The potential of pigeon pea (Cajanus cajan) beverage as an anti-diabetic functional drink

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Abstract. The number of patients with diabetes continues to increase. Diabetes complications might induce serious diseases such as kidney, nervous, cardiovascular diseases and stroke. Diabetic complications can be prevented by keeping blood glucose and cholesterol at normal levels. This study aims to determine the potential of pigeon pea beverage for lowering glucose and total cholesterol plasma levels and increasing the antioxidant status of diabetic-hypercholesterolemia rats. The research was conducted using 18 Sprague Dawley male rats aged 3 months old with an average body weight of 154 g. The rats were divided into three groups: normal group, D-H group (diabetic-hypercholesterolemia group), and pigeon pea beverage group. The results showed that pigeon pea beverage diet showed hypoglycemic and hypocholesterolemic activities, and could improve the antioxidant status of diabetic-hypercholesterolemia rats. Plasma glucose and total cholesterol levels of diabetic-hypercholesterolemia rats decreased 33.86% and 19.78% respectively. The improvement of the plasma antioxidant status was indicated by the decrease of plasma MDA (malondialdehyde) level, reaching 37.16%. The research result provides an alternative to diabetes management by using the local bean as an anti-diabetic functional drink.

Keywords: pigeon pea beverage, hypoglicemic, hypocholesterolemic.

1. Introduction

The number of diabetes patients continues to increase, from approximately 108 million in 1980 to 415 million in 2014. This number is estimated to increase to 552 million by 2035 [1, 2]. Glucose oxidation and non-enzymatic protein glycation which occurs during diabetes leads to an excessive free radical formation and subsequently result in oxidative stress which promotes the development of diabetes complications. Intakes of antioxidants have already been proved to be prospective in the treatment of diabetes [3, 4]. According to Forbes and Cooper [5], diabetes complications cause microvascular diseases, such as kidney, nervous and eye disease, and microvascular diseases, such as cardiovascular disease and stroke. Keeping glucose and cholesterol at normal levels can prevent diabetes complications.

Consumption of legumes provides health benefits such as reduced diabetes risk, obesity and cardiovascular disease [6, 7, 8]. It was related to the components of dietary fiber [9, 10] as well as phenolic compounds [11].
Pigeon pea (Cajanus cajan) is a local commodity that has a complete nutritional composition. When compared to soybeans, this legume has higher levels of dietary fiber, total mineral, and vitamin C, as well as lower fat content [12, 13]. Compared to other legumes, pigeon pea plant has some advantages, such as being the most drought-tolerant legume, resistant to environmental stresses, having high productivity and also contribute to moisture and nutrients of the soil [14, 15]. Pigeon pea is a potential source of antioxidants. It has a higher level of total phenolic and vitamin C content, as well as higher radical scavenging capacity rather than cowpea [16]. Acevedo’s study [17] reported that pigeon pea flour has a low glycemic index value (46-49), with resistant starch content reaching 30 - 33% db. Pigeon pea intake was reported to be capable of providing hypoglycemic effects in humans and diabetic rats [8], improving lipid profile of hypercholesterolemia hamster through its ability to increase HDL cholesterol level, lowering the plasma levels of triglycerides, LDL cholesterol and total cholesterol [19].

In previous research, the authors have successfully produced pigeon pea beverage as a potential functional drink in terms of its sensory and chemical characteristics, and the free radical scavenging capacity. The beverage has low-fat (2.31 g/100 g db) and is rich in dietary fiber (23.03 g/100 g db). It also has VEAC (vitamin C equivalent antioxidant capacity) of 13.72 μmol vitamin C/g db [20]. This study examines the potential of pigeon pea beverages as anti-diabetic functional food through its ability to reduce plasma glucose and total cholesterol levels as well as to increase plasma antioxidant status (reducing MDA levels) of diabetic-hypercholesterolemia rats at doses equivalent to human consumption of 30 grams/day. MDA plasma has known as a key biomarker of oxidative stress and lipid damage mediated by free radicals in diabetes [21]. Some researchers have studied the hypoglycemic activity of pigeon pea, such as pigeon pea roots extract [22], pigeon pea leaves extract [23, 24], pigeon pea roots and leaves extracts [25]. Amalraj and Ignacimuthu [26] studied the effect of roasting on the hypoglycemic activity of pigeon pea. The results showed that unroasted pigeon pea has a hypoglycemic effect on diabetic rats, but roasting eliminates its effect. Habib [27] reported the hypocholesterolemic and hypoglycemic effects of pigeon pea ethanol extracts in diabetic rats. Previous researches [28, 29] have studied the hypocholesterolemic effects of pigeon pea leaves extract. Pigeon pea diets in hypercholesterolemic hamsters at doses of 200-800 (g/kg feed) showed the hypocholesterolemic activity and decreased hepatic MDA levels [19]. However, research on the hypoglycemic and hypocholesterolemic effect and plasma MDA levels reducing of pigeon pea diets on diabetic-hypercholesterolemia rats has not been reported.

2. Material and Method

2.1 Materials

Materials for the preparation of beverages included pigeon pea, ginger, cinnamon, and cloves from the local market, as well as maltodextrin DE 20 (China) from the Bratachem. Assay kits for glucose (GOD-PAP) and cholesterol (CHOD-PAP) were purchased from Diasys (Diagnostic Systems GmbH, Germany). AIN-93M Mineral mix, AIN-93M Vitamin mix, and L-cystine from MP Biochemicals LLC (Santa Ana, CA, USA). TBHQ (tertbutyl hydroquinone (2-(1,1-dimethyl ethyl)-4-benzenediol)), alloxan, cholesterol, choline bitartrate, TEP (tetra-ethoxy propane) and other chemicals were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Casein, soybean oil, CMC (carboxy methyl cellulose), and corn starch were obtained from Food and Nutrition Laboratory (Food and Nutrition Research Center of Gadjah Mada University, Yogyakarta, Indonesia).

2.2 Preparation of pigeon pea beverage

Preparation of the pigeon pea beverage was conducted according to Patent No. P00201304596. Briefly, the legume was dehulled, soaked, boiled, dried and powdered. The powder was allowed through the 60 mesh sieve, further, maltodextrin DE 20 was added to improve the solubility.
2.3 Animals and Diets

Male Sprague Dawley rats (aged 3 months old, weighing 154 ± 16 g) from the Integrated Research and Testing Laboratory of Gadjah Mada University (Yogyakarta, Indonesia). The rats were caged individually in stainless steel cages in uncontrolled lights, at room temperature and adequate ventilation, and ad libitum feeding. The research on animal models was approved by the ethics committee of Sebelas Maret University (Surakarta, Indonesia) No.149/H27/1/17/ER/2010. The animal experiment consisted of four periods, i.e., acclimatization (seven days), hypercholesterolemia induction (five days), diabetic induction (three days) and intervention periods (two weeks), respectively. During acclimatization period, the rats were fed based on American Institute of Nutrition for maintenance (AIN-93M) standard diet [30]. After the acclimatization, the rats were randomly divided into three groups each consist of six rats, set as normal group, diabetes-hypercholesterolemia (D-H) group and pigeon pea group. The normal group rats were fed AIN-93M standard diet during the experimental periods. The pigeon pea and D-H groups were fed hypercholesteroldiets (during hypercholesterolemia induction) or standard diet (during diabetic induction). After hypercholesterolemia induction, the hypercholesterolemia rats were diabetic induced by injecting the fasting rats with alloxan (80 mg/kg body weight). The hyperglycemia was confirmed by determination of plasma glucose 3 days post alloxan injection, and the rats considered to be diabetic if the plasma glucose level was above 200 mg/dL. During intervention periods, the pigeon pea group rats were fed intervention diet and were force feeding of pigeon pea beverage dissolved in distilled water at doses of 2.7 g/kg body weight (equal to human consumption of 30 g/day), whereas the D-H group rats were fed standard diet and were force feeding of distilled water. All diets used in this study were presented in Table 1. Proximate and dietary fiber analysis of pigeon pea beverage was performed according to standard AOAC methods [31, 32]. The result was presented in Table 2. The animal body weights and residual feed intake were recorded. The feed efficiency ratio (FER) was determined as the ratio of body weight gain per unit of feed consumed over a period of time.

Table 1. Diet composition used in the animal study (per 1000 g)

| Ingredient             | Standard diet | Hypercholesterol diet | Intervention diet |
|------------------------|---------------|-----------------------|-------------------|
| Corn starch (g)        | 620.7         | 600.7                 | 605               |
| Casein (g)             | 140           | 140                   | 132.9             |
| Sucrose (g)            | 100           | 100                   | 100               |
| Soybean oil (g)        | 40            | 40                    | 39.2              |
| Carboxy methyl cellulose (g) | 50          | 50                    | 50                |
| Mineral mix (g)        | 35            | 35                    | 34.2              |
| Vitamin mix (g)        | 10            | 10                    | 10                |
| L-cystine(g)           | 1.8           | 1.8                   | 1.8               |
| Choline bitartrate (g) | 2.5           | 2.5                   | 2.5               |
| TBHQ (mg)              | 8             | 8                     | 8                 |
| Cholesterol (g)        | -             | 20                    | -                 |

Table 2. Proximate and dietary fiber of pigeon pea beverage

| Component               | Compositions |
|-------------------------|--------------|
| Moisture (%)            | 7.82         |
| Fat (% db)              | 2.31         |
| Protein (% db)          | 25.18        |
| Crude Ash (% db)        | 2.40         |
| Carbohydrate (by diff) (% db) | 70.11 |
| Dietary fiber (% db)    | 22.25        |
2.4 Blood plasma collection and analysis

The blood samples were collected by retro-orbital puncture at the initial phase (after acclimatization period), after hypercholesterolemia induction and diabetic induction periods, as well as after 1 and 2 weeks intervention periods. Blood plasma isolation was conducted using EDTA (Ethylene diamine tetra acetic acid) as anticoagulants, then centrifugation at 3,000 rpm (1500 × g) for 15 min at 4°C. Plasma glucose and cholesterol levels analyses were conducted using assay kits for glucose (GOD-PAP) and cholesterol (CHOD-PAP) respectively. Plasma antioxidant status was determined by MDA measurement using thiobarbituric acid-reactive substance (TBARS-C18) method [33].

2.5 Statistical analysis

Each measurement was reported as a mean followed by the standard deviation. The IBM SPSS Statistics 22 (SPSS Inc., Chicago, USA) program were used to the research data analyses. The data were analyzed by ANOVA (analysis of variance) and the significant differences between means (p<0.05) were determined using DMRT (Duncan’s multiple range test).

3. Results and Discussions

3.1 Effect of pigeon pea beverage diets on the body weight and feed efficiency ratio (FER) of diabetic-hypercholesterolemia rats

Body weight and FER of all experimental rats groups were presented in figure 1 and 2 respectively. All groups of the rats showed similar body weight (p> 0.05) in the initial phase (before D-H induction), after D-H induction and after 1 week intervention periods (figure 1). After 2 weeks of intervention period, the normal group has significantly higher body weight than the other groups. Body weight gain was observed in both normal group rats (36.67%) and pigeon pea group rats (9.95%) after 2 weeks of intervention period, while the D-H group rats have similar body weight during experimental periods. These results suggest that D-H induction leads to the body weight loss, but it could be suppressed by the pigeon pea beverage administration during the intervention period. It was in line with the feed efficiency ratio (figure 2). The pigeon pea beverage capable to increase the feed efficiency ratio in the diabetic-hypercholesterolemia rats, although it was not as high as the normal group rats. Alloxan induces specific inhibition of glucokinase and increases the ROS (Reactive oxygen species) formation, resulting in reduced insulin secretion due to the specific
inhibition of glucose-induced insulin secretion and necrosis of beta cells [34]. Therefore, the body used protein (protein washing) or fat in adipose tissue (lipolysis) became an energy source. So, the body weight was reduced. Kandulska [35] reported that alloxan treatment induced lipolysis in rats adipocyte which may cause loss of white adipose tissue, and consequently reduce rats body weight. It was attributed by the alloxan capability to form free radicals. In the previous study [20], the author observed that pigeon pea beverage demonstrates radical scavenging activity related to its phenolic compound.

The D-H group rats have negative FER value (figure 2). It was related to the rat's body weight (figure 1) which exhibits decreasing tendency at the end of the experimental period. Bang [36] and [37] reported similar results, i.e. the diabetic rats fed standard diet showed negative FER value and body weight loss.

3.2. Hypocholesterolemic effect of pigeon pea beverage diets on diabetic-hypercholesterolemia rats

Diabetic-hypercholesterolemia rats induced by alloxan and high-cholesterol diets are suitable animal models to simulate clinically diabetics complication with hypercholesterolemia [38, 39]. The effect of pigeon pea beverage supplementation on plasma total cholesterol level of diabetic-hypercholesterolemia rats is presented in table 1. The plasma total cholesterol level of all three groups rat was not significantly different at the initial phase of the experiment. The hypercholesterol diet consisting of 2% cholesterol which was used in the present study significantly elevated the plasma cholesterol level reaching 66% - 71% in both pigeon pea group and D-H group rats. These results were in agreement with Solanski and Bhat’s study [40] which induced hypercholesterolemia with the diet containing 2% cholesterol. A high-cholesterol diet causes cholesterol in the liver to exceed its normal levels, resulting in cholesterol returning to the blood circulation [41]. The plasma cholesterol levels of normal group rats which were fed standard diet before and after hypercholesterolemia induction period were not significantly different.

Table 3. Effect of pigeon pea beverage diet on plasma total cholesterol level of diabetic-hypercholesterolemia rats

| Group               | Plasma total cholesterol levels (mg/dL) | intervention period | 1 week | 2 weeks |
|---------------------|----------------------------------------|---------------------|--------|---------|
| Normal              | 105.58 ± 3.06<sup>AB</sup>             | 107.77 ± 2.72<sup>A</sup> | 110.19 ± 2.63<sup>A</sup> | 106.56 ± 2.97<sup>A</sup> |
| D-H                 | 105.31 ± 4.32<sup>AB</sup>             | 175.76 ± 4.49<sup>B</sup> | 178.70 ± 4.36<sup>B</sup> | 182.68 ± 4.90<sup>C</sup> |
| Pigeon pea beverage | 102.12 ± 5.17<sup>A</sup>              | 175.36 ± 4.34<sup>B</sup> | 164.81 ± 2.05<sup>C</sup> | 140.68 ± 2.47<sup>B</sup> |

Different subscript (capital letters) in the same column and different superscript (lowercase letters) in the same row indicated significant differences (p<0.05).

Table 3 indicates that supplementation of pigeon pea beverage (0.27 g/kg body weight) during the intervention period caused significantly reduced the plasma cholesterol level of diabetic-hypercholesterolemia rats, reaching 6.04% and 19.78% after 1 week and 2 week supplementation respectively. On the contrary, D-H group rats showed significantly elevated plasma cholesterol levels, reaching 1.65% and 3.92% after 1 week and 2 week intervention period. Hypocholesterolemic activity of the pigeon pea beverage may be attributed to the dietary fiber compounds and its antioxidant capacity. Ariviani [20] reported that pigeon pea beverage has soluble, insoluble and total dietary fiber of 8.45 %db, 14.58 %db, and 23.03 %db. The beverage displays radical scavenging capacity equivalent to 13.72 mmol Vitamin C/kg dry weight, due to the vitamin C and tocopherol content as well as the phenolic compound. Dietary fiber provides a hypocholesterolemic effect through the regulation of HMG-CoA reductase expression which lowered cholesterol synthesis and enhanced cholesterol excretion in bile [42]. Asparagus fiber fraction can lower blood plasma cholesterol level of hypercholesterolemia rat [43].
The mechanism of cholesterol reduction by antioxidants is possible due to the antioxidant ability in inhibiting LDL oxidation. The oxidized LDL will be recognized by the receptor scavenger in macrophages and internalized it to form foam cells, resulting in the accumulation of cholesterol in the macrophages [44]. Because the LDL oxidation was inhibited by the antioxidant compounds, so the massive cholesterol uptake in the macrophages will be suppressed. Hypocholesterolemic potentials of the antioxidant compound in pigeon pea beverage are in line with some previous studies. Supplementation of vitamin C [45, 46, 47], tocopherols [45], and flavonoids [43] reduced total plasma cholesterol concentration in hypercholesterolemia rats. Vitamin C is necessary for the transformation of Cholesterol to bile acids (most important pathway of cholesterol catabolism) simultaneous with increased fecal and liver bile acids through activation of 7α-hydroxylase the limiting step of the Cholesterol catabolism in liver [45, 47]. Flavonoids as one of the phenolic compounds are reported to be capable of decreasing blood cholesterol content, down-regulating cholesterol synthesis and increasing the expression of LDL receptors [43].

3.3. Hypoglycemic effect of pigeon pea beverage diets on diabetic-hypercholesterolemia rats

Table 4. Effect of pigeon pea beverage diet on plasma glucose level of diabetic-hypercholesterolemia rats

| Group               | Plasma glucose levels (mg/dL)                  |                  | intervention period |                  |
|---------------------|-----------------------------------------------|------------------|---------------------|------------------|
|                     | initial                                                                                   | after diabetic-hypercholesterolemia induction | 1 week            | 2 weeks          |
| Normal              | 71.68 ± 0.73\textsuperscript{A} \textsuperscript{a}  \hspace{1cm} 73.54 ± 0.89\textsuperscript{A} \textsuperscript{a}  \hspace{1cm} 74.59 ± 0.92\textsuperscript{A} \textsuperscript{a}  \hspace{1cm} 73.93 ± 1.45\textsuperscript{A} \textsuperscript{a} |                  |                  |
| D-H                | 71.79 ± 0.99\textsuperscript{A} \textsuperscript{a}  \hspace{1cm} 226.62 ± 2.26\textsuperscript{B} \textsuperscript{b}  \hspace{1cm} 226.66 ± 4.53\textsuperscript{C} \textsuperscript{c}  \hspace{1cm} 231.97 ± 3.97\textsuperscript{C} \textsuperscript{c} |                  |                  |
| Pigeon pea beverage | 71.57 ± 0.92\textsuperscript{A} \textsuperscript{a}  \hspace{1cm} 227.71 ± 2.80\textsuperscript{B} \textsuperscript{a}  \hspace{1cm} 188.71 ± 3.36\textsuperscript{B} \textsuperscript{c}  \hspace{1cm} 150.60 ± 1.50\textsuperscript{B} \textsuperscript{b} |                  |                  |

Different subscript in the same column and different superscript in the same row indicated significant differences (p<0.05)

Diabetes is a disease marked by high plasma glucose level and the presence of glucose in urine. Alloxan injection at doses of 80 mg/kg body weight proved able to induce diabetes in Sprague Dawley male rats, as indicated by elevated plasma glucose levels from normal levels (71 mg / dL) to hyperglycemia (227 mg / dL) (table 4). Diabetic action mechanism of alloxan was through its ability to induce ROS formation as well as the specific inhibition of glucokinase [34]. Table 4 indicated the hypoglycemic activity of pigeon pea beverage on diabetic-hypercholesterolemia rats. Pigeon pea group rats showed significantly lower plasma glucose level than D-H group, even though it was still higher than those of normal group rats. The results are in line with several previous studies which were reported the hypoglycemic activity of pigeon pea seed, such as [26], [19], and [18].

Capabilities of pigeon pea on suppressing severe hyperglycemia of diabetic-hypercholesterolemia rats were due to the antioxidant and dietary fiber compounds. As described previously, the ones of the diabetic mechanism of alloxan were through its ability on inducing radical (ROS) formation caused oxidative damage of pancreatic beta cells thus impairing the insulin secretion. Radical scavenging ability of pigeon pea antioxidant compound will inhibit the more severe oxidative damage caused by alloxan, thereby insulin secretion enhanced and thus lowering glucose levels. Several study reported the hypoglycemic effect of antioxidant compounds, such as [48], [49], [50]. Dietary fibers showed hypoglycemic effect through several mechanisms such as increasing the viscosity of small intestine juice and hinder diffusion of glucose, glucose binding and decrease the concentration of available glucose in the small intestine, as well as retarding alpha-amylase action through capsuling starch and the enzyme and might directly inhibit the enzyme [51].

3.4. Antioxidant status of diabetic-hypercholesterolemia rats administered with pigeon pea beverage

Oxidative stress is suggested as mechanism underlying diabetes and diabetic complications.
Oxidative stress acts as mediator of insulin resistance and its progression to glucose intolerance, subsequently support the appearance of atherosclerotic complications, and contribute to rising in many micro- and macrovascular complications [52, 21, 3]. Table 5 presents the plasma antioxidant status expressed as plasma MDA level of the normal, D-H and pigeon pea groups rats at the initial, after D-H induction, after 1 week and 2 weeks intervention periods. The Higher plasma MDA level indicates lower plasma antioxidant status. MDA was known as biomarkers of oxidative stress in diabetes mellitus related to lipids peroxidation [3].

**Table 5.** Effect of pigeon pea beverage diet on plasma MDA level of diabetic-hypercholesterolemia rats

| Group                  | Plasma MDA levels (mmol/l) | intervention period |
|------------------------|-----------------------------|---------------------|
|                        | initial                     | after diabetic-     | 1 week | 2 weeks |
|                        |                             | hypercholesterolemia|        |
| Normal                 | 1.45 ± 0.17<sup>a</sup>    | 1.72 ± 0.19<sup>a</sup> |        |
| D-H                    | 1.32 ± 0.18<sup>a</sup>    | 11.88 ± 0.32<sup>b</sup> | 14.08 ± 0.34<sup>c</sup> | 14.28 ± 0.39<sup>c</sup> |
| Pigeon pea beverage    | 1.56 ± 0.24<sup>AB</sup>   | 11.76 ± 0.22<sup>BC</sup> | 13.11 ± 0.25<sup>b</sup> | 7.39 ± 0.17<sup>b</sup> |

Different subscript in the same column and different superscript in the same row indicated significant differences (p<0.05)

Diabetic-hypercholesterolemia induction was significantly reduced plasma antioxidant status due to the lipids peroxidation as indicated by elevated of plasma MDA levels. Supplementation with pigeon pea beverage reduced plasma MDA levels of diabetic hypercholesterolemia rats reached to 37.16% after 2 weeks intervention period. On the contrary, increasing of MDA plasma level (20.20%) was observed in D-H group rats. The plasma MDA levels (table 5) was in line with the plasma cholesterol data (table 3) and plasma glucose data (table 4). These results suggest the potential of pigeon pea beverage as a potential source of antioxidant intake to overcome oxidative stress in diabetics, thus suppressed of diabetic complication.

**5. Conclusion**

The pigeon pea beverages administration at doses equivalent to human consumption of 30 grams/day proven to be capable to reduce plasma glucose and total cholesterol levels, and increase plasma antioxidant status (reducing MDA levels) of diabetic-hypercholesterolemia rats. This result indicated the potential of pigeon pea beverage as an anti-diabetic functional drink. The research result provides an alternative strategy for maintaining plasma glucose and cholesterol at normal levels to prevents diabetes complication by using the local bean as an anti-diabetic functional drink.

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