

Effect of 7-Hydroxy-2-(4-Hydroxy-3-Methoxyphenyl)-Chroman-4-one On Level of Mangan-Superoxide Dismutase (Mn-Sod) and *Superoxide Dismutase 2 (Sod2)* Gene Expression in Hyperlipidemia Rats

Rahmah Dara Ayunda¹, Prasetyastuti^{2*} and Pramudji Hastuti²

¹ Postgraduated Student of Magister of Biomedical Science, Department of Biochemistry Universitas Gadjah mada, Skip Utara 55281 Yogyakarta

² Department of Biochemistry Faculty of Medicine, Public Health, and Nursing Universitas Gadjah Mada, Skip Utara 55281 Yogyakarta

Info Article

Submitted: 22-05-2019

Revised: 16-07-2019

Accepted: 21-08-2019

*Corresponding author
Prasetyastuti

Email:
prasetyastuti.djati@gmail.com

ABSTRACT

Hyperlipidemia is a lipid metabolism disorder characterized by an increase in serum lipid levels. Hyperlipidemia is a major risk factor for many metabolic syndrome diseases because it triggers oxidative stress. Oxidative stress can be reduced by endogenous antioxidant enzymes triggered by exogenous antioxidant compounds, such as 7-OH-2-(4-OH-3-methoxyphenyl)-chroman-4-one isolated from the seeds of *Swietenia macrophylla* King. The aims of this study were to investigate the effects of 7-OH-2-(4-OH-3-methoxyphenyl)-chroman-4-one compounds on cholesterol level, LDL level, Mn-SOD levels and *SOD2* gene expression of hyperlipidemic rats. Thirty rats (*Rattus norvegicus*) were divided into 6 groups, normal group (N), hyperlipidemia group (HL), hyperlipidemia group with simvastatin (P), hyperlipidemic group with 7-OH-2-(4-OH-3-methoxyphenyl)-chroman-4-one with dose 10 (F10), 30 (F30) and 90 (F90) mg/200g body weight (BW). Cholesterol and LDL were analyzed with CHOD-PAP method, Mn-SOD level was analyzed by ELISA method and *SOD2* gene expression was analyzed by qPCR method. The decrease in cholesterol and LDL levels were most prevalent in group F90 with dose 90mg/200g BW of 7-OH-2-(4-OH-3-methoxyphenyl)-chroman-4-one, with average difference each of them was 172.43mg/dL and 36.12mg/dL. The rats fed on high-cholesterol diet exhibited a significant elevation in Mn-SOD levels ($p < 0.05$) compared to normal group. The treated animals with 7-OH-2-(4-OH-3-methoxyphenyl)-chroman-4-one has the level of Mn-SOD is significantly lower ($p < 0.05$) compared with hyperlipidemic group. Expression of *SOD2* in group F90 has value close to normal group ($p > 0.05$). 7-OH-2-(4-OH-3-methoxyphenyl)-chroman-4-one with dose of 90mg/200g BW improved cholesterol levels, LDL levels, Mn-SOD levels and *SOD2* gene expression in hyperlipidemic rats.

Keywords: Hyperlipidemia, flavonoids, Mn-SOD, *SOD2* gene.

INTRODUCTION

Hyperlipidemia is a lipid metabolism disorder characterized by abnormally elevated levels of cholesterol (hypercholesterolemia), elevated levels of triglyceride (hypertriglyceridemia) or a combination of both in the blood. Hyperlipidemia can be a risk factor for various degenerative diseases and metabolic syndrome, such as atherosclerosis, cardiovascular disease, stroke and type 2 diabetes mellitus (Kobayashi *et*

al., 2010; Feng *et al.*, 2011; Li *et al.*, 2013 ; WHO, 2015). Hyperlipidemia is associated with free radical formation (Ismail *et al.*, 2010; Sumardika and Jawi, 2012). The formation of free radicals in hyperlipidemic patients occurs through several mechanisms, including through increased NADH/NADPH oxidase. it leads the formation of more superoxide anion radicals that cause oxidative stress (Ismail *et al.*, 2010; Sumardika and Jawi, 2012; Farris *et al.*, 2005). In addition,

oxidative stress in hyperlipidemic patients can also occur due to uncoupling mitochondrial. It also causes increased production of free radicals (Farris *et al.*, 2005). The compensation mechanism to suppress the rate of oxidative stress is with antioxidants systems. The body has compensation mechanism to the rate of oxidative stress by synthesizing enzymes that act as antioxidants, one of them superoxide dismutase (SOD). In normal condition, the body will balance the formation of free radicals with endogenous antioxidant systems. However, continuous radical formation makes the body unable to inhibits the rate of oxidative stress, thus causing pathological conditions. Therefore, it is necessary to add certain compounds that can help increase endogenous antioxidant activity, such as flavonoid compounds (Manach *et al.*, 2004).

Flavonoids are known to improve hyperlipidemia condition and to increase liver enzyme levels (Prasetyastuti, 2018). Compound 7-OH-2-(4-OH-3-methoxyphenyl)-chroman-4-one was flavanone (one of flavonoid) compound from mahogany seed isolated by Mursiti *et al* (2015). One study found that naringenin, a flavanone in diabetic rat model improved hyperglycemia and hyperinsulinemia condition significantly by restoring lipid level, increasing antioxidant activities, and improving liver function markers (Prasetyastuti, 2018). Based on this background, the aim of this study was to investigate the effects of 7-OH-2-(4-OH-3-methoxyphenyl)-chroman-4-one compounds on cholesterol level, LDL level, Mn-SOD levels and *SOD2* gene expression of hyperlipidemic rats.

MATERIAL AND METHODS

Hyperlipidemia testing was designed by pretest-posttest with control group design and both Mn-SOD level and *SOD2* gene expression were designed by post-test only with control group design. Thirty male Wistar rats weighing between 150 and 200g, 10 weeks old, were used in this study. The location of the research was conducted in two laboratories, Biochemistry Laboratory Faculty of Medicine, Public Health and Nursing Universitas Gadjah Mada and Laboratory of Food and Nutrition Universitas Gadjah Mada. The experiments were carried out according to the guidelines for the use of animals and approved by *Ethical Committee of Research in Medical Health, Faculty of Medicine, Public Health, and Nursing Universitas Gadjah Mada* (No.KE/FK/08/8/EC/2017).

Animal treatments

Six experimental rat groups were established with 5 rats per group. The groups were as follows: group N (normal control group), group HL (hyperlipidemia control group), group P (hyperlipidemia with simvastatin), group F10 (hyperlipidemia with 10mg/200g BW of 7-OH-2-(4-OH-3-methoxyphenyl) chroman-4-one), group F30 (hyperlipidemia with 30mg/200g BW of 7-OH-2-(4-OH-3-methoxyphenyl)-chroman-4-one) and group F90 (hyperlipidemia with 90mg/200g BW of 7-OH-2-(4-OH-3-methoxyphenyl)-chroman-4-one). They were fed with standard AIN 93M for 5 days for adaptation. Induction of hyperlipidemia was done by feeding the animals with laboratory feed enriched with 10g/kg cholesterol and 2g/kg cholic acid for a week. Fasting blood samples were collected to characterization baseline levels of cholesterol and LDL. Group N and group HL were orally administered by infusion with the same volume of water, while the other groups were treated with flavonoid once daily for 4 weeks. At the end of the experiment, all rats were sacrificed, and liver tissues were collected and stored at -80°C for the gene expression study.

Total cholesterol and LDL analysis

Serum was separated by centrifugation at 4000rpm for 15min. Total cholesterol and low-density lipoprotein (LDL)-cholesterol concentrations were measured with enzymatic CHOD-PAP methods by using Cholesterol FS Diagnostic System Kit (German).

Levels of Mn-SOD analysis with Enzyme-Linked Immunosorbent Assay (ELISA)

Levels of Mn- SOD were detected in rat liver samples by sandwich ELISA using a ready kit Fine-Test Kit (Wuhan Fine Biotech Co., Ltd, China) according to the instructions of the manufacturer.

Quantitative PCR (qPCR) analysis of *SOD2* gene expression

Total mRNA was extracted from rat liver with Tri-RNA kits (Favorgen, Biotech Corp, Taiwan) and used to determine the expression level of *SOD2* gene. Total RNA was converted to cDNA High Capacity cDNA Reverse Transcription Kits Protocol (Applied Biosystems, USA). Quantitative PCR was performed with SoFast Evagreen Supermix (BioRad, USA) according to the instructions of the manufacturer. The primer sets used are *SOD2* forward-5'-CCCTGACCTGCCTTACGACT-3' and primer *SOD2*

Table I. Mean of total serum cholesterol levels before and after administration of 7-OH-2-(4-OH-3-methoxyphenyl)-chroman-4-one

Group	Mean \pm SD (mg/dL)		p ^a
	Before	After	
N	80.79 \pm 7.32	83.69 \pm 6.99 ^{#b}	0.087
HL	261.35 \pm 25.19	263.45 \pm 24.29 ^{*b}	0.041
P	311.62 \pm 29.36	106.99 \pm 3.38 ^{*#}	<0.001
F10	303.87 \pm 10.15	167.87 \pm 9.70 [*]	<0.001
F30	274.78 \pm 33.34	135.90 \pm 7.89 ^{*#}	<0.001
F90	286.33 \pm 11.14	113.89 \pm 7.77 ^{*#}	<0.001

Description: N: Normal, HL: Hyperlipidemia, P: HL+Simvastatin, F10, F30, F90: HL+ 7-OH-2-(4-OH-3-methoxyphenyl)-chroman-4-one 10, 30, 90 mg/200g BW respectively. Normality test with Saphiro Wilk; data were tested with Repeated measured ANOVA-test p < 0.05, ^a: tested with paired sample t-test. Notation *: p < 0.05 vs N; #: p < 0.05 vs HL. ^b: p < 0.05 vs P

Table 2. Mean of LDL levels before and after administration of 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chroman-4-one

Group	Mean \pm SD (mg/dL)		p ^a
	Before	After	
N	26.79 \pm 2.07	27.18 \pm 2.26 ^b	0.096
HL	75.06 \pm 4.47	76.24 \pm 5.02 ^{*b}	0.028
P	74.32 \pm 3.10	33.45 \pm 1.91 ^{*#}	<0.001
F10	70.49 \pm 3.39	61.46 \pm 2.11 ^{*#b}	0.010
F30	69.13 \pm 4.09	43.20 \pm 2.03 ^{*#}	<0.001
F90	73.33 \pm 2.52	37.21 \pm 1.68 ^{*#}	<0.001

Description: N: Normal, HL: Hyperlipidemia, P: HL+Simvastatin, F10, F30, F90: HL+ 7-OH-2-(4-OH-3-methoxyphenyl)-chroman-4-one 10, 30, 90 mg/200g BW respectively. Normality test with Saphiro Wilk; data were tested with Repeated measured ANOVA-test p < 0.05, ^a: tested with paired sample t-test. Notation *: p < 0.05 vs N; #: p < 0.05 vs HL. ^b: p < 0.05 vs P

reverse 5'-AGCGACCTTGCTCCTTATTG-3' Beta actin was used as an internal standard, with primer beta actin forward 5'-ACGGTCAGGTCATCACTATCG-3' and beta actin reverse 5'-GGCATAGAGGTCTTTACG GATG-3'. For PCR conditions, PCR reaction procedure was: 95°C for 5min, followed by 40 cycles of 95°C for 30s, and annealing temperature 62°C for 1min and extension 72°C for 30s. Relative expression levels were calculated using the 2^{- $\Delta\Delta$ Ct} method (11) (Livak and Schmittgen, 2001). In the present study, beta actin was the housekeeping gene.

Statistical analysis

The data were represented by Mean \pm SD. A one-way analysis of variance (ANOVA; P < 0.05) was used to determine significant differences among the groups. The variances were homogeneous the individual comparisons were obtained by Tukey's HSD post hoc test. If not, the Games-Howell post hoc test was applied. Pretest and posttest analysis

for total cholesterol and LDL concentration used paired sample t test. Statistical significance was set at P \leq 0.05.

RESULTS AND DISCUSSION

Levels of total cholesterol in hyperlipidemia rats

The results showed that cholesterol-induced rats (HL group rats) had higher cholesterol levels compared with rats in the normal group (N group). The daily administration of 7-OH-2-(4-OH-3-methoxyphenyl)-chroman-4-one for 4 weeks with doses of 10, 30 and 90mg/200g BW can significantly decrease cholesterol (p < 0.05) (Table I).

Levels of LDL in hyperlipidemia rats

The levels of LDL increased after induced cholesterol fed in the HL group compared with the N group (p < 0.05). The results showed a significant decrease after administration of 7-OH-2-(4-OH-3-

methoxyphenyl)-chroman-4-one at a dose of 10, 30 and 90 mg/200g BW in each group (Table II).

C. Levels of Mn-SOD proteins

The rats fed on high-cholesterol diet (HL group) exhibited a significant elevation in Mn-SOD levels compared with N group. After being induced, the administration of 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chroman-4-one for 4 weeks in hyperlipidemic rats liver showed a significant difference between hyperlipidemia group and F30, F90, and treatment group. However, there was no difference between the HL group and F10 group (Figure 1).

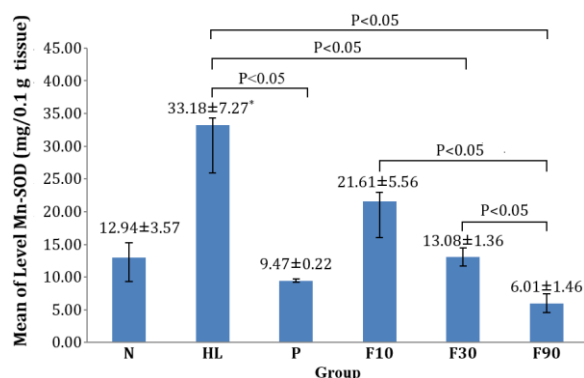


Figure 1. Level of Mn-SOD protein Group, Description: N: Normal, HL: Hyperlipidemia, P: HL+Simvastatin, F10, F30, F90: HL+ 7-OH-2-(4-OH-3-methoxyphenyl)-chroman-4-one 10, 30, 90 mg/200g BW respectively. Normality test with Saphiro Wilk; data were tested with ANOVA-test p < 0.05. Notation *: p < 0.05 vs N; #: p < 0.05 vs HL.

Relative expression of SOD2 gene

The relative gene expression of SOD2 used beta actin for housekeeping gene (Figure 2). The results of the study based on statistics there was no significant difference between the groups. However, in F90 has relative expression value almost approaching with N group.

The results of cholesterol and LDL cholesterol levels showed a significant decrease in cholesterol levels of hyperlipidemia rats before and after administration of 7-OH-2-(4-OH-3-methoxyphenyl)-chroman-4-one in all doses. However, the best decline occurred in group 6 rats, which were given 7-OH-2-(4-OH-3-methoxyphenyl)-chroman-4-one dose of 90 mg/200g BW. Based on the results of statistical analysis, a best dose is 90 mg/200g BW that have same effect with simvastatin 0.18 mg/200g BW to improve lipid level in blood.

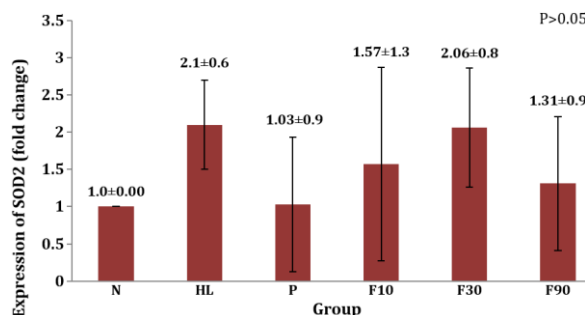


Figure 2. Relative gene expression of SOD2 gene, Description: N: Normal, HL: Hyperlipidemia, P: HL+Simvastatin, F10, F30, F90: HL+ 7-OH-2-(4-OH-3-methoxyphenyl)-chroman-4-one 10, 30, 90mg/200g BW respectively. Normality test with Saphiro Wilk; data were tested with ANOVA-test p > 0.05.

The decreased levels of cholesterol and LDL by daily administration of 7-OH-2-(4-OH-3-methoxyphenyl)-chroman-4-one compound was in accordance with some studies. Study of Gorinstein *et al.* (2007) showed that naringin and hesperidin, which are flavanone compound as 7-OH-2-(4-OH-3-methoxyphenyl)-chroman-4-one, could decrease and preclude the plasma lipid. Jayachitra and Nalini (2011) also revealed that naringenin could hinder high plasma lipid and lipoprotein by alcohol induction. As with Prince and Kannan (2006) that the effects of flavonoid compounds Rutin can reduce cholesterol and LDL levels. The results of Feng *et al.* (2011) also showed that flavonoid content in *Perilla Frutescens* leaves can also lower cholesterol and LDL levels in the blood.

Decrease in cholesterol and LDL levels in the blood occurs through several mechanisms including what were stated by Jayachitra and Nalini (2011) that Flavonoid, such as naringenin could inhibit HMG-CoA reductase activity while HMG CoA is a rate limiting step of cholesterol biosynthesis. Choi *et al.* (2008) conducted that flavonoids can lower cholesterol levels by inhibition and decrease activity of Acyl-coenzyme A: cholesterol acyltransferase (ACAT), is an enzyme involved in cholesterol esterification and has a role in cholesterol absorption in the intestinal. Inhibition of HMG CoA reductase and ACAT reduce cellular hepatic cholesterol level. It induced expression and upregulated LDL Receptor for entered LDL-c as vehicle cholesterol in blood by activating SREBP-2 as transcription factor for LDLR (Duriez, 2003; Wilcox *et al.*, 2001). This mechanisme like naringenin and hesperidine, effect of induced naringenin and hesperidine

inhibited apoB secretion and upregulated expression of LDLR (Wilcox *et al.*, 2001). In addition, according to Koo and Noh (Koo and Noh, 2007) flavonoids also play a role in lowering LDL and cholesterol levels through inhibition of cholesterol absorption in the small intestine and can increase bile excretion (Gorinstein *et al.*, 2005).

The result of analysis levels Mn-SOD proteins in hepatic tissue was highest in group 2 (hyperlipidemia group). Increased protein levels in the hyperlipidemia group compared to the normal group according to Akhgari *et al.* (2003) due to the activated mechanism of body compensation for oxidative stress caused by hyperlipidemia. High lipid level altered lipid properties in lipid raft, and activated NADPH oxidase to produce ROS. High Lipid also decrease antioxidant enzyme activity (Amiya, 2016). The ability of the body to compensate for the presence of free radicals is also influenced by several factors, such as the magnitude of oxidative stress and the dose of the stressor (Chen *et al.*, 2014).

After the administration of 7-OH-2-(4-OH-3-methoxyphenyl)-chroman-4-one for 4 weeks, in the group given 90 mg/200g BW (group 6) there was a significant decrease in Mn-SOD levels compared to the hyperlipidemia group ($p < 0.05$), but the Mn-SOD levels in this group did not significantly differ compared to the normal group and the simvastatin group ($p > 0.05$). This is because flavonoid compounds can act as scavengers by directly donating their hydrogen atoms in order to reduce the reactivity of free radicals without passing through inducing antioxidant enzymes (Redha, 2010). The reduction of free radicals due to direct neutralization by the flavonoid compound causes a decrease in the rate of oxidative stress, so the synthesis of antioxidant enzymes will decrease (Chen *et al.*, 2014)

The results of SOD2 gene expression analysis showed that there was no significant difference between the groups. The highest expression values were in the hyperlipidemic group. This shows that indeed in this study the body is still able to compensate by increasing the level of expression of SOD2 to respond to oxidative stress (Redha, 2010). The presence of oxidative stress signals will cause cell response that is by triggering transcription factor Nrf2 regardless of the Keap1 binding protein. The absence of Nrf2 from Keap1 causes Nrf2 translocated from the cytoplasm to the nucleus. Nrf2 in the nucleus will bind to the antioxidant element response (ARE) thus increasing the expression of the SOD2 gene

(Khaerunnisa *et al.*, 2014; Vomhof-DeKrey and Picklo, 2012).

The analysis compared between the groups given the 7-OH-2-(4-OH-3-methoxyphenyl)-chroman-4-one, the group with the dose of 90 mg/200g BW (group 6) had the lowest fold change value among the other groups, it was close to normal group values and simvastatin. The pattern was similar to its protein content. Decreased levels of gene expression that given 7-OH-2-(4-OH-3-methoxyphenyl)-chroman-4-one compared to hyperlipidemia group according to Feng *et al.* (2011) and supported by Martin *et al.* (2010) that flavonoid compounds in the main role as scavenger, they have the ability to eliminate directly some radicals, such as superoxide anion, hydroxyl radicals and peroxy radicals and indirectly increase expression and activity of endogenous antioxidant for scavenger ROS (Zeka *et al.*, 2017).

The result of Mn-SOD concentration in this study was the same as Khaerunnisa *et al.* studied (2014) using extract from reeds (*Imperata cylindrical*) in hypercholesterolemia rat. The result shows that the induction of hypercholesterol for 3 weeks increases the activity of SOD as a result of the compensation mechanism of the body, after the feeding of extract of reeds the SOD activity has decreased, due to the bioactive compounds in reeds extract play directly in neutralizing superoxide radicals (Vomhof-DeKrey and Picklo, 2012).

However, in the research of Liu and Lin (2011) used a quercetin to treat the animals. After animals were treated for 10 weeks, there was an increase in SOD levels in quercetin group compared with hyperlipidemic rats group. Miler *et al.*, (2016) conducted that naringenin increased expression both mRNA and protein of SOD2 by prooxidant activity of naringenin. Because naringenin can form flavonoid radical and stimulated secretion SOD2 enzyme.

CONCLUSION

Administration of 7-OH-2-(4-OH-3-methoxyphenyl)-chroman-4-one at an effective dose of 30mg/200g BW can decrease cholesterol and LDL levels in serum hyperlipidemia and can also improve the levels of Mn-SOD in rats. But there was no significant effect on gene expression of SOD2 liver in hyperlipidemic rats.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support by The Indonesian Scholarship by LPDP (Indonesian Endowment Fund for

Education) and Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada. And the authors wish thanks to all staff in laboratory Biochemistry Faculty of Medicine, Public Health, And Nursing, Universitas Gadjah Mada and to staff laboratory of Food and Nutrition Universitas Gadjah Mada in preparing the necessary materials during this study.

REFERENCE

- Akhgari M., Abdollahi M., Kebryaezade A., Hosseini R., and Sabzevari O. 2003. Biochemical evidence for free radical induce lipid peroxidation as a mechanism for subchronic toxicity of malathion in blood and liver of rats. *HET*. 22: 205-211.
- Amiya E. 2016. Interaction of hyperlipidemia and reactive oxygen species: Insights from the lipid-raft platform. *World J Cardiol*. 8 (12): 689-694.
- Chen J., Zhang Z., and Cai L. 2014. Diabetic cardiomyopathy and its prevention by Nrf2: current status. *DMJ*. 2014, 38: 337-345.
- Choi JH., Rho MC., Lee SW., Choi JN., Kim K., Song GY., et al. 2008. Bavachin and isobavachalcone, Acyl-coenzyme A: Cholesterol acyltransferase inhibitors from *Psoralea corylifolia*. *Arch. Pharm. Res.* 31(11): 1419-1423.
- Duriez P. 2003. Mechanisms of actions of statins and fibrates. *Therapie*. 58 (1): 5-14.
- Farris MW., Chan CB., Patel M., Van Houten B. and Orrenius S. 2005. Role of mitochondria in toxic oxidative stress. *Molecular Interventions*. 5(2): 94-111.
- Feng FJ., Yu CH., Yin, KJ., Hua J. and Dai XY. 2011. Hypolipidemic and antioxidant effects of total flavonoids of *Perilla frutescens* leaves in hyperlipidemia rats induced by high-fat diet. *Food Res Internationa*. 44: 404-409.
- Gorinstein S., Leontowicz H., Leontowicz M., Krzeminski R., Gralak M., Jastrzebski Z., et al. 2007. Effect of hesperidin and naringin on the plasma lipid profile and plasma antioxidant activity in rats fed a cholesterol-containing diet. *J. Sci. Food. Agric*. 87: 1257-1262.
- Gorinstein S., Leontowicz H., Leontowicz M., Krzeminski R., Grala M., Licon ED., et al. 2005. Changes in plasma lipid and antioxidant activity in rats as a result of naringin and red grapefruit supplementation. *J. Agric. Food Chem*. 53: 3223-3228.
- Ismail M., Al-Naqeep G. and Chan KW. 2010. *Nigella sativa* thymoquinone-rich Fraction greatly improves plasma antioxidant capacity and expression of antioxidant genes in hypercholesterolemic rats. *Free Radical Biology and Medicine*. 48:664-672.
- Jayachitra J. and Nalini M. 2011. Effect of naringenin (citrus flavanone) on lipid profile in ethanol-induced toxicity in rats. *J Food Biochem*. 1 : 1-10.
- Khaerunnisa S., Kuswarini S., Suhartati., Lukitasari L., Humairah I., Arta RBN. and Gwenny IP. 2014. Sari etanol etil asetat alang-alang (*Imperata cylindrical*) terhadap suproksida dismutase (SOD). *IJCP & ML*. 20(2); 128-132.
- Kobayashi Y., Miyazawa M., Kamei A., Abe K. and Kojima T. 2010. Ameliorative effects of mulberry (*Morus alba* L.) leaves on hyperlipidemia in rats fed a high-fat diet: Induction of fatty acid oxidation, inhibition of lipogenesis, and suppression of oxidative stress. *Biosci. Biotechnol. Biochem*. 74 (12): 2385-2395.
- Koo SI. and Noh SK. 2007. Green tea as inhibitor of the intestinal absorption of lipids: potential mechanism for its lipid lowering effect. *J. Nut. Biochem*. 18: 179-183.
- Li H., Horke S. and Forstermann U. 2013. Oxidative stress in vascular disease and its pharmacological preention. *Trends in Pharmacological Sciences*. 34 (6): 313-319.
- Liu C. and Lin JD. 2011. PGC-1 coactivators in the control of energy metabolism. *Acta Biochim Biophys Sin*. 43(4): 248-257.
- Livak KJ. and Schmittgen TD. 2001. Analysis of relative gene expression data using realtime euantitative PCR and the 2^{-ΔΔCt} method. *Methods*. 25: 402-408.
- Manach C., Scalbert A., Morand C., remesy C. and Jimenez L. 2004. Polyphenols: food source and bioavailability. *Am. J. Clin. Nutr*. 79: 727-747.
- Martin MA., Serrano BG., Ramos S., Pulido MI., Bravo L. and Goya L. 2010. Cocoa flavonoids up-regulate antioxidant enzyme activity via the ERK1/2 pathway to protect against oxidative stress-induced apoptosis in HepG2 cells. *J. Nut. Biochem*. 21: 196-205.
- Miler M., Živanović J., Ajdžanović V., Oreščanin-Dušić Z., Milenković D., et al. 2016. Citrus flavanones naringenin and hesperetin improve antioxidant status and membrane lipid compositions in the liver of old-aged Wistar rats. *Exp Gerontol*. 84: 49-60.

- Mursiti S. 2015. *Isolation of Secondary Metabolite Compounds Antihyperglycemia of Mahogany Seeds (Swietenia macrophylla, King* [dissertation]. Gadjah Mada University, Yogyakarta.
- Prasetyastuti, 2018. The Role of isoflavon in reducing blood glucose levels. *J. Med Sci.* 50 (4): 483-492.
- Prince P. and Kannan NK. 2006. Protective effect of rutin on lipids, lipoproteins, lipid metabolizing enzymes and glycoproteins in streptozotocin-induced diabetic rats. *J. Pharm. Pharmacol.* 58: 1373–1383.
- Redha A. 2010. Flavonoid: struktur, sifat antoksidatif dan peranannya dalam sistem biologi. *J. Belian.* 9(2): 196-202.
- Sumardika IW. and Jawi IM. 2012. Ekstrak air daun ubi jalar ungu memperbaiki profil lipid dan meningkatkan kadar SOD darah tikus yang diberi makanan tinggi kolesterol. *MEDICINA.* 43 (2): 67-70.
- Vomhof-DeKrey EE. and Picklo MJ. 2012. The Nrf2-antioxidant response element pathway: a target for regulating energy metabolism. *J. Nutr. Biochem.* 23: 1201-1206.
- Wilcox LJ, Borradaile NM, Dreu LE. and Huff MW. 2001. Secretion of hepatocyte apo B is inhibited by the flavonoids, naringenin and hesperetin, via reduced activity and expression of ACAT2 and , MTP. *J Lipid Res.* 42 : 725-734.
- World Health Organization (WHO). 2015. Top 10 causes of death worldwide. [akses: 23 Februari 2017]. <http://www.who.int/mediacentre/factsheets/fs310/en/>.
- Zeka K., Ruparelia K., Arroo RRJ, Budriesi R. and Mucucci M. 2017. Flavonoids and their metabolites: prevention in cardiovascular disease and diabetes. *Disease.* 5 (19): 1- 18.