Oral Administration of *Caesalpinia bonducella* Seeds Ethanolic Extract Decreased Post Prandial Blood Glucose Level and Prevented the Reduction of Fasting Insulin Level of Diabetic Male Albino Rats (*Rattus norvegicus*)

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Abstract

Caesalpinia bonducella contain flavonoid, tanin, saponin which has antidiabetic activity. The aim of this study was to determine that the *Caesalpinia bonducella* seed extract orally reduces post-prandial blood glucose level and prevent the reduction of fasting insulin level in diabetic male albino rats (*Rattus norvegicus*). This study was a true experimental with pre-test post-test control group design using 24 male albino rats. All samples were induced with streptozotocin which caused the destruction of β cell and nicotinamide which protected the β cell from destructions of that the pancreatic β-cell was not destructed entirely. The selected samples were divided into three groups: the control negative group was given distilled water (P0), the control positive group was given glibenclamide (P1), and the treatment group was given ethanolic extracts of *Caesalpinia bonducella* seed 500mg/kg body weight (P2). Blood glucose and plasma insulin measurements were taken after 14 days of treatment. The result showed that the post-prandial blood glucose level of treatment group (P2) was decreased significantly, from 232,63±10,31 mg/dl to 185,50±16,00 mg/dl ($P<0.001$) whereas in P0 group, post-prandial blood glucose level was rising significantly from 223,75±17.03 mg/dl to 267,25±17,56 mg/dl ($p<0.001$). In P1 group, the reduction of post-prandial blood glucose level was bigger compare to P2 group, which was from 228,50±11,55 mg/dl to 115,88±10,23 mg/dl ($p<0.001$). Post test fasting insulin level of P2 group was significantly different compare to other group ($p<0.05$). The administration of *Caesalpinia bonducella* seed extract increased the fasting insulin level although did not significant statistically ($p>0.05$) whereas the other group showed the reduction of fasting insulin level ($p<0.05$). It can be concluded that the administration of *Caesalpinia bonducella* seed extract orally reduced post-prandial blood glucose level and prevent reduction of fasting plasma insulin level in diabetic male albino rats.

Keywords: *Caesalpinia bonducella* seed, post-prandial blood glucose, fasting plasma insulin, DM

Introduction

The aging process in humans is characterized by the emergence of degenerative diseases due to decreased function of the body's organs. Diabetes mellitus type 2 is the most common degenerative disease. Diabetes mellitus type 2 is a metabolic disease characterized by elevated blood glucose levels and insulin resistance.[1] The cause of this disease is multifactorial, with several risk factors including genetic, free radicals produce by metabolism, hormonal, diet, unhealthy lifestyle and lack of physical activity. Diabetes mellitus can be diagnosed by measuring blood glucose levels.

Several modern medicine has been used to treat diabetes, including insulin, sulphonylurea, biguanide and glitazon.[2] The treatment is primarily intended to control blood glucose within normal range so that complications of the disease does not occur. A very popular example for these is insulin and sulfonyl urea class (glibenclamide) which has been used widely at large scale. However, this synthetic drug such as glibenclamide can cause adverse side effects such as nausea, vomiting,

haematological and dermatological reactions, obstructive jaundice, hyponatremia, and weight gain. In addition, the use of biguanide can cause nausea, vomiting and epigastric pain, lactic acidosis and liver disease.[3] Nevertheless, the benefits of this drugs is far outweigh the possible side effects, then this pharmacological therapy remains the first choice for treating diabetes. The diabetes treatment without side effects remains a challenge that ongoing research is needed to find an alternative antidiabetic drugs without side effects.

Diabetes treatment using herbs has become a worldwide alternative, including Indonesia. Various types of herbs have been identified to have an excellent benefits as treatment for many diseases. One herb that is widely used to treat diabetes is *Caesalpinia bonducella* seed. This herb is believed to have many health benefits, especially as an antidiabetic.[4] *Caesalpinia bonducella* has been used in traditional Ayurvedic medicine for a long time. *Caesalpinia bonducella* derived from Caesalpiniaceae family has also reported to have a therapeutic effect as anthelmintics, antibacterial, antipyretic, and lately known to treat diabetes[5].

The antidiabetic activity of the *Caesalpinia bonducella* seed may resulted from the presence of flavonoids, saponins and tannins[6]. Flavonoids has been known as a natural antioxidant that protects β cells from free radical damage. Flavonoids also protects β cells against damage caused by oxidative stress as well regenerate β cells[7-8]. Morover, saponins can inhibit the transport of glucose in the gut by inhibiting the sodium glucose co-transporter-1 (S-GLUT-1)[7]. It also known that tannins improve glucose and fat metabolism[9].

Previous study conducted by Jana *et al.,* (2012) proved that the hidrometanol extract of *Caesalpinia bonducella* seed can decrease fasting glucose levels, which implied that the *Caesalpinia bonducella* seed extract can stimulate the pancreatic β cells to produce and secrete insulin, and help regeneration of pancreatic β cells. *Caesalpinia bonducella* extract demonstrated antihyperglycemic activity in a chronic model of diabetes mellitus type 2. This extract promotes gluconeogenesis thereby increasing the liver glycogen content. *Caesalpinia bonducella* seed extract also showed antihyperglycemic and hypolipidemic activity in streptozotosin-induced diabetic rat[10]. Antihyperglycemic effects may be caused by blocking the intestinal absorption of glucose[5]. *Caesalpinia bonducella* seed benefits in traditional medicine has been widely recognized empirically. However, the scientific evidence of this plant both to decrease blood glucose levels and increase fasting insulin levels is necessary.

Materials And Methods

This study was a true experimental research using completely randomized pretest-posttest control group design[11]. The samples used were male albino rats (*Rattus norvegicus*), wistar strain, diabetes with post-prandial blood glucose levels ≥ 135 mg / dL[12], aged 2.5-3 months, with around 190-200 grams weight. The number of samples used was calculated using Pocock formula (2008) and obtained a minimum sample of 6 rats/group, so it was used a total of 18 rats.

Rats were adapted for one week, then induced DM type-2 with streptozotocin and nicotinamide. Streptozotocin dose used in this study was 13mg/200g body weight and the dose of nicotinamide was 46mg/kg[13]. On day 15th blood sample were collected 2 hours postpandrial through orbital sinus to examined the glucose and insulin levels before treatment (pretest). After the rats were recognized as diabetes with post-prandial blood glucose levels ≥ 135 mg/dL, the rats were divided into three groups: negative control group was given distilled water 1 cc (P0), the positive control group was given Glibenclamide 5mg/kg (P1), and the treatment group was given ethanolic extract of *Caesalpinia bonducella* seed 500mg/kg (P2) and treated with the appropriate group for two weeks. Blood sample were then collected 2 hours

postpandrial through orbital sinus to examined the glucose and insulin levels after treatment (posttest). Blood glucose levels were measured by glucose oxidase method using gluco tool M[14], while fasting insulin levels were measured using ELISA method using Rat Insulin ELISA kit E0707Ra of Bioassay Technology Laboratory[15].

The data were analyzed statistically using the Statistical Package for Social Sciences (SPSS 17.o Version). *Analysis of Variance* (Anova) test followed by *Least Significant Difference* (LSD) for normally distributed data and *Kruskal Wallis* test followed by *Mann-Whitney* test for not normally distributed data were used to compare the effect of *Caesalpinia bonducella* seed on blood glucose and fasting insulin levels between groups. In addition, *paired sample T-test* for normally distributed data and *Wilcoxon* test for normally distributed data were used to compare the effect of *Caesalpinia bonducella* seed on blood glucose and fasting insulin levels within group.

Results And Discussion

Post Prandial Blood Glucose Level

The mean pretest post-prandial blood glucose level of negative control group (P0) was 223.75 ± 17 036 mg/dL, the positive control group (P1) was 228.50 ± 11,551 mg/dL, and the treatment group (P2) was 232.63 ± 10 309 mg/dL. Anova test result showed that prior to the treatment given, the average of blood glucose levels between groups were not different (p> 0.05). The mean posttest post-prandial blood glucose level of negative control group (P0) was 267.25 ± 17 556 mg/dL, the positive control group (P1) was 115.88 ± 10.232 mg/dL, and the treatment group (P2) was 185.50 ± 16 036 mg/dL. Anova test result showed that after 14 days treatment were given, the average blood glucose levels between groups were significantly different (p <0.001) (Table 1; Fig.1).

The effect of *Caesalpinia bonducella* seed on blood glucose level within group were performed using *paired samples t-test*, showed a significant increase in blood glucose levels in the group given distilled water (P0) (p <0.001). Whereas the other groups, both positive control group was given Glibenclamide 5mg/kg (P1) and the treatment group was given ethanolic extract of *Caesalpinia bonducella* seed 500mg/kg (P2) showed a decrease in blood glucose levels significantly (p <0.001) (Table 2). These implied that administration *Caesalpinia bonducella* seed extract of 500 mg/kg for 14 days can decrease blood glucose levels with an average of 47.13 mg/dL. However, glibenclamide which is the standard drug for diabetes were given for 2 weeks has a better effect in decreasing blood glucose levels of 112.62 mg/dL.

Table 1. Post Prandial Blood Glucose Level

Group	Mean (mg/dL)	SD	*F*	P
Pretest negative control group (P0)	223,75	17.036		
Pretest positive control group (P1)	228,50	11.551	0.893	0,424
Pretest treatment group (P2)	232,63	10.309		
Posttest negative control group (P0)	267,25	17.556		
Posttest positive control group (P1)	115,88	10.232	205.626	0,000
Posttest treatment group (P2)	185,50	16.036		

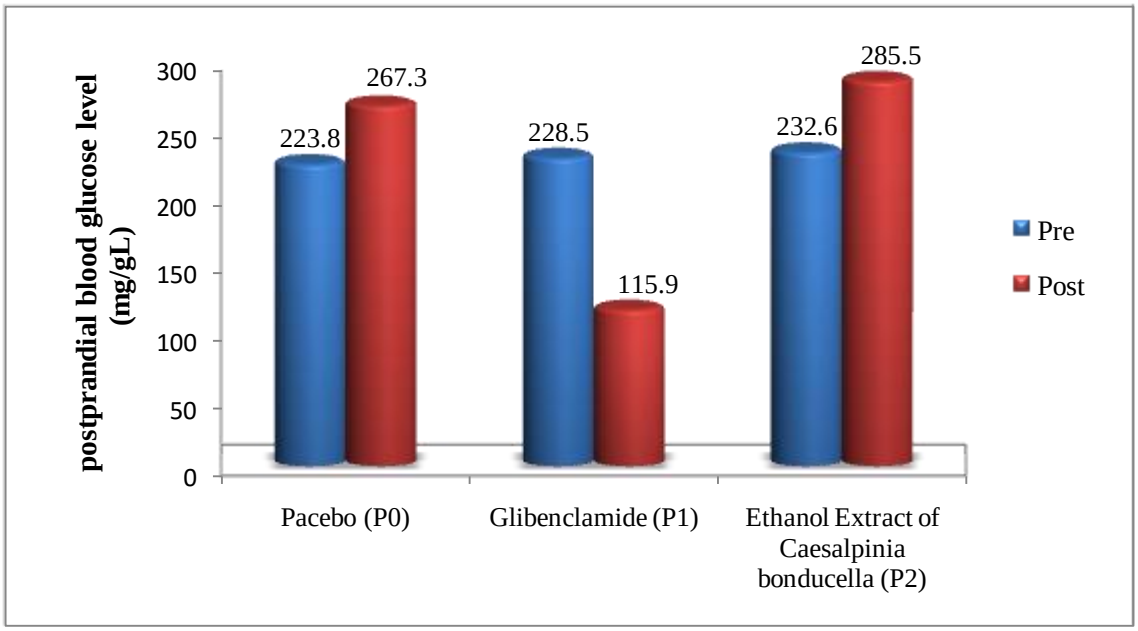

Fig 1. Blood Glucose Levels Before (pretest) and After (posttest) Treatment

Table 2. The Effect of *Caesalpinia bonducella* seed on Blood Glucose Level

Group	Blood Glucose Level (mg/dL)		Mean Difference	t	P
	Pretest	Posttest			
Negative control group (P0)	223,75±17.036	267,25±17.556	43,50	-7,942	0,000
Positive control group (P1)	228,50±11,551	115,88±10,232	-112,62	16,072	0,000
Treatment group (P2)	232,63±10.309	185,50±16.0	-47,13	10,060	0,000

This research showed that the ethanolic extract of *Caesalpinia bonducella* seed can decrease post-prandial blood glucose levels significantly ($p<0.001$) compared to placebo by 20.25%. It is supported by research conducted by Prashant and Bhanudas (2011) which proved that the hydroalcoholic extract of *Caesalpinia bonducella* seed decreased blood glucose levels significantly ($p <0.05$) in normal mice, excess glucose mice and alloxan-induced diabetic mice. Other studies also showed that hidromethanolic extract of *Caesalpinia bonducella* seed decreased fasting blood glucose levels significantly ($p <0.05$)[16].

The antidiabetic activity of the *Caesalpinia bonducella* seed extract may be generated from the active compounds content such as alkaloids, flavonoids, tannins, phenols and saponins. Photochemical analysis results showed that ethanol extract of *Caesalpinia bonducella* seed containing these substances[6]. Flavonoids are known as a natural antioxidant that protects β cells from free radical damage. Furthermore, previous studies have reported that flavonoids protected the β cells against damage caused by oxidative stress, and also regenerate β cells[7]. According to research conducted by Wibudi *et al.*[17], flavonoid compounds play a role in controlling blood glucose level by increasing insulin secretion by pancreatic beta cells by increasing metabolism of Ca2 + and regenerate pancreatic

β cells. The interaction with ATP-sensitive K^+ channels in the membrane of the β cells causing membrane depolarization and promote the opening of Ca^{2+} channel. With the opening of Ca^{2+} channel, the Ca^{2+} will enter β cells and then stimulate granules containing insulin and cause the secretion of insulin.

Other active compounds contained in *Caesalpinia bonducella* seed extract is beneficial saponins that also decrease blood glucose. Saponin containing polycyclic aglykon, which has a distinctive character that is frothy when shaken with water and cause a bitter taste in plants. Saponins can inhibit the increase of blood glucose by inhibiting the activity of α-glucosidase enzyme (enzymes in digestive tract responsible for carbohydrates changes into glucose). Saponins inhibit the absorption of small nutrient molecules such as glucose by inhibiting glucose transporter system [18]. Saponins reported to inhibit the transport of glucose in the gut by inhibiting the sodium glucose co-transporter-1 (S-GLUT-1) [7].

Tannins are fenilik polymer substances capable of tanning leather and gelatin precipitate from the liquid. Tannins can be found in almost every part of the plant. Tannins are divided into two groups, hydrolyzed tannin and condensed tannin. This substance can be used to decrease blood glucose levels by elevating the metabolism of glucose and lipids. Tannins known to stimulate glucose and fat metabolism, so that the pile of these substances can be avoided and ultimately decreasing [9]. Tannins also have antioxidant activity, and having hypoglycemic activity by increasing glycogenesis [19].

Fasting Insulin Level

The mean pretest fasting insulin level of negative control group (P0) was 11.174 ± 1.865 mIU/L, the positive control group (P1) was 8.563 ± 1.327 mIU/L, and the treatment group (P2) was 8.716 ± 2.916 mIU/L. Anova test result showed that prior to the treatment given, the average of fasting insulin levels between groups were significantly different ($p<0.05$). However, there were no differences between positive control group (P1) and the treatment group (P2) ($p>0.05$). The mean posttest fasting insulin level of negative control group (P0) was 6.881 ± 4.125 mIU/L, the positive control group (P1) was 6.564 ± 1.869 mIU/L, and the treatment group (P2) was 8.839 ± 1.114 mIU/L. *Kruskal Wallis* test result showed that after 14 days treatment were given, the average fasting insulin levels between groups were significantly different ($p <0.05$). Fasting insulin level of negative control group (P0) was not different from the positive control group (P1) ($P> 0.05$). Whereas the group treated with *Caesalpinia bonducella* seed extract had the highest level of insulin and significantly different compared to other groups ($P <0.05$) (Table 3; Figure 2).

Table 3. Fasting Insulin Level

Group	Mean (mIU/L)	SD	*F*	P
Pretest negative control group (P0)	11.174[a]	1.865		
Pretest positive control group (P1)	8.563[b]	1.327	3.749	0.041
Pretest treatment group (P2)	8.716[b]	2.916		
Posttest negative control group (P0)	6.881[a]	4.125		
Posttest positive control group (P1)	6.564[a]	1.869	7.745	0.021
Posttest treatment group (P2)	8.839[b]	1.114		

*Difference notation (a,b) showed a statistical different ($p<0.05$)

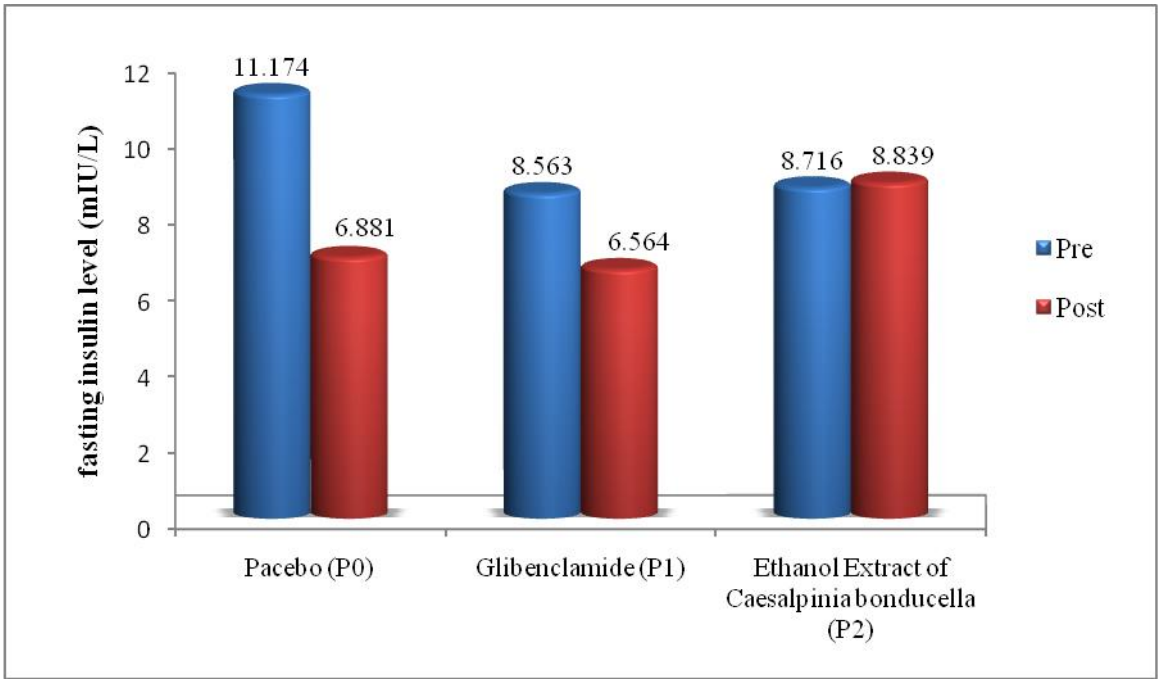

Fig 2. Insulin Levels Before (pretest) and After (posttest) Treatment

The effect of *Caesalpinia bonducella* seed on fasting insulin level within group were performed using *Wilcoxon signed-rank test*, showed a decrease in blood glucose levels in the group given distilled water (P0) and glibenclamide (P1) but not statistically significant (p> 0.05). Whereas the analysis using *Independent sample t-test* in the treatment group was given ethanolic extract of *Caesalpinia bonducella* seed 500mg/kg (P2) showed an increase in fasting insulin levels but not statistically significant (p>0.05) (Table 4).

Table 4. The Effect of *Caesalpinia bonducella* seed on Fasting Insulin Level

Group	Blood Glucose Level (mg/dL)		Mean Difference	t	p
	Pretest	Posttest			
Negative control group (P0)	11,174±1,865	6,881±4,125	-4.2925	-1.540	0,123
Positive control group (P1)	8,563±1,327	6,564±1,869	-1.9988	1.903	0,099
Treatment group (P2)	8,716±2,916	8,839±1,114	0.1137	-0.093	0,928

Polyphenols can protect pancreatic β cells from the toxic effects of free radicals that are produced under chronic hyperglycemia conditions. Polyphenols are also able to increase pancreatic β cell mass and keep the insulin content inside [20].

The antidiabetic mechanism of *Caesalpinia bonducella* seed is remains unclear. According to Shukla *et al.* [21], hyperglycemia condition will lead to increasing number of free radicals through auto-oxidation of glucose, which can lead to stress oxidative and damage liver cells. Previous study proved that *Caesalpinia bonducella* seed extract has a strong antioxidant activity and have a protective effect against DNA damage caused by hydroxyl radicals making it useful in reducing the formation of free radicals as a result of diabetes. It seems that *Caesalpinia bonducella* seed extract can reduce the release of inflammatory cytokines which is causes insulin resistance in diabetes patient [21].

Research has shown that orally administrated of *Caesalpinia bonducella* seed extract can improve histopathologic characteristic of the pancreas, liver and kidneys of alloxan-induced diabetic rats. This was the background of this research that the pancreatic tissue repair after administration of *Caesalpinia bonducella* seed extract will improve β cells function and increase insulin level in mice. This research was also supported by Jana *et al* .[16], proved that streptozotocin-induced diabetic rats showed an improvement in the activity of hexokinase, glucose-6-phosphate dehydrogenase and glucose-6-phosphatase in the liver which are the biomarker controlled by insulin, so it can be predicted that the *Caesalpinia bonducella* seed extract can increase the synthesis or secretion of insulin by the pancreas through the presence of β-cell regeneration [22].

The remarkable results of this study was the increased of fasting insulin level after administration of *Caesalpinia bonducella* seed extract, although not significant statistically. While the posttest fasting insulin level between group are being compared, it can be seen that the fasting insulin level of treatment group (P2) is significantly higher from those negative control group (P0) and the positive control group (P1). This indicates that the administration of *Caesalpinia bonducella* seed extract has a better effect maintain insulin levels rather than Glibenclamide. This result was differed from fact that Glibenclamide is standard antidiabetic drugs that have a hypoglycemic effect by increasing insulin levels. However, the glibenclamide in this study decreased blood glucose levels significantly ($p < 0.001$) with decreasing insulin level. This can be explained as follows: (1) administration of sulfonylureas was initially lead to increased levels of insulin with decrease in blood glucose levels, insulin levels tend to return to the normal value; (2) administration of sulfonylureas may increase the sensitivity of pancreatic β cells to glucose by lowering levels of glucagon. The fall in blood glucose levels during treatment with sulfonylureas was also associated with reduced hepatic glucose production due to the decreasing glucagon level [23].

The different result of this result with previous study in term of insulin levels, can be caused by several factors. Firstly, the difference in content of *Caesalpinia bonducella* seed extract gorek grown in Indonesia and India [16, 22]. Results of phytochemical analysis performed in this study showed no content of triterpenoids on *Caesalpinia bonducella* seed extract while the two previous studies found the substance. Secondly, *Caesalpinia bonducella* seed extract administered orally does not directly affect the levels of insulin but have a protective effect on normal pancreatic β-cells. This was evidenced by the persistence of fasting insulin levels in the group given *Caesalpinia bonducella* seed extract while the other groups tend to decreased.

The mean fasting insulin levels after treated with *Caesalpinia bonducella* seed extract showed an improvement, although not statistically significant. Further research needs to be done with a longer time, more samples and increasing dose so that the results would show a statistically signifikan increase in insulin level.

Conclusion

Based on the results of this study, it can be concluded that the administration of 500 mg/kg *Caesalpinia bonducella* seed extract orally can decrease post-prandial blood glucose levels in diabetic rats by 20.25%. The administration of 500mg/kg *Caesalpinia bonducella* seed extract orally also prevent a decrease in fasting insulin levels of diabetic rats compared to glibenclamide and placebo groups.

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