2011 International Conference on Bioscience, Biochemistry and Bioinformatics
IPCBEE vol.5 (2011) © (2011) IACSIT Press, Singapore

The LDL-cholesterol-lowering Effects of Nano-particled Djulis Grains

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***Abstract*—Djulis containing high amounts of dietary fiber, betanin and polyphenols is an aboriginal cereal plant in Taiwan. The aim of this study is to investigate the biological effects of its grain after nano-processed on the hyperlipidemia hamsters. Blood chemistry of tested animals and antioxidant composition and capacity of samples were investigated. Results showed that a significant decrease in plasma LDL-cholesterol, total cholesterol, accompanied by an increase in HDL-c/LDL-c ratio was observed as compared with those of control animals without supplemented with Djulis grain nano-particles. However, no significant differences of HDL- cholesterol nor triacylglycerides were found. Thus, the supplementation of nano-particles of Djulis grains with high content of dietary fiber, betanin and polyphenols may be beneficial to hyperlipidemia or hypercholesterolemia subjects by lowering their plasma LDL-cholesterol, increasing HDL-c/LDL-c or decreasing total cholesterol levels.**

Keywords-Nano-particled Djulis grain; hypocholesterolemic; dietary fiber; polyphenols; betanin

I. INTRODUCTION

The consumption of dietary food in fruit and vegetables has been correlated with the lowering of coronary heart disease [1]. Epidemiological studies suggested the cholesterol-lowering properties of soluble dietary fiber and their inhibition on the cholesterol stone formation by reducing the biliary cholesterol saturation index [2]. Dietary fiber especially the viscous and highly fermentable fibers was found more effective in the hypocolesterolemic effect on hypercholesterolemic animals than normal ones [3]. Another high potential health-promoting bioactive compound is polyphenol. Tea catechins have been reported to reduce the plasma cholesterol levels by induction of antioxidant enzymes such as SOD, catalase and glutathione S-transferase in rats [4]. Grape seed, red wine polyphenol extract was found to inhibit cellular cholesterol uptake [5]. Polyphenols in hawthorn and Perilla frutescens were found highly effective in decreasing the levels of plasma total cholesterol [6-7] Polyphenols was also reported to be related to dietary fiber in inhibiting the reabsorption of bile acids, cholesterol and dietary fats [3].

Djulis is an aboriginal cereal plants in Taiwan. They contain high amount of protein and dietary fiber. Their phytochemicals including polyphenols and betanin pigment are well-known bioactive compounds and believed to be health protective. However, no information about their effect on animals cholesterol-lowering can be found.

Nano-technology is one of the important scientific approaches presently. It works specifically at the molecular levels in 1-100 nm range. Nutrient with particle size in nm is considered to be more easily absorbed by human body [8]. In this study, Djulis grain powder after nano processed were used to feed the hyperlipid induced hamsters. The changes of their plasma cholesterol (including HDL-c, LDL-c, triglycerides and total cholesterol) were investigated to evaluate the hypo-cholesterolemic effect of Djulis diet. The antioxidant capacities of this feeding sample were also investigated to elucidate the possible relation between the compositions and the results.

II. MATERIALS AND METHODS

A. Djulis sample preparation

The Djulis grains for this study were harvested from Ping Tung County. The grains were dried to Aw=0.6 and ground into micro-particle (MP) by Ultragrinder (Hosokawa Alpine, Germany). Ten grams of MP were dissolved in 1kg water as 1% solution. After gelatinization (70℃, 30minutes), they were further ground into nano-particle (NP) by Nanogrinder (LAB Wet Grinding & Dispersing Mill Batch Type JBM-B035, Just Nanotech Co. Ltd., Taiwan) with 0.1mm Yttria-Stabilized Zirconia Bead (YSZ) for 60 minutes. The particle size of the primary particle and secondary particle of nano-ground Djulis powder was about 20 nm and 75 nm respectively. Then, the samples were concentrated into 50 ml. Therefore, each Djulis feeding sample contained 0.2g Djulis/ml. The compositions of dietary fiber, protein and starch of the samples were analyzed by AOAC. Functional composition such as polyphenols, betanin and antioxidant capacities were analyzed as described in the Material and Method of this study.

B. Animals test

Male specific-pathogen-free (SPF) hamster, 4 weeks old, were obtained from the National Laboratory Animal Center in Taiwan, and maintained on standard animal chow diet and water ad libitum. Permission and approval for animal studies was obtained from the Center for Research Animal Care and Use Committee of the National Pingtung University of Science & Technology. All animal experiments in this study were conducted in accordance with the accepted principles for laboratory animal use and care as described in the US guidelines (NIH publication #85-23, revised in 1985). The animals were fed with a diet containing 10% oil with 2% cholesterol for 4 wks for induction of hyperlipidemia and hypercholesterolemia. After their high levels of triacylglycerol and cholesterol in plasma were confirmed, animals were randomized into control or supplemented groups of 8 animals each. Animals were continuously given the diet containing 10% oil with 2% cholesterol and the treated ones were supplemented with 1% of nano-particle of Djulis grains for 4 wks. Blood samples were taken for examining the levels of triacylglycerol, total cholesterol, LDL-cholesterol (LDL-c) and HDL-cholesterol (HDL-c). In addition, the plasma levels of aspartate aminotransferase (AST; GOT), alanine aminotransferase (ALT; GPT), blood urea nitrogen (BUN) and creatinine were determined, and histopathological changes in liver and kidney tissue were also examined.

C. Blood chemistry assay

Plasma levels of triacylglycerol, total cholesterol, LDL-c, HDL-c, GOT, GPT, BUN and creatinine were determined using commercially available enzymo-colorimetric kits (HUMAN GmbH, Wiesbaden, Germany).

D. Determination of antioxidant compositions

Betanin estimation by HPLC was estimated by using the extinction coefficient (ε) as 61600 through the formula: $A_{\lambda max}$. $= 6.16\times10^4 \times$ concentration (M). Then, the total betacyanin pigment was estimated by calculation from the concentration and peak area percentage as described in Tsai *et al*. [9] Total phenolic content was measured spectro-photometrically (U-2001, Hitachi Ltd.) using the modified Folin-Ciocalteu method described by Chang *et al* [10] and expressed as mg gallate (GAE)/100g. All the Djulis feeding samples were diluted 20 folds before analysis.

E. Determination of antioxidant capacities

The antioxidant capacities including reducing power (ferric reducing ability of plasma, FRAP), DPPH (1,1-Diphenyl-2-picrylhydrazyl) scavenging ability and SOD-like ability. The method of FRAP and DPPH scavenging were described in Tsai *et al*. [9]. For the SOD-like, acetone powder was prepared and dissolved in potassium phosphate buffer (50 mM, pH 7.8). After dialysis (48 hours) and centrifugation (4ºC, 12000 rpm, 30 min), 50μl of the supernatant was mixed with 3 ml reagent including riboflavin, methionine and NBT. The SOD–like activity was measured by the light induced nitroblue tetrazolium /riboflavin assay (A_{560}). A unit of SOD activity was defined as the amount of enzyme that inhibited the 50% of NBT reduction [11]. All the Djulis feeding sample was diluted 20 folds before analysis.

F. Statistical analysis

All results were expressed as mean ± SE. The significance of difference was evaluated with Duncan's multiple range test. A probability level of $p < 0.05$ was considered statistically significant.

III. RESULTS

A. Composition of Djulis fed to the hamster

The composition of the samples used to fed the rat was analyzed and listed in Table 1. Total dietary fiber, protein and starch were 16.3%, 17.5% and 40.8% respectively. After diluted for 20 folds, the Djulis sample exhibited 47.05% DPPH scavenging ability, 1142.8μmole/l FRAP and 9.55 U/mg protein for SOD-Like activity. This might be attributed to their high content of total phenol (827.8 mg/100ml) and betanin (14 μM) in the diet intake, which was equal to 4.97 mg/day/rat for phenols and 0.093mg/day/rat for betanin. The amount of betanin and polyphenols in this nano-particled sample was about 1.8 and 1.7 folds that of original Djulis grain (data not shown).

TABLE I. THE FUNCTIONAL COMPOSITION AND ANTIOXIDANT CAPACITIES OF THE DJLIS SAMPLES USED IN THIS STUDY

Item	concentration
Dietary fiber (%)	16.3
Protein (%)	17.5
Starch (%)	40.8
Total phenol(mg/100ml)	41.39
Betanin	14μM

SOD-like(U/mg protein)	9.55
DPPH scavenging (%)	47.05
FRAP(μmole/l)	1142.8

B. Changes of body weight of the rat during the experiment

The body weights of the hamsters during the experimental period were shown in Fig. 1. Animals gained weights from 80g to 105g in average. No significant differences in the body weight were found between the control and Djulis-supplemented groups during the experimental period.

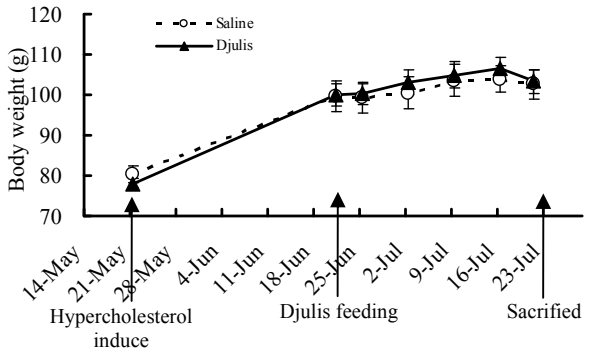

Figure 1. The body weight and Djulis feeding date in rats for 8 weeks.

C. Effect of Djulis on the cholesterol levels

Figure 2 illustrated the level of triglycerides, HDL-c, LDL-c, total cholesterol and HDL-c/LDL-c in the plasma of hamster fed Djulis for 4 weeks after hypercholesterol induced. Feeding the cholesterol diet supplemented with Djulis had no significant effect on the HDL-c and triglyceride concentration. But the plasma total cholesterol and LDL-c concentrations were significantly lower in Djulis supplemented hypercholesterolemic diet than that in control ($p<0.05$). HDL-c/LDL-c ratios were significantly higher in groups fed the Djulis supplemented hypercholesterolemic diet with respect to the control group due to the low LDL-c.

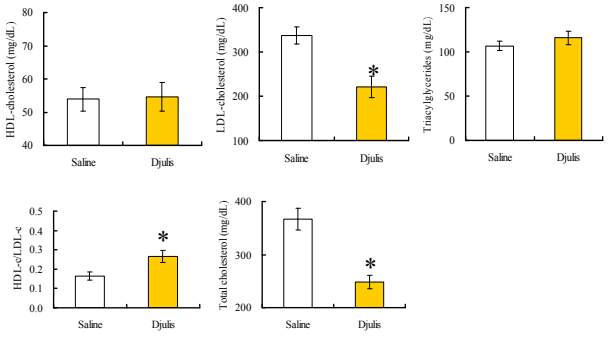

Figure 2. Plasma levels of triglycerides, HDL-c, LDL-c, totalcholesterol and HDL-c/LDL-c in experimental animals.

D. Evaluation of hepatotoxicity and renal-toxicity

Significant decreases in plasma GOT and GPT levels were observed in the Djulis-supplemented group as compared with those in the control group (Fig. 3). Fatty liver was observed in hamsters treated with the diet containing 10% fat and 2% cholesterol for 8 wks. However, the declined plasma GOT and GPT levels of nano-particle of Djulis grain was observed, inferred after assay quantification of slight elevated plasma levels of these two enzymes in all hyperlipidemia/hypercholesterolemia animals. In addition, normal BUN and creatinine levels were observed in all animals (Fig. 4A and 4B). The supplementation of nano-particle of Djulis grains significantly decreased the plasman levels of BUN (Fig. 4A) although no similar effects on creatinine levels were observed.

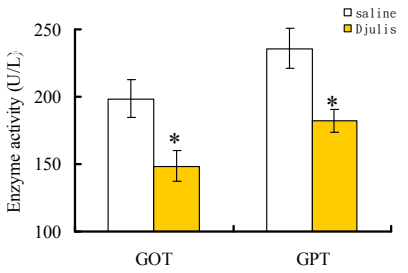

Figure 3. Plasma levels of GOT and GPT in experimental animals.

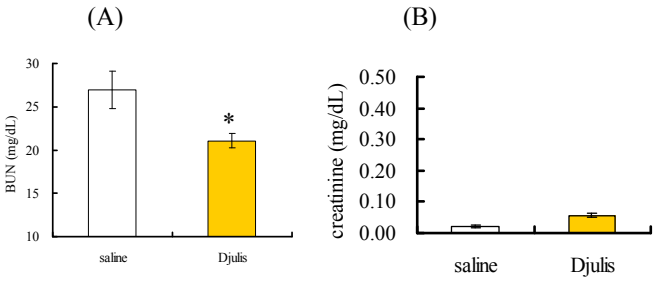

Figure 4. Plasma levels of BUN and creatinine in experimental animals.

IV. DISCUSSION

Many previous studies have demonstrates that dietary fiber and polyphenols rich product will reduce plasma total cholesterol and LDL-cholesterol in hypercholesterolemic rats. Dietary fiber was suggested to inhibit the cholesterol stone formation (schwesinger). This protection appears to be related to the catabolism of cholesterol and a decrease in the cholesterol saturation index. Dietary fiber was found no inhibitory effect on cholesterol synthesis [12], but inhibit cellular cholesterol uptake [5]. They didn't alter either total triglycerides or triglycerides distribution [13]. In this study, 16.3mg/day of dietary intake for the rat (average 105g) is similar to 9.78g/day intake for a man with body weight 60kg. Dietary fibers can also exert hypocholesterolemic effects by increasing fecal excretion of total steroids [1]. Therefore, the high content of dietary fiber in Djulis surely played an important role in the hypolipidemic effect in this study. Since

the capacity of polysaccharides to lower serum cholesterol levels was due to their ability to disperse in water, water soluble fibers was regarded as the major contributing fiber in preventing cholesterol lithogenesis [14]. The investigation of the water soluble fiber content in Djulis is undergoing.

Further, polyphenol was reported to play protective role against oxidative stress in living system. Plasma total cholesterol, triglyceride and LDL-c were decreased in the rat after green tea leaves intake [4]. The mechanism of this hypocholesterol effect was supposed to be related to the effect of dietary fiber and associated with inhibited reabsorption of bile acid and cholesterol [3] as well as the enhanced excretion of total lipids [4]. In this present study, the high intake of polyphenols could not be ruled out for their contribution in the cholesterol-lowering effect. After the rats were supplemented with 4.97 mg/day polyphenols for 4 weeks, the plasma cholesterols especially LDL-c and total cholesterol decreased significantly as compared to saline control group.

Studies of Lin *et al*. [4] also demonstrated that tea polyphenols might increase the activity of SOD activity as well as catalase and phase II enzyme in liver. Many studies reported that LDL oxidation plays an important part in the development of atherosclerosis. Antioxidants naturally occurred in the diet may act as antiatherosclerotic agents. The levels of antioxidant defense enzymes such as SOD of the Djulis-supplement diet was 9.55 U/mg protein, which might exert a synergestic effect with polyphenols and other antioxidant such as betanin. The betanin intake in this study was as high as 0.093 mg/day/rat. In addition, betanin was reported to inhibit linoleate peroxidation induced by cytochrome *c* and was better than that of catechin or α-tocopherol [15]. Purified betanin were even more effective than crude extract in the Inhibition of B16F10 melanoma cell proliferation [16]. Our results also indicated the important role of betanin in the LDL oxidation reducing.

In summary, the present study shows that Djulis supplement diet in nano particle significantly decrease the level of LDL and total cholesterol in the hyperlipid induced hamster which might be attributed to the abundant amount of antioxidant composition and antioxidant enzymes provided by the Djulis after nano-processed. Thus the supplement of adequate form and amounts of Djulis may be beneficial in the prevention of atherosclerosis due to oxidative stress.

ACKNOWLEDGEMENT

We are grateful to the Forestry Bureau of Council of Agriculture Executive Yuan in Taiwan for the financial support.

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