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Synergistic Effect of Curcuminoid and S-methyl Cysteine in Regulation of Cholesterol Homeostasis

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Abstract: Turmeric and garlic have been known to lower cholesterol levels in hyperlipidemic state. Thus, both can be used as an alternative hyperlipidemia therapy to prevent complications of cardiovascular diseases. However, the mechanism of actions of both in lowering cholesterol are still unclear. The purpose of this study was to determine the effect of curcuminoid, S-methyl cysteine and its combination in regulation of cholesterol levels in serum, liver and feces. This study used an animal model of rats with cholesterol metabolism abnormality induced by propylthiouracyl for 7 days. Curcuminoid, S-methyl cysteine and its combination were given on day 8, 1 h before the induction of cholesterol solution. Total cholesterol level in serum, liver and feces were measured during 6 h of observation. The results showed that the curcuminoid, S-methyl cysteine and its combination maintained the serum cholesterol within the normal level by inhibiting cholesterol absorption and lowering cholesterol level in liver. Curcuminoid and its combination with S-methyl cysteine increased the conversion of cholesterol into the feces as much as 3 times higher than the control group. While the S-methyl cysteine alone did not increase the conversion of cholesterol into the feces. We concluded that curcuminoid and S-methyl cysteine have multiple site of actions in lowering cholesterol level in the body. Both also work synergistically to overcome hyperlipidemia.

Key words: Hyperlipidemia, curcuminoid, S-methyl cysteine, total cholesterol level, propylthiouracil

INTRODUCTION

Cholesterol is an important component that plays a role in maintaining normal function of cells (Maxfield *et al*., 2005). Normal level of cholesterol in cells is maintained by regulating the cholesterol through synthesis and dietary absorption. HMG CoA reductase enzyme activity changed rapidly following the changes in plasma cholesterol levels to maintain its homeostasis (Lange *et al*., 2008). Excessive level of cholesterol is toxic to cells. The excess cholesterol in macrophages are associated with atherosclerosis (Attie, 2007).

Various pathways of cholesterol metabolism include the absorption, biosynthesis, catabolism and excretion of cholesterol can be inhibited by cholesterol-lowering drugs currently available through the use of drug combinations. Nowadays, statin is the main choice for lowering cholesterol in serum. These drugs inhibit the synthesis of cholesterol in the liver that is catalyzed by HMG CoA reductase enzyme (Blum *et al*., 2004). However, there are still many people with hyperlipidemia who had not reached the target after therapy with statin. Other mechanism of action of cholesterol-lowering drugs that is potential to be developed is by inhibiting absorption of cholesterol that comes from the diet. Ezetimibe is cholesterol-lowering drug that can inhibit the absorption of cholesterol. However, inhibition of cholesterol absorption causes the increase of synthesis of endogenous cholesterol in the liver. Therefore, the use of ezetimibe is usually combined with statin drugs (Turley, 2004). The combination of statin with ezetimibe is one example of a favorable combination of drugs because it can inhibit the absorption and biosynthesis of cholesterol, making it easier to achieve the goal of lipid therapy (Turley, 2004). Therefore, the effect of drugs on various metabolic pathways of cholesterol represents a highly effective therapeutic strategies and is required to achieve the goals of therapy for patients who do not respond to a cholesterol-lowering drugs currently available. This is an opportunity to seek alternative hyperlipidemia therapy that can affect various metabolic pathways of cholesterol to reduce the risk of cardiovascular disease.

Curcuminoid and S-methyl cysteine are components of turmeric and garlic, respectively that have been known to have a good antihyperlipidemia effect on proclinic and clinical studies (Yeh and Liu, 2001; Sukandar *et al*., 2010). Research on the activity of a combination of garlic and

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turmeric as antihyperlipidemia also been carried out *in vivo* and clinical trials in hyperlipidemic patients (Ashraf *et al*., 2005; Sukandar *et al*., 2010). However, the action mechanisms of curcuminoid and S-methyl cysteine as antihyperlipidemia are still unclear.

The purpose of this study is to investigate the influence of curcuminoid, S-methyl cysteine and its combination on exogenous cholesterol absorption, cholesterol levels in the liver and feces.

MATERIALS AND METHODS

This study was conducted from June-August 2010, in the laboratory of pharmacology and toxicology, School of Pharmacy, Institute of Technology Bandung (ITB), Indonesia. All experimental procedures were approved by animal ethics committee of Hasan Sadikin Hospital, Bandung, Indonesia.

This research was conducted in accordance with the cholesterol homeostasis method as described by Hasimun *et al.* (2011). Briefly, this research was conducted on 24 male Wistar rats aged 3 months with an average body weight of 150-200 g. Animals were divided into 4 groups of 6 rats. all groups were induced by Propylthiouracil (PTU) both orally and in drinking water for 7 days. On day-8, after 16 h of fasting, blood were taken for initial total cholesterol level. Then, group 2 received curcuminoid (purchased from Sigma) of 100 mg kg^{-1} b.wt. dosage, group 3 received S-methyl cysteine (purchased from Sigma) of 100 mg kg^{-1} b.wt. dosage, group 4 received a combination of curcuminoid and S-methyl cysteine of 50 mg kg^{-1} b.wt each dosage while group 1 act as control received carrier only.

One hour after test drug administration, all groups received a solution of cholesterol (purchased from Sigma) in vegetable oil (Bimoli®, obtained from local market) of 400 mg kg^{-1} b.wt. dosage. Blood sampling was performed every hour for 6 h and during observation animals were placed in metabolic cages where feces were collected for total cholesterol analysis. After observation, the animals were sacrificed and the liver were taken for analysis of cholesterol level. Total cholesterol level in serum, liver and feces were measured using commercial enzymatic kits. The data obtained is expressed as Mean±SD. Statistical analysis were using SPSS 14.0 for Windows.

RESULTS AND DISCUSSION

In this study, group received curcuminoid, S-methyl cysteine and its combination showed lower total cholesterol level in serum compared to control group

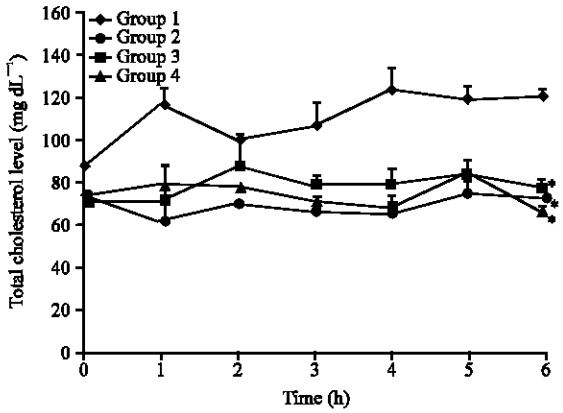

Fig. 1: Profile of total cholesterol levels in serum in all treatment groups for 6 h of observation. *Significant different compared to control group ($p<0.05$)

during the observation period of 6 h (Fig. 1). Generally, within the first or second hour total cholesterol level in serum derived only from the absorption process. The process of cholesterol metabolism started in the next hour. Control group showed the cholesterol absorption profile of about 70% higher than its initial level. Mean while, the group of animals that received curcuminoid, S-methyl cysteine and its combination showed inhibition of exogenous cholesterol absorption in the first 1 hour that were statistically significant compared to the control group ($p<0.05$). Group received combination curcuminoid and S-methyl cysteine showed a decrease in serum cholesterol level that were statistically significant with $p = 0.034$ and $p = 0.001$ compared to group received curcuminoid and group received S-methyl cysteine, respectively. Cholesterol level between group received curcuminoid and group received S-methyl cysteine did not show a statistically significant difference ($p = 0.146$).

Present study showed that curcuminoid, S-methyl cysteine and its combination also affect the level of total cholesterol in the liver. Total cholesterol levels in the liver after 6 hours of cholesterol administration can be seen in Fig. 2. Curcuminoid, S-methyl cysteine and its combination (groups 2-4) lowered total cholesterol in the liver about 65% compared to control group and were significantly different ($p<0.05$). The combination of curcuminoid and S-methyl cysteine (group 4) lowered total cholesterol in the liver of about 25% greater compared to group received curcuminoid ($p = 0.003$) and group received S-methyl cysteine ($p = 0.045$). While the difference in reduction of total cholesterol in the liver between group 2 and 3 showed no statistically significant difference ($p = 0.271$).

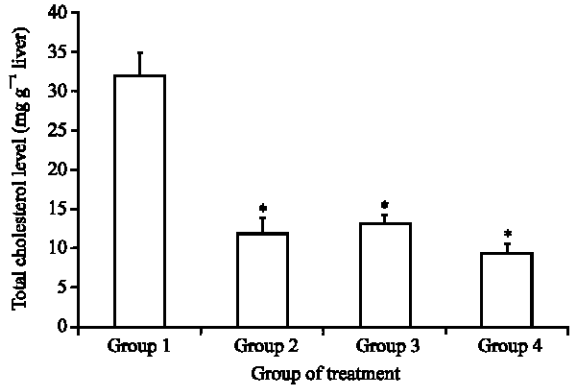

Fig. 2: Total cholesterol levels in the liver after 6 h of administration of exogenous cholesterol for all treatment groups. *Significant difference compares to control group ($p<0.03$)

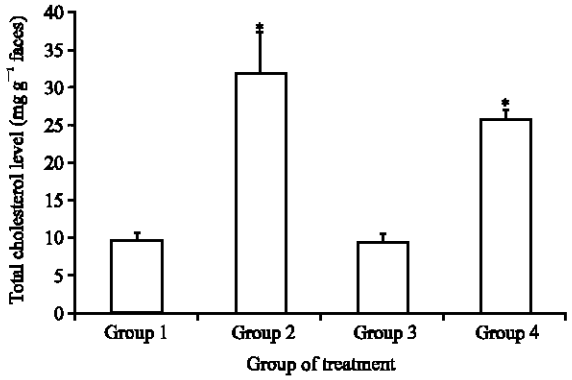

Fig. 3: Total cholesterol levels in the feces after 6 h of administration of exogenous cholesterol for all treatment groups. *Significant difference compares to control group ($p<0.01$)

To understand the effect of the test drug in modulating cholesterol conversion into the feces, the total cholesterol level in feces were collected during 6 h of observation then measured. Total cholesterol levels in the feces can be seen in Fig. 3. Group 2 received curcuminoid and group 4 received combination of curcuminoid and S-methyl cysteine showed an increase of total cholesterol levels in feces as compared to that of control group and were statistically significant ($p<0.05$). While group 3 received S-methyl cysteine showed no increase in total cholesterol level in feces compared to that of control group. Group received curcuminoid significantly increased level of cholesterol in the feces compared to that of group received curcuminoid and s-methyl cysteine combination ($p = 0.002$).

Cholesterol level in the body is tightly controlled through regulation of endogenous cholesterol synthesis, absorption of exogenous cholesterol and elimination through feces to meet the needs of body cells (Princen *et al*., 1997). This regulation prevents excessive cholesterol level in cells that are harmful to the cell physiology (Bonetti *et al*., 2003). Dietary cholesterol has been known to play a role in causing atherosclerosis. This because the dietary cholesterol indirectly increase serum LDL cholesterol level through sterol metabolism in the liver (Dietschy *et al*., 1993).

Liver is the organ that plays a key role in adapting to a high cholesterol diet (Russell and Setchell, 1992). Fasting for at least 24 h (without dietary cholesterol) will increase the biosynthesis of cholesterol in the liver. Conversely, high cholesterol diet inhibits the synthesis of cholesterol in the liver (Jones, 1997). High cholesterol diet also increases the activity of 7α-hydroxylase enzymes that play a role in bile acid synthesis (Horton *et al*., 1995). Therefore, it can be concluded that in general, cholesterol level in serum is influenced by the biosynthesis of endogenous or exogenous cholesterol absorption. Level of cholesterol in the liver is influenced by the biosynthesis of endogenous cholesterol or cholesterol elimination through bile acids. Feces cholesterol level is influenced by the absorption of exogenous cholesterol or cholesterol transport back to the liver as cholesterol catabolism path.

According to Abrams and Grundy (1981), in hypothyroid stated there were an increase absorption of exogenous cholesterol, decrease cholesterol biosynthesis in the liver and decreased excretion of cholesterol through bile acid. Administration of cholesterol will cause an increase cholesterol level in serum and liver while cholesterol level in feces remains low. Present study, using animal-induced hypothyroidism, showed that in the control group there was an increase of cholesterol level in serum and liver while no increase of cholesterol level in feces. This result was in agreement to the finding of Abrams and Grundy (1981).

Present study showed that curcuminoid reduced cholesterol levels in serum and liver. In the first hour after administration of cholesterol, curcuminoid inhibited cholesterol absorption compared to control group that showed an increase in serum cholesterol level. Administration of curcuminoid showed no effect of elevated level of cholesterol in the liver in response to exogenous cholesterol absorption inhibition (unlike ezetimibe). This can be seen in lower cholesterol level in the liver after administration of curcuminoid compared to the control group. Our study supported Godkar *et al*. (1996) study reporting the effects of dietary curcuminoid in rats for 30 days that lowered cholesterol levels in serum and liver.

Present result supported Srinivasan and Sambaiah, (1991) study, reported that curcuminoid increased the

activity of bile acid synthesis that will be secreted into the intestine to emulsify cholesterol. This study showed increased level of total cholesterol in the feces by 3.5 times compared to control group (Fig. 3). The consistency of feces also more flaccid. This result suggest that curcuminoid have a mechanism to increase the conversion of cholesterol into feces. Cholesterol in the feces probably came from exogenous cholesterol that was not absorpted in the intestine and of endogenous cholesterol that was converted into bile acids.

Garlic contains two types of sulfur compounds which are fat soluble and water soluble sulfur compounds (Amagase, 2006). S-methyl cysteine is one of garlic sulfur compounds that are water soluble (Muoio *et al*., 2004). Garlic has been known to have activity in antihiperlipidemia especially lowering total cholesterol and LDL cholesterol levels (Simons *et al*., 1995). One of the action mechanism of garlic in lowering cholesterol levels is through the inhibition of cholesterol biosynthesis in liver (Gebhardt, 1993).

Present study showed that S-methyl cysteine lowered cholesterol levels in serum and liver. This result was in agreement to the finding of Gebhardt (1993). Decreasing in cholesterol level in serum was probably due to S-methyl cysteine inhibited cholesterol biosynthesis in the liver thereby increasing metabolism of cholesterol in serum and VLDL in the liver. Serum cholesterol level in the first hour was higher in the group that only received S-methyl cysteine compared to the group that only received curcuminoid (Fig. 1), although the different was not statistically significant. This showed that in the group that received S-methyl cysteine the absorption of exogenous cholesterol was still occurring so that cholesterol level in the liver was higher than the group receiving only curcuminoid. Interestingly, S-methyl cysteine did not increase the conversion of cholesterol into feces indicated by cholesterol level in the feces that was remained the same as in the control group. S-methyl cysteine focus more in increasing the metabolism of cholesterol in serum and liver resulting in decreased levels of cholesterol in serum and liver. It can be observed in Fig. 1 that between 2nd to 6th h serum cholesterol level for group 3 was lower than group 2.

Administration of a combination of curcuminoid and S-methyl cysteine lowered cholesterol levels in serum and liver and accompanied by increasing in level of cholesterol in feces. This combination showed the combine action mechanisms in inhibiting cholesterol absorption and biosynthesis accompanied by increasing metabolism and conversion of cholesterol into the feces. Total dose of 100 mg kg^{-1} b.wt. in the combination showed a stronger antihyperlipidemic effect. It indicates that curcuminoid and S-methyl cysteine worked synergistically to give a better effect than the use of its single.

The function of the test drug in regulating cholesterol homeostasis in the serum ordered from the strongest to the weakest as followed: combination (curcuminoid and S-methyl cysteine) > curcuminoid > S-methyl cysteine. In the liver: combination (curcuminoid and S-methyl cysteine) > curcuminoid > S-methyl cysteine. Conversion of cholesterol into feces: curcuminoid > combination (curcuminoid and S-methyl cysteine) > S-methyl cysteine.

CONCLUSION

Curcuminoid, S-methyl cysteine and its combination have multiple mechanisms of action in lowering cholesterol levels. Curcuminoid and S-methyl cysteine inhibited the intestinal cholesterol absorption and increased cholesterol metabolism in serum and liver. In addition, curcuminoid also mudulated the conversion of cholesterol into the feces. The combination of curcuminoid and S-methyl cysteine work synergistically in regulating the homeostasis of cholesterol in serum, liver and modulate the conversion of cholesterol into the feces.

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