

The effect of red cabbage extract to serum MDA levels in rats after maximum physical activity

Nadia Husna Haris¹, Trilaksana Nugroho², Astika Widy Utomo², Yora Nindita^{2*}

¹Undergraduate Student of Faculty of Medicine, Universitas Diponegoro, Semarang, ²Department of Pharmacology, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia

<https://doi.org/10.22146/ijpther.1117>

ABSTRACT

Submitted: 27/12/2020

Accepted : 30/09/2021

Keywords:

red cabbage extract;
maximum physical
activity;
MDA;
antioxidant;
oxidative stress;

The maximum physical activity led to generate of free radical levels in the human body called oxidative stress. Red cabbage is well known as the vegetable containing highest anthocyanin among various other head cabbage plants. Anthocyanin is an antioxidant which is proven can prevent oxidative stress through reduction of MDA levels. The study aimed to investigate the effect of red cabbage extract (RCE) on MDA levels in rat after doing maximum physical activity. It was a true experimental study with post-test only control group design. Twenty-four male albino Wistar rats were used in this study. The rats were randomly divided into four groups i.e. the rats receiving maximum physical activity without and with RCE administration at dose of 86 mg/kg BW for eight days and the rats not receiving maximum physical activity without and with RCE administration. A swim test was used for maximum physical activity intervention. Serum MDA levels were measured using TBARS method. Data were analysed using one-way Anova followed by post-hoc LSD. A p value <0.005 was considered to be significant. The results showed that the rats treated with RCE both receiving maximum physical activity or not showed significant MDA reduction compared to rats without treated RCE (p=0.011 and p=0.027, respectively). Meanwhile, there was no significant difference in serum MDA levels between rats treated with RCE receiving maximum physical activity and rats not treated with RCE and not receiving maximum physical activity (p=0.540). Supplementation of RCE can reduce serum MDA levels in rats with and without maximum physical activity. Further study to investigate effective dose of RCE is recommended.

ABSTRAK

Aktivitas fisik maksimal menyebabkan timbulnya kadar radikal bebas dalam tubuh manusia yang disebut stres oksidatif. Kubis merah dikenal sebagai tanaman yang mengandung antosianin paling tinggi sebagai antioksidan di antara berbagai tanaman kubis kepala lainnya. Antosianin adalah antioksidan yang terbukti dapat mencegah stres oksidatif melalui penghambatan peningkatan kadar MDA. Penelitian ini bertujuan untuk mengkaji efek ekstrak kubis merah dalam menurunkan kadar MDA darah tikus setelah melakukan aktivitas fisik maksimal. Penelitian eksperimen murni menggunakan *post-test only control group design*. Dua puluh empat tikus putih Wistar digunakan dalam penelitian ini. Tikus dibagi secara acak dalam empat kelompok yaitu tikus yang menerima aktivitas fisik maksimal tanpa dan dengan pemberian ekstrak kubis merah (RCE) dengan dosis 86 mg/kg BB selama 8 hari dan tikus yang tidak menerima aktivitas fisik maksimal tanpa dan dengan pemberian RCE. Tes berenang digunakan untuk intervensi aktivitas fisik maksimal. Kadar MDA serum diukur menggunakan metode TBARS. Data dianalisis menggunakan Anova satu jalan dilanjutkan *post-hoc* LSD. Nilai p <0,005 dianggap signifikan. Hasil penelitian menunjukkan tikus diberi RCE baik yang menerima aktivitas fisik maksimal ataupun tidak menunjukkan penurunan kadar MDA serum nyata dibandingkan dengan tikus tanpa RCE (p=0,011 dan p=0,027). Sementara itu, tidak terdapat perbedaan bermakna kadar serum MDA antara tikus yang mendapat aktivitas fisik maksimal dengan RCE dan tikus yang tidak mendapatkan aktivitas fisik maksimal tanpa RCE (p=0,540). Suplementasi RCE dapat menurunkan kadar MDA serum tikus dengan dan tanpa aktivitas fisik maksimal. Penelitian lebih lanjut untuk menentukan dosis efektif RCE dianjurkan.

*corresponding author: nindita.yora@fk.undip.ac.id

INTRODUCTION

Humans always conduct physical activity every day. Mild or moderate physical activity has many benefits for health such as reduce the risks of cardiovascular disease and other chronic diseases include diabetes mellitus, cancer, obesity, hypertension, bone, and joint diseases. Meanwhile, strenuous physical activity can cause excessive fatigue, injury, sleep disorder, and overtraining syndrome which is caused by oxidative stress.¹ Oxygen consumption increases 100-200-fold during strenuous physical activity. Increased oxygen consumption results in increased free radicals production.²

Excessive free radical levels in the body cause a phenomenon called oxidative stress. This process has a major role in the development of chronic and degenerative diseases; such as cancer, autoimmune diseases, aging, cataracts, rheumatoid arthritis, cardiovascular disease, and neurodegenerative diseases.³ One of the indicators used to determine oxidative stress is malondialdehyde (MDA) levels which is the result of lipid peroxidation in the body due to free radicals.⁴

The human body has various physiological mechanisms to neutralize oxidative stress.⁵ However, additional antioxidants from outside the body are required to neutralize excess free radicals.⁶ One of which can be obtained from plants containing anthocyanin pigment like red cabbage. Red cabbage contains the highest levels of antioxidants among various other head cabbage plants.⁷ However, the study of red cabbage extract supplementation with maximum physical activity in rats has not been investigated. This study was conducted to evaluate the effect of red cabbage extract supplementation on physical activity in rats.

MATERIALS AND METHODS

Materials

Red cabbage was obtained from supermarkets in Semarang, Central Java,

Indonesia and ethanol 70% were used for the maceration process. Male Wistar rats were used for *in vivo* study. Stat Fax 3300 Spectrophotometry, centrifuge, thiobarbituric acid (TCA) 15% and TBA trichloroacetate (TBA) 0.37% reagent were used for MDA level measurement.

Maceration process

The red cabbage was dried at 45 – 50°C and grounded to obtain dry powder. Extract was prepared by maceration. Twenty-five g of the dry red cabbage powder was placed inside a vessel and added 50 mL of ethanol 70%. The vessel was then closed, kept at room temperature for 24 h and shaken periodically to ensure complete extraction. The mixture was filtered and the filtrate was separated. The residue was then remacerated with ethanol 70% several time until the uncolor filtrate obtained. The filtrates were collected and rotary evaporated to obtain dray extract.⁸

In vivo study

A total of 24 healthy male albino Wistar rats weighing 150-220 g were acclimated for seven days. The rats were divided into four groups randomly; i) Normal rats group (N), rats were given standard feeding and CMC-NA 1% for 8 days, without maximum physical activity; ii) Physical activity control rats group (PC), rats were given standard feeding, CMC-NA 1% for 8 days, and maximum physical activity on day-9th; iii) Negative control rats group, (NC), rats were given standard feeding and 86 mg/100g BW rats cabbage extract (RCE) for 8 days, without maximum physical activity; iv) red cabbage rats group (RC), rats were given standard feeding, 86 mg/100 g BW RCE for 8 days, and maximum physical activity on day-9th. Serum MDA level of rats were measured after the treatment.⁹

Maximum physical activity

The maximum physical activity for rats was swimming. The swimming process was carried out in the pool made of aluminium with a diameter of 1.5 m. The maximum physical activity

of swimming was indicated by signs of fatigue such as almost all the body sinking except the nose, weakened limbs movement, and decreased reaction time. The swimming duration was 50 min.⁸

MDA level measurement

Serum MDA level measurement was done with thiobarbituric acid reactive substances (TBARS) method and carried out at the Biochemistry Laboratory, Faculty of Medicine, Universitas Diponegoro, Semarang. Blood sampling was taken through the medial canthus of the orbital sinus. Three mL of rat blood was put into a centrifuge tube coated with EDTA solution and centrifuged at 3000 rpm for 30 min. Serum was taken and then reacted with TCA 15% and TBA 0.37%. Then it was incubated for 60 min at 95°C. The sample was then centrifuged again at 3000 rpm for 15 min. The supernatant formed was taken and put into a cuvette to read its absorbance on a Stat Fax 3300 spectrophotometer with a wavelength of 545 nm. The serum

MDA level is obtained by the following equation.¹⁰

Data analysis

Data were presented as mean \pm standard deviation (SD) and analyzed using one way analysis of variance (ANOVA) continued by post-hoc LSD by Using IBM SPSS 25 for windows. A p values <0.05 was considered significant.

Ethics statement

The protocol of study had been approved by the Ethical Committee for Health Research, Universitas Diponegoro, Semarang with No.23/EC/H/FK-RSDK/IV/2018.

RESULTS

During the study, one rat in normal group (N) was died. However, until the end of the study, the number of samples still met the WHO requirements stating that at least 5 samples in each group. The serum MDA levels of all groups were presented in the FIGURE 1.

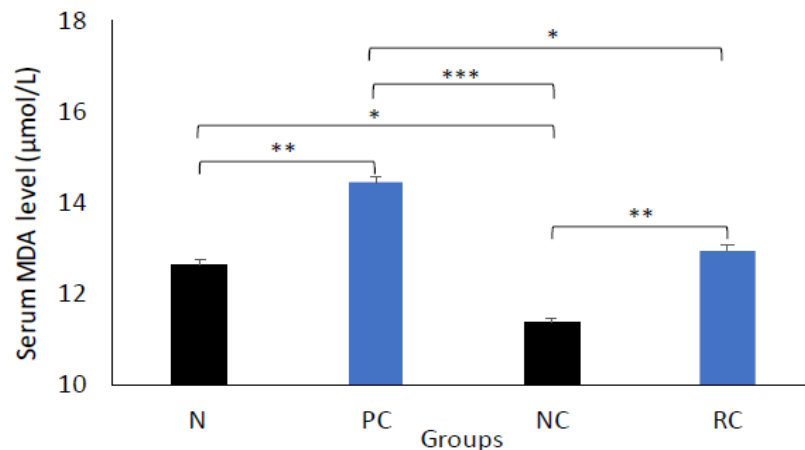


FIGURE 1. Serum MDA level (mean \pm SD) of normal rats group (N), physical activity control rats group (PC), negative control rats group (NC), and red cabbage rats group (RC). * $p<0.05$, ** $p<0.01$, *** $p<0.001$

The highest serum MDA level ($14.45 \pm 0.40 \mu\text{mol/L}$) after intervention was found in the rats receiving maximum physical activity only (PC). In contrast, the lowest serum MDA level after treatment ($11.36 \pm 0.17 \mu\text{mol/L}$) was found in the rats receiving RCE only without maximum physical activity (NC). The serum MDA level of N and RC groups were 12.63 ± 0.38 and $12.95 \pm 0.45 \mu\text{mol/L}$, respectively.

One-way ANOVA analysis showed there was significant difference among group ($p < 0.001$). RC showed significantly higher serum MDA level compared to NC ($p = 0.007$), and RC showed significantly lower serum MDA level compared to PC ($p = 0.011$). No significant difference was found between the N and RC ($p = 0.540$). Meanwhile, NC showed significantly lower MDA level compared to N ($p = 0.027$).

DISCUSSION

This study showed no significant adverse effect and death of rats receiving oral supplementation of 86 mg/100g BW RCE. The previous study has investigated no mortality of RCE up to a dose 5000 mg/kg BW, hence the lethal dose 50% (LD_{50}) of RCE was higher than 5000 mg/kg BW. The study also found non side effect in dose 2000 mg/kg BW for RCE supplementation.¹¹ Therefore, the RCE supplementation is considered safe for use in human.

The NC group showed a significant reduction of MDA level compared to N group. This indicated that red cabbage has the effect to reduce MDA levels in rats without maximum physical activity. The RC group also showed a significant reduction of MDA level compared to PC group after maximum physical activity. This indicated that the red cabbage extract could reduce MDA level in rats with maximum physical activity. Furthermore, the RC and N group showed no significant difference which indicating that RCE could maintain MDA level in rats receiving maximum physical activity similar to normal rats.

A previous study had found that the risk reduction of chronic illness has been

linked with consuming of red cabbage.¹² The red cabbage also showed potential therapy to treat diseases leading cause by oxidative stress.¹³ In our study, red cabbage also has the potential effect to reduce stress oxidative level for daily consumption in normal conditions. Normally, free radicals are produced during physiological cellular function of all living beings.¹⁴

The maximum physical activity led to generation of excessive oxidative stress. A previous study had found that exhaustive swimming led to increase in O_2^- generation in liver, skeletal muscle, and blood respectively. Excessive reactive oxygen species (ROS) generation causes an increase in plasma MDA level.¹⁵ Thus, supplementation of red cabbage may protect organ from damaged due to excessive ROS generation.

RCE has antioxidant properties because it contains high concentration of anthocyanin, vitamin C, beta carotene, and vitamin A.¹⁶ Anthocyanins are water-soluble colored pigments belonging to the phenolic group. Among the anthocyanin pigments, cyanidin-3-glucoside is the major anthocyanin found in most of plants.¹⁷ Previous study had found that cyanidin was only found in red cabbage compared to others head cabbage, 44.53 mg/100 g and the antioxidant activity measuring by DPPH assay of red cabbage reaching for 69.82%.¹⁸

Anthocyanins act directly and indirectly to reduce the risk of several diseases. The direct pathway is through scavenging free radicals in the electron-transfer reaction pathway, it can bind superoxide radical (O_2^-), hydroxyl ($\cdot\text{OH}$), and peroxy-fatty acid radical ($\text{ROO}\cdot$).¹⁶ Moreover, Anthocyanins also scavenge free radicals by attacking hydroxyl groups of the B-ring of the anthocyanin structure and oxonium ion on the C-ring.¹⁹ On the other hand, the indirect pathways of anthocyanin are through down-regulation of cell proliferation and apoptosis.²⁰

A previous study that underlines the anthocyanin effect from another plant such as purple potato showed that it can reduce MDA level, caspase-3 expression,

and improves spatial memory function.²¹ Moreover, study from Herawari *et al.* also showed anthocyanin extract from purple potato could reduce MDA level in blood, liver, and renal system.²² Jawi *et al.*²³ also found significant reduction of MDA level in mice after maximum physical activity receiving purple sweet potato. Thus, the finding of MDA level reduction in our study that underlines anthocyanin from red cabbage supplementation is in coherence with previous research.

Besides anthocyanin, β -carotene acts in liposomes inhibit indirectly radical species and lipid peroxidation.²⁴ Its mechanism is through the addition of chain-propagating peroxy radical to the conjugated polyene system of the carotene. The trapping mechanism can happen under low oxygen pressure.²⁵ Vitamin A can increase immunity and help repair tissue damage so that it can repair the damage caused by free radicals. The antioxidant activity of carotenoids and vitamin A is due to polyene units that has hydrophobic chain. It can suppress singlet oxygen, neutralize radicals and combine with and stabilize peroxy radicals.²⁶ Vitamin C as an antioxidant can protect against lipid peroxidation. Its mechanism is through scavenger of ROS and by one-electron reduction of lipid hydroperoxy radicals. Thus, vitamin C can react directly with superoxide, hydroxyl anion, and fatty acid hydrogen peroxides. The mechanism as an antioxidant is by giving hydrogens from its hydroxyl group.²⁷ For that reason, red cabbage is one of the plants that have strong antioxidant activity and its supplementation may protect from free radicals.

There were several limitations of this study. First, there was no quantitative or qualitative measurement of anthocyanin an antioxidant level of RCE. Second, the MDA level measurement was not determined by current standard curve but by standard laboratory equation.

CONCLUSION

Supplementation of RCE can reduce serum MDA level in rats with and

without maximum physical activity. Further study about the quantitative measurement of ethanolic extract of red cabbage and higher sensitivity measurement of MDA level are needed to improve the study. Moreover, it also needs to investigate the effective dose of red cabbage extract.

ACKNOWLEDGEMENT

We would like to thank all those who have helped and facilitated the implementation of this research.

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