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EFFECT OF CURCUMIN ON THE LEVELS OF TOTAL CHOLESTEROL, LDL-CHOLESTEROL, THE AMOUNT OF F_2-ISOPROSTAN AND FOAM CELL IN AORTIC WALL OF RATS WITH ATHEROGENIC DIET

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ABSTRACT

Death caused by cardiovascular and cerebrovascular disease is still the highest. Atherosclerosis is due to hypercholesterolemia of predisposing factor of both diseases. Curcumin has antioxidant character that can inhibit lipid peroxidase to atherogenesis process. The aim of this study was to prove the effect of curcumin to decrease total cholesterol level, LDL-cholesterol level, the amount of F2-isoprostan and the formation of foam cell in aortic wall in rats receiving atherogenic diet. To determine the effect of curcumin to decrease total cholesterol level, LDL-cholesterol, the number of F2-isoprostan and foam cell in wistar strain white rats, atherogenic diet was given for 10 weeks into 6 groups (n=24): atherogenic diet group (control), atherogenic diet group + curcumin (50 mg/kg bw per day), atherogenic diet group + curcumin (100 mg/kg bw per day), atherogenic diet group + curcumin (200 mg/kg bw per day), atherogenic diet group + curcumin (400 mg/kg bw per day), normal diet group (control). After 10th weeks, total cholesterol level and LDL-cholesterol were measured by spectrophotometry. The number of F2-isoprostan and foam cell was counted semi quantitatively by light microscope. F2-isoprostan staining was done with immunohystochemistry Avidin Biotin Complex method, and foam cell staining with HE-oil Red O. The results showed that the highest total cholesterol level was found in group I (275.15 ± 10.01; mean ± SD), the highest LDL-cholesterol level found in group I (158.15 ± 12.19). Statistically, total cholesterol level and LDL-cholesterol level in group I were significantly higher (p = 0.05) than those in other groups. Total cholesterol level and LDL-cholesterol was found to decrease in group V (93.31 ± 4.07; 19.76 ± 5.25). The highest number of F2-isoprostan was found in group I (5.5 ± 1.29). The highest foam cell was found in group I (3.5 ± 1). Statistically, the number of F2-isoprostan and foam cell in group I were significantly higher (p = 0.05) than those in other groups. The number of F2-isoprostan and foam cell were found to decrease in group V (1 ± 0.82 ; 0.5 ± 0.58). In conclusion, curcumin has effect in decreasing total cholesterol level, LDL-cholesterol, number of F2-isoprostan and the formation of foam cell significantly in rats with atherogenic diet. The administration of curcumin in a dose of 400 mg/kg bw daily is more effective in decreasing total cholesterol level, LDL-cholesterol, number of F2-isoprostan and the formation of foam cell than in other 3 doses of curcumin.

Keywords: *curcumin, total cholesterol, LDL-cholesterol, F2-isoprostan, foam cell and atherogenic diet.*

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INTRODUCTION

Atherosclerosis may cause fatal cardiovascular as well as cerebrovascular diseases, in which hypercholesterol is one of the predisposing factors in the occurrence of atherosclerosis (Yu et al. 2000). F2-isoprostan is an early predictor of early atherosclerotic, in which F2-isoprostan in the tissue is more sensitive and specific compared to that in the plasma (Arjuna 2002). One of toxic products in lipid peroxidation was F2-isoprostan (Halliwell & Gutteridge 1999). F2-isoprostan is an isomer of Prostaglandin F2 (PGF2) and an oxidative non-cyclooxygenase modification product of arachidonic acid resulting from the attack of free radicals towards phospholipide of cell membrane or LDL in the circulation (Mezzetti et al. 2000, Morrow & Roberts 1997). The increase of F2-isoprostan formation is related with the increase of thiobarbituric acid-reactive substance (TBARS) and hydroperoxide levels. This phenomenon can be prevented by Superoxide Dismutase (SOD), an oxygen free-radical scavenger (Mezzetti et al. 2000).

Curcumin is a phytopharmacy found in Turmeric (*Curcuma longa*). Curcumin can reduce lipid peroxidation by maintaining the activity of antioxidant enzymes, such as Superoxide Dismutase (SOD), Catalase and Gluthation Peroxidase in a higher level (Araujo & Leon 2001). Phenol and methoxy group in curcumin are active in scavenging the oxygen free

radicals (Shuran et al. 2000). Previous study found a significant reduction of total cholesterol level, LDL, and peroxidation in rat's blood receiving curcumin for 8 weeks (Babu & Srinivasan 1997). This study investigated whether curcumin had effect on the reduction of total cholesterol level, LDL-cholesterol, the amount of F2-isoprostan and foam cells as the early step towards atherosclerotic control. The objective of this study was to prove the effect of curcumin on the reduction of total cholesterol level, LDL-cholesterol, the amount of F2-isoprostan and foam cells in rats receiving atherogenic diet.

MATERIALS AND METHODS

This study used posttest only control group design in wistar-strain white rats (*Rattus norvegicus*) receiving atherogenic diet and normal diet for 10 weeks. The treatment group was divided as follows:

Table 1. Division of treatment groups

Groups	Treatment
I	Atherogenic diet aterogenik (control)
II	Atherogenic diet with curcumin dose 50 mg/kgBW/day
III	Atherogenic diet with curcumin dose 100 mg/kg BW/day
IV	Atherogenic diet with curcumin dose 200 mg/kgBW/day
V	Atherogenic diet with curcumin dose 400 mg/kgBW/day
VI	Normal diet (control)

Atherogenic diet was given by the addition of 2% cholesterol, 0.2% colic acid, and 10% swine oil in rats' diet (Ali et al. 2001). Blood sample was taken directly from the heart ventricle. Total cholesterol level measurement was carried out at the end of week 10 (Kaniawati 2004) by means of "DiaSys-Cholesterol FS" reagent with "CHOD-PAP Photometric Enzymatic Test" method using spectrophotometer in a wavelength of 500 nm. The LDL level measurement was performed indirectly using Friedewald formula, in which the LDL cholesterol = total cholesterol - TG/5 - HDL.

F2-isoprostan was examined immunohistochemically (Koss 1992). The frozen section of aortic tissue was stained immunohistochemically with ABC (Avidin Biotin Complex) and observed using light microscope in 400 x magnification. The F2-isoprostan was found in subendothelial aorta in brown color. For foam cell examination, the frozen section of aortic tissue was stained with hematoxylin Eosin and Oil Red O. Using light microscope with the same magnitude, the foam cell was also observable in red color with blue nucleus and located at peripheral area. The number of F2-isoprostan and Foam cell count measurement was done semi-quantitatively using binocular microscope with 1000 magnitude in 20 visual fields (Soini et al. 1998).

RESULTS

Table 2. Total Cholesterol Level and Blood LDL Cholesterol

Groups	Total cholesterol level (mg /dl)	LDL-cholesterol level (mg / dl)
I	275.15 ± 10.01	158.84 ± 12.19
II	216.68 ± 14.79	101.47 ± 18.65
III	137.45 ± 510.44	34.74 ± 14.35
IV	109.96 ± 10.18	21.86 ± 5.55
V	93.31 ± 4.07	19.76 ± 5.25
VI	91.14 ± 5.46	38.96 ± 7.82

Notes:
Total Cholesterol : p = 0.000
LDL-cholesterol : p = 0.000

Table 3. The number of aortic subendothelial F2-isoprostan (Mean ± SD) in control and treatment group

Treatment groups	Total subendothelial F_2-isoprostan/lp
I	5.5 ± 1.29
II	4.5 ± 1
III	3.5 ± 1
IV	1.75 ± 0.5
V	1 ± 0.82
VI	1.25 ± 0.96

notes: p = 0.000

Table 4. Foam cell count (Mean ± SD) in aortic wall in control and treatment groups

Treatment groups	Subendothelial foam cell count
I	3.5 ± 1
II	3 ± 1.15
III	1.75 ± 0.5
IV	1 ± 0.82
V	0.5 ± 0.58
VI	1 ± 0.82

notes: p = 0.000

It is apparent that using one-way Anova (a = 0.05), the development of bodyweight in control group was not significantly different (p > 0.05) from that in control group. Table 2 reveals that the mean of total cholesterol level is at the highest in group I, as much as 275.15 mg/dl. The lowest is in group IV, as much as 91.14 mg/dl. The highest mean of LDL cholesterol is in group I (158.84 mg/dl), and the lowest in group V (19.76 mg/dl). The total cholesterol level in group I is statistically significantly higher (p = 0.05) than that in other groups. The LDL-cholesterol level in group I is significantly higher (p = 0.05) that that in other groups. Table 3 shows that the highest mean of F2-isoprostan is in group I (5.5), and the lowest in group V (1). The number of F2-isoprostan in group I is significantly higher (p = 0.05) compared to those in groups IV, V and VI. It is apparent that the highest mean of foam cell count in group I is 3.5, and the lowest is in group V as much as 0.05. The foam cell count in group I was significantly higher (p = 0.05) than those in group IV, V, and VI.

Correlation and Regression

Table 5. Correlation between total cholesterol, LDL-cholesterol, foam cell count and F2-isoprostan

	Total cholesterol	LDL-cholesterol	Foam cells	F_2-isoprostan
Total cholesterol	1.000	0.970**	0.979**	0.961**
P value	-	0.001	0.001	0.002
LDL-cholesterol	0.970**	1.000	0.945**	0.890*
P value	0.001	-.	0.005	0.017
Foam cells	0.979**	0.945**	1.000	0.980**
P value	0.001	0.005	.-	0.001
F_2-isoprostan	0.961**	0.890*	0.980**	1.000
P value	0.002	0.017	0.001	-.

Notes:
* = Significant at alpha = 0.05
** = Significant at alpha = 0.01

Table 6. Correlation between curcumin, with total cholesterol, LDL-cholesterol, and F2-isoprostan number

		Curcumin
Total cholesterol	Pearson correlation	-0.812*
	Sig. (2-tailed)	.039
LDL cholesterol	Pearson correlation	-0.698*
	Sig. (2-tailed)	.049
F_2-isoprostan	Pearson correlation	-0.930*
		.024

Notes:
* = Significant at alpha = 0.05

Table 7. Correlation between curcumin and foam cells

		Curcumin
Foam cells	Pearson correlation	-0.885*
	Sig. (2-tailed)	.036

Notes:
* = Significant at alpha = 0.05

Table 5 shows significant correlation (a = 0.05) between LDL-cholesterol and F2-isoprostan. Other correlation also has high significant value (a = 0.01). Table 6 shows that the correlation between curcumin and total cholesterol as well as F2-isoprostan is highly significant (alpha = 0.05). Correlation between curcumin and LDL-cholesterol is also significant (alpha = 0.05). Table 7 shows that correlation between curcumin and foam cell is highly significant (alpha = 0.05). There was a negative correlation between the increase of curcumin dose and total cholesterol level, and the result of regression test revealed that the total cholesterol level = 193.13 - 0.2869 curcumin with R value = 0.6595. This indicated that the increase of curcumin dose of 1 mg would reduce total cholesterol level as much as 0.2869 times. There was also a negative correlation between the increase of curcumin dose and LDL-cholesterol. The regression test showed LDL = 77.117 - 77,117 - 0,1742 Curcumin with R value of 0.4894, indicating that a 1 mg increase of curcumin dose would reduce LDL level as much as 0.1742 times.

Negative correlation was found between the increase of curcumin dose and the amount of F2-isoprostan and regression test revealed F2-Isoprostan = 4.4891 - 0.0096 Curcumin with a value of R= 0.865, indicating that 1 mg curcumin dose increase would reduce the amount of F2-isoprostan of 0.0096 times. The increase of curcumin dose and foam cell count also had negative correlation, and regression test revealed foam cell = 2.7283 - 0.0062 curcumin with R value of 0.7833, showing that 1 mg increase of curcumin dose would reduce foam cell count of 0.0062 times.

DISCUSSION

From the measurement of total cholesterol level and LDL-cholesterol, it was found that control group with atherogenic diet (group I) was significantly higher (p = 0.05) than the control group with normal diet (group VI). This indicated that atherogenic diet could increase total cholesterol level and LDL-cholesterol in blood. The cause of lower total cholesterol level and LDL-cholesterol in groups IV and V resulted from curcumin administration. Curcumin has a role in the stimulation of the activity of hepatic enzyme cholesterol-7alpha-hydroxylase. The enzyme existing in the hepatic cell

may catalyze the change of cholesterol into bile salt. The increase of enzyme activity indicates an increase of cholesterol catabolism. 7alpha-hydroxylase reaction in cholesterol biosynthesis is the early stages that must be exist in bile acid biosynthesis. Due to enzyme stimulation by curcumin, the alteration of hepatic cholesterol to become bile salt becomes increasing, with the result that hepatic cholesterol level is decreasing. Therefore, to meet the need of cholesterol, the number of LDL receptors in the liver is increased, resulting in an increase of LDL removal in the plasma, which would be followed with the reduction of cholesterol and LDL plasma level (Lennernas & Fager 1997).

There was a positive correlation between atherosclerotic incidence and LDL concentration (Desager & Horsmans 1996, Murray 1999). The foam cell count of group I was significantly higher compared to other groups. Group with atherogenic diet had an increase of cholesterol level and subsequently elevate the number of foam cells. The increase of of total cholesterol and LDL in blood triggered the formation of foam cell. This is important in this study since the formation of foam cell is the early phase of atherosclerotic occurrence (Jialal & Devaraj 1996, Tjokroprawiro 2002).

The reduction of total cholesterol level and LDL by curcumin would reduce the formation of foam cells. Peroxidation in LDL has a role in atherosclerosis, in which anion superoxide plays as an agent that stimulates peroxidation in LDL. Oxidation in LDL can be reduced by antioxidant, so that the formation of foam cell and atherosclerotic lesion (Jialal & Devaraj 1996). Phenolic and methoxy groups in curcumin are active in scavenging oxygen free radicals (Babu & Srinivasan 1997, Sreejayan 1994). These groups in phenyl ring and 1.3-diketone system are observable as an important structure in curcumin chemical structure. They play a role in antioxidant activities (Sreejayan 1994, Venkatesan 2000). Curcumin also has capability in scavenging oxygen free radicals, such as anion superoxide and hydroxyl radicals substantial in commencing lipid peroxidation (Reddy & Lokesh 1992). Cholesterol controlling, particularly LDL, by curcumin can be seen as the controlling of foam cell formation as the early step of atherosclerotic controlling (Jialal & Devaraj 1996). The increase of F2-isoprostan in control group receiving atherogenic diet was significantly higher than that in other groups. The significant reduction of F2-isoprostan was found in curcumin group with dose 4 (group V), in which the reduction was not significantly difference from the F2-isoprostan amount in control group receiving normal diet. Similar to foam cell, the reduction of F2-isoprostan amount was also resulted from curcumin administration as antioxidant that had capability in scavenging oxygen free radicals, such as anion superoxide and hydroxyl radical substantial in commencing lipid peroxidation (Venkatesan 2000, Reddy & Lokesh 1992).

Curcumin is also able to defend the activity of antioxidant enzymes, such as superoxide dismutase (SOD), Catalase and Gluthation Peroxidase (GPx), in higher level, in which these enzymes have important role in lipid peroxidation regulation (Reddy & Lokesh 1992). The increase of F2-isoprostan formation is related with the increase of TBARS (Thiobarbituric Acid-Reactive Substance) and hydroperoxide levels. This phenomenon can be prevented with SOD, a scavenger of oxygen free radicals (Halliwell & Gutteridge 1999). With its antioxidant nature, curcumin can reduce foam cell count and F2-isoprostan as the early step of atherosclerotic controlling.

CONCLUSIONS

Curcumin has effect in reducing total cholesterol level, LDL-cholesterol, number of F2-isoprostan, and foam cell count in in rats receiving atherogenic diet. This study proved that the administration of curcumin in a dose of 400 mg/kg/BW daily was more effective in reducing total cholesterol level, LDL-cholesterol, number of F2-isoprostan, and foam cell count compared to other three doses. Further studies are needed to determine optimal dose and administration frequency of curcumin. Clinical test in human is also needed if it is intended to be used clinically.

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