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The effect of purple passion fruit juice on superoxide dismutase and malondialdehyde levels in hypercholesterolemic rats

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ABSTRACT

BACKGROUND
Hypercholesterolemia due to a high cholesterol diet can increase free radicals resulting in oxidative stress. Superoxide dismutase (SOD) and malondialdehyde (MDA) have been used as the study markers of oxidative stress in cases of hypercholesterolemia. Purple passion fruit contains various compounds that may reduce free radicals. This study aimed to determine the effect of purple passion fruit juice on SOD and MDA levels in hypercholesterolemic rats.

METHODS
An experimental analysis with post-test only control group design involving 28 male Wistar rats. They were divided into 4 groups: normal control (K1), hypercholesterolemic control (K2), purple passion fruit juice treatment at 4.2 mL/200 gBW/day (K3), and simvastatin treatment at 0.018 mg/200 gBW/day (K4). The purple passion fruit juice at 4.2 mL/200 gBW/day was administered for 14 days. SOD levels were examined by enzymatic colorimetric methods using the Ransod kit and MDA levels by the TBARS method.

RESULTS
The Kruskal-Wallis test showed a significant difference in SOD levels between the tested groups ($p<0.05$). One-way ANOVA test for MDA levels showed a significant difference ($p<0.05$). Post Hoc test (Mann-Whitney for SOD and LSD for MDA levels) also showed significant differences: K1 vs. K2, K2 vs. K3, K2 vs. K4, and K3 vs. K4 ($p<0.05$).

CONCLUSION
This study demonstrated that purple passion fruit juice significantly increases the SOD and lowers the MDA level in hypercholesterolemic male Wistar rats. Consumption of purple passion fruit juice may help to modulate oxidative stress caused by hypercholesterolemia in rats.

Keywords: Hypercholesterolemia, malondialdehyde, oxidative stress, Passiflora edulis var edulis, superoxide dismutase, rats

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INTRODUCTION

Hypercholesterolemia is a condition where the level of cholesterol in the blood exceeds the normal level due to an increase in cholesterol and low-density lipoprotein (LDL).[1] This condition can be caused by a high-cholesterol diet, increased body weight, age, genetics, and decreased estrogen levels in postmenopausal women.[2] Hypercholesterolemia is one of the main risk factors for cardiovascular disease.[3] In 2016, 17.9 million people died from cardiovascular disease.[4] According to the Indonesian Ministry of Health in 2018 of the Indonesian population aged 15 years there were 21.2% with borderline cholesterol (200-239 mg/dL) and 7.6% with high cholesterol (≥240 mg/dL).[5] Hypercholesterolemia causes an increase in free radicals, which modify the amino acids in low density lipoprotein (LDL) apolipoproteins so that lipid peroxidation occurs in LDL. Macrophages engulf the modified LDL causing further free radical formation. This increase in free radicals triggers oxidative stress,[6,7] which is an imbalance between antioxidants and pro-oxidants, the amount of pro-oxidants being greater than that of antioxidants.[8] Oxidative stress can be reduced by increasing the number of antioxidants. One of the main chain-breaking antioxidants is superoxide dismutase (SOD), which acts by scavenging superoxide free radicals.[9] Superoxide dismutase catalyzes the conversion of the superoxide free radicals into hydrogen peroxide (H_2O_2) or molecular oxygen.[10,11]

According to the American Heart Association (AHA) and the American College of Cardiology (ACC), hypercholesterolemia therapy is divided into two therapeutic modalities: 1) non-pharmacological therapy through lifestyle changes, and 2) pharmacological therapy with fat-lowering drugs.[12] One of these pharmacological therapies are the statins, the most potent drugs in lowering LDL cholesterol.[13] Statins act by lowering cholesterol through inhibition of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase and increasing the production of high-density lipoprotein (HDL).[14] These statin drugs can cause the side effects of myalgia, rhabdomyolysis, diabetes mellitus, and drug interactions.[13] Meanwhile, non-pharmacological therapies in the management of hypercholesterolemia include increased fruit and vegetable consumption, aerobic physical activity, weight loss, and smoking cessation.[12] Consumption of fruits and vegetables is very important, since in addition to containing fiber components to lower cholesterol, they also have phytochemical components and vitamins with antioxidant properties.[15]

Indonesia has a variety of antioxidant-containing fruits that are beneficial for health.[16] These fruits are a source of natural antioxidants because they contain phytochemical compounds that can reduce pro-oxidants.[17] One of these fruits is the purple passion fruit from the Passiflora family.[18] The purple passion fruit (*Passiflora edulis*) is a plant originating from Brazil that can live in tropical and sub-tropical areas,[19] and is widely cultivated in Indonesia, especially in Sulawesi and Sumatra.[20] The passion fruit plant is easy to grow but the fruit is often not used because of its sour taste. Previous studies have explored the contents of various parts of this fruit (seeds, peel, and juice). Purple passion fruit contains flavonoids, alkaloids, phenols, cyanogenic compounds, glycosides, vitamins, minerals, and polyphenol antioxidants.[21] Flavonoid antioxidants in fruits are reported to play a role in preventing cell damage caused by free radicals.[22] Behind its exotic taste, it turns out that purple passion fruit contains various nutrients and non-nutritive phytochemicals that are beneficial for health. Many pharmacological studies have been carried out, where various components of purple passion fruit show antimicrobial, antihypertensive, antidiabetic, antioxidant, and other activities.[23]

This study is a part of our research roadmap. In the last study, we explored the antihypercholesterolemic potential of purple passion fruit juice at various doses.[24] Then we studied the effectiveness of this fruit juice against

simvastatin in the hypercholesterolemic rat model. Research on lipid profiles, which is closely related to this study, has been previously published by Muntafiah et al.[25] Furthermore, the present study describes the effect of purple passion fruit (*Passiflora edulis* var *edulis*) juice on the levels of superoxide dismutase (SOD) and malondialdehyde (MDA) in experimental animal models of hypercholesterolemia. We choose SOD as our research parameter because it is the marker enzyme against oxidative stress that plays a key antioxidant role in living cells against the toxicity of superoxide free radicals. In hypercholesterolemia it is known that high cholesterol in cells results in an altered cell membrane due to lipid peroxidation. Malondialdehyde (MDA) is the marker of lipid peroxidation generating free radicals. Excess generation of free radicals in hypercholesterolemia depletes body antioxidants leading to oxidative stress.

Several studies have reported the effect of Passiflora fruit on antioxidants and oxidative stress, but different results were obtained. The plant materials used in these studies are also different. Our study is consistent with a study by Silva et al.[26] which states that treatment with a polysaccharide fraction from the peel of *Passiflora edulis* fruit significantly reduced MDA concentration and increased glutathione (GSH) levels in mice. This is also consistent with the study on the antioxidant activity of *Passiflora alata* fruit extract by Medeiros et al.[18]

In contrast, another study by de Souza et al.[27] evaluated the effects of *Passiflora edulis* f. *flavicarpa* Degener (yellow passion fruit) juice on the lipid profile and oxidative stress status of Wistar rats, showing that the levels of triglycerides and very-low-density lipoprotein-cholesterol, superoxide dismutase activity (SOD), and total glutathione concentration were not statistically different between the two groups.

This research is a continuation of a previous study that explored the potential of purple passion fruit juice as an anti-hypercholesterolemic agent, where the fruit juice at a dose of 4.2 mL/200 g BW could significantly reduce total cholesterol levels.[24]

METHODS

Research design

This experimental study used a completely randomized post-test only control group design. This research was conducted at the Biochemistry Laboratory and the Pharmacology & Experimental Animal Laboratory, Faculty of Medicine, Jenderal Sudirman University (FK UNSOED), Purwokerto, Central Java, Indonesia, from October 2016 to November 2016.

Experimental animals

The research subjects were healthy male albino rats (*Rattus norvegicus*) of the Wistar strain, 2-3 months old, and weighing 180-220 g, obtained from LPPT III, Gadjah Mada University (UGM), Yogyakarta. The minimum total number of subjects in this study was 28 based on the 'resource equation' approach.[28] The animals were maintained at 22"25°C with 12 h/12 h light /dark cycle. All animals were fed rat pellets and provided with tap water ad libitum.

Establishment of rat hypercholesterolemia model

Induction of hypercholesterolemia was carried out after the experimental animals underwent a seven-day acclimatization period. Induction was carried out in groups K2, K3, and K4 for 10 days with duck egg yolk at a dose of 2 mL/200 g BW/day and pork oil at 3 mL/200 gBW/day orally through a probe. After the induction period, the experimental animals were fasted for 12 hours and 2 ml of blood was drawn from the infra-orbital vein to confirm the hypercholesterolemia.[25] Induction was declared successful if the total cholesterol level in the blood exceeded 54 mg/dL. Normal rat plasma total cholesterol level is 10-54 mg/dL.[24]

Treatment of the hypercholesteremia rats

The experimental animals were randomly divided into 4 groups: normal control (K1), hypercholesterolemic control (K2), purple passion fruit juice treatment at a dose of 4.2 mL/200 gBW (K3), and simvastatin control (K4). The experimental animals were put into cages by group. During the treatment period, the experimental animals received treatment every morning. The healthy/normal control group (K1) and the hypercholesterolemic control group (K2) were treated with distilled water orally through a probe. The K3 group received purple passion fruit juice at 4.2 mL/200 g BW/day by oral probe. A dose of 4.2 mL/200 g BW is the maximum dose that can be accepted by these animals. Based on our previous study, this dose can significantly reduce total blood cholesterol.[24] The K4 group received simvastatin at a dose of 0.018 mg/200 g BW. Before the treatment period, the experimental animals were acclimatized for 7 days, placed in the same environment, in cages of the same size, shape, material, and location, and received food and drinking water ad libitum.[29]

The dose of simvastatin used in this study was based on the usual dose given to adult humans, which is 10 mg/day. The conversion factor from humans with a bodyweight of 70 kg to rats (*Rattus norvegicus*) with a body weight of 200 grams is 0.018 mg/200 g BW/day.[25] Therefore, the dose of simvastatin for rats equals the conversion factor for rats multiplied by the dose of simvastatin in humans = 0.018 x 10 mg/day, giving the dose of 0.18 mg/200 g BW/day.

Plant materials

This study used ripe purple passion fruit (with dark purple and smooth or slightly grooved skin). We used fresh fruit juice directly taken from ripe fruit. The fruit was taken in a season with sufficient rainfall and sunshine. The preparation of purple passion fruit juice begins with washing of the fresh fruit, after which the fruit is cut and the fruit flesh is made into juice then filtered through a filter cloth so that the seeds are separated from the fruit juice. The fruit juice is made every day to obtain fresh fruit juice.[30] Determination test was carried out at the Biology Laboratory, Jenderal Soedirman University.

Determination of SOD and MDA

Posttest blood sampling was carried out at the end of the study period, using a 3 mL hematocrit pipette through the infra-orbital vein. Previously, the experimental animals had been fasted for 12 hours. Whole blood samples were put into an EDTA tube, centrifuged at a speed of 1300 rpm for 10 minutes at room temperature, then examined for antioxidant levels of SOD and MDA.[27] The SOD antioxidant levels were measured by the colorimetric enzymatic method using the Ransod kit, while MDA levels were determined by the thiobarbituric acid reactive substance (TBARS) method. The absorbance was read on a spectrophotometer (Robert Riele GmbH & Co KG, Germany), at a wavelength of 540 nm.

Statistical analysis

Data were processed and analyzed using the SPSS 10 for Windows statistical software. The data distribution was tested for normality with the Shapiro-Wilk test and continued with the Levene's test for homogeneity. The data on SOD and MDA levels were found to be normally distributed ($p>0.05$), but the data variance was not homogeneous ($p<0.05$), so that data transformation was carried out. After transformation, the data variance of SOD was still non-homogeneous ($p<0.05$) so the Kruskal-Wallis non-parametric test was used. Meanwhile, the data variance of MDA was homogeneous after transformation so it was tested with One-Way ANOVA.[31]

Ethical clearance

This research has received ethical approval from the Faculty of Medicine Research Ethics Committee, Jenderal Soedirman University under No. Ref: 120/KEPK/2016.

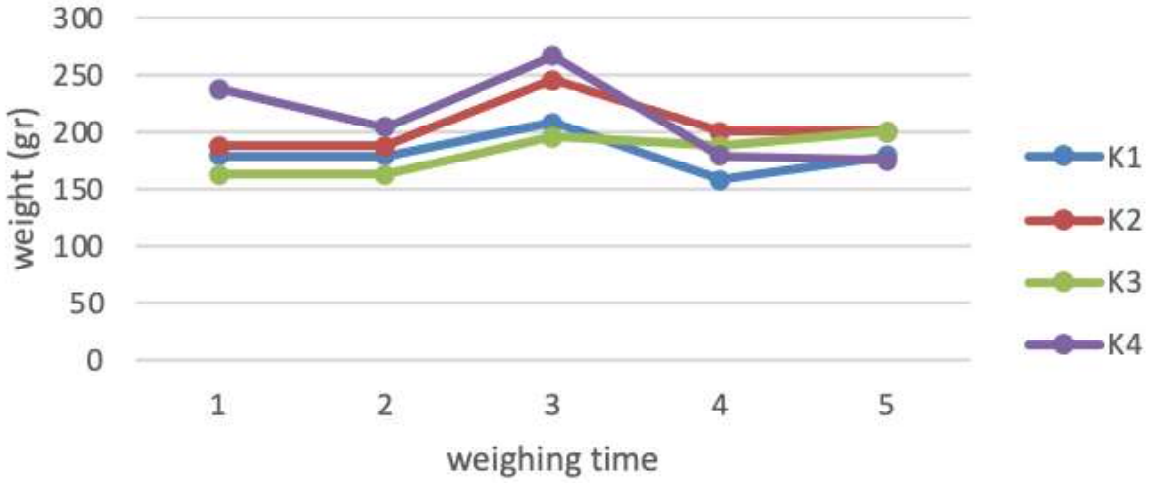

Figure 1. Body weight of the experimental animals

RESULTS

As shown in Figure 1, there was an increase in body weight in all groups at week 3 of the study. The normal control group (K1) and the hypercholesterolemic group (K2) experienced weight loss at weeks 4 and 5. The treatment group receiving purple passion fruit juice (K3) had relatively stable body weight until the 5th week. The treatment group on simvastatin experienced weight loss.

The data of SOD and MDA levels determined at the end of the study period are presented in Table 1. The hypercholesterolemic control group (K2) had the lowest SOD and highest MDA levels compared to other groups. The purple passion fruit juice group (K3) and the simvastatin group (K4) had higher SOD and lower MDA levels than the hypercholesterolemic control group (K2). The SOD level in the simvastatin group (K4) was higher than in the hypercholesterolemic control group (K2) but was still lower than in the normal control group (K1). Administration of purple passion fruit juice at 4.2 mL/200 g BW to the K3 group could increase SOD to levels that were higher than in the normal control group (K1), where the levels increased about 3-fold if compared to the hypercholesterolemic control group (K2). The increase in SOD levels was in line with the decrease in MDA levels in that group.

Table 1. Distribution of mean SOD and MDA levels by treatment group

	Treatment group				p value
	K1 (n=6)	K2 (n=6)	K3 (n=6	K4 (n=4)	
SOD (U/mL)	17.10 (11.70 - 24.00)[a]	9.15 (7.00 – 10.60)b	22.25 (12.10 -63.90)[a,c]	12.45 (8.90 – 16.50)[a]	< 0.05*
MDA (µmol/L)	0.61 ± 0.03[a]	2.45 ± 0.12[b]	0.78 ± 0.07[c]	1.20 ± 0.08[d]	< 0.05**

Statistical test using 95% confidence intervals.
*Kruskal-Wallis test, Followed by Mann-Whitney test shows a significant difference between K1 vs. K2 (p=0.004), K2 vs. K3 (p=0.004), K2 vs. K4 (p=0.020), and K3 vs. K4 (p=0.045).
**One-Way ANOVA. Followed by post hoc LSD showed a significant difference between K1 vs. K2 (p=0.00); K2 vs. K3 (p=0.00); K2 vs. K4 (p=0.00); and K3 vs K4 (p=0.00).
[a,b] Different notations on the same column indicate significant differences.
K1: normal control; K2: hypercholesterolemic control; K3: treatment with purple passion fruit juice at 4.2 mL/200 gBW/day; and K4: treatment with simvastatin at 0.018 mg/200 gBW/day.
SOD: Superoxide dismutase, MDA: Malondialdehyde, SD: standard deviation
Data presented as SOD: Median (Min-Max) for SOD and MDA:Mean ± SD

The Kruskal-Wallis nonparametric test showed a significant difference between the treatment groups (p=0.002). Therefore, the post hoc test with Mann-Whitney was continued to find out which groups had these significant differences. Mann-Whitney test showed a significant difference in group K1 vs. K2 (p=0.004), group K2 vs. K3 (p=0.004), group K2 vs. K4 (p=0.020), and group K3 vs. K4 (p=0.045). Meanwhile, the K1 vs. K3 and K1 vs. K4 groups has no significant differences, with p-values of 0.262 and 0.150, respectively.One-Way ANOVA showed significant differences between the treatment groups (p<0.05). Post hoc LSD showed significant differences in K1 vs. K2 (p=0.00), K2 vs. K3 (p=0.00), K2 vs. K4 (p=0.00), and K3 vs. K4 (0.00).

DISCUSSION

This study was conducted to determine the effect of purple passion fruit juice (*Passiflora edulis* var *edulis*) on SOD and MDA levels in experimental hypercholesterolemic model animals. The results of this study showed that the hypercholesterolemic control group (K2) had the lowest levels of SOD antioxidants and highest levels of MDA compared to the other groups. This is in line with the research conducted by Silva et al.[26] on the effect of the polysaccharide fraction from the peel of *Passiflora edulis* fruit on MDA in mice. Research by Panelli et al.[32] on the benefits of the bark of *Passiflora edulis* under conditions of oxidative stress in obese mice also showed that it was effective in improving antioxidant capacity and reduced malondialdehyde levels. Hypercholesterolemia is characterized by an increase in total cholesterol and LDL cholesterol. The cell membrane is composed of cholesterol as a structural component that plays a role in the structural and functional integrity of the cell. The presence of high levels of cholesterol in hypercholesterolemia results in changes in the physical properties of the cell membranes, which may facilitate leakage of reactive oxygen species (ROS) from the mitochondrial electron system or activation of NADPH oxidase. These reactive free radicals cause lipid peroxidation in the cell membranes that produce lipid peroxide radicals and other free radicals.[6,33] Increased lipid peroxidation (which can be seen from the high levels of MDA causes oxidative stress due to an imbalance between peroxides and oxidants.[33]

Superoxide dismutase is a marker enzyme against oxidative stress in cells. This enzyme degrades superoxide into ordinary oxygen molecules or hydrogen peroxide. It also acts as a key antioxidant in living cells that protects against the toxicity of superoxide free radicals.[6,11] In hypercholesterolemic conditions, SOD antioxidants are at their lowest levels because the increased production of free radicals exceeds the body's capacity to handle them. The increase in free radicals in the blood occurs because of the increased low-grade systemic inflammatory response, where this systemic inflammation increases the production of ROS.[34] These free radicals modify the amino acids in LDL apolipoproteins so that lipid peroxidation occurs in LDL. The macrophages then engulf the modified LDL causing further free radical formation. The continuous increase in free radicals triggers oxidative stress.[6,7] Under normal conditions, the body can reduce oxidative stress conditions with endogenous (enzymatic) antioxidants, one of which is SOD, but in hypercholesterolemic conditions, the increase in free radicals exceeds the body's capacity to fight oxidative stress conditions, resulting in a decrease in SOD levels in the K2 group.[32]

The results of this study showed a significant increase in SOD levels in the K3 group, about 3 times higher than in the hypercholesterolemic control (K2). This is in line with the research conducted by Kandandapani et al. [35] where administration of purple passion fruit at 500 mg/kg of leaf, peel, and fruit extract in streptozotocin-induced diabetic rats showed a significant increase in SOD and a reduction in the TBARS levels in the heart, liver, and kidney. In the present study, the increase in SOD levels in the K3 group

was made possible by various phytochemical compounds in purple passion fruit juice. The increase in superoxide dismutase levels may be explained by several mechanisms. Purple passion fruit juice contains flavonoid compounds that act directly by donating hydrogen ions to neutralize the toxic effects of free radicals. Flavonoids also act indirectly by increasing endogenous antioxidant genes through the activation of nuclear factor erythroid 2 related factor 2 (NRF2). Activation of this gene plays a role in the synthesis of antioxidant enzymes such as the SOD gene.[36,37] Purple passion fruit besides having flavonoid compounds that can reduce free radicals, also contains vitamin C and beta carotene, which act as antioxidants by donating electrons or hydrogen atoms to free radicals to neutralize these free radicals. Neutralization of these free radicals can reduce oxidative stress.[38] Therefore, the flavonoid compounds, vitamin C, and beta carotene contained in purple passion fruit may increase SOD levels in the K3 group.[39] Research by Doungue et al.[36] showed that the administration of the flavonoid extract fraction to rats with aluminum chloride-induced Alzheimer's disease was able to significantly increase SOD levels in the hippocampus and cortex of the rats. The study also stated that flavonoids were the main cause of the increase in SOD levels, although other bioactive compounds also played a role. Our study is also in line with research conducted by Hu et al.[37] which showed that administration of pure purple passion fruit anthocyanin extract, which is a type of flavonoid in purple passion fruit, could significantly increase SOD activity at doses of 400 mg/kg and 600 mg/kg in the skeletal muscle of rats with induced muscle fatigue.

SOD antioxidant levels in the K4 group also showed a significant increase, but the levels were still lower than in the healthy control group (K1). Research conducted by Eger et al.[40] on the effect of simvastatin administration to Wistar rats for 1 month on the increase in free radicals induced by lisdexamfetamine dimesylate (LDX) showed that simvastatin could prevent a decrease in SOD activity in the cerebellum of the rat brain. Research by Yang et al.[41] on Sprague Dawley rats subjected to smoke inhalation showed that simvastatin in doses of 25 mg/kg to 100 mg/kg could increase SOD activity in a dose-dependent manner, but the increase had not yet reached the SOD levels in the healthy groups. Clinical studies show that statins affect plasma SOD levels, while research shows that statins have antioxidant effects through cell antioxidation and increased free radical elimination. Statins have anti-inflammatory properties by lowering C-reactive protein (CRP) and pro-inflammatory cytokines.[42]

SOD enzymes are important in reducing the effects of oxidative stress by breaking down free radicals into hydrogen peroxide which will then be broken down by catalase or glutathione peroxidase into non-toxic products, namely water, and oxygen. Conditions that cause a decrease in the SOD enzyme can cause cell damage due to oxidative stress.[43-45] Purple passion fruit acts as an antioxidant that can reduce hypercholesterolemia-induced oxidative stress by increasing SOD activity and neutralizing free radicals. The present study showed that purple passion fruit juice contains antioxidants that are effective in increasing plasma SOD and reducing MDA levels in Wistar rats with induced hypercholesterolemia.

One limitation of this research is the short duration of the trial period in which the study has not been able to show the antioxidant or vitamin content of the purple passion fruit extract, so that further research is planned for advanced stages of the study in humans.

CONCLUSIONS

Administration of purple passion fruit juice (*Passiflora edulis* var *edulis*) could significantly increase SOD antioxidant levels and reduce MDA levels in male Wistar rats (*Rattus norvegicus*) induced by hypercholesterolemia. Thus, our results reinforce the ethnopharmacological use of *Passiflora edulis* in popular medicine.

CONFLICTS OF INTEREST

The author(s) declared no potential conflicts of interest concerning this article's research, authorship, and/or publication.

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CONTRIBUTORS

All authors take public responsibility for the content of the manuscript submitted to Universa Medicina. AM is the main researcher who is responsible for the entire research process, analyzing and interpreting data, and writing of the manuscript. JHS played a role in helping draft the manuscript. SH, DN, and HH played a role in assisting the research process. All authors have read and approved the final manuscript.

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