Original Article

Difference in Level of Malondialdehyde, Total Cholesterol, and Triglyceride After Administration of Passion Fruit Seed's Ethanol Extract in Wistar Rats

Dina Keumala Sari [1]*, Marianne [2], Sri Lestari [3] and Lidya Imelda Laksmi [4]

[1] Nutrition Department, Faculty of Medicine, Universitas Sumatera Utara ; dina@usu.ac.id
[2] Pharmacology Pharmacy Department, Faculty of Pharmacy, Universitas Sumatera Utara; marianne80@usu.ac.id
[3] Public Health Department, Faculty of Medicine, Universitas Sumatera Utara; srilestari@usu.ac.id
[4] Anatomy Pathology Department, Faculty of Medicine, Universitas Sumatera Utara; lidya.imelda@usu.ac.id
* Correspondence: dinaridha@yahoo.com, dina@usu.ac.id; Tel.: +62 81397177693

Abstract: High oxidative stress in cells due to inflammation process or excessive cell proliferation would produce oxidants or free radicals with biomarkers, one of which is malondialdehyde (MDA). Passion fruit seed's contain high antioxidant and are expected to decrease the level of cholesterol and MDA. The objective is to identify the effect of passion fruit seed's ethanol extract in Wistar rats that have been fed with atherogenic feed. The method was preclinical trial (post-test control group design) in rats, by administering passion fruit seed's ethanol extract for 14 days. This study used 26 male rats aged two months, divided into 5 groups. The result showed significant difference in MDA level which was found in group that was given passion fruit seed extract 10mg/kg BW with positive control group that was given standard feed. Passion fruit seed's extract showed significant difference in level of triglyceride, which was found in negative control group that was given atherogenic feed with group that was given passion fruit seed's extract 5mg/kg BW (mean±standard deviation: 1.09±0.30 mg/dL vs 0.77±0.25mg/dL; p=0.048). This study showed that passion fruit seed's ethanol extract had significant lowering effect in level of MDA, total cholesterol, and triglyceride for 14 days.

Keywords: atherogenesis, passiflora edulis sims, lipid profile, free radicals, pre-clinical trial

1. Introduction

High incidence of atherosclerosis disease worldwide has caused numerous studies aiming to find more effective treatment and prevention of cardiovascular disease. High incidence of this disease is also seen in developing countries that have different dietary habit and lifestyle from developed countries, so various types of therapy are found including the use of food products that are easily found in the country or herbal products [1, 2].

Theories explaining the cause of atherosclerosis process are developed from high fat consumption, inflammation process, and oxidative stress theory. Oxidative stress occurs due to metabolic reaction that uses excessive oxygen resulting in impairment of prooxidant and antioxidant homeostasis in cells. High fat consumption and excessive oxidative stress could cause increased lipid peroxidation process and produce reactive aldehydes such as malondialdehyde (MDA) [3, 4].

Plants containing phenolate compound (bioflavonoid) could inhibit lipid peroxidation and have strong antioxidant effect by cutting off peroxy radical chain reaction through scavenger effect. Bioflavonoid mixture in food intake and supplement have role as antioxidant and protecting effect,

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one of which is passion fruit. Studies about passion fruits that contain bioflavonoid have developed with focus on passion fruits but there are still a few studies addressing passion fruit seed [5-7].

Passiflora edulis Sims (Passifloraceae) is a woodbine frequently found in tropical countries. This plant is found in several colors, for example yellow, red, and purple and fruit flesh is the form that is consumed the most frequent [8, 9]. There are a lot of published studies about fruit flesh and rind of passion fruit but not about passion fruit seed, which is a typical plant grows in tropical region which is North Sumatera, Indonesia. Passion fruit seed's extract is reported to contain polyphenol compound that could affect as antioxidant better than fruit flesh or rind of passion fruit.

Compound of polyphenol contained in passion fruit showed effect in body metabolism such as lipid metabolism. Polyphenol compound contained in passion fruit is piceatannol which has antioxidant effect analog with resveratrol [5]. Previous studies have shown that piceatannol in passion fruit has effect of insulin sensitivity improvement, improvement in lipid profile, vasorelaxant effect, inflammation, and oxidative stress reduction [5].

Passion fruit that grows in tropical regions especially in North Sumatera, Indonesia, certainly has different growing place with geographic environment and season that differ from other regions. With variation of passion fruit, difference in activity of antioxidant contained in passion fruit is expected. Objective of this study is to identify the effect of administration of passion fruit seed's ethanol extract towards level of lipid profile, malondialdehyde, and blood vessel's histopathological feature in atherogenic Wistar rats for 14 days. The result of this study is expected to provide product of passion fruit seed's ethanol extract that could be used to decrease level of lipid profile and malondialdehyde in Wistar rats, this product could increase improvement of body metabolism and to utilize passion fruit product in North Sumatera as local product utilization.

2. Materials and Methods

This preclinical trial used experimental trial design (post-test control group design). Study samples were 25 white rats Rattus norvegicus Wistar strain aged ± 2 months and weighed 150-200 grams. The rats were divided into 5 groups which were treatment group with normal diet (C0), treatment group with atherogenic diet (C1), treatment group with atherogenic diet with administration of purple passion fruit seed's ethanol extract with different doses (P2, P3, P4). Purple passion fruit seed's ethanol extract was given orally with orogastric tube once daily for 14 days. Passion fruit seed's ethanol extract was made previously at Pharmacy Laboratory, Faculty of Pharmacy, University of North Sumatera, to be further given orally to the experimental rats.

2.1. Sample collection

2.1.1 Purple Passion Fruit

Purple passion fruits or Passiflora edulis in Latin have round egg shape or full round and have diameter of approximately 4 – 6 cm. This variety of passion fruit is the most cultivated because of its most delicious taste and flavor. Usually these types of passion fruit can be found in plateau area with wet climate [5,10].

Passion fruits have thin rind (0.5 mm) like a hard cork and they easily break when they are still raw, then they become flexible when they are ripe. In the fruit's cavity, there are dozens of black-colored flat seeds 0.5 cm, with very hard seed coat. The seed itself is in two pieces and white-colored. The seed coat is covered with thin pulp. This pulp is light yellow to orange-colored. Fruit size, pulp thickness, flavor and acidity level have become standard to determine the quality of passion fruit. The bigger the fruit size, the thicker the pulp with high level of flavor and acidity, the more qualified the passion fruits are. In Indonesia there are 4 (four) types of cultivated passion fruits which are purple passion fruit (Passiflora edulis var. edulis), konyal passion fruit (Passiflora lingularis), yellow passion fruit (Passiflora edulis var. flavicarpa), and erbis passion fruit (Passiflora quadrangularis) [11,12] (**Figure 1**).

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Figure 1. Passiflora edulis Sims

Purple passion fruit with Latin name (Passiflora edulis var. edulis) is sour purple passion fruit, that has round oval shape, raw fruit is green-colored but the ripe one is brown-purple, with fresh sour taste and good flavor. Purple passion fruit is included into Passiflora edulis species, Passiflora genus, Malpighiales ordo, Spermatophyta division, and Plantae Kingdom, with binomial name of Passiflora edulis. This fruit takes 1.5 years to grow from seeding until first harvest in plateau. This plant is cultivated at altitude of 1000 m above the sea level, but with certain treatment purple passion fruit can also be cultivated in middle ground or lowland, this type is commonly processed into syrup or other processed product that has high economic value. The breeding method is the same as other type of purple fruit, which is by propagating it in trees or in loft or fence [9,13,14]. This study used passion fruit obtained from Berastagi Plantation, located 66 km south of North Sumatera's capital city which is Medan City, at 1300 m above the sea level, with latitude of 3.1853oN and longitude of 98.5049oE.

2.1.2 Passion fruit seed's ethanol extraction process

Passion fruit seed's extract is made using maceration method with 96% ethanol diluent and was conducted in Pharmacy Laboratory, University of North Sumatera, Indonesia. The sample used was seed of Passiflora edulis var Sims weighed 10 kg (gross weight). Passion fruits were sliced in two to remove the seeds to be collected and cleaned from dirt (wet sorting), then they were washed with running water until they were clean and drained, the aim was to obtain passion fruit's seeds that were free from fiber and fruit flesh. The seeds were then dried in open air and protected from direct sunlight and then continued with drying using drying cabinet (Indotrading, Indonesia) and then dried with oven at temperature of 40oC (**Figure 2**). Dried simplicia was then crushed using blender (Miyako, Indonesia) until became powder simplicia and sifted with 20 mesh sieves.

Figure 2. The process of drying passion fruit seeds

Extract of passion fruit seed was made using maceration method, which was by using 96% ethanol that had been previously distilled for as much as 10 times the weight of passion fruit seed powder. An amount of 1340 g simplicia powder was put into container and poured with 96 % ethanol, closed and left for three days protected from light while repeatedly stirred. After three days it was then sifted, the left-over extract was then dried. The result of maceration was then contained in container and was then distilled using rotary evaporator device (Hei-VAP Rotary Evaporators, Heidolph, Germany) at temperature of 45° C that aimed to separate solution and steam so that an almost viscous extract was obtained. The almost viscous extract was then steamed in waterbath (Griffin) until a

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viscous extract was obtained. The extract's net weight was then weighed and the extraction process resulted a yield of 134.15 grams.

2.1.3 Preparation of passion fruit seed sample

Preparation of passion fruit seed's ethanol extract with dosage of 5mg/kg body weight for rats was conducted by weighing 5 mg passion fruit seed's ethanol extract. It was then put into mortar, and slowly crushed. Solution of CMC Na 0.5 % was added little by little, crushed until homogenous. The suspension then was put into 10 ml volumetric flask, and volume was added by adding CMC Na solution until limit mark. The same applied to the creation of passion fruit seed's ethanol extract dosage of 5 mg/kgBW, 10 mg/kgBW and 20 mg/kgBW. The extract was given by orogastric tube once daily in rats by creating suspension of passion fruit seed's ethanol extract using solution of Carboxymethyl Cellulose Natrium (CMC Na) 0.5%.

2.2. Preparation of Wistar Rat sample

2.2.1 Ethical approval

This study had been approved by ethical principle in experimental trial study with experimental animal and this study was approved by The Animal Research in Biology Faculty of University of North Sumatera, with ethical number 112/KEPH-FMIPA/2018. In total 26 young Wistar rats (Rattus norvegicus) weighed 150-200 g were obtained.

2.2.2 Conditioning of experimental animals

All experimental rats underwent acclimatization in experimental cage for two weeks to uniform the way of living, eating, and condition of the experimental cage. Rats were put in experimental cage with room temperature with 12 hours exposure of bright light and darkness alternately. All rats were fed with commercial standard feed and water ad libitum. Bedding for rats came from sterilized coarse sawdust and was changed twice a week. Lighting used natural light (from window) with room temperature (normal). Cage was also equipped with exhaust fan to keep air flow and to remove excess heat.

2.2.3 Grouping of experimental animals

All experimental rats were divided into five groups which were negative control (C-neg) consisted of 6 rats, whereas positive control (C-pos), treatment 1 (P1), treatment 2 (P2), and treatment 3 (P3), were each consisted of 5 rats. C-neg group was given 1 additional rat to anticipate the death of rats due to atherogenic feed. Rats in C-pos group were only given standard feed, rats in C-neg group were only given atherogenic standard feed. Grouping for C group was based by hypothesis that administration of atherogenic feed in C negative group would result in negative effect whereas the group that was given standard feed would result in positive effect. Meanwhile the treatment group was divided into three treatment groups which were 1, 2, and 3 and were given atherogenic feed and one quail egg yolk daily for 14 days, this was conducted in order to induce atherogenic rats. After induction time, control group was still given standard feed, whereas group 1 was given only atherogenic feed, group 2 was given atherogenic feed and passion fruit seed's ethanol extract 5 mg/kg body weight, group 3 was given atherogenic feed and passion fruit seed's ethanol extract 10 mg/kg body weight, and group 4 was given atherogenic feed and passion fruit seed's ethanol extract 20 mg/kg body weight. Administration of passion fruit seed's ethanol extract was conducted for 14 consecutive days non-stop. The rats' body weights were weighed at the beginning of study and during blood sampling.

2.2.4 Data Collection Method

Collected data consisted of rat's body weight obtained by weighing rat using weight scale (Sartorius Melter brand) with accuracy of 0.1 kg once a week. Data of daily food intake was measured

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by weighing the left-over feed given to experimental animals every day using weight scale (Sartorius Melter brand) with accuracy of 0.1 kg.

After 14 days of treatment, the rats were fasted for 10 hours, then blood sampling was performed by cardiac puncture. Before blood sampling, the rats were anesthetized with ether solution to be further euthanized. Blood was withdrawn as much as 3 mL and centrifuged with speed of 3500 rpm for 5 minutes. Laboratory tests of total cholesterol, LDL, HDL, triglyceride, and MDA were further conducted by taking blood samples. Lipid profile and MDA were checked using spectrophotometry's monochromator method using spectrophotometer device (Thermo ScientificTM MultiskanTM GO Microplate Spectrophotometer, Thermo Fisher Scientific Laboratory Equipment (LPG), United States). Meanwhile, examination of blood vessel was performed by histopathology examination of formation of sponge cells. This examination was performed by taking the aortic arch to conduct histopathological examination of formation of sponge cells. The tissue was stained using Oil Red-O (OR-O) and Hematoxylin Eosin (HE).

2.2.5 Rat Feed Intake

Standard feed given contained isocaloric standard food, whereas atherogenic feed contained high level of fat, as addition one quail egg yolk was also given daily to increase cholesterol level in rat's blood (**Table 1**). Egg yolk could increase level of lipid in blood so condition of hyperlipidemia in experimental animals could be achieved. It is known that cholesterol from egg yolk is lipid component consists of 65.5% triglyceride, 5.2% cholesterol and 28.3% phospholipid. Additional egg yolk was given during acclimatization period and treatment period which was 1 egg yolk/rat/day given ad libitum in the morning and evening.

Table 1. Composition of Rat's Food

Ingredients	Standard Intake	Atherogenic Intake
Confeed PAR-S (gr)	200	200
Wheat(gr)	100	100
Glucose (gr)	-	80
Cholesterol (gr)	-	8
Cholic Acid (gr)	-	0,8
Coconut Oil (gr)	-	40
Water (ml)	71.2	71.2

2.3. Content analysis

2.3.1 Phytochemical screening and antioxidant test (DPPH method)

Phytochemical screening was conducted by performing test, whereas antioxidant test was performed by method of Phytochemical analysis and antioxidant test IC50-DPPH conducted in Central Laboratory of Biopharmaceutical Study, Research and Community Service Body, Bogor Agricultural Institute, Indonesia, with certificate number 405.013/LPSB/IPB/V/2018.

2.4. Statistical Analysis

Data obtained from research result would be analyzed and processed using SPSS 20. Data analysis was started by data normality test using Shapiro Wilk test. To identify difference from each treatment, One-way Anova statistical test was performed, followed by Least Significant Differences (LSD) test. For abnormal distributed data, Kruskal Wallis test would be performed and followed by double comparison which was Mann Whitney test. Significance is determined with p value <0.05.

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3. Results

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation as well as the experimental conclusions that can be drawn.

3.1. Phytochemical screening and antioxidant analysis

Flavonoid test results showed the presence of flavonoids in passion fruit seed extract, besides that, tannin, saponin and triterpenoid substances were also present, presented in **Table 2**. Furthermore, antioxidant activity of passion fruit seed extract was tested using DPPH radical (2.2 diphenyl-1-pikrilhidrazil), with the mechanism of antioxidant compounds that will react with DPPH radicals. The mechanism of action is the donation of hydrogen atom which will cause DPPH discoloration from purple to yellow measured at a wavelength of 517 nm (Molyneux, 2004). The parameter of this DPPH method is the 50% inhibitory concentration (IC50) or the concentration which can reduce free radical activity by 50%.

Table 2. Phytochemical Screening Analysis

Sample	Identity and sample	Parameter		Results	Unit	Technique of Analysis
Passiflora edulis Sims. seed ethanol extract	Solids	Phytochemical:				Color visualization
		Flavonoid		Positive	-	
		Alkaloid	Wagner	Negative	-	
			Mayer	Negative	-	
			Dragendrof	Negative	-	
		Tannin		Positive	-	
		Saponin		Positive	-	
		Quinone		Negative	-	
		Steroid		Negative	-	
		Triterpenoid		Positive	-	

Passion fruit extract was made into several concentrations and tested using DPPH radicals. The purpose of making some of these concentrations is to find IC50 values using mathematical equations obtained through the correlation between inhibition and extract concentration. Inhibition is a presentation of purple discoloration and can be calculated from its absorbance. At each extract concentration, free radicals will be given and allowed to react for 30 minutes, with the effective time for reaction of the test sample and DPPH is 30 minutes due to entering propagation stage. The relationship of concentration and inhibitory percentage of passion fruit seed extract using ethanol solvent is shown in **Figure 3**.

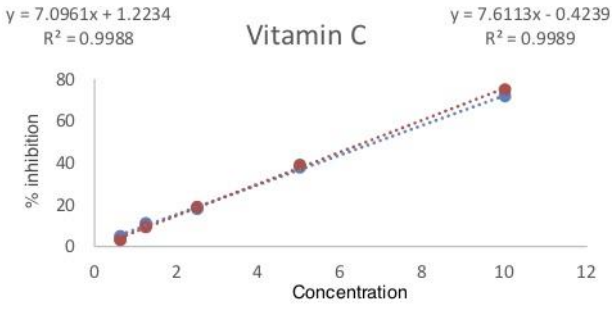

Figure 3. Comparison Between Concentration and Inhibition of Free Radical Absorbance

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The results obtained by the extract concentration value are directly proportional to the inhibitory value. The higher of concentration, the higher of inhibitory value. It also shows that the greater the concentration, the more antioxidant content in the extract which can reduce free radical activity (marked by the discoloration of purple color from DPPH). Antioxidant testing was also carried out on vitamin C (ascorbic acid) as a positive control and comparison. Control is intended to test the validity of a method (comparing the results of research with other studies that have been done). The correlation between concentration and inhibition in vitamin C is illustrated in Figure 3 and has a high correlation of 0.998.

The effectiveness of a sample to counteract free radicals from the DPPH method is named IC50. The definition of IC50 is a concentration that can reduce 50% DPPH free radicals. The smaller the IC50 value, the greater the antioxidant activity. IC50 values from passion fruit seed extract using ethanol solvent and vitamin C are presented in **Table 3**.

Table 3. Analysis of antioxidants using the DPPH method

Sample	Sample's condition	Parameter	Results	Unit	Technique of Analysis
Ethanol	Solids	Antioxidant IC_{50}-DPPH	< 31.25	ppm	Spectrophotometry
Standard vitamin C	Solids	Antioxidant IC_{50}-DPPH	6.75	ppm	Spectrophotometry

A compound is said to have a very strong antioxidant activity if the IC50 value is less than 50 ppm, the strong group, IC50 is in between 50-100 ppm, the medium group, if IC50 value is 101-150 ppm, and the weak group, if IC50 value is 150-200 ppm (Molyneux, 2004). Based on the statement, it can be said that passion fruit seed extract using ethanol solvent and vitamin C has a very strong antioxidant activity, equivalent to vitamin C in counteracting free radicals.

3.2. Weight

At the beginning of the study, the rat's body weight of each group showed significant differences in each group. The mean body weight of all rats before treatment was 189.8 516.34 grams and after treatment 219.08 26.99 grams, with significant differences for each group for body weight before treatment, while for body weight after treatment also showed significant differences in each group ($p = 0.003$ and $p = 0.006$).

The results of this study showed the knj percentage of feed intake of rats fed by standard food, atherogenic food, atherogenic food added with passion fruit extract of 5 mg / kg body weight, atherogenic food added with passion fruit extract 10 mg / kg body weight, and atherogenic food added with passion fruit extract 20 mg / kg body weight, increase body weight in each group. In this study, the weight of all rats before the study showed differences in all groups with mean body weight 189.85 16.34 grams, after the treatment period there was an increase in rat body weight to 219.08 26.99 grams ($p = 0.001$), with an average difference in body weight before and after the treatment was 29.23 19.89 grams.

The body weight of the rat was weighed every day to monitor the progress of the rat's body weight. In **Table 4**, it is shown that the difference in body weight between the beginning and the end of the study to see the relationship between changes in body weight and rat food intake. The results showed a significant difference ($p < 0.05$) between the initial and final body weight of the study in four groups, there was no significant increase in the K + group, namely the group which was given standard food. Each group experienced an increase in body weight, which was most apparent in the P2 group, after being tested by statistics, the most significant difference was seen in the K + and P2 groups ($p = 0.001$).

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Table 4. Mean values of body weight at the beginning and end of the study

Group	n	Body weight at the beginning (gr)	Body weight at the end (gr)	*p*
C-	6	189.40±16.18	221.20±19.25	0,006*
C+	5	167.0±5.74	185.20±6.45	0,05
P1	5	199.20±15.05	230.60±16.01	0,014*
P2	5	196.17±13.12	238.5±33.6	0,001*
P3	5	196.20±7.66	216.0±17.07	0,032*

*significant difference
Paired t-test
C-: negative control group
C+: positive control group
P1: 50-gram treatment group
P2: 100-gram treatment group
P3: 200-gram treatment group

This difference in body weight is influenced by the intake of atherogenic food which is higher in fat composition than standard food. This increase in fat levels affects changes in body weight, levels of lipid profiles, and MDA. Fat composition in atherogenic food intake is greater, which is equal to 47.3% of total energy compared to standard food intake which only contains 8% of total energy. The addition of quail egg yolks for 14 days continuously, gave additional cholesterol to the intake of the group of rats with atherogenic food which caused the condition of dyslipidemia which triggered the acceleration of the atherogenic process in experimental rats.

The administration of passion fruit seed extract showed no significant difference, it was seen from the treatment group and the atherogenic food fed group all showed significant differences, only the group which was given standard food had an increase but did not show significant differences before and after treatment.

3.3. Effects on MDA and lipid profiles

Wistar rats were given treatment for 14 days, and on the last day of the study, rats were fasted for 12 hours by taking out all food and drinks from their cages, then taking blood and evaluating them according to the research protocol. The results obtained showed that the results of statistical analysis on MDA serum showed there were differences in MDA levels of wistar rats' serum in various groups (ANOVA test, p = 0.021). The post hoc tukey test showed that there were two doses of passion fruit seeds which showed significant differences. This difference was seen in the group given standard food with P2 group. This proves that the passion fruit extract given gives a significant difference with standard food (K +) but not with atherogenic food (K-).

The administration of passion fruit seed extract of 20 mg / kg body weight (P3) showed a significant difference with the standard food group. This illustrates that the administration of passion fruit seed extract of 10 mg / kg for 14 days, decreases oxidative stress compared to the standard group. This significant difference illustrates the low emphasis on MDA levels in the 10mg / kg body weight of passion fruit seed extract group accompanied by the atherogenic diet compared with standard food (mean MDA levels 1.38 0.12 vs 1.83 0.4μM, respectively). However, a significant difference was not found between each group which were given atherogenic food, from the results obtained, passion fruit seed extract had not been able to show a significant difference in decline in the K- and P1 groups, although the lowest level of MDA was found in P2 group. In this case, the administration of passion fruit seed extract at a dose of 10 mg / kg has shown significant differences compared to other doses (**Table 5**).

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Table 5. Mean values of total cholesterol level before and after treatment

Parameter \ Group	C-	C+	P1	P2	P3	*p*
Triglyceride (mg/dL)	1.09±0.30	0.75± 0.17	0.77±0.25	0.74±0.23	0.48±0.25	0.014*
Total cholesterol (mg/dL)	84.54±13.69	73.39±5.5	74.51±4.31	68.04±6.17	68.83±8.42	0.029*
HDL (mg/dL)	60.58±13.47	52.50±10.85	53.91±8.04	48.28±4.78	45.32±12.64	0.199
LDL (mg/dL)	21.17±4.31	23.62±5.79	21.05±3.45	20.36±2.14	18.83±4.72	0.502
MDA (µM)	1.62±0.07	1.83±0.41	1.59±0.17	1.38±0.12	1.44±0.12	0.021*

The results of this study indicate that atherogenic food given to rats for 14 days gave an increase in all lipoproteins including triglycerides, total cholesterol and HDL, but not LDL. Atherogenic food given contains high fat content ranging from 25-35% of total energy. High fat intake for 14 days affected lipoprotein levels, a significant difference was seen in total cholesterol and triglycerides, whereas in HDL only a significant difference was seen between K + and K- groups (52.51 10.85 mg / dL vs 60.59 13.47 mg / dL). In the treatment group, the administration of passion fruit seed extract showed the highest mean value in group P1, namely by giving 5 mg / kg body weight (**Table 5**).

On examination of LDL levels also showed no significant difference in all groups, the highest increase was seen in the group with standard feed (K +). Interestingly, the lowest LDL level was seen in the treatment group, especially in the P3 group, which was given 20mg / kg body weight. The treatment group, P1 and P2 showed a decrease in LDL levels, but did not show significant differences with the K-group. The duration of treatment might be the possible factor of the no differences shown in each group. In addition, despite that all the treatment groups were given passion fruit seed extract, atherogenic food was still given, and with this treatment design, low LDL levels can be seen in all treatment groups (**Table 5**).

The group that showed the biggest difference in total cholesterol levels was in the P2 and P3 groups with the administration of passion fruit seed extract of 5 and 10 mg / kg body weight compared with the K-group (atherogenic food feeding). The lowest average cholesterol level was found in P2 group. While the highest total cholesterol level was found in the K-group. The administration of passion fruit seed extract showed a decrease in the mean value total cholesterol level in the groups according to the number of doses given. The larger the dose, the lower the average total cholesterol level obtained, the minimum value found in the P3 group was 56.56 mg / dL (**Table 5**). Passion fruit seed extract containing antioxidants can inhibit the rate of increase in total cholesterol and triglyceride levels, especially in rats given atherogenic food within 14 days. Flavonoids contained in passion fruit seed extract can inhibit an increase in total cholesterol levels by a mechanism that inhibits the activity of the enzyme HMG CoA reductase which plays an important role in cholesterol biosynthesis.

Based on the LSD test, there were significant differences in each parameter, the treatment group that showed the most significant difference indicating the effect of passion fruit seed extract for 14 days on atherogenic food was in the P2 group (10mg / kg body weight dose of passion fruit seed extract). The most significant results showed the P3 group (dose of passion fruit extract 20mg / kg body weight) with the atherogenic food group with parameters of triglyceride levels (**Table 6**).

Table 6. Significant changes in triglycerides, total cholesterol and MDA levels

Parameter	Different Groups	*p*
Triglyceride	C+ and C-	0.036
	C-and P1	0.048
	C- and P2	0.025
	C- and P3	0.001
Total cholesterol	C- and C+	0.044
	C- and P2	0.003
	C- and P3	0.007
MDA	C+ and P2	0.002
	C+ and P3	0.009

3.4. Blood vessel histopathology

At the end of the treatment, namely on the 15th day, the rats were terminated, the aortic arc was taken and histopathological examination was performed to see the formation of plaque which would describe the process of atherosclerosis. Tissue was stained using Red-O (OR-O) and Hematoxylin Eosin (HE) Oils.

Histopathological examination of the aortic arc, showed that in the K-group, plaques and thickening of the aortic arc wall were formed which described the process of atherosclerosis (**Figure 4**). In group P3, atherosclerosis was seen in part of the arterial wall, but congestion was seen on the lumen, where erythrocyte cell accumulation occurred, no plaque was found inside (**Figure 5**).

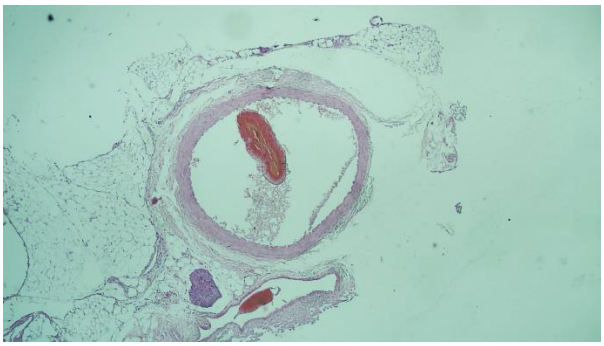

Figure 4. plaque and atherosclerosis in C negative rats aorta

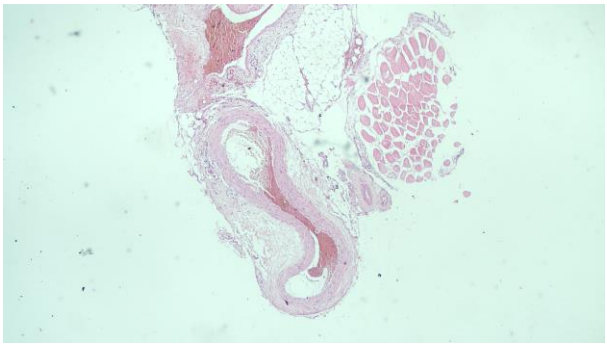

Figure 5. Normal artery wall in P2 group, filled with erithrocyte

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4. Discussion

Passion fruit is a fresh fruit in the Asian region, especially Indonesia (North Sumatra), which can be used as a drug because it contains high flavonoids, tannins and antioxidants. Seeds, leaves and mesocarp of passion fruit can be used in the pharmaceutical industry, but in North Sumatra, purple passion fruit (Passiflora edulis) is generally only used as raw material for making syrup. This new study shows the presence of phenol levels in passion fruit seed extracts, not yet measuring how much the phenol content is.

Previous research stated that passion fruit rinds showed higher antioxidant activity than passion fruit pulp especially in effect on the production of reactive oxygen species (ROS) and myeloperoxidase (MPO) activity, which play a role in the inflammatory process [15]. The study did not examine passion fruit seeds, the pulp was made by separating porridge and seeds [8,15]. The active ingredient in passion fruit seeds is piceanntanol, in all types of passion fruit variants including yellow passion fruit [16].

Piceanntanol's work activities in inhibiting linoleic acid peroxidation is more optimal than other antioxidants, including beta carotene and show a variety of biological activities, including antioxidant activity, anticarcinogenic, antiatherogenic, and anti-inflammatory. Phenol compounds inhibit lipid peroxidation and the lipooxygenation process by terminating peroxyl radical chain reactions through cleaning effects (scavenger), such as the working power of vitamin C, beta carotene, and vitamin E [16].

The results of this study indicate that antioxidant activity has a correlation with flavonoid levels, DPPH capture activity by passion fruit seed extract showed a very strong relationship between the content of polyphenols and free radical capture activities. The working power of the antioxidant components of phenolic compounds by cleaning reactive oxygen species (ROS), with inhibition is better than other forms such as beta carotene [16,18].

Previous research showed that the bioavailability of passion fruit seeds is influenced by the content of the matrix contained in them, the form of passion fruit seed extract is better than other forms. It is important to emphasize that the food matrix is complex and there are synergistic or antagonistic interactions of each compound [16-19]. Other antioxidant activities or vitamins in them also influence each other, but in this study, it has not shown other antioxidant content or vitamins contained in it.

Lipid peroxide level measurement is used as an indicator of cell and tissue oxidative stress. Lipid peroxides are unstable and decomposed resulting in a number of compounds including reactive carbonic compounds. Polyunsaturated fatty acids decompose to produce malondialdehyde (MDA) [20-22]. Results of this study indicates a difference in MDA levels between groups of rats, especially in the administration of passion fruit seeds. With this difference, it gives an illustration of the inhibition of fat peroxidation by giving passion fruit seed extract. The role of piceanntanol in passion fruit extract is similar to resveratrol as an antioxidant (5), the antioxidant's working power is strong and the possible pathway is to terminate the peroxyl radical chain reaction through a cleansing effect on ROS like OH *, ONOOH *, and HOCl * [18,23].

This study showed a significant difference in MDA levels with a dose of 10mg / kg body weight within 14 days in rats given atherogenic food compared to standard food. This will provide an overview for human studies taking into account the dosage and duration of administration. MDA levels can be used as a marker of oxidative stress in various inflammatory reactions in the body, both in total or free form MDA [24]. Pathological changes caused by various types of diseases indicate endogenous and exogenous MDA in living cells. There is an increase in MDA levels due to stimulation by H2O2 in a high oxidative pressure environment [25]. Differences in MDA levels in experimental rats can illustrate that there is a decrease in oxidative stress levels.

This study showed a difference in total cholesterol and triglyceride levels. Differences in total cholesterol levels after supplementation of passion fruit seeds in experimental rats can be reflected in the metabolism of the human body. The human body with a lifestyle of a high intake of saturated fat, trans fat, and cholesterol causes fat buildup and an inflammatory reaction that triggers the process of atherogenesis which will eventually fall into atherosclerosis [26,27].

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Differences in cholesterol levels at a dose of 20mg / kg body weight within 14 days can make the active ingredient in passion fruit seed extract can be used to reduce total cholesterol levels in the body. High cholesterol concentrations, especially LDL levels, are expressed as a cause of atherosclerosis. Atherosclerotic lesions begin with the oxidation of LDL which causes endothelium to express monocyte attachment and produce monocytic chemotactic proteins and stimulation factors for macrophage colonies and to attach to blood vessel walls to form foam cells and end with the formation of initial lesions known as plaques [27-29]. In this study, the presence of plaque in the histopathological picture and the process of blood vessels that have formed atherosclerosis were seen, but in the administration of passion fruit seed extract, minimal plaque formation and no blood vessel formation with atherogenesis are seen.

In previous studies, studies were conducted on human subjects, passion fruit seed extract was given to non-overweight and overweight men and women without other metabolic disorders. In a study, it can be seen that the role of piceanntanol contained in passion fruit seeds, on insulin levels, HOMA-IR, blood pressure, and heart rate; the results showed a decrease especially in male subjects with overweight [5]. The results of the study did not show any effect on the markers of oxidative stress and lipid profiles, this is probably due to the small number of samples, the number of short administration (8 weeks), and the absence of effective doses.

Several previous studies have looked at the role of nutrition in the prevention of cardiovascular disease. Research in people who consume red wine proves that these people rarely suffer a heart attack compared to people who don't consume it. It is estimated that red wine contains resveratrol which has a cardio-protective effect, piceanntanol is stated to have similar working power with resveratrol [5,30,31]. This study has shortcomings, namely the short duration of the trial period so that it has not able to show a difference in levels for LDL, the antioxidant or vitamin content in the passion fruit seed extract and the level of piceanntanol have not been known, further research is planned for advanced stages in humans.

5. Conclusions

Passion fruit extract (Passiflora edulis Sims) has a potential effect in reducing MDA, total cholesterol and triglycerides levels in rats. Administration of passion fruit extract can be used as an alternative in holding back the rate of increase in total cholesterol and MDA levels with atherogenic intake. With further research, it is hoped that the role of passion fruit seeds will be further known when a low fat and cholesterol food is given, so that it can be applied to prevent the process of atherosclerosis.

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