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Beetroot Powder *(Beta vulgaris L.)* Decrease Oxidative Stress by Reducing of Malondialdehyde (MDA) Levels in Hyperuricemia

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ABSTRACT

Introduction: Hyperuricemia is a metabolic disease associated with lifestyle habits, age, sex, and inherited factors. High uric acid levels in a hyperuricemia state cause oxidative stress that can lead to increased malondialdehyde (MDA) levels. Beetroot powder has the potential as a functional food rich in antioxidants that can reduce MDA levels. This study aims to determine the beetroot powder effect on MDA level reduction in hyperuricemia rat models. **Methods:** This research was an experimental study with fifteen hyperuricemia rats were divided into three groups by randomized pre-post control group design. They were divided into three groups: P1 were healthy rats + standard feed (comfeed), P2 were hyperuricemia rat models + standard feed (comfeed) and P3 were hyperuricemia rat models + beetroot powder 3,12 g/kg/bw for 14 days. The MDA level examinations were conducted on day 0 and day 14. **Results:** After 14 days of treatment, the P3 group has a significant decrease in MDA levels compared to day 0 ($p = 0.042$). Groups P1 and P2 have a significant increase in MDA levels ($p = 0.043$). MDA levels between groups were significantly different ($p = 0.002$) on day 14. **Conclusion:** Giving beetroot powder for 14 days with dose 3,12 g/kg/bw for 14 days significantly reduced MDA levels in hyperuricemia.

Keywords: Beetroot Powder, Malondialdehyde, Hyperuricemia

JEL Classification Codes: I10, I19, I12

INTRODUCTION

Indonesia is a country that has the highest prevalence of hyperuricemia in Asia with a prevalence of 24.35%, followed by Thailand 18.4%, China 15.4%, and Saudi Arabia 8%. This figure can increase with age(Gosling et al., 2014; Mehmood et al., 2017). The purine catabolic metabolic process in the human body produces uric acid as a byproduct. Due to changes in consumption patterns of purine-rich foods, genetic, and environmental variables, the prevalence of hyperuricemia is gradually increasing over the world (Chatzipavlou et al., 2014; von Lueder & Girerd, 2016)

Hyperuricemia is a metabolic illness that is linked to age, sex, lifestyle behaviors, and genetic factors. Hyperuricemia has been linked to high blood pressure, diabetes, dyslipidemia, kidney failure, obesity, and liver disease. Hyperuricemia can be caused by an unhealthy lifestyle, such as a diet high in purine nucleotides, protein, alcohol, and carbohydrates. (Zhou et al., 2018).

Increased inflammatory protein expression was triggered by high uric acid levels, which triggered a complex pro-inflammatory cascade that can damage cells and tissues. In this process, oxidative stress is quite important. Through complicated inflammatory signaling

pathways, uric acid contributes to the activation of inflammatory responses in vascular endothelial cells (Cai et al., 2017; Chen & Lan, 2017).

Increased uric acid levels can cause oxidative stress and the formation of reactive oxygen species (ROS) in vascular endothelial cells. Massive ROS can boost TNF-expression and IL-6 regulation. MDA and uric acid are linked. MDA is a lipid peroxidation product that may be easily identified in blood or plasma and is used as an indicator of oxidative stress (Q. Li et al., 2017; Sarvaiya et al., 2015). Antioxidant activity has been shown in several studies to alleviate oxidative stress. Disorders associated with oxidative stress, such as hyperuricemia, can be prevented by reducing oxidative stress because of their antioxidant and anti-inflammatory action in healing oxidative damage.

Ascorbic acid, carotenoids, phenolic acids, flavonoids, betalains, saponins, polysaccharides, and tannins are among the phytochemicals found in beetroot. Several highly bioactive phenolics found in beets, such as rutin, epitaxin, and caffeic acid, are also recognized to be effective antioxidants. (Chawla H, Parle M, Sharma K, 2016; Clifford et al., 2015). Beetroot is processed into powder products because beetroot powder contains Vitamin C and Total Antioxidant Potential (TAP) (Pierucci et al., 2016).

Beetroot powders (95.31%), was reported to have a higher total antioxidant content when compared to other processed beet products such as cooked beetroot (85.79%) and beetroot juice (80.48%) (Chhikara et al., 2019). To make tomato pastes, soups, sauces, sweets, jams, jellies, ice creams, and morning cereals redder, beetroot powder or extracted pigments are utilized. (Gamila et al., 2013).

Research conducted by El Gamal et al., (2014) In gentamicin-induced rats, beetroot ethanol extract reduced oxidative stress (MDA and uric acid) and increased antioxidant activity. Furthermore, betalain-containing beetroot ethanol extract has been shown to block the xanthine oxidase enzyme (Putri et al., 2021). This study was conducted to determine the effect of giving beetroot powder on MDA levels in hyperuricemia rats induced by potassium oxonate.

RESEARCH METHOD

This research is a laboratory experimental study with a randomized pre-post test control group design. The sample in this research used was male rats *Sprague-Dawley strain with* inclusion criteria (the rats were 7 to 8 weeks of age and body weight from 180-220 grams) and exclusion criteria (the rats were sick and died during the treatment).

The rats cage made of stainless steel (long; 20 cm, wide; 30 cm, high; 17 cm) was regulated by room temperature (24 – 28^{o}C) and humidity (70 - 75%). The light reception in the maintenance room is set for 12 hours of light and 12 hours of darkness. The rats were kept in a room with a temperature of 24-28^{o}C and a humidity of about 70-75%. The light reception in the maintenance room was set for 12 hours of light and 12 hours of darkness. The feed given was standard *comfeed* and drinking water *ad libitum.* The conditions for rats to be used as experimental animals are; 1) Mice must be free from pathogenic germs, because if there are pathogenic germs it will interfere with the course of the experiment in the study, 2) have the ability to provide a good immune reaction, 3) sensitivity to a disease 4) nutrition, maintenance, hygiene, and health of mice must be maintained properly (Tolistiawaty et al., 2014).

The procedure for making hyperuricemia rats was carried out by injecting potassium oxonate at a dose of 250 mg/kg body weight/day intraperitonial for 14 days. The number of samples for each treatment group was determined using the Federer formula (Aprillinda et al., 2018). A total of fifteen hyperuricemia rats were divided into three treatment groups based on the simple random sampling method as follows: (P1) healthy rats + standard

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feed (comfeed), (P2) hyperuricemia rats + standard feed (comfeed), (P3) hyperuricemia rats + beetroot powder 3,12 g/kg body weight.

The beetroots used in this reasearch was obtained from Boyolali and age of 6 weeks. Making of beetroot powder began by sorting, trimming, washing, reducing size, drying, milling and sieving with an 80-mesh sieve size. Beetroot powder is given via gastric swab once a day in the morning at a dose of 3,12 g kg body weight for 14 days. The stages of making beetroot powders are as follows:
1) Sorting
Sorting is the process of selecting materials according to the desired quality.
2) Trimming
Trimming aims to separate the outer skin. This process uses a knife
3) Washing
The washing process uses running water which aims to separate the dirt that sticks to the beetroot

A simple spectrophotometric process converts one molecule of MDA into two molecules of 2-thiobarbituric acid, which is the basis for the TBARS test. At a pH of 2-3, this reaction happens. TBA produces a chromogen-pink color that can be spectrophotometrically evaluated. In addition to evaluating MDA levels produced by the lipid peroxidation process, the TBA test can also detect additional aldehyde products, such as non-volatile compounds produced by the heat generated during the measurement of actual serum MDA levels. Plasma, tissue, and urine can all be tested for MDA levels. The colorimetric approach with a spectrophotome is a frequently used TBA measurement method. The TBA test in addition to measuring the levels of MDA formed due to the lipid peroxidation process, the TBA test can also measure other aldehyde products including non-volatile products that occur due to heat generated when measuring the actual serum MDA levels. MDA levels can be checked in plasma, tissue, and urine. The TBA measurement method that is often used is the colorimetric method with a spectrophotometer.

The Measurement of MDA levels can be done using the TBARS method in the following way:
1) A total of 4 ml of blood samples were put into the tube and centrifuged at 3000 rpm for 30 minutes at 400C.
2) The blood sample that has been centrifuged is then taken as much as 200 l of serum and put into an empty centrifuge tube.
3) Add 2000µl of 15% TCA solution.
4) Add a solution of 0.37% TBA in 0.25 N HCl as much as 2000µl
5) Heat in a water bath at 95oC for 60 minutes.
6) Cool at room temperature on an ice bath for 15 minutes.
7) Centrifuge for 15 minutes at 3000 rpm.
8) Read the absorbance of the supernatant with a spectrophotometer at a wavelength of 532 nm

The MDA level measurement was conducted twice, namely before the 0^{th} day of treatment and after the 14^{th} day of treatment. The MDA level examinations were conducted at the Laboratory of the Center for Food and Nutrition Studies, UGM, Yogyakarta (which is ISO/IEC 17025: 2000 certified). The data were analyzed using the *Willxocon* test and *Kruskal Wallis*.

RESULTS AND DISCUSSION

MDA Levels in Hyperuricemia Rats

Based on Table 1, on day 14 the result shows that the P3 group has a significant decrease in MDA levels compared to day 0 ($p = 0.042$). Groups P1 and P2 have a significant increase in MDA level ($p = 0.043$). MDA levels between groups were significantly different

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(p = 0.002) on day 14. The average reduction in MDA levels in the P3 group ranged from -2.09 ± 0.28 nmol/mm, while in the P1 group there was an increase of 0.13 ± 0.07 nmol/mm. In the P2 group, there was an increase of 0.25 ± 0.10 nmol/mm.

Table 1. Results of MDA Level on Day 0 and Day 14

Group	Day 0 (Mean ± SD) (nmol/mm)	Day 14 (Mean ± SD) (nmol/mm)	ΔMDA (nmol/mm)	P[a]
P1	1.02±0.12	1.11±0.15	0.13±0.07	.042*
P2	7.55±0.32	7.81 ±0.21	0.25±0.10	.043*
P3	7.77±0.23	5.67±0.14	-2.09±0.28	.043*
P[b]	0.007*	0.002*	0.004*	

Note: * There is a significant difference as p<0.05 (*Willxocon*[a] test and *Kruskal Wallis*[b] test)

Beetroot, known by the scientific name Beta vulgaris, is a plant belonging to the dicotyldenous family. The following is a taxonomy of red beet plants (Chawla H, Parle M, Sharma K, 2016).

Kingdom : Plantae
Subkingdom : Tracheobionta
Super division: Spermatophyta
Division : Magnoliophyta
Class : Magnoliopsida
Subclass : Caryophyllidae
Order : Caryophyllates
Family : Chenopodiaceae
Genus : Beta
Species : B. vulgaris

Beets are native to the Mediterranean coast (Sea beets). Beet was produced by the Romans in the 15th century, and the leaves were frequently consumed. Beets ate not only the leaves but also the roots at the end of the 15th century. Seeds for beets are planted 1.5 inches deep and spaced 3-5 inches apart. It takes 5-10 days for seeds to germinate. Beets are picked in the summer after the leaves have dried out and the roots have grown to a diameter of 2.53 inches. Beetroot may be kept for a long time in cold, humid conditions. (Chawla H, Parle M, Sharma K, 2016).

Table 2. Beetroot Content in 100 Grams

Nutrient Content	Content per 100 g	Unit
Energy	43	Cal
Carbohydrate	9,56	g
Fat	0,17	g
Protein	1,61	g
Fiber	2,80	g
Cholesterol	0	mg
Calcium	16	mg
Copper	0,075	mg
Iron	0,80	mg
Magnesium	23	mg
Manganese	0,329	mg
zinc	0,35	mg
Potassium	325	mg
Sodium	78	mg

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Vitamin A	33	IU
Thiamine (Vitamin B1)	0,031	mg
Riboflavin (Vitamin B2)	0,057	mg
Niacin (Vitamin B3)	0,334	mg
Folic acid	109	µg
Vitamin C	4,9	mg
Vitamin E	0,04	µg
Vitamin K	0,02	µg
pantothenic acid	0,155	mg
Pyridoxine	0,067	µg
Beta carotene	20	µg
Betaine	128,7	mg
Lutein-zeaxanthhin	0	µ

Source: Chawla *et al.,* (2016).

By stimulating rats with potassium oxonate, a rat model of hyperuricemia was created. Potassium oxonate is a reagent that inhibits the enzyme urate oxidase, resulting in hyperuricemia. The mechanism of potassium oxonate in increasing uric acid levels is to prevent uric acid from becoming allantoin. Allantoin is water soluble so that by inhibiting the uricase enzyme by potassium oxonate, uric acid will accumulate and not be eliminated in the form of urine (Kensara, 2013). Injections of potassium oxonate resulted in a decrease in total serum antioxidant capacity and an increase in blood MDA levels in rats.

The sample of this study used male white rats of the Sprague-Dawley strain. Male rats were chosen with the consideration that male rats do not have the hormone estrogen. The hormone estrogen helps excrete uric acid through the urine so that uric acid levels are generally normal. Men do not have high levels of the hormone estrogen in their blood so that uric acid is difficult to excrete through urine and the risk is that their blood uric acid levels can become high. Hormonal conditions in male rats are more stable than female rats, because female rats experience changes in hormonal conditions during certain periods such as during pregnancy, the estrus cycle and breastfeeding which can affect the psychological condition of the test animals. The stress level in female rats is also higher than that of male rats, which can interfere with the test. Animals were adapted for 7 days to reduce stress levels (Suhendi et al., 2011).

Hyperuricemia is divided into two types, according to Singh, V Gomez, (2014): primary or idiopathic hyperuricemia and secondary hyperuricemia.
a. Primary or Idiopathic Hyperuricemia is hyperuricemia whose cause is unknown, it can be due to genetic disorders, and there are no obvious physiological or anatomical abnormalities. When hyperuricemia occurs without the presence of a concurrent condition or medicine that affects uric acid synthesis or excretion, it is referred to as primary hyperuricemia, and it is usually lifelong.
b. Secondary hyperuricemia is a situation in which other diseases, medicines, food products, or toxins cause excessive urate production (increased production) or impaired renal clearance.

The following factors affect hyperuricemia:
1) Food Factor
Purines are organic base compounds that make up nucleic acids and are included in the amino acid group, the building blocks of proteins. Foods that contain high purines (150 – 180 mg/100 g) such as organ meats, sea food, beans, spinach, mushrooms, cauliflower, and sardines, alcoholic beverages. Men who consume beef or goat meat can increase the risk of gout by 21% (Chilappa et al., 2010).

Drinks containing fructose and chemical sweeteners may increase the risk of gout, while dairy products, coffee, and vitamin C may help to prevent gout. By increasing purine nucleotide degradation, fructose serves as a substrate for uric acid synthesis. Alcohol is considered to alter uric acid levels by producing a purine metabolic substrate in the form of guanosine (specifically beer), increasing nucleotide turnover, and interfering with renal urate production through lactic acidosis (Roddy and Choi, 2014).

2) Medicines

Diuretic drugs (furosemide, thiazides, low-dose salicylates, cyclosporine, pirinamide, ethambutol, levodopa and nicotinic acid and hydrochlorothiazide), cancer drugs, vitamin B12 can increase the absorption of uric acid in the kidneys and can decrease the excretion of uric acid in the urine. Several antihypertensive treatments have been shown to have an effect on serum urate levels. -blockers raise urate levels in the blood (Roddy & Choi, 2014).

3) Metabolic Syndrome

Hypertension, obesity (BMI 25kg/m2), insulin resistance, dyslipidemia, and hyperuricemia are all markers of the metabolic syndrome, which can raise uric acid levels.

4) Age.

Adult men aged 30 years and women who have experienced menopause or aged 50 years are at risk for hyperuricemia because at that age women experience impaired production of the hormone estrogen.

High uric acid levels can increase the expression of inflammatory proteins by causing a complex proinflammatory cascade to occur, resulting in cell and tissue damage In this process, oxidative stress is thought to play a significant role(Chen & Lan, 2017). Through complicated inflammatory signaling pathways, uric acid leads to the development of inflammatory reactions in vascular endothelial cells. Increased uric acid levels in the blood can improve the shape and function of vascular endothelial cells, causing leukocyte and hormone adhesion as well as the production of proinflammatory cytokines, which can activate inflammatory chain reactions that lead to endothelial dysfunction (Cai et al., 2017).

Purine catabolism and production are largely consistent between 300 and 400 mg per day. In humans, the end product of purine metabolism is uric acid, which is excreted in the urine. A significant number of enzymes are involved in the conversion of two purine nucleic acids, adenine and guanine, to uric acid. Adenosine monophosphate (AMP) is transformed to inosine by one of two mechanisms: Inosine monophosphate is generated by combining two amino groups (IMP), which is then dephosphorylated with a nucleotidase to form inosine, or nucleotidase reduces a phosphate group to form adenosine, which is then deaminated to form inosine. Guanine monophosphate (GMP) is converted to guanosine by nucleotidase. Purine nucleoside phosphorylase convert the nucleosides inosine and guanosine to purine bases hypoxanthine and guanine (PNP). The enzyme xanthine oxidase converts hypoxanthine to xanthine, whereas the enzyme guanine deaminase converts guanine to xanthine. When xanthine is oxidized by xanthine oxidase, uric acid is produced (Jin et al., 2012). Humans are unable to convert uric acid to the more soluble compound allantoin due to a lack of the enzyme uricase. The kidneys are in charge of excreting the majority of uric acid. In other mammals, the enzyme uricase (urate oxidase) transforms uric acid to allantoin. The uricase gene most likely experienced a functional mutation during the early stages of hominoid evolution. As a result, humans and a number of other primates have uricase deficiency (Jin et al., 2012).

Uric acid is a strong Reactive Oxygen Species (ROS) scavenger and antioxidant, as well as a peroxynitrite scavenger. High levels of uric acid can be found in the cytosol of normal human and animal cells, particularly in the liver. If the concentration of urate increases in the blood, the formation of uric acid crystals will also increase. Premenopausal women have normal uric acid levels around 2.6-5.7 mg/dl (155-339 mol/liter) while men and postmenopausal women have uric acid levels around 7.0 mg/dl (208-416 mol/liter) . Mammals such as mice have normal blood uric acid levels of around 1.18-3.56 mg/dl

(Chen & Lan, 2017). Uric acid has a low water solubility; in humans, the typical uric acid concentration in the blood is near to the solubility limit (6.8 mg/dL). Uric acid crystals develop as monosodium urate (MSU) crystals when the uric acid level reaches 6.8 mg/dL. Due to a lack of the enzyme uricase, humans are unable to convert uric acid to the more soluble molecule allantoin. Approximately 75% of uric acid is excreted through the kidneys and 25% is excreted through the gastrointestinal tract (Mehmood et al., 2017).

The inflammatory response triggered by a range of harmful chemicals is complicated by oxidative stress. The majority of Reactive Oxygen Species (ROS) are created in the body. The ability to eliminate ROS is hampered, resulting in an imbalance in the oxidative and antioxidant responses, leading to oxidative stress in a variety of systems. . Elevated uric acid can induce oxidative stress and ROS production in vascular endothelial cells. Massive ROS can upregulate IL-6 and TNF- expression (Newsholme et al., 2016).

Increased lipid peroxidation causes an increase in free radicals and results in an increase in the MDA formation as a free radical marker. Increased uric acid production is also associated with reactive oxygen species (ROS) formation and results in increased lipid peroxidation. Lipid peroxidation produces MDA as a cell membrane damage markers (Sukrama, 2015).

The results of this study showed a decrease in MDA levels in hyperuricemia rat models that were given the intervention of beetroot 3.12 g/kg body weight for 14 days. The antioxidant activity in beetroot is relatively strong so that it can reduce MDA levels (Novatama et al., 2016). MDA is a lipid peroxidation product that may be easily identified in blood or plasma and is used as an indicator of oxidative stress. (L. Li et al., 2017; Sarvaiya et al., 2015). In oxonate-pretreated control rats, a significant increase in serum MDA levels. In rats, potassium oxonate is a selectively competitive uricase inhibitor that inhibits hepatic uricase and causes hyperuricemia (Haidari et al., 2011).

Several studies have identified the active antioxidants within beetroot (*Beta vulgaris L.)* including flavonoids, ascorbic acid, carotenoids, betalains, saponins dan p.henolic acids (Clifford et al., 2015; Wong & Siow, 2015). The betacyanin content in beetroot is 37.64 mg /100 g with antioxidant activity of 79.73 ppm. From these results, it can be concluded that beetroot has relatively strong antioxidant activity.10 The total phenol content in beetroot ranges from 98.08 mg/100 ml and flavonoids 83.34 mg /100 ml. Research reports that beetroot juice contains 98.08 mg/100 ml of phenol and 83.34 mg /100 ml of flavonoids (Olumese & Oboh, 2017). Polyphenols are a group of compounds that are widely revealed in plants. Structurally, this compound is characterized by one or more phenol units. Flavonoids are a component of polyphenols. Hence, flavonoids have the identic antioxidant activity mechanism. The antioxidant activity of polyphenol and flavonoid compounds depends on the number and location of phenolic groups (-OH), which have a role to neutralize free radicals by donating hydrogen atoms (electron donors or hydrogen atoms). Polyphenols and flavonoids donate their hydrogen atoms to reduce free radicals such as superoxide, alkoxyl, peroxyl, and hydroxyl radicals (Musa et al., 2015).

Polyphenols and flavonoids have health benefits and are used in nutraceutical and functional food formulation. Polyphenols and flavonoids from various plants have been reported to have the potential to reduce hyperuricemia disorders by reducing uric acid synthesis by inhibiting xanthine oxidase, *susspress* urate renal reabsorption, and improving uric acid secretion (Mehmood et al., 2017).

Beetroot powder has a vitamin C content of 925.68 mg/100 g. According to in-vivo study, vitamin C has uricosuric (uric acid reducing) properties. Uricosuric characteristics can hold back uric acid reabsorption in the renal tubules, permitting the kidneys to eliminate uric acid more quickly. Vitamin C has been shown to inhibit the lipid peroxidation process, reducing MDA levels significantly (Popovic et al., 2015). This study's results are consistent

with that of Krajka-Kuźniak et al., (2013) and Kujawska et al., (2009) who reported that administering beetroot juice (dosage 8 ml/kg BW/day for 28 days) protects rats from oxidative stress by lowering lipid peroxidation caused by liver injury. El-Gamal et al. found that rats administered beetroot ethanol extract (doses of 250 or 500 mg/kg BW/day for 28 days) had considerably lower levels of proinflammatory mediators, reduced oxidative stress, and enhanced endogenous antioxalate production.

CONCLUSIONS

Giving beetroot powder dose 3.12 g/kg/bw for 14 days significantly reduced MDA levels in hyperuricemia rat model.

Ethical Consideration

The Ethical Committee, Faculty of Medicine, Universitas Sebelas Maret (No.468/UN27,06/KEPK/EC/2019) has given their approval to this study.

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Conflict of Interest

There are no conflicts of interest reported by the author in this study.

Author Contribution

Anggraini Wulandari, the initial author, conceptualizes and designs research, drafts articles, observes experimental animals, and collects and analyzes data. Paramasari Dirgahayu is in charge of field data collection, evaluates and interprets the data, and assists in the compilation and revision of the article. Budiyanti Wiboworini examines the data, does data analysis and interpretation, and assists in the preparation and revision of the manuscript.

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