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# A Potential of *Coleus Tuberosus* Crackers Rich in Resistant Starch Type 3 Improves Glucose and Lipid Profile of Alloxan –Induced Diabetic Mice

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**Abstract**

This study aims to determine the consumption effect of *Coleus tuberosus* flour and crackers rich in resistant starch type 3 to glucose and lipids profiles in diabetic mice. The study was conducted with four treatments (normal mice with AIN 93 diet, diabetic mice with AIN 93 diet, diabetic mice with *Coleus tuberosus* flour diet, diabetic mice with *coleus tuberosus* crackers rich in resistant starch type 3). The analysis of blood glucose levels and lipids profile were determined using an enzymatic colorimetric method with a commercial kit. The results showed that the consumption of *Coleus tuberosus* flour and crackers rich in resistant starch type 3 can improve glucose and lipids profile (total cholesterol, triglycerides, low-density lipoprotein, high-density lipoprotein) in alloxan-induced diabetic mice. The atherogenic index was smaller on the diet treatment of *Coleus tuberosus* flour rich in resistant starch type 3 and *Coleus tuberosus* crackers rich in resistant starch type 3 compared to the AIN 93 feed. This study shows that the raw material (*Coleus tuberosus* flour rich in resistant starch type 3) and *Coleus tuberosus* crackers that are rich in resistant starch type 3 can potentially be consumed as a functional food to improve glucose and lipid profiles in diabetes mellitus condition.

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**Keywords**

*Coleus tuberosus* crackers,
Diabetes mellitus,
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## Introduction

*Coleus tuberosus* is one vegetable that belongs to the family *Lamiaceae* and sub family *Nepetoideae*. *Coleus tuberosus* originated in tropical and sub tropical Asia, Africa and the Pacific Islands. *Coleus tuberosus* is a small herbaccous plant, 15-30 cm high, with a succulent stem, dark brown tubers produced in clusters[1]. However, it is now grown in tropical Asia. There are some *Coleus tuberosus* size, shape and color. *Coleus tuberosus* is a potential food crop as a source of carbohydrate food alternatives and drugs. *Coleus tuberosus* is included in the Clade 1b which are exploited as food and medicinal uses (diarrhea, gastric pain)[2].

Diabetes mellitus is a condition with hyperglycemia features that occurs due to abnormalities of insulin secretion or insulin[3]. This condition is caused by

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factors that prevent insulin work or decrease in the amount of insulin produced by the pancreas.

It is predicted that the number of people with diabetes mellitus in Indonesia will increase, from 7.3 million in 2011 to 11.8 million in 2030, and 90 % including type 2 diabetes[4]. Diabetes mellitus is closely related to eating habits. Dietary management can effectively control blood glucose level, blood pressure, and lipid profile of people with type 2 of diabetes mellitus. Dietary changes and exercise could improve the management of blood glocose for people daignosed with type 2 diabetes.

Management profile of glucose can be done by setting the diet, one of which is through the selection of food to control the release of glucose and increase insulin sensitivity[4]. Diet modification may decrease postprandial glucose, that impacts on the reducing the risk of diabetes mellitus. Some research suggests that dietary fiber may improve glucose control in healthy individuals and those diagnose with diabetes[5].Resistant starch include as insoluble fiber, so it has no effect on glucose absorption. However, resistant starch has benefits associated with increased insulin sensitivity in humans, thus having a positive impact for the uptake of glucose at manusia[6].

*Coleus tuberosus* is one of tubers as a source of carbohydrates and alternative medicines. As a source of carbohydrates, it can be modified by the processing to increase the levels of resistant starch type 3 (RS3). RS3 is the most interesting because it can maintain the nutritional characteristics of food when it is added to the food. RS3 is also preferred because it is relatively heat-resistant than other types, so that it can maintain its nature during food processing. RS3 is a type of resistant starch that is most widely used as a raw material-based functional food resistant starch. Generally, RS3 content in foods is low; the content of RS3 can increase when the food is baked or in the form of pasta and cereal products[7].

Crackers are a thin, crisp wafers or biscuits, are usually made from unsweetened and unleavened dough, a long shelf life and are well known by the public. Generally, crackers are made from wheat flour. However, this research will use *Coleus tuberosus* rich in RS3 as one of the ingredients, a wheat flour substitution for crackers, it is expected that it will impact positively on the glucose and lipids profile.

This research aims to identify the influence of the consumption of crackers and *Coleus tuberosus* flour rich in RS3 on glucose and lipids profile of alloxan induced diabetic mice. This information is expected to be utilized as functional foods for the prevention and management of degenerative diseases i.e. diabetes mellitus.

**Materials and Methods**

This research used *Coleus tuberosus* obtained from farmers in Clereng, KulonProgo Yogyakarta, Indonesia. Enzymes for analysis of RS consist of α-amylase enzymes (EEC 232-560-9) and amyloglucosidase (EEC 232-880-2). Analysis glucose profile used glucose kit (Diagnostic Bavaria Germany). Analysis lipids profile i.e.cholesterol kit, triglycerides kit, low density lipoprotein (LDL) kit, high-density lipoprotein (HDL) kit from Dyasis Diagnostic system, Holzheim, Germany. All reagents are of analytical reagent grade.

**Preparation Materials of *Coleus Tuberosus* Rich in RS3**

The process of making *Coleus tuberosus* rich in RS3 were *Coleus tuberosus* stripping, weighing, excision, soaking, and steaming at a temperature of 100 °C for 15 minutes and followed by cooling process on temperature 4 °C for 24 hours[8]. After the cooling process, then the *Coleus tuberosus* dried with cabinet dryer (50 °C for 14 hours). The dried *Coleus tuberosus* then ground and sifted with 100 mesh tyler sieve. The process of making crackers was done by replacing 20 % part of wheat flour with *Coleus tuberosus* flour rich in RS3, so obtained ratio between wheat flour with flour *Coleus tuberosus* rich in RS3 was 1:4.(Table 1). This formulation was chosen based on hedonic sensory test, favoured by 80 non-trained panelists in terms of aroma, flavor, color, texture and overall[8].

**Table 1: Recipe of *Coleus tuberosus* crackers based on hedonic sensory test by 80 non-trained panelists**

| Ingredient | Amount (g) |
|---|---|
| Wheat flour | 150 |
| *Coleus tuberosus* flour rich in RS3 | 116.88 |
| Yeast | 9.56 |
| Salt | 1.25 |
| Baking soda | 1.15 |
| Cream of tartar | 1.1 |
| Hot water | 166.67 |
| White butter | 26 |
| Butter | 26 |
| Molase | 1.25 |

**Analysis of Proximate Analysis and Resistant Starch in *Coleus Tuberosus* Flour and *Coleus Tuberosus* Crackers**

The crude protein, fat, ash, moisture of the samples were determined by the AOAC method[9]. The carbohydrate content was estimated by difference was calculated. Analysis of resistant starch was performed[10].

**Maintenance of Experimental Animals**

In vivo evaluation was done by setting up an animal treatment conducted in the Laboratory of Animal Experiment, Center for Food & Nutrition Studies, Gadjah Mada University. The experimental animals were 24 male wistar type white mice with a weight of 110-150 grams. They were kept in a closed condition. The enclosure that includes the light is not controlled; air vents in the cage are enough; the air temperature at a room temperature ranges from 28 °C- 32 °C and humidity 58 ± 4 %. Standard feed was given for three days by using standard AIN 199311.

*Coleus tuberosus* flour rich in RS3 was made into pellet form and used as rat feed for 28 days of treatment. Intraperitoneal injection of alloxan was done with a dose of 125 mg/kg rat body weight to make the mice became diabetic. The mice were given standard feed. In the third day,an evaluation of blood glucose levels was performed. On the third day after injection of alloxan, the mice revealed positive diabetes mellitus. The next process is mice were divided into four groups.

The number of experimental animals used federer formula.

$(t - 1)(n - 1) \geq 15$

t = number of treatment; n = number of animals[12].

The amount of treatment are 4, so the number of mice every treatment there are six mice, and the number of the required are 24 mice.Twenty four mice were divided in four groups each contain 6 mice : I- 6 non diabetic mice with standard diet (AIN 93), II-6 diabetic mice with standard diet(Ain 93); III-6 diabetic mice with the diet of Coleus tuberosus crackers rich in RS3, and IV- 6 diabetic mice with the diet of *Coleus tuberosus* flour rich in RS3.They were given water ad libitum. Cages were cleaned on a daily basis; residual feed was weighed every day. Feed was given each morning.

Blood glucose analysis was conducted with the method GOD Glucose PAP: enzymatic reactions photometric test. Blood samples were taken from the eyes of mice and centrifuged at 4000 g (3 minutes, 4 °C) to get the serum. Serum obtained was then kept at a temperature of -20 °C, up lipid serum will be analyzed. Concentrations of Total Cholesterol (TC), Total triacylglycerol (TG), low density lipoprotein (LDL) and high density lipoprotein (HDL) were determined by enzymatic colorimetric method using commercial kits[13].

Athesclerosis index (AI) calculated by = LDL/ HDL[14].

**Statistical Analysis**

The data consist of four treatments and six replicates. The data were analyzed with SPSS version 16.0 (SPSS Inc., South Wacker Drive, Chicago, United State of America). Anova of one line was used to analyze the difference between the average of the sample with the least significant difference at 95% significance. The difference was expressed as meaningful if $p \leq 0.05$. All values obtained from the results of the research are presented in the form of the mean ± SD.

## Results and Discussion

### Chemical Composition of *Coleus tubersus* flour and *Coleus tuberosus* crackers

*Coleus tuberosus* flour and crackers have a different chemical content (Table 2). *Coleus tuberosus* crackers contain higher protein than *Coleus tuberosus* flour. The higher protein content was due the use of wheat flour that containing proteins of 10-18%15. Crackers contain resistant starch higher than the *Coleus tuberosus* flour. This proves that the existence of the incremental processing will increase the content of resistant starch. Baking process on crackers making can cause increased levels of resistant starch on crackers. This is in line with the research of Sajilata *et al.,*[16] and Amaral *et al.,*[17] that proves that the process of baking can increase the levels of resistant starch.

**Table 2: The chemical composition of *Coleus tuberosus* flour and crackers rich in RS3**

| Parameter | crackers (%) | flour (%) |
|---|---|---|
| Moisture | 6,10 ± 0.27[a] | 8,67 ± 0.30[b] |
| Ash | 2,76 ± 0.15[a] | 3,46 ± 0.10[b] |
| Protein | 11,26 ± 0.97[b] | 4,81 ± 0.24[a] |
| Carbohydrate | 70,96 ± 0.26[a] | 77,63 ± 0.42[b] |
| Fat | 8.92 ± 0.17[b] | 5.43 ± 0.12[a] |
| Amylose | 10,67 ± 0.23[a] | 11,78 ± 0.47[b] |
| Resistant starch type3 | 15,37 ± 0.08[b] | 9,51 ± 0.96[a] |

Note: the difference letter indicates in the same row a significant difference at the level of significance of 5%

### Experimental Animals Consumption

The consumption of standard feed, *Coleus tuberosus* flour rich in RS3 and *Coleus tuberosus* crackers rich RS3 (see Table 3),which shows that the percentage of a given feed consumption varies between 82 – 96 %, meaning that the animals could still try to adapt to the type of feed given.

**Table 3: Consumption of standard feed, *Coleus tuberosus* (flour and crackers) rich RS3**

| Kind of feed | Animal condition | Feed (gram) | Rest of the feed (gram) | Percentage of consumption (%) |
|---|---|---|---|---|
| Standard (AIN 93) | Non diabetic | 20 | 0.52±0.18[a] | 98.17[d] |
| Standard (AIN 93) | Diabetic | 20 | 0.74±0.29[b] | 96.28[c] |
| *Coleus tuberosus* flour rich in RS3 | Diabetic | 20 | 2.78±0.55[c] | 86.10[b] |
| *Coleus tuberosus* cracker rich in RS3 | Diabetic | 20 | 3.45±1.07[d] | 82.75[a] |

Note: the difference the letter indicates a significant difference at the level of significance of 5%

Experimental animals injected with alloxan which led to increased glucose profile. The mice experiencing diabetes mellitus then given feed *Coleus tuberosus* flour rich in RS3 and *Coleus tuberosus* crackers. The results showed that occurred decreased the amount of feed consumed. The decline in the amount of feed-based *Coleus tuberosus* consumed by rats caused by unpleasant scents from a *Coleus tuberosus* flour that became one of the constituent components of the feed. This unpleasant scent caused by bioactive compounds in *Coleus tuberosus* flour namely phenols, flavonoids and triterpenic acid. Typical aromas are thought to have an impact on the decreasing percentage of their consumption. In addition, the texture especially on *Coleus tuberosus* crackers is harder than standard feed and *Coleus tuberosus* flour. So, it is thought to also lower the percentage of their consumption. The aroma and the texture of the feed which are different from those of the standard feed allegedly can affect the level of pleasure or animal appetite.

Decrease the amount of feed consumed caused by alloxan injection that cause pain and less comfortable, thus affecting the appetite of mice. But the next day, and on the 28$^{th}$ day of almost all groups of mice show increased consumption of feed. Begg and Woods[18] reported that increased appetite (poliphagia) is one of the clinical symptoms of diabetes mellitus. Poliphagia can be caused due to a decrease in glucose utilization in cells nuclei ventromedialis hypothalamus.

Weight of experimental mice feed with *Coleus tuberosus* flour rich in RS3 or *Coleus tuberosus* crackers rich in RS3 are presented in Fig 1. The diet treatment of *Coleus tuberosus* flour rich in RS3 and *Coleus tuberosus* crackers rich in RS3 can increase the weight of mice suffering from diabetes mellitus with significant differences ($p < 0.05$) compared to the standard feed diet. The mice which did not suffer from diabetes mellitus gained weight despite the diet with a standard feed; however, mice suffering from diabetes mellitus and using standard feed diet experienced weight loss.

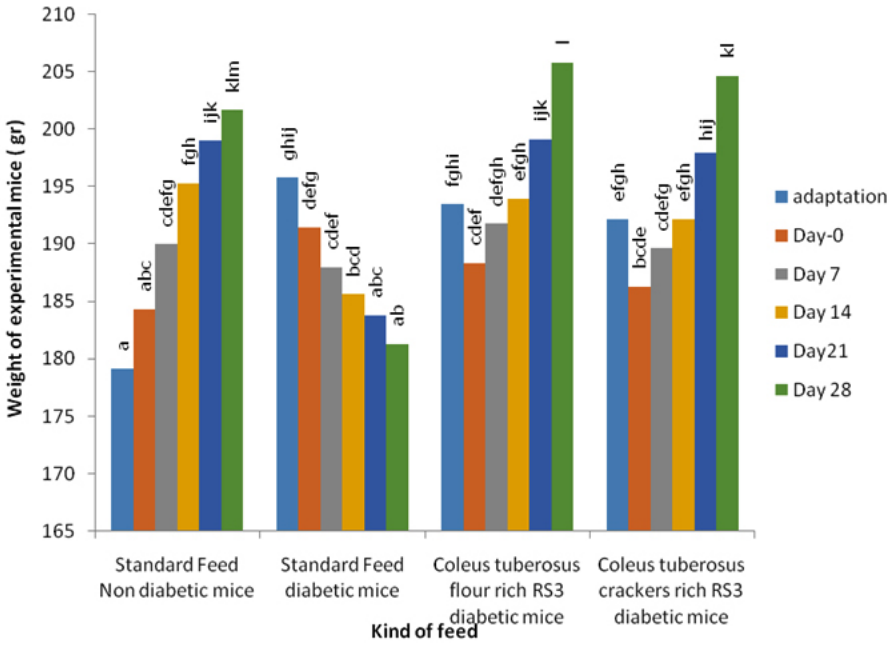


**Fig. 1: Weight of experimental mice which feed with *Coleus tuberosus* flour rich in RS3 or *Coleus tuberosus* crackers rich in RS3 for 28 days**

**Note: The difference letter on kind of feed indicates a significant difference weight of experimental mice at the level of significance of 5% due to the different types of the given feed and long time treatment on mice**

The weight gain of mice showed that mice can adapt to the given feed and improve the condition of mice with a induced condition of diabetes.In addition to weight loss because of a decline in the intake, it was also caused by metabolic disorder of carbohydrates with the induction of diabetes by using injection of alloxan. Weight loss is possible due to proteolysis and lipolysis as well as fairly severe dehydration. Diabetics glucose uptake decreased; thus, to maintain the balance of energy, it used energy reserves sourced from protein and fat, thus causing proteolysis and lipolysis, eventually leading to dehydration. The conditions that cause weight loss. The weight gain shows that it happened to repair metabolism and condition of the mice with the consumption of *Coleus tuberosus* flour rich in RS3 and *Coleus tuberosus* crackers rich in RS3. The content of resistant starch on crackers and flour of *Coleus tuberosus* can improve insulin sensitivity which have an impact on the improvement of the metabolism so that it can increase the weight gradually mice[6].

**The Level of Glucose Serum**

The influence of feeding standard diet (corn starch), *Coleus tuberosus* flour rich RS3 and *Coleus tuberosus* crackers rich RS3 can be seen in Table 4. Mice injected with alloxan and suffering from diabetes mellitus are characterized by blood glucose levels above 200 mg/dl.Serum glucose testing was conducted on the third day after injection of alloxan (day 0) and the 28 day after injection of alloxan.

Diabetic mice were grouped and each group was given different feeds namely feed standard, *Coleus tuberosus* flour rich RS3 and *Coleus tuberosus* crackers rich RS3, which showed a decrease in serum glucose levels significantly after treatment for 28 days. Based on statistical analysis, it can be known that there is a significantly difference between mice that were given standard feed diet with *Coleus tuberosus* flour rich in RS3 and *Coleus tuberosus* crackers rich in RS3. A decrease in blood glucose levels in the group of mice which were given standard feed are not significant.

Table 3 shows that glucose level of group with *Coleus tuberosus* flour rich in RS3 decreased by 43.65 % (from 215.50± 7.85 to 121.43 ± 5.91). Glucose levels of group with Coleus tuerosus crackers rich in RS3 decreased by 48.90 % (from 213.26± 2.70 to 108.99 ± 3.52).

Decreased of glucose level related with the levels of resistant starchin *Coleus tuberosus* flour rich in RS3 and *Coleus tuberosus* crackers rich in RS3. Table 2 shows that *Coleus tuberosus* crackers rich in RS3 have the levels of resistant starch higher than *Coleus tuberosus* flour rich RS3.

The results of this study showed that consumption of *Coleus tuberosus flour* rich in RS3 and *Coleus tuberosus* crackers can lower glucose levels in the diabetic mice. One of the factors that is thought to play a role in lowering glucose levels is resistant starch. High levels of resistant starch have the potential to lower blood glucose. This is in line with this research, that the processing of flour *Coleus tuberosus* be crackers will cause an interaction component constituent there by increasing levels of resistant starch.The making of crackers, imperfect gelatinization occurs on starch, because of low water availability. So when followed by the cooling process, then the starch will experience a retrogradation and, the starch will be crystalline shaped and resistant to enzyme digest and called with resistant starch[16]. The retrogradation level is directly proportional to the levels of resistant starch in starch. Sajilata *et al.,*[16] reported that the higher levels of resistant starch the higher probability of retrogradation. The decrease in glucose level in mice fed with *Coleus tuberosus* crackers rich in RS3 is higher than those fed with *Coleus tuberosus* flour rich in RS3. It is directly proportional to the levels of RS on a diet.

Resistant starch (RS), that is a small fraction of the starch that resistant against hydrolysis by the enzyme $\alpha$-amylase enzyme and pululanase, was given in vitro. RS was not hydrolyzed after 120 min incubation[19]. Cumming and Bingham[20] stated that resistant starch is starch that is resistant on digestion by digestive enzymes on healthy individuals. The starch up to the colon was fermented by intestinal microflora. Therefore, now that RS is defined as the fraction of the starch that escapes digestion can be in the intestine[16].Due to the resistance in the digestive process, then the glucose produced is too little, so that it impacts on the postprandial response to the low level of food containing high amylose or included in resistant starch. Resistant starch has

been reported also hypoglycemic. Resistant starch is identified with levels of amylose because it is hard to digest by digestive enzymes. Resistant starch lowers the glycemic response due to its viscous fiber in food as well as water soluble so it inhibits the absorption of glucose.

Sajilata *et al.,*[16] stated that foods containing RS will go through a slow process of digestion. This gives implications on controlling the release of glucose. The study used 10 healthy people who were given a diet of 50 g of starch free RS (0 % RS) or 50 g of starch containing high RS (54 % RS), suggesting that a diet with high levels of RS significantly lowered postprandial blood glucose concentration, insulin and epinephrine. Consumption of RS in animals increases the regulation of expression of glucagon-like peptides in the colon which have an impact on the improvement of glucose control. Resistant starch can increase insulin sensitivity and regulate blood sugar levels[21,22].

The research indicates that *Coleus tuberosus* flour rich in RS3 and *Coleus tuberosus* crackers rich in RS3 are capable of controlling and lowering glucose levels significantly in the normal range. The mice had normal blood sugar levels < 120 mg/dl). Based on this research, *Coleus tuberosus* flour rich in RS3 can be used as s raw material for making food that has the purpose of managing the profile of glucose.

**The levels of total cholesterol**

Levels of total cholesterolin mice, the mice were suffering from diabetes (day 0) and 28 days of treatment with a standard feed, Coleus tuberosus flour rich RS3 or *Coleus tuberosus* crackers rich in RS3 is shown in Table 4. Diabetic mice after consumption of standard feed, *Coleus tuberosus* flour rich in RS3 and *Coleus tuberosus* crackers rich in RS3 for 28 days, showed the total cholesterol levels decreased. Statistical analysis showed a significant difference between diabetic mice that were given standard feed, *Coleus tuberosus* flour rich in RS3 and *Coleus tuberosus* crackers rich in RS3. Mice fed with the standard diet experienced a decline of total serum cholesterol levels of 20.47 % (from 216.07 ± 10.05 to 171.83 ± 2.91), while the decrease occurred in *Coleus tuberosus* flour rich in RS3 of 41.37 % (from 206.90 ± 5.50 to 121.30 ± 2.72) and Coleus tuberosus crackers rich in RS 3 of 50 % (from 209.96 ± 4.15 to 105.03 ± 4.31). Cholesterol levels in diabetic mice that consumed *Coleus tuberosus* flour rich RS3 and *Coleus tuberosus* crackers rich in RS3 showed a normal range (normal cholesterol levels < 200 mg/dl).

Diabetes has an impact on the metabolism of lipids where one of the impacts of diabetes is the occurrence of elevated levels of both lipid profile i.e. total cholesterol, triglycerides, LDL[23]. Experimental animals used in the study were given standard feed diets, *Coleus tuberosus* flour rich in RS3 and *Coleus tuberosus* crackers rich in RS3. Decreased cholesterol in mice fed with *Coleus tuberosus* flour rich in RS3 and *Coleus tuberosus* crackers rich in RS3 is related to the levels of RS3. *Coleus tuberosus* crackers that contain higher levels of RS3 has the capability of decreasing total cholesterol greater than a diet of *Coleus tuberosus* flour rich in RS3. This is in line with the research of Han *et al.,*[24] which proved that resistant starch from the nut (*Vignasabatia*), kintoki (*Phaseolus vulgaris*) and tebou (*Phaseolus vulgaris*) given to animals can lower the total cholesterol in animals compared to the standard feed (corn starch).

**The level of Triglycerides**

The level of triglycerides after being given the diet i.e. *Coleus tuberosus* rich in RS3 and *Coleus tuberosus* crackers rich in RS3 to 28 days, the mice indicated that triglycerides showed a decline compared to controls fed standard diet (Table 4). Table 4 show that diabetic mice that consumed standard feed, *Coleus tuberosus* flour rich in RS3 or *Coleus tuberosus* crackers rich in RS3 showed decreased triglyceride levels significantly. The percentage decreases in triglyceride levels in mice fed with standard feed, *Coleus tuberosus* rich in RS3 and *Coleus tuberosus* crackers rich in RS3 were 7.23 % (from 120.94± 8.88to 112.19 ± 3.29), 30.60 % (from 113.63± 5.79 to 78.86 ± 2.09) and 43.63 % (from 115.61± 3.21 to 65.17 ± 2.90).

The diabetic mice suffering from diabetes after treatment with standard feed, *Coleus tuberosus* flour rich in RS3 and *Coleus tuberosus* crackers rich in RS3 showed a decrease of triglyceride levels significantly. Based on the analysis of the statistics, it can be known that there is a significant difference between mice given standard feed diet, *Coleus*

*tuberosus* flour rich in RS3 or *Coleus tuberosus* crackers rich in RS3. The results showed that the levels of triglyceride of diabetic mice with the diet of *Coleus tuberosus* flour rich in RS3 or *Coleus* tuberousus crackers rich in RS3 were in the normal range < 145 mg/dl.

**Table 4: Levels of blood glucose, total cholesterol, triglycerides, LDL and HDL in mice, the mice were suffering from diabetes (day 0) and 28 days of treatment with a standard feed (AIN 93), *Coleus tuberosus* flour rich RS3 or *Coleus tuberosus* crackers rich in RS3**

| Kind of feed | Glucose | | Total Cholesterol | | Trigliserida | | LDL | | HDL | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Day 0 | Day 28 | Day 0 | Day 28 | Day 0 | Day 28 | Day 0 | Day 28 | Day 0 | Day 28 |
| Standard feed (healthy mice) | 65.24 ± 3.08[a] | 66.36± 2.85[a] | 87.29 ± 4.31[a] | 88.07 ± 4.37[a] | 75.33 ± 5.64[b] | 76.44 ± 5.39[b] | 31.54 ± 1.88[a] | 33.86 ± 1.71[b] | 64.08 ± ±4.67[c] | 63.43 ± 4.50[c] |
| Standard feed ( diabetic mice) | 217.56± 6.75[d] | 217.02± 5.20[d] | 216.07± 10.05[f] | 171.83± 2.91[d] | 120.94± 8.88[d] | 112.19± 3.29[c] | 142.36± 7.20f | 156.42± 6.10[d] | 49.52± 2.48[b] | 28.52± 4.18[a] |
| *Coleus tuberosus* flour rich in RS3 (diabetic mice) | 215,50± 7,85[d] | 121.43± 5.91[c] | 206.90± 5.50[e] | 121.30± 2.72[c] | 113.63± 5.79[c] | 78.86± 2.09 | 134.45± 4.43[e] | 48.07± 2.62 | 49.73± 1.76[b] | 63.78± 1.87[c] |
| *Coleus tuberosus* Crackers rich in RS3 (diabetic mice) | 213.26± 2.70[d] | 108.99± 3.52[b] | 209.96± 4.15[d] | 105.03± 4.31[b] | 115.61± 3.[21c] | 65.17± 2.90[a] | 135.27± 5.26[e] | 31.87± 1.37[a] | 51.56± 4.03[b] | 74.01± 2.95[d] |

Note: difference letter on the same treatment and parameter indicates a significant difference at the level of significance of 5%

A decrease in the levels of triglycerides is allegedly related to the existence of the content of resistant starch. Resistant starch binds bile acids and increases the excretion of bile acids via the stool so that the amount of bile acids used is less[25]. To compensate the excretion of bile acids, liver syntezid bile acid from Cholesterol and it reduce cholesterol levels. Resistant starch replacesthe pool of bile acids from cholic acid which becomes chenodeoxycholic acid. Chenodeoxycholic acid is an inhibitor of 3-hydroxy-3-methylglutaryl CoA reductase (HMG), a regulatory enzyme required for the biosynthesis of cholesterol. The activity of HMG CoA reductase is low; the production of cholesterol will decrease. It causes serum cholesterol to go down. Feed containing RS increases m RNA hepatic cholesterol 7a-hydroxylase, steroid and bile acid excreted along with feces[26].

**The Level of High Density Lipoprotein (HDL)**

The level of HDL after being given the diet i.e. *Coleus tuberosus* rich in RS3 and *Coleus tuberosus* crackers rich in RS3 to 28 days, the mice indicated that HDL showed a raising compared to controls fed standard diet (Table 4). Based on statistical analysis on Table 4, it is known that there were significant differences between mice fed with a standard diet of *Coleus tuberosus* flour rich in RS3 and *Coleus tuberosus* crackers rich in RS3. The increased level of HDL on groups with *Coleus tuberosus* flour rich in RS3 was 28.25 % (from 49.73± 1.76 to 63.78 ± 1.87), while *Coleus tuberosus* crackers rich in RS3 was 43.54 % (from 51.56± 4.03 to 74.01 ± 2.95). However, in the standard feed diet, the HDL level decreased to 42.40 % (from 49.52± 2.48 to 28.52 ± 4.18).

High Density Lipoprotein (HDL) is one of the lipoproteins that takes cholesterol from the liver and removes it from the body. HDL is often called good cholesterol. Based on the results of this research, it is found that the HDL levels are in the high category. This is in line with Jae[27] stated that levels of HDL 60 mg/dl > are regarded as high. When compared to normal mice which did not suffer from diabetes mellitus, the results show that a diet

*Coleus tuberosus* flour rich in RS3 and *Coleus tuberosus* crackers rich RS3 can improve HDL profile to be better. Bronkowska *et al.,*[28] proved that diet with resistant starch type 4 can raise HDL on experimental animals compared to control. Trinidad *et al.,*[29] showed that the consumption of sweet potato and cassava that contain resistant starch for 90 days can increase HDL levels in healthy people.

The feed has given an effect in lowering the profile of lipid through the mechanism of increasing bile acids and sterols excreted along with feces; bile acid production increased as a result of increased bile acids excreted along with feces as well as an increase in the production of fermented like propionate, which can hamper the synthesis of cholesterol. The increased molar ratio of butyric and propionate will lower the molar ratio of acetate. So, the number of cholesterol that is synthesized also declined, because acetic acid is a precursor for synthesis of cholesterol in the body.

**The Level of Low Density Lipoprotein (LDL)**

The level of LDL after being given the diet i.e. *Coleus tuberosus* rich in RS3 and *Coleus tuberosus* crackers rich in RS3 to 28 days, the mice indicated that LDL showed decreased compared to controls fed standard diet (Table 4). Table 4 shows that there were significant differences between mice fed with a standard diet (non diabetes mellitus), the diabetic mice diet *Coleus tuberosus* flour rich in RS3 and *Coleus tuberosus* crackers rich in RS3. The decrease percentage of LDL levels in *Coleus tuberosus* flour rich in RS3 was 64.25 % (from 134.45 ± 4.43 to 48.07 ± 2.62). The decrease percentage in *Coleus tuberosus* crackers rich in RS3 was 76.44 % (from 135.27 ± 5.26 to 31.87 ± 1.37). Meanwhile, the standard feed increased by 8,99% (from 142.36 ± 7.20 to 156.42 ± 6.10)

Low Density Lipoprotein (LDL) is a lipoprotein that carries cholesterol to the network including the arteries. LDL is often referred to as the bad cholesterol. Most of the blood is cholesterol in the form of LDL. Based on the research results, it is found that the categories include normal LDL. This is in line with the Jae[27] who proposed that LDL < 100 mg/dl is normal. The decline in the level of LDL in this research is in line with the research of Humeera and Rahila[35] which showed that the consumption of corn starch that contain resistant starch can lower LDL profile in animals. This decline is associated with the increased expression of LDL receptor in the liver of animals, SR-B1, and cholesterol 7alpha-hydroxylase mRNAs[30].

**Atherosclerosis Index**

Atherosclerosis index is an indicator to know the risk of atherosclerosis. Atherosclerosis index shown in Table 5. Atherosclerosis index on *Coleus tuberosus* flour rich in RS3 diet in diabetic mice was 0.75 ± 0.02, *Coleus tuberosus* crackers rich in RS3 diet in diabetic mice was 0.43 ± 0.01, standard feed diet was 3.55 ± 0.12 in diabetic mice and standard feed diet in non diabetic mice was 0.53 ± 0.08. Atherosclerosis index on *Coleus tuberosus* flour rich in RS3 in diabetic mice and *Coleus tuberosus* crackers rich in RS3 diet in diabetic mice showed smaller than diabetic with standard feed or non diabetic mice with standard feed. This showed that the risk of atherosclerosis disease is smaller on the diet treatment with *Coleus tuberosus* flour rich in RS3 or *Coleus tuberosus* crackers rich in RS3.

**Table 5: The atherogenic index in non diabetic and diabetic mice after 28 days treatment**

| Kind of diet | LDL/HDL ratio |
|---|---|
| Standard feed (non diabetic) | $0.53\pm0.08^{b}$ |
| Standard feed (diabetic mice) | $3.55\pm0.12^{d}$ |
| *Coleus tuberosus* flour high in RS3 (diabetic mice) | $0.75\pm0.02^{c}$ |
| *Coleus tuberosus* crackers rich in RS3 (diabetic mice) | $0.43\pm0.01^{a}$ |

The difference letter indicates a significant difference at the level of significance of 5%

Atherosclerosis index (AI) can be used as a risk the possibility of the formation of atherosclerosis plaque. The results of this study showed that feeding *Coleus tuberosus* flour rich in RS3 and *Coleus tuberosus* crackers can lower the index atherogenic. Low atherogenic index value may indicate that the possibility of a *Coleus tuberosus* flour rich in RS3 and *Coleus tuberosus* crackers can prevent the formation of plaque of atherosclerosis. The results of this study are in accordance with the opinion of Mayes and Botham[31]who stated that atherosclerosis is associated with the high ratio of LDL and HDL. This relationship can be explained in terms of the role of HDL which is the major vehicle for the transport of cholesterol from peripheral cells to the liver for excretion and catabolism. Fernandez and Webb[32] state that the risk of death from cardiovascular disease in humans can occur when the ratio of LDL/ HDL levels has reached a value of 4.3.

Diet of *Coleus tuberosus* flour rich in RS3 or Coleus tuberosus crackers rich in RS3 gives a positive impact against the profile and glucose profile lipid in diabetic mice. It is known from the trend of parameters of levels of glucose, total cholesterol (TC), triglyceride (TG) and LDL that showed declining trend and approaching normal levels in mice. Meanwhile, the HDL parameter indicates an increase approaching normal HDL levels.

The improvement of glucose and lipids profile in diabetic mice is associated with the functional properties of *Coleus tuberosus* flour or *Coleus tuberosus* crackers that contain high RS3. The level of RS3 on *Coleus tuberosus* crackers is higher than the raw material (*Coleus tuberosus* flour). This has an implication on the profile of glucose and lipids on experimental animals. Experimental animals with diet of *Coleus tuberosus* crackers rich in RS3 have better lipid and glucose profiles than those diet with *Coleus tuberosus* flour, because RS3 play a role in decreasing levels of glucose and total cholesterol, triglyceride, and LDL.

**Conclusion**

The consumption of raw materials namely *Coleus tuberosus* flour high in RS3 can control profile lipids on experimental animals suffering from diabetes mellitus. *Coleus tuberosus* flour high in RS3 can lower total cholesterol, triglycerides and LDL: 41.37 %, 30.60 %, 64.25 %, respectively and raise the HDL 28.25 %. Meanwhile, the *Coleus tuberosus* crackers rich in RS3 can lower total cholesterol, triglycerides and LDL: 50 %, 43.63 % and 76.44 %, respectively and raise HDL 43.54 %. The risk of coronary heart disease is smaller on the diet treatment of *Coleus tuberosus* flour rich in RS3 and *Coleus tuberosus* crackers rich in RS3 compared to the standard feed. The results of this research proves that *Coleus tuberosus* cracker has the ability of glucose and lipids control in diabetes mellitus condition. This research provide information that *Coleus tuberosus* crackers can be utilized as functional food for people with diabetes mellitus or people who have a problem in the manage glucose and lipids profile.

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