ABSTRACT

Metabolic syndrome is associated with abnormalities of lipid levels in the blood such as hyperlipidemia. Hyperlipidemia conditions can increase the risk of atherosclerosis and hypertension. Beetroot (Beta vulgaris L.) is a plant that contains high antioxidants. Beetroot has the potency to be used as a functional food that can reduce the potential for atherosclerosis and blood pressure. The aim of this study was to examine the effect of beetroot-enriched feed on the cholesterol level, atherogenic index and blood pressure of rats (Rattus norvegicus Berkenhout, 1769) induced by high fat and fructose diet. As many as 25 rats were divided into control, hyperlipidemia, and three treatment groups. The hyperlipidemia and treatment groups were induced to become hyperlipidemia using AIN93-M modified high fat and fructose feed for 8 weeks. The treatment groups were followed by intervention with 6, 9, and 12% beetroot enriched feed for 6 weeks. The feed was prepared by mixing beetroot flour in the pellets. Blood pressure, total cholesterol, and HDL level measurement was conducted after hyperlipidemia induction and after the intervention. The data analyzed with one-way ANOVA, DMRT, and T-Test. The results showed that 6% beetroot intervention have the highest increasing of HDL-cholesterol than other groups. The 9% beetroot intervention significantly decrease total cholesterol lower than normal baseline, and 12% beetroot intervention significantly decrease blood pressure than other groups. The atherogenic index of all treatment group was decreased. The 9% beetroot enriched feed was seen as an optimum dose to reduce total cholesterol, atherogenic index and blood pressure and increase HDL-cholesterol.

INTRODUCTION

Modern lifestyle encourages continuous consumption of high-calorie foods. Moreover, the high-calorie consumption in this community is not balanced with regular physical activity. According to WHO in 2016, obesity sufferers reached 13% of the world's population (World Health Organization 2021). Obese people have a high possibility to suffer from hyperlipidemia (Purnell et al. 2018). Hyperlipidemia is a group of disorders characterized by excess lipids in the bloodstream which include cholesterol, phospholipids, and triglycerides (Nelson 2013). Increased levels of
fat in the blood can be caused by unhealthy lifestyles such as eating foods that have high fat levels or foods that contain free radicals. Continuous hyperlipidemia will increase the risk of cardiovascular diseases such as atherosclerosis (Pan et al. 2018).

Atherosclerosis can lead to advanced cardiovascular diseases such as strokes and heart attacks. The chance of someone suffering from atherosclerosis can be calculated by the atherogenic index (Othman et al. 2019). Atherosclerosis is also influenced by blood pressure, if blood pressure increases (hypertension) it will increase the potency of atherosclerosis (Lu et al. 2015).

Hyperlipidemia and obesity give rise to a group of other disorders called metabolic syndromes. Metabolic syndrome is a group of disorders that includes central obesity, decrease high density lipoprotein (HDL) levels, hypertension, increase triglyceride levels and fasting blood sugar levels (Rochlani et al. 2017). Metabolic syndrome, especially the rise of blood pressure is closely related to chronic cardiovascular diseases such as coronary heart disease, stroke, and kidney failure (Scuteri et al. 2005).

Treatment of hyperlipidemia and hypertension still rests on the consumption of drugs that have several side effects such as vision problems, erectile dysfunction, fatigue, and insomnia (Tedla & Bautista 2016). Functional foods exist as a therapeutic solution for a disease with minimal side effects. Functional food is food that is not only digested to meet ordinary nutritional needs but contains ingredients that can play a role in preventing or treating certain diseases (John & Singla 2021). Functional food ingredients that can prevent hypertension and hyperlipidemia are those that contain high antioxidants such as beetroot.

Table beet is a common subspecies of beet which is primary used as vegetables. Beetroot is a temperate crop, thus has optimal growth condition in cold weather (Goldman & Navazio 2008). Based on Fardiaz (2014), cultivation of beetroot in Indonesia is carried out in highlands area such as Lembang, Batu, Kopeng, and Pengalengan. However, in Indonesia beetroot belongs to unpopular vegetables, even though it contains several bioactive compounds such as carotenoid, betaine, flavonoid, and polyphenols that could provide many benefits to its consumer. Beta-lain, an antioxidant, is the pigment contained in beetroot (Gokhale & Lele 2014).

Previous research showed that ethanol extract from beetroot can reduce cholesterol levels by inhibiting HMGCR activity. The antioxidants contained in beetroot also have the potential to lower blood pressure by inhibiting ACE. These two antioxidant roles could decrease the risk of atherosclerosis (Al-Dosari et al. 2011). Other study concludes that red beet juice consumption has potential effect as cardiovascular protective agent through reducing inflammation and suppressing oxidative stress mechanism (Singh et al. 2015). As a functional food application, semi-finished material like flour is recommended because it is longer to store, easy to mix and easy to apply (Lingling et al. 2018). Therefore, it is important to study the supplementation of beetroot flour by mixing it into food. The aim of this study to evaluate the effects of beetroot enriched feed in total cholesterol, atherogenic index, and blood pressure of rats induced by high fat and fructose diet.

**MATERIALS AND METHODS**

**Feed Preparation**

Standard feed was composed of modified AIN93-M. In 100 g standard feed contained 62.10 g corn starch, 14 g casein, 10 g sucrose, 4 g corn oil, 5 g α-cellulose, 3.5 g mineral mixture, 1 g vitamin mixture, 0.18 g DL-
methionine, 0.25 g choline chloride, and 0.008 g tertbutyl-hydroquinone. High fat and fructose feed formulated by Lozano (Lozano et al. 2016) contained 21% of trans fat and 25% of fructose for induction phase (HFFD). Beetroot was obtained from traditional market. The beetroot criteria was a fresh, red, and ripped tubers. The clean beetroot was thinly sliced and then heated in oven (50 °C, 8 hours). The dry beetroot was crumbled by disk mill to become a beetroot flour. Beetroot enriched feed (BEF) contained beetroot flour in various percentage compared to the total weight of feed by modifying the standard feed. BEF contained 6% of beetroot flour for dose 1, 9% for dose 2 and 12% for dose 3.

**Animals**
Twenty-five male wistar rats (100-120 g, 4 weeks old) were obtained from Faculty of Pharmacy, Universitas Gadjah Mada. The rats were maintained in a standard environment (22-25 °C and 12 hours light cycle). The rats were acclimatized and fed using standard feed and water *ad libitum* during one-week before beginning the study. Ethics Committee of the Integrated Research and Testing Laboratory, Universitas Gadjah Mada approved all procedures in this study with registration number: 00011/04/LPPT/V/2019.

**Experimental Study**
The rats were divided into 5 groups: N (normal), HFFD (high-fat fructose diet), HFB1 (high-fat beetroot dose 1), HFB2 (high-fat beetroot dose 2), HFB3 (high-fat beetroot dose 3). The normal group (N) was fed by standard diet for 14 weeks. HFFD group was fed by high fat and fructose feed for 14 weeks. HFB1, HFB2, and HFB3 was fed by high fat and fructose feed for 8 weeks of induction then were given oral feed of BEF6% for HFB1, BEF9% for HFB2, and BEF12% for HFB3 during 6 weeks of treatment. In the all-phase rats was feed *ad libitum*

**Lipid Profile and Blood Pressure Analysis**
Blood samples were obtained from sinus retro-orbital, before (pre) and after (post) intervention with injected 50 mg ketamine and 50 mg xylazine per kg body weight intramuscular for anesthesia. Total cholesterol and HDL-cholesterol of serum were analysed with DiaSys Diagnostic Systems GmbH. Blood pressure was analysed with CÖDA Non-invasive Blood Pressure System. Analysis procedure was conducted according to manufacturer’s protocol. Atherogenic index (AI) was measured by HDL-cholesterol (HDL.C) and total cholesterol (TC) level calculated using formula: (TC – HDL.C) / HDL.C (Suanarunsawat et al. 2014)

**Statistical Analysis**
All values are presented as mean ± standard deviation. One-Way ANOVA, DMRT, and T-test were used to analyse total cholesterol, atherogenic index, and blood pressure between each group.

**RESULTS AND DISCUSSION**
Feeding high lipid and fructose for eight weeks was carried out to make hyperlipidemia condition. The induction of hyperlipidemia with the addition of fructose will cause oxidative stress so that it reaches hyperlipidemic conditions faster. It is expected that total cholesterol levels will increase, HDL levels will decrease, the atherogenic index will increase, and blood pressure will increase (Hannou et al. 2018).
HDL-Cholesterol
Beetroot-enriched feed intervention gave a significant increase in HDL levels. It is shown by significant different between pre and post-test of HFB1, HFB2, and HFB3 (Figure 1). HFB1, HFB2, and HFB3 groups were recovered the HDL level close to normal group. Based on Figure 1, the HFB2 and HFB3 groups have no significant difference in HDL level compared to normal group in post intervention phase.

Figure 1. The average of HDL-cholesterol in rats pre and post intervention in Normal (N), High Fat - Fructose Diet (HFFD), High Fat - Fructose and BE-F6% (HFB1), High Fat - Fructose and BEF9% (HFB2), High Fat - Fructose and BEF12% (HFB3) treatments. abwxyz Different letters within the same color bars indicate significant difference between before and after treatment (p < 0.05). Star symbol (★) indicates significant difference between pre- and post-test in the same group.

High consumption of fat in HFFD treatment cause a high cholesterol storage in peripheral tissues. In this condition, LDL act as inducer of cholesterol breakdown in peripheral tissue (Wang et al. 2017). A rise in HDL level in beetroot intervention treatment is consistent with the research of Singh et al. (2015) found that beetroot supplementation could increase HDL levels. Based on Ayala et al. (2014) the increase of HDL levels after high antioxidant food treatment could reduce the lipid deposition in peripheral tissues, the most contributor for rise of HDL levels. The structure of flavonoids has a big impact on antioxidant activity. Hydroxyl group of flavonoids is the most significant determinant to pro-oxidant scavenging mechanism by donating hydrogen and an electron (Santos-Sánchez et al. 2019)

Total Cholesterol
Beetroot-enriched feed intervention gave a significant difference in total cholesterol levels. Based on Figure 2, the group which treated by 6% beetroot enriched feed (HFB1) had total cholesterol levels after intervention which was lower than HFFD treatment but not significantly different, while HFB3 treatment had total cholesterol levels close to normal treatment. HFB2 treatment experienced a significant reduction in total cholesterol and was lower than the normal treatment.

The decrease in total cholesterol levels is due to the content of antioxidant compounds in beetroot such as flavonoids, betalains, and carotenoids (Clifford et al. 2015). Previous study showed that flavonoid compounds such as flavanone, flavone, and isoflavone could decrease total
cholesterol level by cholesterol synthesis inhibition and increase LDL receptor expression (You et al. 2008). A high level of total cholesterol may be caused by free radicals in the blood. Antioxidant compounds in beetroot could have a role as free radical scavengers (Chen et al. 2021).

**Figure 2.** The average of total cholesterol in rats pre and post intervention in Normal (N), High Fat - Fructose Diet (HFFD), High Fat - Fructose and BE-F6% (HFB1), High Fat - Fructose and BEF9% (HFB2), High Fat - Fructose and BEF12% (HFB3) treatments.

Different letters within the same color bars indicate significant difference between before and after treatment (p < 0.05). Star symbol (★) indicates significant difference between pre- and post-test in the same group.

Diets enriched with 9% beetroot (HFB2) is better than 6% (HFB1) and 12% (HFB3). Previous study by Nahla et al. (2018) reported that a higher concentration of beetroot ethanol extract increases the antioxidant activity. Carotenoid was the one of antioxidant compound contained in beetroot (Ceclu & Nistor 2020). On another hand several compounds of carotenoid in high concentration has been reported could affect prooxidant activities in cell models (Lee et al. 2003).

**Atherogenic index**

In this study, high fat and fructose diet in induction phase accomplish to increase atherogenic index. Figure 3. shows that atherogenic index of induced group (HFFD, HFB1, HFB2, and HFB3) was significantly increased than normal group. Consumption of a high amount (more than 25% of energy requirement per day) fructose could affect lipid dysregulation, visceral adiposity, and decreased insulin sensitivity. These conditions were associated with cardiovascular disease and type 2 diabetes (Stanhope et al. 2009). Beetroot enriched feed intervention gave a significant difference in the atherogenic index. The group with high fat and fructose feed treatment was then given 6% beetroot enriched feed (HFB1), high fat and fructose then given 9% beetroot enriched feed (HFB2), high fat and fructose then given 12% beetroot enriched feed (HFB3) which has an atherogenic index approaching normal treatment.

A high cholesterol storage in peripheral tissues while a level of HDL is low makes lipid deposition in peripheral tissue cannot be decreased. This condition makes the potency of atherosclerosis increase. Potential of atherosclerosis measured by atherogenic index (Suanarunsawat et al. 2011). In this study beetroot enriched feed could affect the HDL level ant total cholesterol. Based on Murphy et al. (2012)
in cardiovascular system, HDL acts as anti-atherogenic agent by preventing and curing the inflammatory of blood vessel peripheral tissue.

**Figure 3.** The average of Atherogenic index in rats pre and post intervention in Normal (N), High Fat - Fructose Diet (HFFD), High Fat - Fructose and BEF 6% (HFB1), High Fat - Fructose and BEF 9% (HFB2), High Fat - Fructose and BEF 12% (HFB3) treatments.

\(^{abcxy}\) Different letters within the same color bars indicates significant difference between before and after treatment (p<0.05).

Star symbol (★) indicates significant difference between pre- and post-test in the same group.

**Blood Pressure**

Beetroot enriched feed intervention gave a significant difference in blood pressure. The high fat and fructose feed treatment was then treated with 6% and 9% beetroot enriched feed (HFB1 and HFB2) has a blood pressure close to normal group. Based on Figure 4, the blood pressure of 12% beetroot enriched feed intervention group decreased below normal. Post-intervention blood pressure from high lipid treatment was then treated with 9% and 12% beetroot enriched feed (HFB2 and HFB3) experienced a significant reduction.

**Figure 4.** The average of blood pressure in rats pre and post intervention in Normal (N), High Fat - Fructose Diet (HFFD), High Fat - Fructose and BEF 6% (HFB1), High Fat - Fructose and BEF 9% (HFB2), High Fat - Fructose and BEF 12% (HFB3) treatments.

\(^{abcxyz}\) Different letters within the same color bars indicates significant difference
between before and after treatment (p<0.05)
Star symbol (★) indicates significant difference between pre- and post-test in the same group.

A decrease in blood pressure in the three treatment groups could occur due to the content of antioxidant compounds in beetroot such as carotenoids, phenols, and flavonoids. Previous study reported that antioxidant compounds reduced oxidative stress in the peripheral tissue of blood pressure. Thus, blood pressure decreased significantly (Parik et al. 1996; Murphy et al. 2012; Sorriento et al. 2018). Previous study reported that beetroot antioxidant compound like flavonoids could inhibit angiotensin converting enzyme (ACE) by holding the NADPH oxidation in renin angiotensin aldosterone system (RAAS). A low activity of ACE could affect the arteries vasodilatation and lower blood pressure (Aviram et al. 2004). In this study, a higher dose of beetroot enriched feed given a higher effect to reduce blood pressure.

Correlation analysis
There is a positive correlation between the atherogenic index and blood pressure. Based on Figure 5, an increase in the atherogenic index have implications for an increase in blood pressure. A high level of non-HDL cholesterol and low HDL will cause oxidative stress. Oxidative stress conditions result in damage of blood vessel of peripheral tissue and increased blood pressure (Riccioni et al. 2009). This result is in line with the research Kazemi et al. (2018), which found that the atherogenic index and blood pressure were positively correlated.

![Figure 5. Correlations between atherogenic index and blood pressure (R² = 0.474)](image)

Based on the simple linear correlation analysis, the atherogenic index affected blood pressure in the moderate level (R² = 0.474).

Based on this study, beetroot should be promoted as a key component of a healthy lifestyle to control blood pressure and hyperlipidemia. For hypertension and hyperlipidemia sufferer beetroot could be alternative for non-medicine treatment. However, more research about optimal doses for humans, side effects, and proper composition with other ingredients should be conducted in the future.
CONCLUSION
Beetroot enriched feed could decrease total cholesterol, atherogenic index and blood pressure in rats induced by high fat and fructose feed. Intervention of 9% feed enriched beetroot was seen as an optimum dose to reduce total cholesterol, atherogenic index and blood pressure and increase HDL. Atherogenic index and blood pressure have a positive correlation with moderate level.

AUTHORS CONTRIBUTION
A.E.H. collected and analysed the data and wrote the manuscript. S. designed the trial and supervised research process, L.H. analysed the data, S.W. supervised the research process and manuscript.

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CONFLICT OF INTEREST
There is no conflict of interest of this study.

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