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Research Article

Hypocholesterolaemic Effects of *Rhodobacter capsulatus* on Rat

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ABSTRACT

Rhodobacter capsulatus (*RC*) is a photosynthetic purple bacterium that able to reduce serum cholesterol and triglycerides concentrations in rats and pigs as well as egg-yolk of laying hens and Japanese quails and in broiler meat. The study was designed to investigate the hypochlesterolemic effects of *Rhodobacter capsulatus* on rats as experimental animal. Total 63 Wistar-Imamichi male rats were randomly divided into three dietary groups. The rats were fed a basal diet (BD), high cholesterol (1%) diet (HCD) and HCD plus 0.02% *RC* (HCD+*RC*) diet for 4 weeks. Enzymatic analysis of serum, liver tissues and faces samples, bile acid determination, histological study of hepatic lipid accumulation, and incorporation of ^{14}C-glucose into lipid fractions were performed. The concentration of LDL-cholesterol and triglycerides in serum were significantly ($P<0.05$) reduced in the rats fed HCD+*RC* than HCD diet. However, there was no significant ($P>0.05$) difference in case of total cholesterol, HDL-cholesterol and glucose concentrations in serum of the rats fed experimental diets. Compared to the other experimental diets, the HCD+*RC* potentially counteracted the accumulation of lipid droplets in the liver cells observed after staining by Oil Red O and Sudan III stains. Total cholesterol and triglycerides concentrations were significantly decreased ($P<0.05$) in liver and increased in faces of the rats fed HCD+*RC* than that of HCD diet. The concentrations of bile acids were increased in both liver and faces of the rats fed HCD+*RC* diet. Incorporation of ^{14}C-glucose among into lipid fractions of liver tissues, triglycerides were increased ($P<0.05$) where cholesterol, cholesterol esters, fatty acids and phospholipid were slightly ($P>0.05$) decreased in the rats fed HCD+*RC* diet. Therefore, the study concludes that the dietary *RC* has the overall potential hypocholesterolemic effects on rats.

Key words: *Rhodobacter capsulatus*, Hypocholesterolemic effects, Incorporation of ^{14}C-glucose, Lipid metabolites, Rat

INTRODUCTION

The *Rhodobacter capsulatus* (ATCC 11166) is a photosynthetic purple bacterium introduced as a highly beneficiary agent in the production of single-cell protein, water purification, and fish culture (Kobayashi and Kurata, 1978). Besides these, *Rhodobacter capsulatus* (*RC*) is known as a hypocholesterolemic agent able to reduce serum cholesterol and triglycerides concentrations in rats and pigs (Tsujii *et al.*, 2007; 2008). It is also a potential agent for lowering cholesterol concentrations not only in serum, but also in egg-yolk of laying hens and Japanese quails, as well as in broiler meat (Salma *et al.*, 2007a, b, c). Moreover, the dietary supplementation of *RC* improved the ratio of unsaturated fatty acids to saturated fatty acids in egg-yolk of laying hens and Japanese quails and in broiler meat (Salma *et al.*, 2007a, b, c; Sadia *et al.*, 2010; Salma *et al.*, 2011). Lipids, such as cholesterol and triglycerides are insoluble in plasma. Circulating lipid is carried in lipoproteins that transport the lipid to various tissues for energy utilization, lipid deposition, steroid hormone production, and bile acid formation (Mahley *et al.*, 2008). Plasma lipoproteins are divided into 5 major classes based on their relative density: chylomicrons, very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) (Kwiterovich *et al.*, 2000). Bile acids are the end products of cholesterol catabolism in the liver (Chiang, 2009). In addition to the classic function of bile acids in facilitating intestine absorption and transport of nutrients, drugs, and steroids, bile acids also play important roles in regulating the lipids, drugs, glucose, and energy metabolism (Lefebvre *et al.*, 2009). The bile acid–binding resins improved glycemic control in patients with type 2 diabetes and dyslipidemia (Garg and Grundy, 1994; Kawabata *et al.*, 2006), indicate a close link of bile acid metabolism with glucose and lipid metabolism and suggest a novel

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therapeutic agent of obesity and diabetes. Type 2 diabetes and dyslipidemia are more frequently associated with each other than by chance, pointing to a possible common underlying mechanism(s) in their etiology (Taskinen, 2005). From the clinical point of view, dyslipidemia in patients with type 2 diabetes has several features: predominance of remnant particles and small dense LDL and elevation of plasma triglycerides, especially in a postprandial state, as well as low HDL cholesterol (Garg and Grundy, 1990). These are highly atherogenic and, thus, predispose patients with diabetes to atherosclerotic disease, such as coronary artery disease and stroke, which not only accounts for 70% of mortality in patients with diabetes, but also places a social and economical burden in many countries (Taskinen, 2005). Therefore, therapeutic strategies that are beneficial for both conditions are strongly warranted. Bile acids are increasingly recognized for their function as metabolic regulators. Via the activation of different signaling pathways, they participate in the control of bile acid, lipid and glucose metabolism. However, the effect of dietary *RC* on hepatic accumulation of lipids and metabolism of lipid fractions are still in dark. Therefore, the present study was designed to examine the hypocholesterolemic effects of *RC* in rats fed high cholesterol diet.

MATERIALS AND METHODS

Animals, diet and management

A total of 80 male pathogen free Wistar-Imamichi rats were purchased from Japan SLC Inc., Shizuoka, Japan and acclimatized for one week with *ad libitum* basal diet (Table 1) and clean drinking water. At five-week old, total 63 uniform (125−127 g) rats were selected and randomly divided into three groups having 21 in each group. The rats were housed, cared and sacrificed in accordance with the 'Guidelines for Regulation of Animal Experimentation, Faculty of Agriculture, Shinshu University, Japan'. They were housed individually in suspended stainless steel cages in a light-controlled room with a 12 h light-dark cycle beginning at 06.00 hours and with a temperature of 21±1°C. The rates under control groups were fed basal diet (BD) and high cholesterol diet (HCD), and treatment group was fed HCD+0.02% of *RC*. The BD was formulated according to the Lab MR stock, Nihon Nosan Kogyo Co., Ltd., Kanagawa, Japan. Cholesterol (1% of the BD, Wako Pure Chemical Industries, Tokyo, Japan) was added to the BD. The *RC* cells were grown in outdoor culture under natural illumination as previously described (Kobayashi and Kurata, 1978) and nutrient composition was maintained according to Salma *et al.* (2011). The rats were fed daily HCD or BD for 4 weeks *ad libitum*. The dose of 0.02% *RC* was decided based on our previous study (Tsujii *et al.*, 2007).

Body weight and feed intake

The rats were monitored daily for general health and weighed individually prior to blood collection at the beginning and end of each week. Their daily feed intake was recorded and feed efficiency (feed intake: body weight gain) was calculated during 4 weeks of the experimental period.

Table 1: Nutrient composition of the basal diet

Proximate components	Amount (%)	Mineral	Amount (%)
Crude protein	18.37	Calcium	0.96
Crude Fat	3.81	Chlorine	0.40
Crude Fiber	6.45	Magnesium	0.25
Crude Ash	6.74	Phosphorus	0.78
Nitrogen free extracts	53.44	Potassium	0.95
Vitamin	Amount (%)	Sodium	0.28
Vitamin A	16,150 (IU)	Selenium	0.00
Vitamin D3	3,168 (IU)	Cobalt	0.00
Vitamin E	0.0044	Iron	0.02
Vitamin K3	0.0015	Manganese	0.01
Vitamin B1	0.0010	Zinc	0.01
Vitamin B2	0.0009	Copper	0.00
Vitamin B6	0.00180	Amino acids	Amount (%)
Vitamin B12	0.000003	Arginine	1.07
Vitamin C	0.0048	Histidine	0.45
Choline	0.1800	Isoleucine	0.66
Folic acid	0.0003	Leucine	1.33
Niacin	0.0099	Lysine	0.87
Pantothenic acid	0.0025	Methionine	0.25
Biotin	0.00003	Phenylalanine	0.82
Inositol	0.0010	Threonine	0.63
Carotene	0.0001	Tryptophan	0.21
		Valine	0.77
		Cysteine	0.27

Blood, liver, kidney, heart and droppings collection

At the end of the experimental period, after 4 h of fasting the rats were anesthetized, blood was collected from the abdominal aorta and centrifuged at 3,000×g for 10 min to collect the serum. After blood collection, the rats were killed with an ethyl-ether inhalation and their liver and heart were collected and washed with normal saline, blotted dry on filter paper and weighed. On the other hand, fresh droppings samples from each rat in each group were collected. Care was taken to collect fresh droppings that had no contact with other things. All droppings samples were dried in a forced-air oven at 60°C until reaching constant weight. The serum, liver, heart and droppings were then stored at -80°C until analysis.

Liver and droppings sample preparation

Hepatic and droppings lipids were extracted from 300 mg of the liver and droppings of rats with chloroform: methanol (2:1, v/v), according to the method of Folch *et al.* (1957). After 24 hours of shaking, it was filtered and centrifuged at 1,000×g for 10 minutes with 20% distilled water to recover the lower layer. Then they obtained samples were stored at -80°C until further analysis.

Enzymatic analysis

The total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol and glucose concentrations in the serum samples were measured spectrophotometrically at 570 nm by using commercially available reagent kits (Wako Pure Chemical Industries, Tokyo, Japan) according to the manufacturer's instructions. The cholesterol and triglycerides concentrations in liver and droppings samples were determined using the same reagent kits as those used for the serum.

Oil red O staining

Oil red O staining for neutral lipids was performed on fresh frozen tissue cut in 8-μm sections. Slides were air

dried for 30−60 minutes and fixed in ice-cold 10% formalin for 10 minutes. Slides were rinsed three times in distilled water, placed in propylene glycol for 5 minutes, and stained with 0.5% oil red O solution for 8 minutes at 60°C. After rinsing in 85% propylene glycol solution for 30 minutes, slides were washed with distilled water and stained in Gill's hematoxylin solution for 30 seconds. After two rinses in distilled water, slides were mounted with aqueous mounting medium and examined under a microscope.

Sudan III stain

Sudan III is a lysochrome (fat soluble dye) predominantly used for staining triglycerides in animal tissues (frozen sections). The frozen section slides were fixed in 10% phosphate buffered formalin for one minute and rinsed sections carefully in two changes of distilled water and again rinsed in ethyl denatured 70% alcohol. Then stained in Sudan III Stain for 10 minutes and removed excess stain by dipping in ethyl denatured 70% alcohol and then washed thoroughly in distilled water. Depending on preference of nuclear stain intensity, counterstained with hematoxylin stain for 2−3 minutes, then washed gently in several changes of tap water. Blue slides were dipped 10 times in supersaturated lithium carbonate and washed gently in several changes of tap water; blotted excess water from slides and mounted by coverslip with Mount-Quick Aqueous Mounting Medium.

Electron microscopy

At the time of tissue removal, pieces of rat liver were fixed in Trump's fixative. After fixation in osmium tetroxide, the samples were embedded in epoxy resin and thinly sliced (0.12 µM). The sections were stained with a solution of uranyl acetate followed by aqueous lead citrate and viewed using a JEOL (Tokyo, Japan) transmission electron microscope. Thicker sections (1 µM) of the embedded tissue that had been fixed in osmium tetroxide were counterstained with methylene blue and viewed under a regular light microscope.

Incorporation of ^{14}C-glucose

Fresh hepatic tissue (100 mg) was placed into a tube containing phosphate buffer, and homogenized at room temperature for 1 min. After pre-incubation for 10 min, ^{14}C-glucose (370 MBq/mM; specific activity 230 µM/mM) was added and samples were incubated with shaking incubator for 3 hours at 37ºC. The liver tissues were centrifuged 12,000×g at 4ºC for 10 min. Lipids in liver were extracted with chloroform and methanol (2:1) according to the method of Bligh and Dyer (1959). After the separation from alcoholic layer, chloroform layer was collected and evaporated to dryness. The lipid extract was dissolved in 1.0 ml hexane and aliquots of this extract were used for further analysis. The distribution of ^{14}C-glucose in different neutral and polar lipids was carried out by thin layer chromatography (TLC). TLC was carried out by using aluminium sheet silica gel 60 thin-layer plates (Merck, Darmstadt, Germany) 2.5×7.5 sq. cm. For the fractionation of neutral lipids, the lipid extract was developed in hexane/diethylether/glacial acetic acid (80:20: 2 in volume) at room temperature. On the other hand, for the characterization of the polar lipid fractions, development in methyl acetate/propan-1-ol/chloroform/ methanol/0.25 % aqueous KCl 825:25:25:10:9 in volume) was made appropriate non-labeled lipid standards (purchased from Supelco, Inc., Minesota, USA) were added to the liver lipids to provide enough amount of materials for the detection on thin layer plates. Spots were visualized and scraped from the plates and collected separately in scintillation vials containing 1 ml water and 10 ml scintillation cocktail and their radioactivity was measured by the liquid scintillation counter (LS 6500; Beckman Instruments, inc., USA) as counts per minutes (CPM).

Statistical analysis

Data were analyzed as a one-way ANOVA by the general linear model procedures of SAS. The means for treatments showing significant differences in the ANOVA were compared using Fisher's protected least significant difference procedure. Values were expressed as mean±SEM. Differences were considered significant at level of $P<0.05$.

RESULTS

Dietary effects of *RC* on body weight, feed efficiency and organ weight of rats fed BD, HCD and HCD+*RC* for 4 weeks are shown in Table 2. There were no significant dietary effects ($P>0.05$) on final body weight, body weight gain, feed intake, feed efficiency, as well as organs weight (liver and heart) among the rats during 4-wk experimental period. However, there was a little ($P>0.05$) effect of HCD+*RC* on final body weight, body weight gain, feed intake, feed efficiency, as well as organs weight. Faces excretion was significantly ($P<0.05$) increased in the rats fed HCD+*RC* than BD diet.

Effect of 4-wk feeding with BD, HCD and HCD+*RC* on lipid metabolites and glucose concentration in serum are shown in Table 3. The concentration of LDL-cholesterol and triglycerides in serum were significantly ($P<0.05$) reduced in the rats fed HCD+*RC* than HCD diet. But there was no significant ($P>0.05$) difference in case of total cholesterol, HDL-cholesterol and glucose concentrations in serum of the rats fed experimental diets. However, little higher values of HDL-cholesterol and glucose concentrations were observed in the rats fed HCD+*RC* compared to the other diets.

Dietary effects of *RC* on lipid metabolites and bile acids (mg/g) in liver and faces of the rats are shown in Table 4. Total cholesterol and triglycerides concentrations were significantly decreased ($P<0.05$) in liver and but increased in faces of the rats fed HCD+*RC* than those of HCD diet. The concentration of bile acids were increased in both liver and faces of the rats fed HCD+*RC* compared to BD or HCD diet.

Effects of *RC* supplemented diet on incorporation of ^{14}C-glucose into lipid fractions in liver tissues of the rats are shown in Table 5. There was a greater ($P<0.05$) incorporation of ^{14}C-glucose into hepatic triglycerides in the rats fed HCD+*RC* than that of HCD diet. But incorporation of ^{14}C-glucose into hepatic cholesterol, cholesterol esters, fatty acids and phospholipids were slightly ($P>0.05$) decreased in the rats fed HCD+*RC* diet.

Table 2: Effect of dietary *Rhodobacter capsulatus* on body weight, feed efficiency and organ weight of rats.

Parameters*		Control (BD)	HCD	HCD+*RC*
Body weight (g)	Initial	127.1±0.9	127.0±2.0	125.3±2.0
	Final	306.6±9.6	312.4±4.6	319.8±7.9
Weight gain (g)		179.5±8.1	185.4±4.3	194.5±6.9
Total feed intake (g)		865.9±40.4	851.6±13.1	853.4±23.7
Feed efficiency[#]		0.207	0.218	0.228
Feces weight (g/day)		12.8±0.8[a]	16.1±0.9[b]	17.0±1.4[b]
Weight (g/kg body weight)	Liver	5.20±0.02	5.40±0.13	5.43±0.22
	Heart	0.41	0.39	0.38

[a,b] Means within a row without common superscripts differ significantly (P<0.05); *All measurements were taken as fresh basis; values are Means±SEM for 21 rats per group; [#]Feed efficiency = Weight gain / intake feed (g).

Table 3: Effect of dietary *Rhodobacter capsulatus* on lipid metabolites and glucose concentration in serum (mg/dl)

Metabolites*	Control (BD)	HCD	HCD+*RC*
Total cholesterol	60.16±0.7	65.71±2.1	65.69±2.0
Triglycerides	60.12±1.6[a]	69.49±4.8[c]	65.22±1.9[a]
HDL-cholesterol	35.49±0.6	36.59±0.9	37.16±0.8
LDL-cholesterol	12.65±0.1[a]	19.34±1.8[b]	14.77±0.7[a]
Glucose	145.14±0.7	148.48±8.7	155.98±8.1

[a,b] Means within a row without common superscripts differ significantly (P<0.05); *All measurements were taken as fresh basis; values are Means±SEM for 21 rats per group

Table 4; Effect of dietary *Rhodobacter capsulatus* on lipid metabolites and bile acids (mg/g) in liver and faces

Parameters	Control (BD)	HCD	HCD+*RC*
Liver			
Total cholesterol	4.9±1.3[a]	12.3±3.1[c]	8.5±0.8[b]
Triglycerides	16.6±1.0[a]	24±0.6[b]	14.2±1.6[a]
Bile acids	1.2±0.5[a]	2.2±0.6[b]	3.4±0.9[c]
Faces			
Total cholesterol	6.1±2.1[a]	33.3±3.4[b]	42.5±4.7[c]
Triglycerides	2.1±1.3[a]	4.2±1.3[b]	5.4±2.4[c]
Bile acids	1.7±0.6[a]	4.0±1.7[b]	5.7±1.8[c]

[a,b,c] Means within a row without common superscripts differ significantly (P<0.05); *All measurements of liver and faces were taken as fresh and dry basis, respectively; values are Means±SEM for 21 rats per group.

Table 5: Effect of dietary *Rhodobacter capsulatus* on incorporation of ^{14}C-Glucose into lipid fractions in liver of the rats

Lipid fractions (%)	HCD	HCD+*RC*
Cholesterol	4.21±1.2	3.72±0.7
Triglycerides	3.77±2.0*	7.13±5.1
Fatty acids	83.18±2.3	81.42±4.8
Cholesterol ester	0.87±0.4	0.85±0.3
Phospholipid	7.98±1.5	6.89±1.0

[a,b] Means within a row without common superscripts differ significantly (P<0.05); *All measurements were taken as fresh basis; values are Means±SEM for 21 rats per group.

Effect of 4-wk feeding with BD, HCD and HCD+*RC* on rat liver tissue stained with Oil red O and Sudan III are shown in Figure 1. Liver tissue of the rat fed HCD stained by Oil red O showed red color where, there between the liver tissues of the rats fed BD and HCD+*RC* diet. Sudan III is predominantly used for staining triglycerides in animal tissues. The liver of rat fed HCD was diazo color (lysochromes, fat-soluble dye). Sudan staining of liver of the rat fed HCD+*RC* was a little reddish than that of BD diet. On the other hand, heart tissue of the rat fed HCD stained by Oil red O showed red color where, there is no differences between the liver tissues of the rats fed BD and HCD+*RC* diet. A large number of brown colored fat globules and cavity are seen in the heart muscle of the rats fed HCD and HCD+*RC* diet (Figure 2 K).

Compared to the other experimental diets, the HCD+*RC* potentially counteracted the accumulation of lipid droplets in the liver cells observed by electron microscopy (Figure 3 B).

DISCUSSION

The present study revealed that dietary *RC* (0.02%) reduced LDL-cholesterol and triglycerides in serum of the rats fed HCD diet. Our previous study reported that the *RC* (2.0%) reduced not only LDL-cholesterol and triglycerides concentrations, but also total cholesterol in serum of the rats fed HCD (Tsujii *et al.*, 2007). This variation regarding total cholesterol concentration between the two studies might be due to the inclusion level of *RC* diet. This triglycerides-reducing effect is important because an elevated serum triglycerides concentration has long been linked to the prevalence of small, dense LDL particles that are known to promote atherosclerosis and other cardiovascular diseases (Havel and McCollum, 1993).

Dietary cholesterol is absorbed in the proximal small intestine and transported to the liver. In the liver, dietary cholesterol regulates the expression of many genes, including some that influence plasma cholesterol levels (Maxwell *et al.*, 2003). Differences in regulation of some of these genes could account for the variation in plasma cholesterol response to dietary cholesterol between species or between individual humans. A candidate gene for this role is cholesterol 7α-hydroxylase (Cyp7a1), a liver enzyme that governs the rate-limiting step in the classical pathway of bile acid synthesis (Cohen *et al.*, 1992). Dietary cholesterol can upregulate mouse and rat Cyp7a1 through activation of the liver X receptor (LXR) transcription factor, which binds to an LXR response element in the Cyp7a1 promoter (Peet, *et al.*, 1998). The human Cyp7a1 gene contains a mutated LXR response element, and it has been hypothesized that the failure of cholesterol to up-regulate this gene may account for the relatively greater response of plasma cholesterol to dietary cholesterol in humans compared to mice and rats (Agellon *et al.*, 2002). In the liver, cholesterol is converted to bile acids, thus removing it from the active cholesterol pool and resulting in up-regulation of hepatic LDL receptors and decreased plasma cholesterol levels. In addition, bile acids are excreted into the small intestine, where they act as detergents to facilitate the absorption of dietary cholesterol, lipids, and fat soluble vitamins. Most of the bile acids are reabsorbed in the distal ileum and returned to the liver, but in each cycle, 5% are excreted into the feces (Russell and Setchell, 1992). Thus, conversion to bile acids represents a major pathway whereby cholesterol can be removed from the body, which may also influence serum cholesterol levels. After 4-wk feeding period, total cholesterol and triglycerides concentrations were increased in faces but decreased in liver of the rats fed *RC* supplemented HCD. Our previous studies also observed

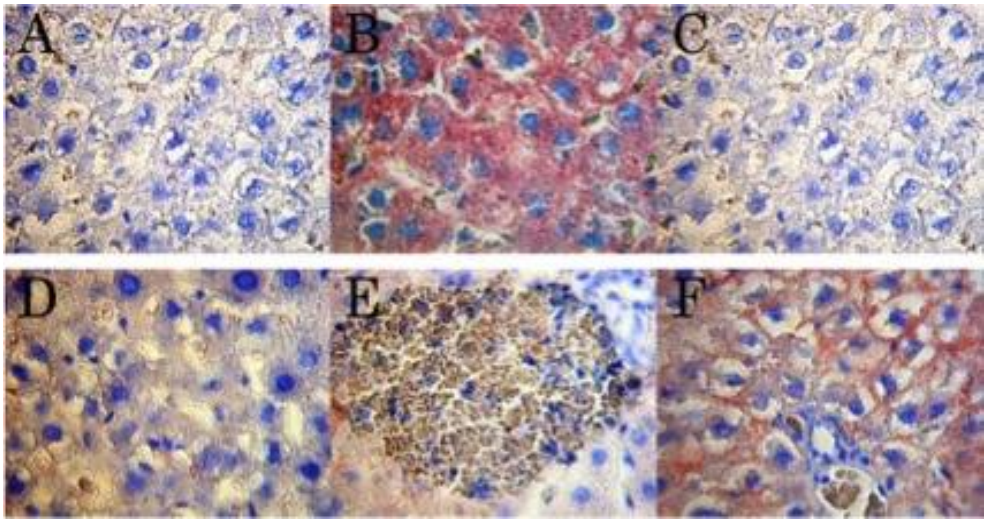

Fig. 1: Effect of dietary *Rhodobacter capsulatus* on rat liver tissue after Oil red O and Sudan III staining. Histological structure of liver tissues of the rats stained by Oil red O (Fig. A, B, C), and Sudan III (Fig. D, E, F), fed basal diet (BD), high cholesterol diet (HCD) and high cholesterol diet + *Rhodobacter capsulatus* (HCD+*RC*).

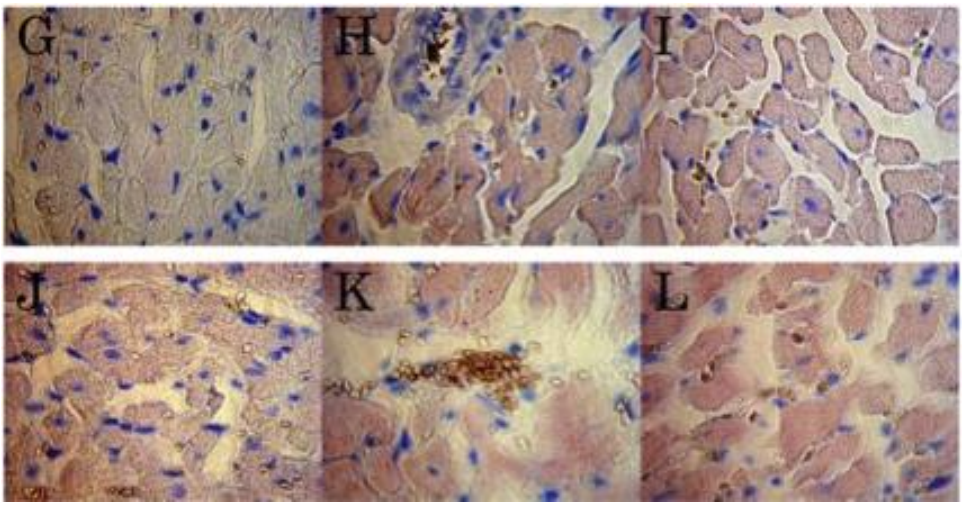

Fig. 2: Effect of dietary *Rhodobacter capsulatus* on rat heart tissue after Oil red O and Sudan III staining. Histological structure of heart tissues of the rats stained by Oil red O (Fig. G, H, I), and Sudan III (Fig. J, K, L), fed basal diet (BD), high cholesterol diet (HCD) and high cholesterol diet + *Rhodobacter capsulatus* (HCD+*RC*).

that dietary supplementation of *RC* increased total cholesterol and triglycerides concentrations in faces by decreasing their concentrations in liver of the laying hens (Sadia *et al.*, 2010; Salma *et al.*, 2011). The liver plays a major role in maintaining whole-body cholesterol homeostasis, that is, it is the major site for the elimination of cholesterol from the body via bile, either through converting cholesterol into bile acids or by direct biliary cholesterol secretion. The liver also produces VLDL and it is a major catabolic site for LDL through the LDL receptor mediated pathway (Spady *et al.*, 1983; Spady and Dietschy, 1985). A hepatic free cholesterol concentration was suggested to be a signal to trigger the transcriptional regulatory pathways in the cholesterol metabolism through sterol-regulatory element binding protein (Brown and Goldstein, 1997). It was shown that VLDL formation increases as hepatic cholesterol ester synthesis when induced (Huff *et al.*, 1994). In this way, the mechanisms that influence hepatic free cholesterol and cholesterol ester levels in the liver are important for maintaining body cholesterol homeostasis. The present study revealed that the accumulation of lipid droplets appeared in hepatocytes of the rats fed HCD, but there was no lipid droplets were appeared in hepatocytes of the rats fed HCD supplemented with dietary *RC*. Though, the question remains how much of the lipid deposited in the liver and how much is contributed in blood stream. Therefore, the present study investigated the incorporation of ^{14}C-glucose on hepatic total lipids and lipid fractions to estimate the hepatic biosynthesis of phospholipids, cholesterol and triglycerides in rats. The incorporation of ^{14}C-glucose was higher on hepatic triglycerides of the rats fed *RC* supplemented HCD diet. Salma *et al.* (2011) also demonstrated that the incorporation of 1-^{14}C-palmitic acid on hepatic lipids and lipid fractions were higher in the laying hens fed diet supplemented with dietary *RC*. It has been proposed that cholesterol arises mainly in the serum which ultimately originated in liver. The hypothesis is that the liver is largely responsible for serum lipid synthesis is supported by the work of Griffin (1992), which indicates that lipoproteins formed in liver.

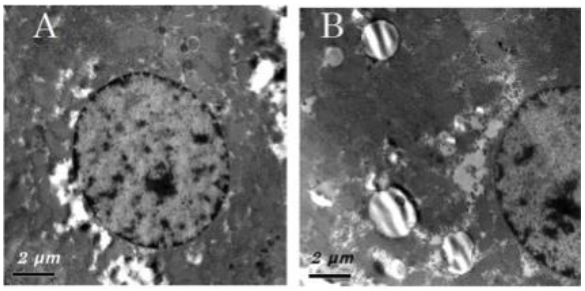

Fig. 3: Electron microscopic structure (A and B) of liver cells of rats fed basal diet (BD) and high cholesterol diet + *Rhodobacter capsulatus* (HCD+*RC*). Outline of the mitochondria was clear in the liver cells of the rats fed HCD+*RC*.

The dietary supplementation of *RC* might be reduced hepatic cholesterol effectively by increasing fecal excretion of cholesterol. The reduction of cholesterol concentrations in serum might be attributed to the reduced hepatic cholesterol content and increased excretion of cholesterol through excreta, which was because of the inhibition of dietary cholesterol absorption and the excretion of endogenous cholesterol by bile acids and inhibition of their reabsorption (Salma *et al.*, 2011). Paik *et al.* (2005) reported that *Bacillus polyfermenticus* was effective in improving LDL-cholesterol, hepatic total cholesterol, and triglycerides levels in serum, while increasing the fecal excretion of total cholesterol and triglycerides of hypercholesterolemic rats by exerting its hypocholesterolemic effects. The present study speculates that the level of serum lipid metabolites was reduced in rats fed *RC* supplemented diets by the inhibition of dietary cholesterol absorption, resulting in excreting the cholesterol through feces. Our previous study also indicated that *Rhodobacter palustris* and *RC* have important roles in reducing the risk of atherosclerosis, because the ratio of HDL-cholesterol to total cholesterol was significantly higher in the rats fed HCD diet. The serum cholesterol content in the body is balanced by dietary cholesterol absorption, cholesterol synthesis in the body and biliary cholesterol excretion (Tsujii *et al.*, 2007). In the present study, cholesterol-lowering effects of the dietary supplementation of *RC* were observed probably due to increasing bile acid excretion, which occurs through interference with the absorption of cholesterol. It is reasonable to speculate that a reduced ability to convert cholesterol to bile acids would lead to body cholesterol overload, with the subsequent development of atherosclerosis (Assmann *et al.*, 2006).

Animals that were able to excrete large amounts of cholesterol did not develop hypercholesterolemia, whereas those with a less efficient excretion had increased plasma levels of cholesterol (Lofland *et al.*, 1972). Moreover, the degree of hypercholesterolemia was inversely correlated with the rate of bile acid elimination. These animal experiments suggest that the atherogenic effect of the cholesterol-rich diet is highly dependent on the animal's ability to eliminate cholesterol in the form of bile acids (Guorong *et al.*, 2004).

Conclusion

The study found that triglycerides and LDL cholesterol were decreased in the serum of the rats fed *RC* supplemented diet. The *RC* supplemented diet might reduce the risk of atherosclerosis, as the ratio of HDL cholesterol to total cholesterol become higher in the treatment group than in the control group. Thus, the results may suggest that *RC* has hypocholesterolemic effects on health benefits of the experimental rats.

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