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Combination of Capsaicin and Vitamin E Increase HDL and Decrease LDL Levels in Hypercholesterolemic Rats

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Abstract: Cardiovascular disease was the most lethal disease in the world. Atherosclerosis was one of the factor that can lead to the development of cardiovascular disease. Low density lipoprotein (LDL) and high density lipoprotein (HDL) were often associated with atherosclerosis. LDL and HDL concentration can be modified by diet, such as capsaicin and vitamin E. In this study, 25 male Sprague Dawley rats were divided into 5 group, A, B, C, D and E. Each group had 5 rats. Group B, C, D, and E were rendered high fat diet (A.D. II standard diet, 10% lard, olive oil and yolk) for 14 days while group A was given standard diet. Group B was positive control and get no treatment, group C was given 1 mg/kg capsaicin for 14 days, group D was given 22 IU vitamin E. LDL and HDL concentration were measured before and after the treatment. LDL concentration decreased significantly and HDL concentration increased insignificantly after capsaicin treatment. LDL and HDL concentration were significantly increase after given 22 IU vitamin E. Combination of 1 mg/kg capsaicin and 22 IU vitamin E could also improve HDL concentration and reduce LDL concentration. Vitamin E gave the best effect when compare with capsaicin alone and the combination of capsaicin and vitamin E in increasing HDL concentration and reducing LDL concentration.

Key words: Hypercholesterolemic, LDL, HDL, capsaicin

INTRODUCTION

Cardiovascular disease is the most lethal disorder in the world. In 2030, it is predicted that 23.6 million people will die due to cardiovascular disease. There are many factors that contribute in the development of cardiovascular disease. One of them is atherosclerosis. Atherosclerosis is a complex pathological process and influenced by many factors. Blood cholesterol level is one of many causes (Walldius and Jungner, 2004).

Cholesterol availability in circulation highly depends on how lipoprotein conveys it throughout the body. Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL) are two lipoproteins that often get special concern. HDL transport lipid from peripheral part of the body to the liver. That ability has contradictory relations with atherosclerosis related diseases (Daniels *et al*., 2009). On the other side, increment of LDL often associated with higher incidence of cardiovascular diseases (Briel *et al*., 2009).

Body concentration of LDL and HDL can be improved by consumption of food that has cholesterol-lowering ability (Mooradian *et al*., 2006). Chilli pepper (Capsicum frutescens) is one type of vegetable that can easily get and frequently used by Indonesian as a seasoning which can give hot and burnt sensation. One important active ingredient that contained in chilli pepper is capsaicin (Anonym, 1997). Another nutrition that also considered having the ability to suppress malicious effect of cholesterol is vitamin E (Hennig and Boisson, 1988).

Several previous studies described the ability of capsaicin to modify the level of HDL and LDL. Nevertheless, the ability of combination of capsaicin and vitamin E in modifying HDL and LDL level has never been studied. According to that fact, a study that intended to investigate the effect of combination of capsaicin and vitamin E is important to conduct.

The objectives of this study is to understand the difference between LDL and HDL concentration in hypercholesterolemic Sprague Dawley rat after administration of capsaicin, vitamin E and combination of capsaicin and vitamin E.

MATERIALS AND METHODS

Animal treatment: This study used 25 sprague dawley (SD) rats which are obtained from Laboratorium Penelitian dan Pengujian Terpadu Universitas Gadjah Mada (LPPT UGM). We used male, adult, look-healthy rats, with weight ranging from 150-200 g. The rats were given standard diet and drank *ad libitum*. Rats were situated in cages with room temperature and light-dark cycle setting consisting 12 h each.

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Diet: Standard diet that used in this study are A.D. II diet which consist of (%): water, 12; protein, 15; fat, 37; fiber, 6; ash, 7; calcium, 0.9-1; and phosphor, 0.6-0.9. High fat diet (HFD) used in this study is A.D. II standard diet supplemented with 10% lard, yolk and olive oil. Standard diet and HFD were obtained from Pusat Studi Pangan dan Gizi (PSPG) UGM.

Capsaicin: Capsaicin was obtained by extracting chili pepper (*Capcisum frutescens*) with ethanol using maceration technique. Extraction was done in the Laboratorium Farmakologi dan Toksikologi UGM. Capsaicin dose used was 1 mg/ kg/body weight in liquid form. The usage of this dose was based on the consumption of capsaicin commonly consumed by the Thai people (Kawada *et al*., 1986).

Vitamin E: The dose of vitamin E used was 22 IU. This dose was based on the recommended dietary allowance issued by NIH (NIH, 2011). Vitamin E was derived from PSPG UGM.

Steps: SD rats were divided into 5 groups: A, B, C, D and E. After 3 days of adaptation, group B, C, D and E were fed HFD orally for 14 days. One group was given A.D.II as standard diet. Blood concentration of total cholesterol (TC) was measured to determine the success of induction of hypercholesterolemia. When group B, C, D and E had been induced, subsequent groups of mice were given appropriate interventions. Group A and B were not given any intervention and perceived as negative and positive controls, respectively. Rats in group C were given 1 mg/kg/body weight capsaicin for 14 days, group D were given 22 IU of vitamin E for 14 days and group E received a combination of capsaicin 1 mg/kg/body weight and 22 IU of vitamin E for 14 days. Parameters such LDL and HDL were measured before and after the intervention.

Parameter measurement: Blood total cholesterol, HDL and LDL of mice was measured by Randoux Cholesterol Test method and performed in PSPG UGM. Blood sampling was done via the orbital plexus of rats.

Statistical analysis: This study used computerization data analysis. In order to prove the differences between decrease of LDL level and increase of HDL level before and after the intervention, paired t-test was used. One-Way ANOVA used to find the affectivity of intervention between each group in decreasing the levels of LDL and increasing the level of HDL.

RESULTS

Administration of HFD was successful to induce hypercholesterolemia in rats. After 14 days, levels of LDL and HDL were measured as a pretest.

Table 1 shows rats total cholesterol levels after administration of HFD. Through this table, it could be seen that total cholesterol levels of the four groups of rats were above 200 mg/dL. It indicated the induction of hypercholesterolemia had been successfully carried out. Rats which had been declared being in hypercholesterolemia state were given intervention based on their group for 14 days.

Standard diet administration on the negative control group led to an increase in LDL level, from 27.92 to 30.62 mg/dL, p = 0.001 (Table 2). LDL level in the positive control group also experienced a significant increase (p = 0.007) after administration of HFD. Increased level of LDL that occurred in the negative control was greater than that occurred in the positive control. Administration of capsaicin on capsaicin group was able to significantly lower LDL level to 54.07 mg/dL (p = 0.000). This study showed that vitamin E reduce LDL level in hypercholesterolemic rats to 36.05 mg/dL (p = 0.000). SD hypercholesterolemic rats LDL level reduced significantly to 44.44 mg/dL after administration of capsaicin and combination of capsaicin and vitamin E (p = 0.000).

Table 3 demonstrates the standard diet administration on rats lowered HDL to 117.25 mg/dL (p = 0.566). There is a decrease of HDL level in positive control group to 57.25 mg/dL (p = 0.026). The decline that occurred in the positive control greater than the decline that occurred in the negative control. Administration of capsaicin caused insignificant raise in HDL level of hypercholesterolemic mice to 75.82 mg/dL (p = 0.09). HDL level of hypercholesterolemic mice experienced a significant increase to 106.80 mg/dL (p = 0.000) after the administration of vitamin E. The combination of capsaicin and vitamin E increased HDL levels in combination group to 88.37 mg/dL (p = 0.000).

This study showed that capsaicin, vitamin E and combination of both significantly reduced LDL. Effect of administration of capsaicin, vitamin E and combination of both on LDL levels were analyzed using a One Way ANOVA to determine whether there were any differences between them (Table 4). It can be seen from the table that there was significant difference in after treatment LDL level among the three intervention groups (p = 0.000). To determine which interventions provided the best reduction in LDL, a post hoc test was performed (Table 5).

Table 5 shows that the administration of vitamin E lowered LDL level better than the capsaicin and combination of both. This could be seen from the average post test levels of LDL in vitamin E group that was lower than those in capsaicin group and combination group.

Rats HDL levels increased in the three intervention groups. Capsaicin, vitamin E and combination of both increased HDL level significantly. Further analysis using One Way ANOVA was done to determine whether there were any differences in after-intervention HDL level in all three groups.

Table 1: Average of rats total cholesterol level before intervention

Group	Total cholesterol (mg/dL)
Negative control	108.76
Positive control	235.66
Capsaicin	240.64
Vitamin E	239.20
Combination	241.60

Table 2: Average rats LDL level in control and intervention group

	---- LDL (mg/dL) ----			
Group	Before	After	Δ[a] (mg/dL)	P[b]
Negative control	27.92	30.62	2.70	0.001*
Positive control	70.53	72.96	2.43	0.007*
Capsaicin	73.40	54.07	-19.33	0.000*
Vitamin E	71.18	36.05	-35.13	0.000*
Combination	72.15	44.44	-27.71	0.000*

[a]: LDL before-LDL after
[b]: P was counted with paired t test
*: Significant with p<0.05

Table 3: Average rats HDL level in control and intervention group

	---- HDL (mg/dL) ----			
Group	Before	After	Δ[a] (mg/dL)	P[b]
Negative control	117.42	117.25	-0.17	0.566
Positive control	60.13	57.25	-2.88	0.026*
Capsaicin	67.07	75.82	8.75	0.09
Vitamin E	61.55	106.80	45.25	0.000*
Combination	61.55	88.37	26.82	0.000*

[a]: LDL before-LDL after
[b]: P was counted with paired t test
*: Significant with p<0.05

Table 4: Analysis of mean LDL level of each after-intervention group

Group	Mean (mg/dL)	P[a]
Capsaicin	54.07	0.000*
Vitamin E	36.05	-
Combination	44.44	-

[a]: analyze using One Way ANOVA,
*: Significant with p<0.05

Table 5: Comparison of the effect of intervention on after-intervention LDL level mean

Group		Mean difference LDL post test	P[a]
Capsaicin			
	Vitamin E	18.02	0.000*
	Combination	9.63	0.000*
Combination			
	Capsaicin	-9.63	0.000*
	Vitamin E	8.39	0.000*
Vitamin E			
	Capsaicin	-18.02	0.000*
	Combination	-8.39	0.000*

[a]: Analyzed using Turkey post hoc test
*: Significant with p<0.05

Table 6: Analysis of mean LDL level of each after-intervention group

Group	Mean (mg/dL)	P[a]
Capsaicin	75.82	0.000*
Vitamin E	106.80	-
Combination	88.37	-

[a]: Analyze using One Way ANOVA
*: Significant with p<0.05

Table 6 describes that there were significant differences on after-intervention HDL levels in all three intervention groups (p = 0.000). To determine which interventions provide the best increase in HDL level, a post hoc test was performed.

Table 7: Comparison of the effect of intervention on after-intervention LDL level mean

Group	Post test HDL mean difference	P[a]
Capsaicin		
Vitamin E	-30.98	0.000*
Combination	-12.55	0.000*
Combination		
Capsaicin	12.55	0.000*
Vitamin E	-18.43	0.000*
Vitamin E		
Capsaicin	30.98	0.000*
Combination	18.43	0.000*

[a]: Analyzed with Turkey post hoc test *: Significant with p<0.05

Based on Turkey post hoc test appeared on Table 7, it is shown that the administration of vitamin E was able to increase HDL level better than those occurred in capsaicin group and combination group. This could be seen from the average post test levels of HDL in vitamin E group that was higher than those in capsaicin group and combination group.

DISCUSSION

This study showed capsaicin reduced LDL levels in hypercholesterolemic rats significantly (Table 2). This result fits with previous studies indicating that capsaicin could reduce blood LDL levels of turkeys given HFD (Negulesco *et al*., 1987). Capsaicin was also proven to reduce LDL and raise phospholipids, which works to increase solubility of cholesterol in micelle. This effect was associated with an increase of cholesterol 7-α hydroxylase enzyme. This enzyme was closely related to anti lithogenic effect of capsaicin (Shubha *et al*., 2011). Increased thermogenesis caused by capsaicin improved fat oxidation (Westerterp-Platenga *et al*., 1999). Thermogenesis effect arising from the administration of capsaicin on people who are obese could last for 8 weeks (Belza *et al*., 2007). Increasing thermogenesis by capsaicin occurred because capsaicin is able to increase the secretion of catecholamines from the adrenal medulla of mice, primarily through the activation of the central nervous system and the transient receptor potential vanilloid receptor 1 (TRPV1) (Hursel and Westerterp-Platenga, 2010). Increased fat oxidation due to thermogenesis, such as those found in some of the studies above, was likely to be one of the causes of the decrease in LDL levels in this study.

The administration of capsaicin 1 mg/kg/body weight capsaicin for 14 days also increased HDL, but this increase was not significant (Table 3). In a previous study, administration of capsaicin (0.015%) for 10 weeks on hypercholesterolemic rats increased HDL but the increase was not statistically significant (Shubha *et al*., 2011). Another study by Ma *et al*. (2011) showed that there was no meaningful increase in HDL from mice fed HFD after administration of capsaicin (0.01%) for 24 week. In that study, gene producing Apo E was removed. Still in that study, administration of capsaicin activated

TRPV1. *In vitro*, activation of TRPV1 receptor reduced lipid accumulation in vascular smooth muscle cells due to increased cholesterol efflux and decreased uptake of cholesterol.

Different results from this study demonstrated by Negulesco *et al.* (1987) which proved that there was an increase in HDL levels in turkeys which were fed HFD after given capsaicin and dihydrocapsaicin, one of capsaicinoid derivatives. The study also showed a significant increase in HDL levels in turkeys fed by cholesterol-free diet. The mechanism of HDL increase was likely to be caused by increased activity of the lipoprotein lipase enzyme by capsaicin. The enzyme lipoprotein lipase could produce surface remnant, which is a major source of HDL (Eisenberg, 1984). Another possible mechanism to explain the ability of capsaicin in increasing HDL was capsaicin can increase lecithin cholesterol acyltransferase enzyme (LCAT). LCAT worked as an agent that turn discoid shaped young HDL into spherical HDL particles through the conversion of free cholesterol into cholesterol esters. This spherical cholesterol served as a scavenger of tissue cholesterol, which can maintain cholesterol homeostasis (Mahley *et al*., 1984).

LDL levels of hypercholesterolemic rats decreased significantly after administration of vitamin E (Table 2). The same finding was experienced by some previous studies. Vitamin E was proven to reduce level of oxidized LDL and also improved the quality of LDL in baboons. Oxidized LDL was a risk factor for the emergence of cardiovascular disorders (Rainwater *et al*., 2007). Study by Hodis *et al*. (2002) also showed that vitamin E decreased circulating LDL and also decreased the chances for LDL to be oxidized.

In the group of rats fed with standard diet and not given HFD (negative control), there was a significant increase in LDL (Table 2). Through this result, we could conclude that LDL levels might rise even on a standard diet and HFD was only one factor that could increase levels of LDL. This was consistent with previous studies that showed the concentration of LDL in the blood will rise due to several factors such as diet high in fat or carbohydrate and less physical activity (Sola *et al*., 2012; Mangravite *et al*., 2011; Anonym, 2010).

Vitamin E administration in hypercholesterolemic mice successfully raised HDL cholesterol level significantly (Table 3). Several studies showed an opposite result. One of the studies was a study comparing the effects of vitamin E supplementation, with alpha-tocopherol as the main composition, with the HDL levels. Research conducted by Rainwater *et al*. (2007) showed two contradictory effects of vitamin E on HDL, which on the one hand reduced the size of HDL and on the other hand increased the levels of apo-AI, without significant changes in HDL levels. Small diameter HDL had negative effects for cardiovascular whereas apo-AI positively affect the blood vessel.

Shargorodsky *et al*. (2010) in a study showed that vitamin E, vitamin C, coenzyme 10 and selenium increased HDL, but the increase was not significant. Other studies had shown the use of tocotrienols proven to increase the ratio of HDL/total cholesterol through the mechanism of inhibition of HMG-CoA reductase (Chin *et al*., 2011).

Administration of combination of capsaicin and vitamin E significantly lowered LDL levels in hypercholesterolemic mice (Table 2). These results were consistent with the effect of administration of capsaicin and vitamin E when given separately which could lower LDL levels. The mechanism of this decrease was probably caused by a combination of vitamin E antioxidant ability and the aptitude of capsaicin to induce the cholesterol 7-α hydroxylase enzyme and increase thermogenesis (Rainwater *et al*., 2007; Shubha *et al*., 2011; Belza *et al*., 2007).

This study showed that the combination of capsaicin and vitamin E could significantly increase HDL (Table 3). It could be associated with other findings in this study which state that an increase in HDL may occur in the administration of vitamin E alone and capsaicin alone, although the administration of capsaicin gave a non-significant increase in HDL. This increase was associated with the ability of vitamin E in the inhibition of HMG-CoA reductase (Chin *et al*., 2011), the ability of capsaicin in activating the lipoprotein lipase enzyme (Negulesco *et al*., 1987) and LCAT (Mahley *et al*., 1984). Through analysis using Turkey post hoc test, it could be seen that the reduction in LDL levels and the increase of HDL occurred with the administration of vitamin E (Table 5 and 7). When combined with capsaicin, the ability of vitamin E in lowering LDL and raising HDL reduced. Mechanism that might lead to this was the ability of capsaicin to inhibit plasma membrane NADH oxidoreductase (PMOR). PMOR inhibition would lead to increased reactive oxygen species (ROS), which then induced apoptosis (Wolvetang *et al*., 1996). ROS contradicted the effect of endogenous antioxidants and vitamin E. The mechanism of capsaicin, vitamin E and a combination of capsaicin and vitamin E in lowering LDL and raising HDL described in this study was confined to the hypothesis (Piao *et al*., 2011; Yanai *et al*., 2008).

Conclusion: There was significant reduction of LDL levels in hypercholesterolemic rats after administration of capsaicin at a dose of 1 mg/kg/body weight for 14 days. HDL levels of rats given capsaicin increased, but not significant. After administration of 22 IU of vitamin E for 14 days, there was significant reduction of LDL levels and increased HDL levels in hypercholesterolemic rats. There was significant reduction of LDL levels and increased HDL levels in hyper cholesterolemic rats after administration of the combination of capsaicin 1 mg/kg/body weight and 22 IU of vitamin E for 14 days. The reduction of LDL levels and the increased of HDL levels are best in the group of rats given vitamin E.

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