



THE PROTECTIVE EFFECTS OF RED BEETROOT (*Beta vulgaris L.*) AGAINST OXIDATIVE STRESS IN RATS INDUCED BY HIGH FAT AND FRUCTOSE DIET

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ABSTRACT

Background: One of consequence high-fat and fructose diet is oxidative stress. Consumption of antioxidants from red beetroot may increase antioxidant defense.

Objectives: This study aimed to evaluate red beetroot administration on improving antioxidant defense in rats induced high fat and fructose diet.

Methods: A total 20 male Wistar rats were divided into 4 groups: 1) normal control group (N), received standard diet; 2) High fat and fructose diet (HF), received high fat and fructose diet (HFFD); 8 weeks induction with HFFD and received 9g red beetroot (BA); and combination of HFFD and 9g of red beetroot from beginning of the study (HFBA). At the end of the study the levels of circulatory oxidized LDL (ox-LDL) were determined using enzyme-linked immunosorbent assay (ELISA) method. Superoxide dismutase 2 (SOD2) and catalase (CAT) gene expressions were determined by quantitative polymerase chain reaction (qPCR) method.

Results: Induction HFFD increased the levels of circulatory ox-LDL levels compared to normal control (10.00 ± 0.29 vs 12.69 ± 0.57). Administration of red beetroot for 6 weeks and combination HFFD with red beetroot during the study significantly decreased ox-LDL levels compared to high fat and fructose group (12.69 ± 0.57 vs 9.66 ± 0.46) and (12.69 ± 0.57 vs 8.59 ± 0.18), respectively. The decreased circulatory ox-LDL levels were found negatively correlated with upregulation SOD2 ($r = -0.548$; $P = 0.012$) and CAT ($r = -0.460$; $P = 0.041$) gene expression in the liver tissues.

Conclusion: Administration of red beetroot may ameliorate oxidative stress in rats induced high-fat and fructose diet through increasing antioxidant defense.

Keywords : Antioxidant, Gen expressions, Oxidative stress, Ox-LDL, Red beetroot.

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INTRODUCTION

Excess consumption of fat and fructose are known to induce oxidative stress.[1,2] Oxidative stress condition caused by overproduction of reactive oxygen species (ROS) has important role in metabolic syndrome development.[2,3] In addition, increasing ROS production also induces oxidation of LDL and generates oxidized LDL (ox-LDL).[4]

Ox-LDL is a marker of lipoprotein-associated oxidative stress.[5] Elevating concentration of ox-LDL in circulation has correlation with metabolic syndrome[6] and also marker component at present of cardiovascular disease, mainly atherosclerosis.[4] Meisinger et al.[7] reported that increased of ox-LDL levels in healthy men are associated with four times greater risk to cardiovascular disease risk. Frijhoff et al.[8] showed that plasma ox-LDL in patients with cardiovascular disease are found consistently to be elevated. Furthermore, Ali et al.[5] showed that circulatory ox-LDL in subjects with type 2 diabetes mellitus are higher compared to normal subjects.

Reducing circulatory of ox-LDL is one of promising strategy to minimize cardiovascular disease risk and many other diseases.[9] It is suggested that consuming fruits and vegetables containing antioxidants may prevents diseases related to oxidative process.[10] Antioxidant play important role in the regulation and maintenance our body metabolism against oxidative stress.[11] As ox-LDL is one of biomarkers of oxidative stress, antioxidant consumption and supplementation may have beneficial effects to decline circulatory ox-LDL levels.[12] Several antioxidant enzymes, including NAD(P)H oxidase, endothelial nitric oxide synthase, xanthine oxidase, myeloperoxidase, superoxide dismutase, catalase, and

glutathione peroxidase are formed to tackle the production of ROS. Superoxide dismutase alters superoxide anion into hydrogen peroxide (H_2O_2). While, catalase converts H_2O_2 into and O_2 .[13,14]

Red beetroot (*Beta vulgaris L*) is one of vegetables that contains several important bioactive compounds, such as carotenoids, polyphenols, flavonoids, betaine, and betalains which are responsible for the antioxidant properties.[15] Previous study showed that administration of red beet juice has potential effect as cardioprotective agent through reducing inflammation and suppressing oxidative stress mechanism.[16] Wang et al.[17] reported that consumption beverage as source of polyphenol such as cocoa drink, green tea, and grape juice may decline ox-LDL levels in circulation. Another study reported that strawberry consumption, a beverage containing flavonoid with high fat meal, could significantly reduce ox-LDL levels.[18] Since the consumption of red beetroot are still very rare to be used as preventive agent for metabolic diseases, here we elucidate that administer red beetroot in rat has effect on increasing superoxide dismutase 2 (SOD2) and catalase (CAT) gene expression level. Thus, red beetroot could be useful and healthful fruit to be developed as functional food for nutrition therapy for metabolic syndromes. The aim of our study to evaluate the effects of red beetroot in rats induced oxidative stress by high fat and fructose diet.

MATERIAL AND METHODS

Animals

A total 20 male Wistar rats, weighing approximately 100-120g and aged 4 weeks, were obtained from Faculty

of Pharmacy, Universitas Gadjah Mada. The rats were housed in individual cage and maintain in standard environment (22-25°C room temperature and 12 h day/night cycle). Before beginning the study, rats were acclimatized using a modified AIN-93 formulation (L-Cystine was replaced by DL-Methionine and Choline bitartrate by Choline chloride) and water *ad libitum*. All of the procedures in this study was approved by Ethics Committee of the Integrated Research and Testing Laboratory, Universitas Gadjah Mada.

Experimental Study

After one-week acclimatization, the rats were divided into 4 groups, normal control (N) that fed by standard diet; high fat and fructose diet (HF) group received high fat and fructose diet (HFFD) formulated by Lozano et al.[19] until at the end of the study. To study the effect of antioxidants from red beetroot, after 8 weeks fed by HFFD, BA group received 9g of red beetroot for 6 weeks. In order to study the protective effect of antioxidants from red beetroot in HFFD, HFBA group received combination of HFFD and 9 g of red beetroot during the study.

In this study 100g standard diet contained 62.10g corn starch, 14g casein, 10g sucrose, 4g corn oil, 5g α -cellulose, 3.5g mineral mixture, 1g vitamin mixture, 0.18g DL-methionine, 0.25g choline chloride, and 0.008g tertbutyl-hydroquinone. Meanwhile, BA and HFBA diet was prepared with substitution of corn starch with red beetroot.

Biochemical analysis

Blood samples were obtained from retro-orbital sinus, after intervention. The serum of ox-LDL level was analyzed using ELISA method (FineTest, Wuhan, China), analysis procedure was conducted according to manufacturer's protocol.

Isolation of RNA and quantitative polymerase chain reaction (qPCR)

Total RNA was extracted from frozen-liver tissue using TRNzol reagent (Tiangen, Beijing, China). Reverse transcription of 1 μ g total RNA was done using Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific, USA). Analysis of qPCR was done using SYBR Green master mix (Bio-rad, United Kingdom). The total reaction in this protocol was 10 μ L. The results were normalized against beta actin. Primer sequences used in this study were shown in Table 1. The thermocycling condition in this protocol were: 5 min at 95°C, 1 min at 95°C, followed by 59°C and 40 cycles.

Table 1. Primer sequences

Gene	Primer	Ref.
SOD2	Forward	5'-Sadi et al. [20]
	GCACATTAACGCGC	
	AGATCA-3'	
	Reverse	5'-
CAT	AGCCTCCAGCAACT	
	CTCCTT-3'	
	Forward	5'-El-Bahr [21]
	ACGAGATGGCACAC	
Beta actin	TTTGACAG-3'	
	Reverse	5'-
	TGGGTTTCTCTTCTG	
	GCTATGG-3'	
Beta actin	Forward	5'-Kelm-Nelson
	TGTGGATTGGTGGC et al. [22]	
	TCTATC -3'	
	Reverse	5'-
	AGAAAGGGTGTA	
	AACGCAG -3'	

Statistical analysis

All values are presented as mean \pm standard error of mean (SEM). We used One-Way ANOVA to analyze serum ox-LDL levels and also SOD2 and CAT gene expression between each group. Pearson correlation was used to analyze correlation between ox-LDL levels and hepatic SOD2 and CAT genes expression. The difference

was considered statistically significant at $P < 0.05$.

RESULTS

In Figure 1 we showed ox-LDL levels after intervention in circulation. Compared to N group, HF group showed high ox-LDL levels and has significance statistically different ($P = 0.001$). On the other hand, administration of beetroot in BA group and HFBA group have low ox-LDL levels compared to HF group and also compared to N group there is no significant difference ($P = 0.930$; $P = 0.103$), respectively.

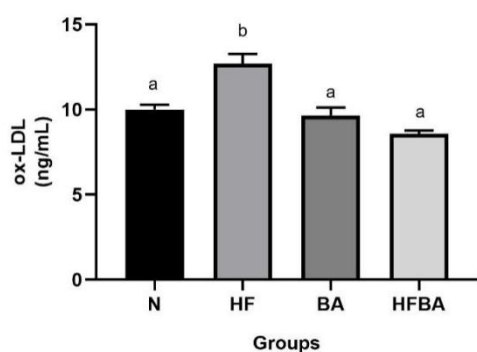


Figure 1. Circulatory levels of ox-LDL levels after intervention. N: normal control group; HF: high fat and fructose diet (HFFD) group; BA: HFFD induction and 9g red beetroot group; HFBA: HFFD combination with 9g red beetroot. Superscript ^a and ^b indicate $P < 0.05$ compared to N group by One-Way ANOVA test followed by Tukey HSD test.

The gene expression of endogenous antioxidant enzymes, SOD2 and CAT, were quantified using qPCR method. Figure 2 showed that intervention of red beetroot could modulate SOD2 and CAT gene expression in liver tissues. Compared to N group, HF group has no statistically significant difference ($P = 0.915$). Moreover, compared to N group SOD2 and CAT gene expression in BA has no significant difference ($P = 0.983$), yet, surprisingly in HFBA group shows significant difference ($P = 0.040$).

We also obtained the correlation based on Pearson correlation test. Ox-LDL serum level and SOD2 gene expression in liver tissues have a weak and negative correlation ($r = -0.548$; $P = 0.012$). This was also observed in ox-LDL serum levels and CAT gene expression in liver tissue ($r = -0.460$; $P = 0.041$) (Figure 3).

DISCUSSION

Our study showed that induction of high fat and fructose diet triggers excessive ROS production and leads to oxidative stress, confirmed by elevated of circulatory ox-LDL level compared to N, BA, and HFBA groups (Figure 1). Our results have similar with previous study that reported high fat diet may induce circulatory ox-LDL levels.[23] Ox-LDL levels is one of oxidative stress biomarker related to atherosclerosis and cardiovascular disease.[24] Enhancing of circulated ox-LDL levels may contribute to the risk of cardiovascular diseases through promoting inflammatory response[25], conversely reducing in circulated ox-LDL levels may reduce risk of cardiovascular diseases.[26] In this study, we reported that group receiving red beetroot has low ox-LDL levels compared to high fat and fructose diet group, but interestingly, in group that fed by high fat and fructose diet and red beetroot from beginning of the study has the lowest circulated ox-LDL level. We suspect synergetic effect between antioxidant from red beetroot and endogenous antioxidant from body metabolism during simultaneous administration of high-fat and fructose diet. Bouayed and Bohn [27] said that endogenous and exogenous antioxidants could act synergically to maintain redox homeostasis, for instance, regeneration of vitamin E by GSH and

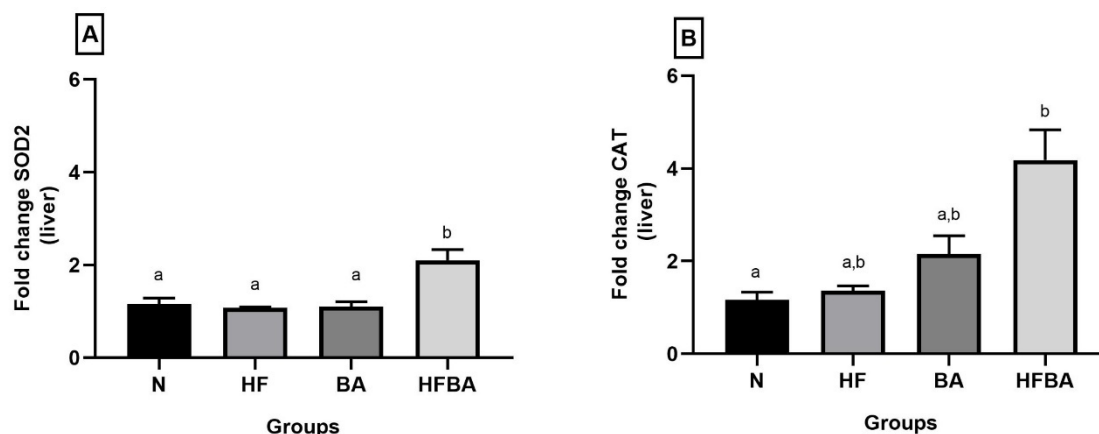


Figure 2. SOD2 and CAT genes expression in liver tissues after 6 weeks intervention.

N: normal control group; HF: high fat and fructose diet (HFFD) group; BA: HFFD induction and 9g red beetroot group; HFBA: HFFD combination with 9g red beetroot. Superscript ^a and ^b indicate $P < 0.05$ compared to N group by One-Way ANOVA test followed by Tukey HSD test. Superscript ^{a,b} indicate no difference between ^a nor ^b.

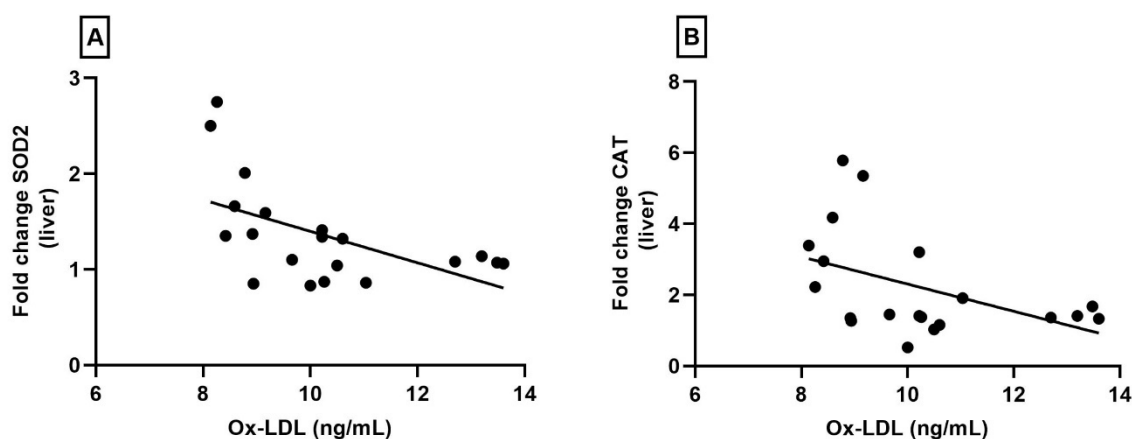


Figure 3. Correlation between SOD2 and CAT genes expression in liver tissues and ox-LDL serum levels.

vitamin C to prevent lipid peroxidation. Thus, further we need more investigation to elucidate the role of antioxidants from red beetroot with endogenous antioxidants, such as SOD2 and CAT.

Red beetroot is one of vegetables that is rich of carotenoids, polyphenols, flavonoids, betaine, and betalains.[15] Study reported that red beetroot has potential antioxidant effects for treatment therapy related to oxidative stress[28], also Sinaga et al.[29] showed that red beetroot

juice administration in athletes reduces malondialdehyde (MDA) levels during training. Previous study suggested that betanin, an antioxidant extract from red beetroot, has a hepatoprotective effect in rats induced with high fat diet.[30] Di Renzo et al.[10] reported diets contain polyphenols and flavonoids also have beneficial effects in health, such as inhibition of LDL oxidation thus reducing risk cardiovascular disease.

Supplementation of red beetroot powder may increase the activity of enzymatic antioxidants such as SOD2 and CAT in diabetic rats.[31] In this study, we also evaluated gene expression level of SOD2 and CAT in liver tissue. In this study we reported that SOD2 and CAT gene expression between normal and HF group were not significantly different. This finding similar with Girard et al.[32] that showed no difference Cu/Zn-SOD gene expression between control group and high fructose diet group, although these activities were observed increased in rats with high fructose diet. Our results showed that rat induced by high fat and fructose diet reduces hepatic SOD2 and CAT gene expression (Figure 2). As mentioned before, high fat and fructose diet reduced the mRNA enzymatic antioxidant expression, including SOD2 and CAT.[33, 34] SOD2 is one of SOD isoenzyme that has function to protect against oxidative damage. SOD catalyzes dismutation of superoxide into hydrogen peroxide (H_2O_2) and oxygen, then CAT or glutathione peroxidase would metabolize H_2O_2 into oxygen and water. Therefore SOD, CAT, and glutathione peroxidase have important function to protect the cell from oxidative damage caused by ROS.[35] Lack of SOD2 expression leads to oxidative damage in the liver, meanwhile overexpression of SOD2 generally has a protective role.[36]

In this study we suggested that red beetroot supplementation ameliorates the endogenous antioxidant through the modulation of antioxidant-associated genes (Figure 2). Meanwhile, a negative correlation was observed between SOD2 and CAT gene expression and circulated ox-LDL level (Figure 3). In line with our result, research conducted by Chan et al.[37] showed that antioxidant supplementation induces antioxidant-

associated gene expression. Elevating antioxidant status via upregulation SOD2 and CAT gene expression can ameliorate oxidative stress.[11] A study reported the inverse relationship of SOD2 levels with circulated ox-LDL, after administration cacao bean extract.[38]

Antioxidant supplementation has beneficial effect to modulate antioxidant-associated genes, including SOD2 and CAT.[39] One of the possible mechanisms is the upregulation of SOD2 and CAT gene expression due to bioactive components in red beetroot that may increase the activation of factor E2-related factor 2 (Nrf2). According to Cardozo et al.[40] bioactive compounds from food have ability to induce Nrf2. Activated Nrf2 will bind with antioxidant-responsive element (ARE) in the nucleus and upregulate the antioxidant-associated genes.[37,41] Overall, our data in this study provide about the beneficial red beetroot in suppressing oxidative stress through modulation of antioxidant-associated gene expression. However, limitation in our study, we did not measure SOD2 and CAT activity in circulated plasma to support our results, thus further study is needed..

CONCLUSION

The present study showed that red beetroot has ability to reduce high-fat and fructose diet induced oxidative stress. The possible mechanism in reducing oxidative stress is via upregulation of antioxidant-associated genes expression in the liver tissue.

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