis of Total Cholesterol and Fecal Total Fat

In the last three days, the number of fecal total cholesterol was measured by spectrophotometry 30 IKM, and total fat was measured by graphimetry. [20]

Statistical Analysis

Statistical analysis was performed with statistical system. Values are expressed as mean ±SD. Results were analysis by one-way ANOVA and differences among the treatments were determined by the least significant differences' test (LSD). Alpha 0.05 was used to determine statistically significant differences.

RESULTS AND DISCUSSION

Results

Body Weight of Wistar Rats

Data of body weight gain of rats every week can be seen in Table 1 and Figure 1.

Table 1. Mean body weight gain of wistar rats

Parameters	Group		
	Control	High Fat	DFF
Body weight (Start)	148.94±8.95^{b,c}	194.93±9.13^{a}	187.09±8.70^{a}
Body weight (Week 1)	152.28±9.89^{b,c}	203.26±12.00^{a,c}	180.17±6.82^{a}
Body weight (Week 2)	159.89±13.88^{b,c}	206.74±12.86^{a,c}	178.56±5.53^{b}
Body weight (Week 3)	163.74±16.22^{b,c}	210.46±12.41^{a,c}	173.64±5.47^{b}
Body weight (Week 4)	167.10±15.01^{b,c}	212.23±12.45^{a,c}	170.75±5.41^{b}

Mean ±SD (n = 9) which was followed by different superscripts in the same line, showed significant difference ($p<0.05$) ; group 2 (high fat diet) ; group 3 (High fat diet plus dragon fruit flesh extract). ^{a} showed control group ($p<0.05$); ^{b} showed significant difference from high fat diet ($p<0.05$); and ^{c} showed significant difference from high fat diet plus dragon fruit flesh extract ($p<0.05$).

According to Table 1, it can be seen that body weight of rats in group 1 was significantly different at the beginning ($p<0.05$) compared to group 2 and group 3. Mean of body weight for group 1 was 148.94±8.95 gram ; group 2, 194.93±9.13 gram and group 3, 187.09±8.7 gram.

It showed there was an increase body weight of rats in group 2, but there was decrease of body weight in group 3. Body weight change can be seen in Figure 1.

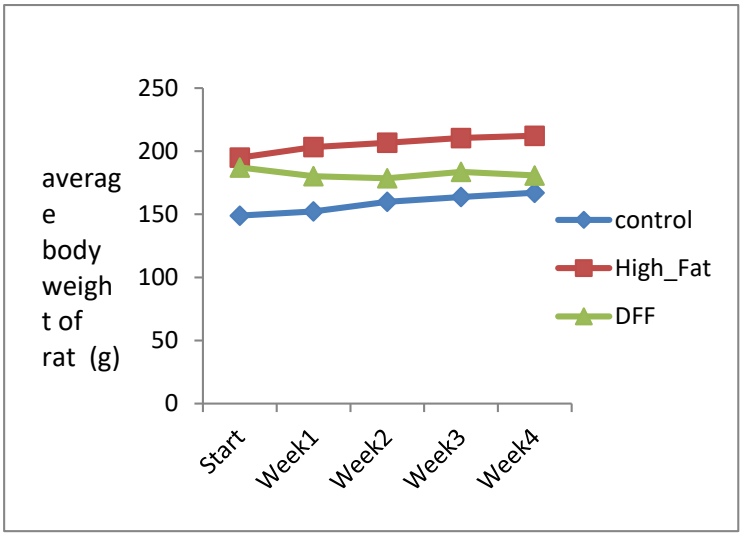

Figure 1. Body weight change of rats in four weeks

The Influence of Red Dragon Flesh Extract on Lipid Profile of Serum and Fecal

After the rats were given treatment for 4 weeks, the feeding was stopped for 12 hours and then blood samples were taken and analyzed according to research protocol. Comparison of the mean total cholesterol, LDL cholesterol, HDL cholesterol, TG, VLDL, the ratio of total cholesterol gains HDL cholesterol of blood serum, fat and cholesterol of rats fecal can be seen at Table 2 and Figure 2.

Table 2. Mean Concentration of profile lipid in blood serum and fecal rats

Parameters	Groups		
	Control	High Fat	DFF

Serum Total Chol. (mg/dL)	74.00±3.89^{b,c}	140.83±11.58^{a,c}	83.00± 4.00^{a,b}
LDL (mg/dL)	19.93±3.49^{b,c}	81.08±4.70^{a,c}	13.37±2.03^{a,b}
HDL (mg/dL)	38.83±2.72^{b,c}	22.50±1.66^{a,c}	47.13±1.37^{a,b}
TG (mg/dL)	74.17±12.42^{b,c}	200.50±18.36^{a,c}	112.50±13.09^{a,b}
VLDL(mg/dL)	14.83±2.48^{b,c}	40.10±3.67^{a,c}	22.50±2.62^{a,b}
Total Chol. /HDL	1.91±0.15^{b}	6.25±0.12^{a,c}	1.77±0.10^{b}
LDL/HDL	0.53±0.09^{b}	3.54±0.12^{a,c}	0.28±0.05^{b}
Fecal Total Chol mg/100g Total Fat mg/100g	0.28±0.03^{c} 2.77±0.35^{b,c}	0.29±0.04^{c} 1.85±0.08^{a,c}	0.49±0.01^{a,b} 4.86±0.11^{a,b}

Mean ±SD (n = 9) which was followed by different superscripts in the same line showed significant difference ($p<0.05$).
[a] showed control group ($p<0.05$); [b] showed significant difference from high fat diet ($p<0.05$); and [c] showed significant difference from high fat diet plus dragon fruit flesh extract ($p<0.05$).

Total cholesterol (total chol) ; Trigliseride (TG) ; Low density lipoprotein (LDL); Very low density lipoprotein (VLDL); High density lipoprotein (HDL).

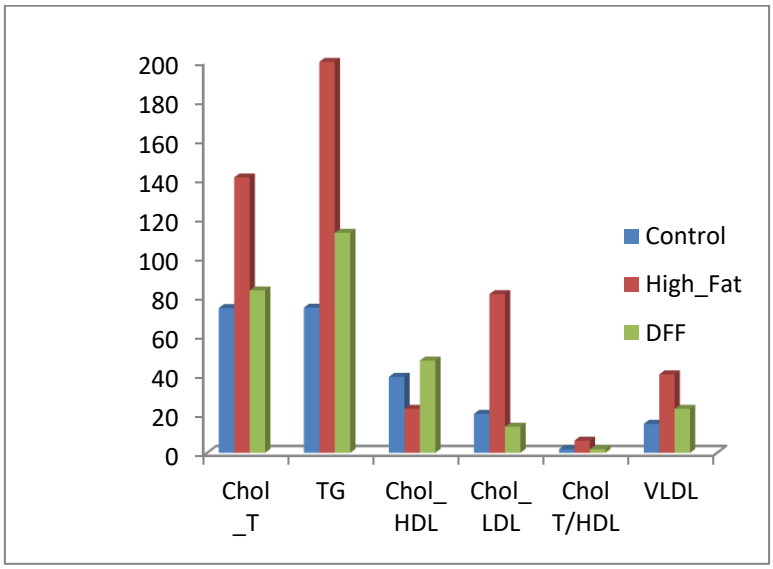

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Figure 2. Means concentration of lipid profile blood serum of the rats

The Influence of Dragon Fruit Flesh Extract to The Organ Weight

The weight of rats was observed with operation according to the research protocol. The data can be seen Table 3.

Table 3. Data of rats' organs' weight.

Parameter	Group		
	Control	High Fat	DFF
Kidney (g)	1.08±0.04^{c}	1.42±0.16^{c}	0.92± 0.01^{a,b}
Heart (g)	0.54±0.02	0.76±0.03	0.55±0.03
Lymph (g)	0.63±0.02	0.36±0.03	0.36±0.03
Liver (g)	5.59±0.11^{b,c}	7.49±0.04^{a,c}	4.72±0.28^{a,b}

Mean ±SD (n = 9) which followed by different superscript in the same line showed significant difference.
[a] showed control group ($p<0.05$); [b] showed significant difference from high fat diet ($p<0.05$); and [c] showed significant difference from high fat diet plus dragon fruit flesh extract ($p<0.05$).

The Influence of Dragon Fruit Flesh Extract to The Weight Visceral Fat

Data from influence of dragon fruit flesh extract to weight of visceral fat can be seen in Table 4.

Table 4. The weight of visceral fat

Parameter	Group		
	Control	High Fat	DFF
Retroperitonial (g)	1.48±0.03[b,c]	7.65±0.44[a,c]	0.63± 0.02[a,b]
Perirenal (g)	0.67±0.01[b]	3.71±0.12[a,c]	0.63±0.02[b]
Perianal (g)	0.64±0.02[b]	3.67±0.10[a,c]	0.53±0.02[b]
Ovarium (g)	0.93±0.02[b]	2.20±0.08[a,c]	0.87±0.04[b]

Mean ±SD (n = 9) which followed by different superscript in the same line showed significant difference ($p<0.05$).
[a] showed control group ($p<0.05$); [b] showed significant difference from high fat diet ($p<0.05$); and [c] showed significant difference from high fat diet plus dragon fruit flesh extract ($p<0.05$).

The Influence of Dragon Fruit Flesh Extract to The physical characteristic of rats

The observed of physical characteristic of rats can be seen in Table 5

Table 5. The physical characteristic of rats

Parameter	Group		
	Control	High Fat	DFF
Stomach girth (cm)	16.75±0.42[b]	20.91±0.33[a,c]	16.49±0.27[b]
IOL	0,28±0.01[b]	0.33±0.01[a,c]	0.28±0.01[b]
Feces weight (g)	2.40±0.32[b]	0.36±0.03[a,c]	2.36±0.03[b]
Food Intake (g)	17.28±0.23[b]	25.35±0.19[a,c]	16.77±0.61[b]

Mean ±SD (n = 9) which was followed by different superscripts in the same line showed significant difference ($p<0.05$).
[a] showed control group ($p<0.05$); [b] showed significant difference from high fat diet ($p<0.05$); and [c] showed significant difference from high fat diet plus dragon fruit flesh extract ($p<0.05$).
Index Obesity Lee (IOL) ; Dragon Fruit Flesh (DFF)

Discussion

According to Table 1 and Figure 1, it can be seen that after for four weeks of the experiment there was an 8.73% decrease in the body weight in group 3. This was caused by a decrease in food intake ; stomach girth ; and index obesity Lee about 33.85% ; 21.14% ; and 15.15%, respectively and the amount of excreted stools increases about 555.55% compared to high fat diet group (Table 5). The weight loss of liver (36.98%) and kidney (35.21%) (Table 3) and decrease of visceral fat (Table 4) were also occurred in 3 groups with significant difference ($p < 0.05$) compared to high fat diet group. There was a significant increase in rats of group 1 and group 2 with regard to body weight about 12.2% and 9.28% respectively. This was due to an increase in food intake. Obesity was induced by high fat diet for 8 weeks. This is well established that fat over diet lead to obesity in a number of animal models. [22, 23]

In group 2 (high fat) there was an increase in total cholesterol about 140.83 mg/dl, TG (200.50mg/dl) ; LDL (81.08 mg/dl) ; VLDL (40.10 mg/dl) and decrease in HDL (22.50mg/dl) in blood serum and fat (1.85 mg/100g) and cholesterol (0.29 mg/100g) in fecal. High fat diet elevates profile lipid, decreases HDL in blood and decreases fat, and cholesterol in fecal by altering the hepatic lipid metabolism. [4] This high fat diet model has been used as a screening method for antiobesity activity and elucidating lipid metabolism. [4] Dragon fruit flesh extract exhibited antiobesity activity and the maximum effect was observed at 100 mg/kg b.wt. Consumption of the high fat diet led to obesity because it facilitates the development of a positive energy balance leading to an increase in visceral fat deposition, and this led to abdominal obesity. High fat diet is the source of the increase in body weight. Rat consuming the high fat diet, received more kilocalories, so more weight, and had larger fat pads than rats fed based on the standard diet. [4, 23] The increased concentration of total cholesterol could cause the risk of atherosclerosis, and in this case the increase of total cholesterol in serum was above maximum standard (130 mg/dL). The elevation of total cholesterol, TG, ratio of cholesterol/over HDL cholesterol and decrease of HDL cholesterol may cause atheroclerosis and the risk factor of coronary heart disease. [20, 24-27]. The increase of total cholesterol and LDL cholesterol also happened in rabbit experiment with high cholesterol diet during 30 days. [25] The significant elevation ($p<0.05$) of total cholesterol, LDL, TG and decrease of HDL in blood serum of chicken layer with high cholesterol diet were observed in 60 days. [28]

Based on the data in the Table 2 and figure 2, feeding of dragon fruit flesh extract 100 mg/kg b.w., could prevent the increase of total cholesterol (41.06%) ; LDL (83.51%) ; TG (43.89%) ; VLDL (51.44%) ; ratio of total cholesterol

over HDL (53.44%) and the decrease of HDL cholesterol (109.47%) ; fecal fat (162.70%) and fecal total cholesterol (68.97%) compared with high fat diet group. The elevation of HDL cholesterol in DFF group was higher than high fat group. The decrease of profile lipid blood serum which was caused by crud fiber, phenolic, polyphenols and flavonoid content in dragon fruit flesh extract, was a good source of antioxidants, being able to inhibit the absorption cholesterol in intestine then excrete through the feces. [9, 29] This caused content of capacity antioxidant, antocyanin, phenol and fiber which could inhibit absorption of fat in intestine so that more crud fiber can be excreted together with feces. [30, 31] According to Jamilah, at al. (2011), red dragon fruit extract contains a lot of flavonoids such as antocyanin. Shipp and Abdel-Aal (2010) also stated that antocyanin could improve blood profile lipid and had a protective effect. [18] Antocyanin also had anti-inflammatory effect which inhibits cytocin such as tumor necrosis factor α (TNF-α). Decreasing of TNF-α could elevate fatty acid in liver and inhibit cholesterol synthesis by liver cell.

Feeding dragon fruit flesh extract could decrease total cholesterol, LDL, TG, and ratio of total cholesterol over HDL cholesterol, body weight, index obesity Lee and also could increase serum HDL cholesterol, fecal total cholesterol and fat significantly ($p<0.05$) compared to high fat diet (group 2). From this study, it was shown that dragon fruit flesh extract had biological activities of antiobesity and hypolipidemic which could prevent atherosclerosis. Intake of extract dragon fruit flesh not only could bind cholesterol and fat from the feed, but also bound cholesterol originated from liver, through excretion of line to intestine, and then into fecal, thereby increasing the concentration of feces cholesterol and fat.

CONCLUSION

From the above results and discussion, it can be concluded that feeding of dragon fruit flesh extract could be regarded as antiobesity and hypolipidemic by decreasing body weight, IOL, total cholesterol, LDL, TG, VLDL, ratio of total cholesterol over HDL cholesterol and increasing blood serum HDL cholesterol and fat, and cholesterol of rats fecal which given high fat diet.

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