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The Effect of Red Dragon Fruit (*Hylocereus polyrhizus*) Peel Ethanol Extract on Oxidative Stress in Sprague Dawley Rats (*Rattus norvegicus*)

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ABSTRACT

Heat stress can induce DNA, lipid, and protein damage by releasing inflammatory mediators and metabolic reactive oxygen species. The antiradical activity of red dragon fruit (*Hylocereus polyrhizus*) has been reported in several studies, but there is little information on the effect of its extract on heat stress exposure. Therefore, this study was conducted to investigate the effects of red dragon peel ethanol extract on acute heat stress in Sprague Dawley rats (*Rattus norvegicus*). Ethanol extract was prepared from red dragon fruits. Thirty rats were divided into five groups and treated for 14 days. Rats were fed a standard diet in the negative and positive control groups (K1 and K2, respectively). Furthermore, treatment groups P1, P2, and P3 received dragon fruit peel ethanol extract on the 14th day at doses of 400, 800, or 1,600 mg/kg BW, respectively. On the 15th day, heat exposure was used to create stress in K2, P1, P2, and P3. The oxidation state was determined by analyzing malondialdehyde (MDA) levels. The results of the MDA levels in rats in the K1, K2, P1, P2, and P3 groups were 19.50 ± 0.85, 49.30 ± 3.70, 20.65 ± 4.19, 20.37 ± 2.63, and 22.44 ± 6.68 ng/mL, respectively. Furthermore, significant ($p < 0.001$) values were observed in the K2 group, as well as nominal differences between the treatment groups P1, P2, and P3 ($p > 0.05$). Based on these findings, red dragon fruit extract improves an oxidative stress biomarker in acute heat-induced stress rats. However, the impact is not dose-dependent.

Keywords: Fruit peel extract, Heat stress, Malondialdehyde, Oxidative stress, Red dragon.

Introduction

Heat stress causes an increase in reactive oxygen species (ROS), which results in the formation of extracellular superoxide. It upregulates the modulation of transcription nuclear factor (NF-κB), which can lead to the synthesis of hormones, inflammatory cytokines, and metabolic changes in the body.[1] As a result, pro-inflammatory cytokines and inflammatory mediators will increase in response to this condition.[2] Antioxidants help to reduce lipid peroxide generation and eliminate free radicals, which helps to reduce the inflammatory reactions to heat stress. Therefore, oxidative stress is prevented. Exogenous nutritional supplementation is also often utilized to counteract the harmful effects of heat stress.[3] Red dragon fruit (*Hylocereus polyrhizus*) was first discovered in Mexico and was reported to have significantly high antiradical activity due to the presence of phenolic compounds. Betacyanin, anthocyanins, and other flavonoids are also important characteristics of plant pigment.[4] This fruit is common in Indonesia because of its colour, taste, and ease of cultivation. Betacyanin is highest in the peels of the red dragon, which is responsible for the red-violet color. The antioxidant activity of this fruit's extract is higher than that of its white counterpart.[5]

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There is evidence of the red dragon's metabolic benefits, such as lower MDA levels in a variety of metabolic disorders.[6] The betacyanin content in the peels has increased its application in food products.[7] However, there is still a lack of information on the effects of this fruit on a heat stress-induced oxidative marker called malondialdehyde.

Therefore, this study was aimed at investigating the effects of red dragon peel extract on malondialdehyde levels after acute heat stress in Sprague Dawley rats (*Rattus norvegicus*).

Materials and Methods

Source of plant material

Red dragon fruits were purchased from the local market in March 2019. The identity was authenticated by the Laboratory of Biology, Faculty of Medicine, Diponegoro University, Semarang, Indonesia with an ID number: 112/IV/2019.

Source of animals

Sprague Dawley rats weighing 150-300 grams on average, 2-3 months old before adaptation, were obtained from the animal laboratory, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia. There was no anatomical abnormality in the rats. Each rat was kept in a cage in a room with a temperature of 25°C and a 12:12 hour light/dark cycle. They were then acclimated for seven days with a semi-pure laboratory rat diet based on the AIN-93M formulation and water *ad libitum*.

Ethical approval

The ethics committee of the Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia, approved this study before the commencement of the experiments (Approval No.: 122/EC/H/KEPK/FK-UNDIP/X/2019).

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Preparation of red dragon fruit peel extract

The red dragon fruits (4.8 kg) were macerated in a distilled solvent with a pH of 5 and then adjusted with citric acid. It was peeled by hand, sliced into little pieces (2 mm), and dried for 24 hours at 50°C. Furthermore, it was mixed with 96% ethanol every 6 hours for 60 minutes. After that, the solution was separated from the rest of the mixture. This drying operation was repeated 3 times before filtering with Whatman No. 4 filter paper. The filtrate was dried at 56–60°C for 4 hours to remove the solvent and extract material, then stored at 4°C, and protected from light until further tests.[8]

Experimental grouping

This was a randomized post-test-only control group study. The rats were randomly divided into five groups of six rats each. The treatment was administered on the first day after adaptation. For 14 days, groups K1 and K2 were given standard feed. Red dragon fruit peel extract was given in graded doses of 400, 800, and 1,600 mg/kg BW to groups P1, P2, and P3, respectively, for 14 days. On the 15th day, groups K2, P1, P2, and P3 were exposed to heat stress for 70 min at a temperature of 43°C using a 20-watt incandescent bulb. The dosage was established using data from a previous study in which red dragon fruit peel extract at 500 mg/kg BW was found to be an effective treatment for acetate lead poisoning.[9]

Collection of blood samples

The rats in this study were euthanized on day 15 using 100 mg/kg BW of pentobarbital. Subsequently, blood samples were collected and sent to a laboratory for analysis.

Laboratory analysis

Lipid peroxidation was measured by determining the reaction between MDA and thiobarbituric acid (TBA). The MDA colorimetric assay kit (Sigma Aldrich) employed a colorimetric (532 nm)/fluorometric (λex = 532/λem = 553 nm) approach, proportionate to the MDA present.

Statistical analysis

To compare and identify the level and difference of MDA between treatment groups, the data were statistically analyzed using one-way ANOVA and post hoc analysis. These analyses were performed using the statistical package for social sciences (SPSS; version 21), and a *p-value* of 0.05 was considered significant. Furthermore, the data are presented as mean ± SD with 95% confidence intervals.

Results and Discussion

Abnormalities such as infection, thermoregulatory problems, or decreased body weight were not noticed during the experiment. At the end of the experiment, the body weight of the animals was increased. Meanwhile, five dead rats were excluded, and the remaining 25 samples were randomized into 5 groups. As shown in Table 1, the difference in body weight between the groups of Sprague Dawley rats was insignificant ($p > 0.05$). Table 2 demonstrates the MDA level in heat-stressed Sprague Dawley rats after administration of red dragon fruit peel extract in graded doses. The results revealed that the distribution was normal. The results of the subsequent comparison between groups are shown in Table 3. There was a significant difference ($p < 0.05$) between groups K1, P1, P2, and P3 compared to K2 in the level of MDA. Therefore, the results showed that the administration of red dragon fruit dried extract improves MDA levels induced by heat stress. However, no reduction in MDA levels was observed when the fruit extract dose was increased to 400, 800, or 1,600 mg/kg BW. Heat stress can impair cellular antioxidant enzyme activity and oxidative stress markers, leading to dehydration and cell damage.[10] Furthermore, the activation of heat stress-induced receptors results in tissue damage.[11] Protein carbonyl due to secondary lipid peroxidation causes high lipid oxidative stress and affects protein oxidation.[12] Damage to the cell membrane and lipid peroxidation can increase oxidative stress. Meanwhile, an elevated level of MDA is one of the established biomarkers of oxidative stress.[13]

Table 1: Average body weight of Sprague Dawley rats.

Group	Body weight (g)	Normality test	*p**
K1	198.20 ± 19.318	0.716	
K2	205.20 ± 36.465	0.481	
P1	201.20 ± 12.153	0.653	0.064
P2	167.00 ± 19.455	0.836	
P3	205.60 ± 15.852	0.388	

K1: Standard feed; K2: Standard feed, heat exposed; K3: Heat exposed, RDF 400 mg/kg BW; K4: Heat exposed, RDF 800 mg/kg BW; K5: Heat exposed, RDF 1,600 mg/kg BW; RDF: Red dragon fruit peel extract treatment; *: One-way ANOVA.

Table 2: Level of MDA between experimental groups

Group	Level of MDA (ng/mL)	Normality test	*p**
K1	19.50 ± 0.85	0.716	
K2	49.30 ± 3.70	0.481	
P1	20.65 ± 4.19	0.653	0.064
P2	20.37 ± 2.63	0.836	
P3	22.44 ± 6.68	0.388	

P1: Dragon fruit peel extract (400 mg/kg BW) treatment group; P2: Dragon fruit peel extract (800 mg/kg BW) treatment group; P3: Dragon fruit peel extract (1,600 mg/kg BW) treatment group. The treatment was administered for 14 days. On the 15th day, the K2, P1, P2, and P3 groups were subjected to heat-stress exposure; *: One-way ANOVA.

Table 3: MDA level of difference between groups

Variable	K1	K2	P1	P2	P3
K1	-	<0.001*	0.968	0.946	0.854
K2	-	<0.001*	<0.001*	0.001*	0.001*
P1	-	1.000	0.984	P1	0.984
P2	-	-	-	-	0.960
P3	-	-	-	-	-

K1: Standard feed; K2: Standard feed, heat exposed; K3: Heat exposed, RDF 400 mg/kg BW; K4: Heat exposed, RDF 800 mg/kg BW; K5: Heat exposed, RDF 1,600 mg/kg BW; RDF: Red dragon fruit peel extract treatment; *: Post Hoc analysis, significant level at p<0.05

The levels of MDA in rats exposed to heat stress in the K2 group were higher than in the K1 group, according to the measurements. The K2 group had a 252.82% higher average MDA level than the K1 group. This study shows that heat stress can induce the production of lipid peroxidation as an inflammatory response due to free radicals. Under this condition, lipid peroxidation and protein oxidation occurred in large amounts in the experimental animals. The generation of peroxyl radicals is mediated by lipid hydroperoxides, which are formed when oxygen is coupled with lipids.[14] The cells of heat-stressed rats showed signs of malfunction. The antioxidant components in red dragon fruit, namely phenol and flavonoid compounds, efficiently inhibit lipid oxidation by counteracting free and peroxide radicals. Therefore, the high content of betacyanins, polyphenols, and ascorbic acid in red dragon fruit boosts oxidative defense.[15] MDA levels were higher in the K2 group exposed to heat stress without red dragon fruit peel extract than in the P1 group. The K2 group differs significantly in MDA levels from those in the P2 and P3 groups. This result indicates that administration of this extract to Sprague Dawley rats in groups P1, P2, and P3 can reduce MDA levels to levels similar to those in group K1. This implies that

MDA, a lipid peroxidation product, is capable of destroying DNA, proteins, enzyme activity, and the initiator of cell death. Lipid peroxidation, which occurs in two ways, nonenzymatic and enzymatic, is a threat to the cell membrane.[16] MDA levels were greater and lower in the P1 group than in the P2 and P3 groups. Furthermore, the MDA levels in the P2 group were lower than in the P3 group. There was no significant difference between groups P1, P2, and P3. This implies that at 400 mg/kg BW, the administration of red dragon fruit peel extract significantly reduced the MDA levels in the heat-stressed group.

In another study, the red dragon fruit peel extract was employed to ensure optimum filtration and concentration.[17] The phenolic and flavonoid components may be optimally bound using 96% ethanol as a solvent. Antioxidant activity is enhanced by increased phenolic and flavonoid content. This is the first study to look at the impact of different red dragon fruit extract dosages on MDA levels after acute heat stress. Therefore, the effect of red dragon fruit on heat stress established in this study may have clinical implications in the future. However, there are some limitations to this study. First, the presence of phenolic acid compounds was not investigated before the study. This is because the red dragon fruit's crude ethanolic extract has not been determined. Second, this study focused particularly on oxidative biomarkers to better understand how stress is produced. Also, it needs to include more markers to fully comprehend the role of oxidative stress in heat-stressed rats. Finally, histological evaluation post-treatment was not performed. As a result, the histological and immunohistochemical examinations can be used to further investigate the oxidative process and offer more quantitative measurements.

Conclusion

The findings of this study reveal that the red dragon fruit extract improves lipid peroxidation MDA in acute heat stress-induced rats, however, the effect is not dose-dependent.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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