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Effect of Consumption of *Coleus tuberosus* on the Lipid Profile of Alloxan-induced Diabetic Rats

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Abstract: *Coleus tuberosus* is a minor vegetable belonging to the *Lamiaceae* family. *C. tuberosus* and processed products have been evaluated on resistant starch content and effect of consumption of *C. tuberosus* and processed products on the lipid profile has been studied in rats with diabetes mellitus. Resistant starch was analyzed using the megazyme method. Analysis of lipid profile was performed in experimental alloxan-induced animals. Such lipid profile as Total Cholesterol (TC), Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL) are determined enzymatically by the Cholesterol Oxidase-oxidase-Phenol Aminophenazone (CHOD-PAP) method. Triglyceride levels are determined by the enzymatic Glycerol-3-Phosphate Oxidase-Phenol+Aminophenazone (GPO-PAP) method. The results showed that the treatment process can increase the levels of resistant starch. *C. tuberosus* consumption and processed products can lower the lipid profile of TC, TG and LDL and increase HDL in experimental animals. Resistant starch contained in *C. tuberosus* and processed products is one of the factors that affect the lipid profile of experimental animals with diabetes mellitus.

Keywords: *C. tuberosus*, diabetes mellitus, lipid profile, resistant starch

INTRODUCTION

The number of adults who suffer from diabetes is expected to increase from 171 million in 2000 to 366 million in 2030. Numerous studies have shown that the risk and incidence of coronary heart disease and vascular disease in patients with diabetes mellitus was higher than non-diabetics (Barakat *et al*., 1996). Diabetes is a disease prone to such complications as heart, kidney and eye disease and is characterized by hypercholesterolemia, hypertriglyceridemia and hyperglycemia. One cause of diabetes is the presence of free radicals that cause damage to the β-pancreatic cells. Attempt to minimize complications in diabetes mellitus is to consume foods that contain bioactive compounds (Yadav *et al*., 2008) or foods that contain resistant starch amylose or high resistant starch (Zhang *et al*., 2007).

Diabetes association recommends people with diabetes to increase their consumption of complex carbohydrates to approximately 50% of the total energy and more fiber foods (Vessby *et al*., 1994). Health benefits can be obtained from foods containing carbohydrates are relatively resistant to digest in the small intestine. Amount of carbohydrate cannot fully be a reference for the same amount of carbohydrates from different sources, can provide different responses. Carbohydrates in different foods can have different glycemic responses. Carbohydrates with high amylase and much resistant starch content has a low glycemic index.

Resistant Starch (RS) is the amount of starch and starch degradation products not absorbed in the small intestine of healthy people, because it is resistant to digestive enzymes. Resistant starch certain kinds of carbohydrates that resist digestion in the small intestine and enter into the large intestine. Resistant starch consumption was also associated with decreased post-prandial glycemic and insulinemic responses, which may have beneficial implications in the management of diabetes (García-Alonso *et al*., 1999) and is associated with decreased levels of cholesterol and triglycerides (De Deckere *et al*., 1993). Another effect of RS consumption is increasing the frequency and bulk fecal excretion (Tharanathan, 2002). Including the Resistant Starch 3 (RS3) is a non-granular starch derived from the digestion-resistant material. Resistant Starch (RS3) is generally formed during starch granules retrograded (Wepner *et al*., 1999). Resistant Starch (RS3) is

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retrograded starch, which can be formed in cooked foods stored at room temperature. Resistant Starch type 3 (RS3) is of particular interest, because of the thermal stability. This allows it to be stable in most normal cooking operations and allows its use as an ingredient in a wide variety of conventional foods (Haralampu, 2000). Food processing, which involves heat and humidity, in many cases destroys RS1 and RS2 but it can cause the formation of RS3 (Faraj *et al*., 2004). Resistant Starch (RS3) showed a higher water storage capacity of granular starch (Sanz *et al*., 2008). Some examples of RS3 are cooked and cooled potatoes and corn flakes (Wepner *et al*., 1999).

Coleus tuberosus is one of Indonesia's agricultural crops as a source of carbohydrates and alternative medicine. *C. tuberosus* contain flavonoids, ascorbic acid, which can increase the activity of antioxidant enzymes: superoxide dismutase, catalase, glutathione peroxide and peroxide products decreased in rats fed high-fat diet. Extracts of *C. tuberosus* contain triterpene compounds (maslinic acid) can inhibit the expression of EBV early-antigen in Raji cells (Mooi *et al*., 2010). *C. tuberosus* contain two triterpenic acids such as oleanolic acid and ursolic acid (Nugraheni *et al*., 2011). Its bioactive compounds have an effect on the treatment of diabetes in rats. Study of consumption of processed *C. tuberosus* and its impact on the prevention of diabetes mellitus has not been done.

Research *C. tuberosus*, particularly in relation to products based on *C. tuberosus* that can be consumed and the effect of product consumption on the prevention or treatment of diabetes has not been done.

Studies in vivo is expected to provide information aboutpotential *C. tuberosus* as a source of resistant starch in biological systems, as well as encouraging people to re-plant *C. tuberosus* in order to support food security and not to assume that *C. tuberosus* as the only source of carbohydrates, but it has potential as a functional food beneficial in maintaining a healthy body. The purpose of this study is to investigate the levels of resistant starch in *C. tuberosus* and processed products and effects of the consumption of processed products to lipoprotein profile in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Triglycerides kit, cholesterol kit, High-Density Lipoprotein (HDL) kit, Low-Density Lipoprotein (LDL) from Diasys diagnostic system kit, Holzheim, Germany. Standard feed (American Institue of Nutrition, AIN 1993), Alloxan monohydrate, pancreatic α-amylase, amyloglucosidase enzyme from Sigma Aldrich (St. Louis, MO, USA). All other reagents and solvents were of analytical reagent grade.

Preparation of resistant starch analysis in *C. tuberosus*: *C. tuberosus* was obtained from farmers in Bantul regency, Yogyakarta, Indonesia when the plant was at the age of 3 months. The required cultivation conditions such as low temperature of 15 to 20°C, enough sunlight and humidity 80 to 90%, in 2500-3300 mm of rainfall per year and grow well in acidic soils (pH) 4.9 to 5.7) was met in this study. *C. tuberosus* was selected and processed in two different methods. It was the boiled and made flakes (steamed and baked). Stages of boiling processing were carried out by washing and then boiling the tubers of *C. tuberosus* for 30 min. Boiled *C. tuberosus* was then ready to be analyzed for resistant starch content (with skin). *C. tuberosus* flakes compositions were *C. tuberosus* flour (with skin), tapioca flour, soy flour, margarine and sorbitol. Flakes processing stages were performed by mixing all ingredients, then flattened into sheets, then steamed. After steaming for 30 min the dough was shaped into slabs of rectangular cut and baked at a temperature of 170°C for 25 min.

Resistant starch analysis: To 100 mg samples (boiled *C. tuberosus* or *C. tuberosus* flakes) 10 mL solution of pancreatic enzyme α-amylase (500 U) 0.1 mol/L tris-maleate buffer solution (calcium chloride 4 mL) was added. It was allowed for 16 h at 37°C. After that, 40 mL of ethanol was added, allowed for 1 h and then centrifuged. The residue obtained was washed twice with 80% ethanol and dried at 60°C. The residue was dried and 1.56 mL of H_2O and 1.5 mL 4 mol/L KOH were added and allowed to stand for 0.5 h at room temperature. After that, 12 mL H_2O was added. Obtained dispersion was taken and1.5 of 0.65 mL 2 mol/L acetic acid (to obtain pH 4.5) and 0.1 mL amyloglucosidase enzyme (0.1 mL of 0.1 mol/L Na acetate buffer pH 4.5) were added. It was shaken for 90 min at 65°C. Glucose was determined using glucose oxidase test. The result is a resistant starch (Champ *et al*., 1999).

Animal diet: Animal diet refers to the standard feed composition AIN 1993 (Reeves, 1997). One kilogram of feed is made of the standard composition of corn starch (560.70 g), casein (>85% protein, 200 g), saccharose (100 g), corn oil (40 g), Carboxy methyl cellulose (50 g), mineral mix (AIN 93-MX, 35 g), vitamin mix (AIN 93-VX, 10 g), L-cysteine (1.8 g) and choline bitartrate (2.5 g). Another diet is boiled *C. tuberosus* and *C. tuberosus* flake, without the addition of other materials.

Eighteen male white rats (wistar) weighing 130-180 g were supplied by the Animal Care laboratory rats experiments carried out by Gadjah Mada University, Yogyakarta, Indonesia. Rats were kept under controlled temperature of 20-24°C and 12-h light/dark cycle. They were fed with standard laboratory feed and water ad-libitum. Induction of diabetes mellitus was done by intraperitoneal administration alloxan monohydrate (120 mg/kg body weight) dissolved in normal saline. Diabetes was confirmed after 72 h by measuring blood glucose using the GOD-PAP. The rats with fasting blood glucose levels above 250 mg/dL were considered diabetic.

Eighteen male white rats (wistar) weighing 130-180 g were included in the enclosure with sufficient light, sufficient air vents, at room temperature in line with refers to the standard feed AIN 1993. Boiled *C. tuberosus* and *C. tuberosus* flakes were used to feed rats for 28 days maintenance. The rats were divided into three groups consisting of six animals respectively (n = 6). The rats were treated for 28 days as follows: Group 1 received the standard diet, Group 2 received boiled *C. tuberosus* diet and Group 3 received *C. tuberosus* flakes diet. Drinking water was provided ad libitum. Rats were fed every morning. Weighing was performed every 2 days. After 7 days since the start of the experiment, rats fasted for 12 h and blood samples were collected. Blood was collected retro-orbitally from Anthus in the eye with ether anesthesia using capillary tubes (Hoff, 2000). Total cholesterol was determined enzymatically with the CHOD-PAP method (Richmond, 1973). LDL-cholesterol was determined enzymatically with the CHOD-PAP method (Wieland and Siedal, 1983). HDL cholesterol was determined enzymatically with the CHOD-PAP method. Triglycerides were determined using GPO-PAP method (McGowan *et al*., 1983).

Statistical analysis: The results are presented as the average deviation and standard of six experiments. One-way ANOVA was used to analyze differences in means between the samples followed by Least Significant Difference multiple comparison test to compare mean values at $p<0.05$. The value of $p<0.05$ indicates was considered a significant difference $p<0.05$. SPSS version 16.0 (SPSS Inc., South Wacker Drive, Chicago, United State of America) was used.

RESULTS AND DISCUSSION

Determination of resistant starch content: The results of the analysis of Resistant Starch (RS) on raw *C. tuberosus*, boiled *C. tuberosus* and *C. tuberosus* flakes are contained in Table 1. Table 1 show that the processing can increase the levels of RS. The processing causes gelatinization. Heating starch with water will result in excessive starch gelatinization, a process that includes hydration and dissolution of starch granules. The process continued after the gelatinized starch e.g., refrigeration, freezing, baking or frying will result in retrogradation of starch that can alter the structure leading to the formation of new crystals do not dissolve. Gelatinization and retrogradation which often occurs in starchy materials processing affects starch digestibility in the small intestine.

Table 1 shows the processing by boiling can increase levels of resistant starch than raw potatoes. Boiling process causes to be perfect on starch gelatinization and when placed at room temperature retrogradation occurs that affects the formation of resistant starch in the small intestine. RS levels in

Table 1: Resistant starch content on raw *C. tuberosus*, boiled *C. tuberosus* and *C. tuberosus* flakes

Starch	Resistant starch content (%)
Raw *C. tuberosus*	10.2±0.4
Boiled *C. tuberosus*	15.4±1.0^{b}
C. tuberosus flakes	44.1±0.1^{c}

Values were expressed as mean±standard deviation (n = 6); Means with different letters in same column were significantly different at level of $p<0.05$

Table 2: Weight of wistar rats during feeding with standard diet, boiled *C. tuberosus* and *C. tuberosus* flakes in vivo

	Weight (g)	
Treatment	0 day	28 days
Standard feed	181.50±4.97^{a}	167.83±4.71^{a}
Boiled *C. tuberosus*	183.83±7.94^{a}	188.17±9.20^{b}
C. tuberosus flakes	181.17±6.91^{a}	186.17±6.34^{b}

Values were expressed as mean±standard deviation (n = 6); Means with different letters in same column were significantly different at level of $p<0.05$

C. tuberosus flake higher than raw and boiled *C. tuberosus*. This influence by the steaming process that causes starch gelatinization and proceeds with the roasting process which can increase levels of RS. This is similar with the results of Marsono (1999) who have found that the process continued after starches had gelatinized, for example refrigeration, freezing, baking or frying which leads to the formation of new crystals do not dissolve. The treatment process consists of several stages of heating can provide resistant starch content is higher. Potatoes boiled and then cooled would increase levels of Resistant Starch (RS3) (Sajilata *et al*., 2006). These results indicated that treatment with high temperature can increase levels of resistant starch in which it has a beneficial effect in relation to physiology: preventing colon cancer in the presence of short chain fatty acids (Ferguson *et al*., 2000), hypoglycemic effect (Reader *et al*., 1997), as a prebiotic effect (Brown *et al*., 1996) and hypocholesterolemic (Martinez-Flores *et al*., 2004).

Body weight: Food consumption was monitored every day by feeding every morning as much as 15 g. Processed products from *C. tuberosus* were given after the rats were injected alloxan and declared diabetes. Rats that are grouped in three treatments can be given feed. Table 2 shows that the weight of rats when not suffered from diabetes mellitus were not significantly different. However, after the rats had diabetes mellitus, weight rats given standard feed diet was significantly lower than rats fed diet boiled *C. tuberosus* and *C. tuberosus* flakes. Meanwhile, the weight of the rats with diet boiled *C. tuberosus* and flake *C. tuberosus* were not significantly different (Table 2).

Diabetes mellitus rats fed standard diet suggests that weight loss may be due to carbohydrate metabolism disorders caused by alloxan injection. Weight loss is possible as well as proteolysis and lipolysis and severe dehydration. Patients with diabetes

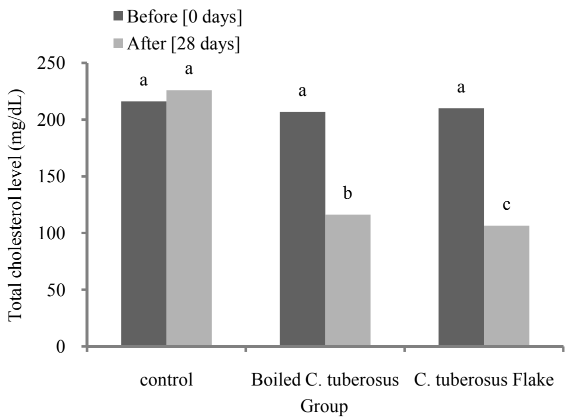

Fig. 1: Total cholesterol level on diabetic rat fed different diets
Control group: Fed standard diet; Boiled *C. tubersosus* group: Fed boiled *C. tuberosus* daily diet (15 g); *C. tuberosus* flakes group: Fed *C. tuberosus* flake daily diet (15 g); data are presented as mean±S.D. from six independent experiments

there is a decrease of glucose absorption, so to maintain the energy balance, energy derived from protein and lipid is used, resulting in proteolysis and lipolysis which ultimately lead to dehydration (Kim *et al*., 2010). While diabetes mellitus rats fed flake and boiled *C. tuberosus* were not lose weight. It's showed rats can adapt to the feed and indicates an improvement of carbohydrate metabolism. This is presumably because of the content of antioxidants and RS on boiled *C. tuberosus* and *C. tuberosus* flakes.

Levels of total cholesterol: Diabetes is linked to problems with lipid metabolism because the hormone insulin is involved in starting the lipid metabolism process. Good blood cholesterol levels are below 200 mg/dL. This research uses experimental animals receiving a standard feed diet of boiled *C. tuberosus* and C. *tuberosus* flakes. Ratsfed with boiled *C. tuberosus* and *C. tuberosus* flake sup to 28 days showed significant decrease in total cholesterol levels (below 200 mg/dL) compared with rats receiving a standard feed diet (remained above 200 mg/dL) (Fig. 1), whereas rats receiving a standard feed diet did not show decreased levels of total serum cholesterol. Decrease in total cholesterol in the groups receiving boiled *C. tuberosus* and *C. tuberosus* flakes were 43.8 and 49.3%, respectively.

Decrease in total cholesterol levels caused by the RS content of the diet given to the rats was raw, boiled and *C. tuberosus* flake. Differences in the level of cholesterol reduction caused by different levels of resistant starch (Table 1). Based on Table 1 obtained information that a high content of resistant starch significantly reduced cholesterol levels in experimental animals. Low levels of cholesterol in the digest likely due to high feed intake resistant starch that blocks cholesterol absorption in the small intestine. Resistant starch Contributed to the Decrease in cholesterol. The mechanism of cholesterol lowering by resistant starch is through the binding of bile acids and increasing fecal excretion of bile acids, so the amount of recycle is less. Therefore, to compensate the bile acid excreted, the liver synthesizes new bile acids from cholesterol and may lower the level of cholesterol. This is according to research (Hashimoto *et al*., 2006) which proves that the Rats feed a diet containing 15 g of potato retrograded starch in potato pulp from potato contains significant Benimaru serum (TG) is lower than the control group. In the Benimaru group, fecal bile acid content was significantly higher than the control group. These results suggest that pulp Benimaru Promoted the excretion of bile acids, resulted in lower roomates Concentrations of serum cholesterol.

Other research proved that mechanism of cholesterol lowering by resistant starch by replaces the bile acids cholic acid pool becomes Chenodeoxycholic acid. Chenodeoxycholic acid is an inhibitor of 3-Hydroxy-3-Methylglutaryl (HMG) CoA reductase, an enzyme necessary for cholesterol biosynthesis. HMG CoA reductase activity resulted in the decrease of cholesterol production and it causes a decrease in serum cholesterol (Anderson *et al*., 1994). Decrease in cholesterol levels in this study similar to Levrat *et al*. (1996) who have found that dietary resistant starch is effective in lowering plasma cholesterol and triglycerides. Consumption of food containing resistant starch can cause a decrease in cholesterol levels in experimental animals (Ranhotra *et al*., 1996; Chang *et al*., 2004; Mitra *et al*., 2007; Han *et al*., 2003).

Nugraheni *et al*. (2011) have shown that *C. tuberosus* containing UA and OA (data not shown). Ursolic acid and oleanolic acid are bioactive compounds that have a possible effect of lowering total cholesterol. UA and OA can decrease cholesterol profile in hamsters via inhibition of ACAT (AcylCoA + cholesterol acyltransferase). OA and UA lower cholesterol absorption through primary inhibition of ACAT activation of the small intestine and play an important role in absorbing cholesterol (Lin *et al*., 2011). Another research was found that UA significantly decreased total cholesterol levels (Azevedo *et al*., 2010).

Triglycerides: Triglycerides are forms of fat found in blood and food. Triglycerides are produced by the liver (liver). Triglycerides should be below 100 mg/dL. High levels of triglycerides can lead to heart disease, such as coronary heart disease. Figure 2 shows that rats with diabetes and given diet boiled *C. tuberosus* and *C. tuberosus* flakes showed decreased triglyceride at day 28^{th}. Statistical analysis showed significant differences between rats fed a standard feed diet with boiled *C. tuberosus* and *C. tuberosus* flakes. Rats fed a standard diet showed no decrease in serum triglyceride levels (remain above 100 mg/dL) whereas rats fed diet

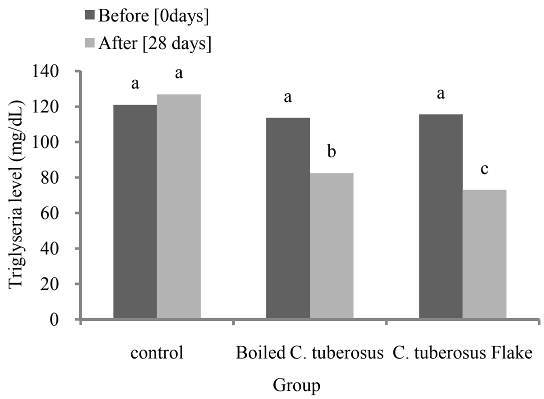

Fig. 2: Total triglyceride level on diabetic rat fed different diets
Control group: Fed standard diet; Boiled *C. tubersosus* group: Fed boiled *C. tuberosus* daily diet (15 g); *C. tuberosus* flakes group: Fed *C. tuberosus* flake daily diet (15 g); Data are presented as means±S.D. from six independent experiments

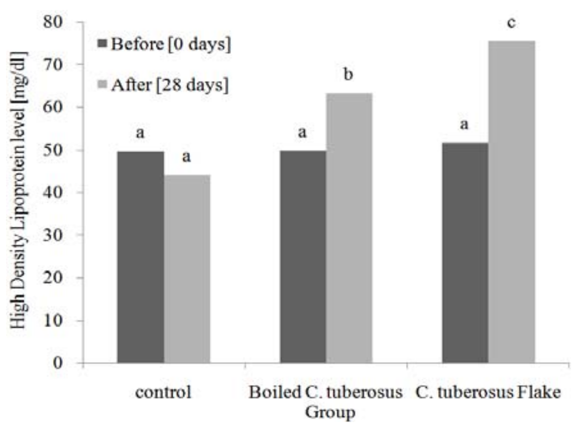

Fig. 3: Total HDL level on diabetic rat fed different diets
Control group: Fed standard feed; Boiled *C. tubersosus* group: Fed boiled *C. tuberosus* daily diet (15 g); *C. tuberosus* flakes group: Fed *C. tuberosus* flakes daily diet (15 g); Data are presented as mean±S.D. from six independent experiments

boiled *C. tuberosus* and *C. tuberosus* flakes showed decreased serum triglyceride levels (below 100 mg/dL). Decrease in triglycerides in the diet group of *boiled C. tuberosus* was 27.5%, while *a C. tuberosus* flake was 36.8%.

Decreased levels of triglycerides are influenced by the consumption of resistant starch on *C. tuberosus*. Feed containing high resistant starch (*C. tuberosus* flake) was able to reduce triglycerides level, greater than the raw and boiled *C. tuberosus*. Mechanism resistant starch in lowering triglycerides is reduce the hepatic mRNA levels of Fatty Acid Synthase (FAS) in experimental animals that correlated with the mRNA levels of Sterol Regulatory Element-Binding Protein-1c (SREBP-1c). This is consistent with studies (Hashimoto *et al*., 2006) who found that the Rats fed a diet containing 15 g of potato retrograded starch in potato pulp from potato Hokkaikogane have serum TG Significantly lower than the control group. In the Hokkaikagane group, the hepatic mRNA levels of Fatty Acid Synthase (FAS) were significantly lower than that in the control group. The FAS mRNA levels correlated with the mRNA levels of Sterol Regulatory Element-Binding Protein-1c (SREBP-1c), a regulator of expression of FAS, positively. These results suggested that pulp Hokkaikagane inhibited the synthesis of fatty acids at the mRNA levels of FAS and SREBP-1c; roomates might lead to a reduction of the serum TG level. Hamsters fed a diet of rice bran, white rice and brown rice, raw or processed, will have lower levels of TG. A possible mechanism for lowering TG is to increase of the excretion of sterols (Kahloon *et al*., 2000).

High Density Lipoprotein (HDL): HDL is the one taking charge of throwing lipoprotein cholesterol from the liver tissue out of the body. HDL is often called good cholesterol. HDL levels should be above 60 mg/dL. Levels of HDL below 40 mg/dL can cause heart disease. Figure 3 showed that rats with diabetes mellitus after diet with boiled *C. tuberosus* and *C. tuberosus* flakes showed an increase in HDL cholesterol after 28th days above 60 mg/dL. Statistical analysis showed a significant difference between rats fed a standard feed with diet of boiled *C. tuberosus* and *C. tuberosus* flakes. An increased level of HDL in the feed boiled *C. tuberosus* was 27.2%, while *C. tuberosus* flake was 46.4%.

Nugraheni *et al*. (2011) have shown that *C. tuberosus* contain UA and OA (data not shown), thought to be bioactive compounds that affect the increase in HDL. UA dan OA increases HDL in rats (Liu, 1995; Azevedo *et al*., 2010). So that the UA is mainly thought to be useful in prevention strategies of the people at risk for diabetes and cardiovascular complications associated with increased levels of plasma glucose and lipid profiles, as well as to promote the deposition of liver glycogen.

This study indicates that boiled *C. tuberosus* and *C. tuberosus* flakes contain RS (Table 1). RS content in *C. tuberosus* thought to affect the increase in HDL levels of rats. This result similar to the research result conducted by Shallan *et al*. (2010), which states that an administration of the rice diet containing high amylase (resistant starches) can raise HDL in streptozotocin-induced diabetic rats.

The result of other study conducted (Mohamed and Afifi, 2011) was known that rats fed potato starch may have increased level of (HDL) significantly. Potato starch is resistant enzyme, which is associated with a large granule size, higher phosphate levels, type B crystals, the different chain length and chain length distribution, as well as different molecular weight and weight distribution, as compared to cereals and other starches. Viscous polysaccharides can inhibit sterol

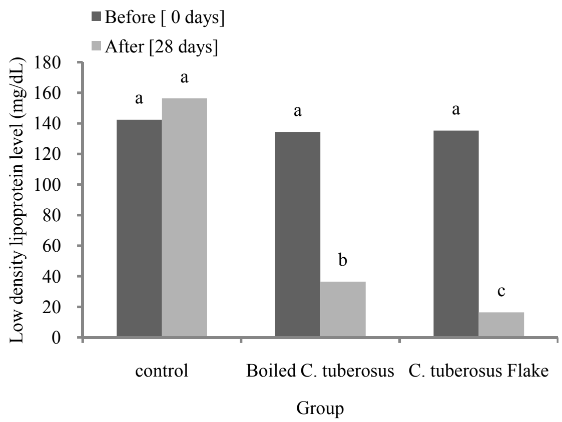

Fig. 4: Total LDL level on diabetic rat fed different diets Control group: Fed standard feed; Boiled *C. tubersosus* group: Fed boiled *C. tuberosus* daily diet (15 g); *C. tuberosus* flakes group: Fed *C. tuberosus* flakes daily diet (15 g); Data are presented as mean±S.D. from six independent experiments

absorption in the intestine. Viscous is believed to increase the thickness of the layer between the surface of the food and the border of the small intestine that absorb nutrients prevented. The yogurt mixture soba increased significantly compared to control HDL in 20 hypercholesterolemia subjects. The mechanism can be attributed to low protein digestibility buckwheat, buck wheat good nutritional value, or the specific effects of photochemical found in buckwheat (Shakib *et al*., 2011).

Low Density Lipoprotein (LDL): LDL is a lipoprotein that carries cholesterol to tissues including the arteries. LDL is often referred as bad cholesterol. Most blood cholesterol is in the form of LDL. Blood LDL should be below 100 mg/dL. If the LDL level is above 130 mg/dL, then it can lead to heart disease. Figure 4 shows that rats suffering from diabetes after consuming either a standard feed, boiled *C. tuberosus* and *C. tuberosus* flakes showed reduced levels of LDL until below 100 mg/dL. The statistical analyses suggest that there are significant differences between rats fed a standard diet with boiled *C. tuberosus* and *C. tuberosus* flakes. Decrease in LDL levels in the diet group of boiled *C. tuberosus* was 72.9%, while *C. tuberosus* flakes was 87.8%.

C. tuberosus contain OA and UA (Nugraheni *et al*., 2011). It is possible to reduce levels of LDL by the contents of OA and UA. UA and OA can lower LDL in the blood in rats (Liu, 1995). OA and UA can reduce LDL in rats with diabetes mellitus (Somova *et al*., 2003; Gutierrez *et al*., 2009).

Diet of boiled *C. tuberosus* and *C. tuberosus* flakes has a positive effect on lipid profile in rats with diabetes. It can be seen from the parameters of a declining trend in the near-normal LDL in rats. This is similar with the results (Sajilata *et al*., 2006) who found that rats with dietary RS can increase the size of the caecum and caecum pool of SCFA and SCFA absorption and lowers plasma cholesterol and TG. The study reported that diet in rats fed RS from Adzuki bean starch and Tebou bean starch can lower serum cholesterol, SR-B1 (Scavenger Receptor class B1) and cholesterol 7 alpha-hydroxylase mRNA (Han *et al*., 2003). Bean starch lowers LDL, increases the concentration of caecal SCFA (particularly butyric acid concentration) and increases fecal sterol excretion.

Other research found that rats fed RS kintoki can improve liver mRNA of cholesterol 7 α-hydroxylase and bile acids and steroids are excreted with the feces (Han *et al*., 2004). Kintoki having characteristics similar to dietary fibre can reduce the bile acids absorbed into the liver via the enterohepatic circulation mechanism. RS dietinrats that have liver LDL receptor mRNA was higher when compared with controls, thus increasing LDL receptor activity in the liver and SRB1. The resistant starch digestibility can lower LDL levels by increasing LDL receptor mRNA liver (Fukushima *et al*., 2001).

CONCLUSION

This study suggests that the processing steps increase the levels of RS in *C. tuberosus*. RS content in boiled *C. tuberosus* and *C. tuberosus* flakes can lower lipid profile such as TC, LDL and TG and increase HDL. The percentage decrease in TC levels in boiled *C. tuberosus* and *C. tuberosus* flakes were 43.8 and 49.3%, respectively. Standard feed diet did not lower TC. The percentage reduction in LDL levels in rats with diet boiled *C. tuberosus* and *C. tuberosus* flakes were 72.9 and 87.8%, respectively. The percentage decrease in triglyceride levels in rats with diet of boiled *C. tuberosus* and *C. tuberosus* flakes were 27.5 and 36.8%, respectively. Standard feed diet did not lower TG. The percentage increase in HDL on diet boiled *C. tuberosus* and *C. tuberosus* flakes were 27.2 and 46.4%, respectively. Based on this study, *C. tuberosus* has the potential to be developed as a functional food in the prevention of diabetes mellitus because of its ability in controlling the lipid profile.

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