

Ethanollic Extract of *Caesalpinia bonducella* f. Seed Ameliorates Diabetes Phenotype of Streptozotocin-Nicotinamide-Induced Type 2 Diabetes Rat

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Caesalpinia bonducella F. (Leguminosae) is widely used medicinal plant which contains flavonoid, tannin, saponin, and a potent antioxidant activity. However, the antihyperglycemic effect of the seed of *C. bonducella* is remained to be evaluated. This study used 24 male Wistar albino rats that were induced for type 2 diabetes with streptozotocin (STZ) and nicotinamide (NA). The rats were divided into three groups: the distilled water-treated group (NC group), glibenclamide-treated group (10 mg/kg/d, oral; PC group), and *C. bonducella* seed extract-treated group (500 mg/kg/d, oral; T group). Blood glucose and plasma insulin measurements were done after 14 days of treatment. The results showed that the postprandial blood glucose (PPBG) level of both PC and T groups were decreased significantly ($p < 0.01$ for both), whereas in NC group, the PPBG level was rising ($p < 0.01$). Glibenclamide was found to be more effective to decrease the PPBG level than *C. bonducella* seed extract ($p < 0.01$). The post-test fasting insulin level of T group was higher than other groups ($p < 0.05$). In summary, our results suggest that ethanollic extract of *C. bonducella* seed possesses antidiabetic activity against experimentally-induced type 2 diabetes.

Keywords: *C. bonducella* seed, postprandial glucose, insulin, diabetes, rat.

The aging process is characterized by the emergence of degenerative diseases due to the declining normal physiological function. Type 2 diabetes mellitus (T2DM) is the most common age-related degenerative disease, as the incidence and prevalence of this disease increase with age (Cowie *et al.*, 2006). The T2DM is a metabolic disease characterized by elevated blood glucose levels resulting from defects in insulin secretion, insulin action, or both (Committee and Classification, 2010). The cause of this disease is multifactorial,

with several risk factors including genetic, free radicals, hormonal, diet, unhealthy lifestyle and lack of physical activity (Wu *et al.*, 2014).

Several modern medicines have been widely used to treat diabetes, including insulin, sulphonylurea, biguanide, and glitazones (Chaudhury *et al.*, 2017). Most of the diabetes management are primarily intended to control blood glucose levels within normal range so that complications of the disease does not occur. The oral agent management of T2DM is the most common,

and in some settings, they are the first option when drug treatment is required. Sulfonylurea class (such as glibenclamide) is the class of drug that has been used widely at large scale becoming the most common treatment since 2007 (Camelo Castillo *et al.*, 2014). However, this synthetic drug can cause adverse side effects such as nausea, vomiting, hematological and dermatological reactions, obstructive jaundice, hyponatremia, weight gain, epigastric pain, lactic acidosis and liver disease (Sola *et al.*, 2015). Nevertheless, the benefits of this drugs far outweigh the possible side effects, then this pharmacological therapy remains the first choice for treating diabetes. The diabetes management without side effect remains a challenge that needs to be further investigated.

Diabetes treatment using herbs has become a worldwide alternative, including in Indonesia. Various types of herbs have been identified to have excellent benefits as a treatment for many diseases. *Caesalpinia bonducella* F. seed is believed to have many health benefits, as it has been used in traditional Ayurvedic medicine for a long time. *C. bonducella* derived from Caesalpinaceae family has also reported having a therapeutic effect as anthelmintics, antibacterial, antipyretic and analgesic, and lately known to treat diabetes (Chakrabarti *et al.*, 2003; Kannur *et al.*, 2006; Parameshwar *et al.*, 2002). *C. bonducella* seed benefits in traditional medicine have 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

Animals

Twenty-four 8-week-old experimentally naïve male Wistar albino rats with an average initial body weight of 190 ± 13 g (Animal House of Faculty of Veterinary Medicine, Udayana University, Denpasar, Indonesia) were used. Animals were housed under environmentally controlled condition (12 hr light/dark cycle; 22 – 24 °C), food and water were available *ad libitum* throughout the experiment. Animals were allowed to adjust to a new condition for seven days. The protocols used conformed to guidelines of animal studies and were approved by the committee on

the ethics of animal experiments in Faculty of Veterinary Medicine, Udayana University.

Preparation of the extract and phytochemical analysis

The *C. bonducella* seed extract was prepared according to the previously described method (Lilaram and Ahmed, 2013). In summary, the dried seed was coarsely powdered using a grinder and then extracted with 95% ethanol in the Soxhlet apparatus. The crude extract from the previous step was filtered by using Whatman paper and the solvent was dried by vacuum rotary evaporator under reduced pressure at a maximum temperature of 50 °C. The final fraction was stored at -20 °C until further use.

The crude ethanolic extracts of *C. bonducella* seed was tested for the presence of alkaloids, phenolic, flavonoids, steroids, tannins, saponins and triterpenoid glycosides were performed using previously described method (Iqbal *et al.*, 2015). The qualitative results are expressed as (+) for the presence and (–) for the absence of phytochemicals. The total phenolic content was performed by the Folin–Ciocalteu colorimetric method expressed in $\mu\text{g GAE/mg}$ (GAE= Gallic Acid Equivalent), and the total flavonoid content was examined using the aluminum colorimetric method expressed in $\mu\text{g CE/mg}$ (CE= Catechin Equivalent) (Iqbal *et al.*, 2015). The free radical-scavenging activity of the *C. bonducella* ethanolic seed extract was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical 1-1-diphenyl 2-picryl hydrazyl (DPPH) as previously described (BLOIS, 1958).

Streptozotocin-nicotinamide-induced type 2 diabetes

Type 2 diabetes was induced by intraperitoneal injection of 100 mg/kg BW nicotinamide (Sigma Chemical Co, St. Louis, MO, USA) dissolved in 0.9% normal saline followed after 15 minutes by freshly prepared streptozotocin (Sigma Chemical Co, St. Louis, MO, USA) of 45 mg/kg BW dissolved in 0.1 M citrate-phosphate buffer (pH = 4.5). Rats were considered diabetic when their postprandial plasma glucose concentration was greater than 150 mg/dL (Abdellatif *et al.*, 2017).

Experimental design

After 48 h of diabetes induction, diabetic rats were randomly divided into three groups,

containing eight rats in each group as follows: negative control (NC) group that was induced with streptozotocin (STZ)–nicotinamide and treated with oral distilled water, positive control (PC) group that was induced with STZ–nicotinamide and treated with oral Glibenclamide (Sigma Chemical Co, St. Louis, MO, USA) of 10 mg/kg/d, and the treatment (T) group that was induced with STZ–nicotinamide and treated with *C. bonducella* seed ethanolic extract of 500 mg/kg/d. The rats were orally administered once a day with Glibenclamide and *C. bonducella* seed ethanolic extract by dissolving in distilled water by intragastric tube. The rats were treated daily for a period of 14 days.

Biochemical Analysis

Plasma glucose concentrations were determined by colorimetric methods after an enzymatic reaction with peroxidase (Randox, CO, Antrim, UK). The plasma insulin levels were determined using a rat insulin ELISA kit (Bioassay Technology Laboratory Shanghai, China) according to the manufacturer's instructions. Blood was sampled for postprandial glucose measurements at 2 hours after the meal and for fasting insulin measurements at 8 hours after the meal.

Statistical analysis

Data are presented as group mean \pm standard deviation (SD). One way ANOVA with Least Significance Difference (LSD) post hoc test was performed to detect the significant differences between groups. The paired *t*-test was performed to detect the significant differences between pretest and posttest within the same group. Experimental differences were considered statistically significant if $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical screening of *C. bonducella* seed ethanolic extract

Phytochemical analysis and antioxidant activity of *C. bonducella* were carried out in this research. The preliminary phytochemical screening revealed the presence of alkaloids, phenolics, flavonoids, steroids, tannins, and saponins. The triterpenoid glycosides were not detected in the *C. bonducella* ethanol extract. The total amount of phenolic content present in *C. bonducella* seed ethanolic extract was 26.99 μg GAE/mg, and the total flavonoid content was 16.28 μg CE/mg. The

DPPH radical-scavenging activity revealed that the IC50 value of *C. bonducella* seed ethanolic extract was 78.41 $\mu\text{g}/\text{ml}$ (Table).

Postprandial Blood Glucose (PPBG) Level

Postprandial hyperglycemia is the most phenotype of type 2 *diabetes* and a critical therapeutic target for optimizing glycemic control. Our study showed that prior to the treatment, the average of PPBG level between groups was not different ($p > 0.05$). In contrast, after 14 days of treatment, both the Glibenclamide (PC) group and the *Caesalpinia bonducella* seed ethanolic extract (T) group had a lower PPBG level compared to distilled water-treated group ($p < 0.01$ for both). The blood glucose of NC group was elevated by 14 days STZ-nicotinamide treatment by 19.5% ($p < 0.01$), while both Glibenclamide and ethanol extract of *Caesalpinia bonducella* seed decreased postprandial blood glucose level of STZ-nicotinamide-induced diabetes rats by 49.3 and 20.3% respectively ($p < 0.01$ for both) (Fig 1).

It is supported by previous research which proved that the hydroalcoholic extract of *Caesalpinia bonducella* seed decreased blood glucose levels significantly in normal mice, hyperglycemia mice and alloxan-induced diabetic mice (Nadaf, 2017). Another study using a hydroethanolic extract of *Caesalpinia bonducella* seed showed that it decreased fasting blood glucose levels (Ghosh *et al.*, 2012). The antidiabetic activity of the *Caesalpinia bonducella* seed extract may be generated from the chemically active compounds content such as alkaloids, flavonoids, tannins, phenols, and saponins. Phytochemical analysis showed that ethanol extract of *Caesalpinia bonducella* seed containing these substances (Table 1).

Several pathologies, including type 2 diabetes, are related at some point to a pancreatic cell deregulation. Beta cell apoptosis is a common feature of both type 1 and type 2 diabetes, although the mechanism activating it in each case may be different. Therefore, the antiapoptotic activity of the *Caesalpinia bonducella* might be involved in beneficial effects against diabetes. Flavonoids are known as a natural antioxidant that protects beta cells from oxidative damage, as well as regenerate damaged-beta cells (Pinent *et al.*, 2008). Several experiments proved that flavonoids can exert antiapoptotic effects in pancreatic beta cells (Han,

2003). Other active compounds contained in *Caesalpinia bonducella*, saponin, can inhibit the elevation of blood glucose by inhibiting the activity of the \pm -glucosidase enzyme. Saponin inhibits the absorption of small nutrient molecules such as glucose by inhibiting glucose transporter system (Li *et al.*, 2015). Saponin was reported to inhibit the transport of glucose in the intestine by inhibiting the

sodium glucose co-transporter-1 (S-GLUT-1) (Li *et al.*, 2018). *Caesalpinia bonducella* also contains tannin has also been proven can be used to decrease blood glucose levels by stimulating glucose and fat metabolism. Tannin also has a hypoglycemic activity by inducing phosphorylation of the insulin receptor and Akt, as well as translocation of glucose transporter 4 (GLUT 4) (Liu *et al.*, 2005). Previous

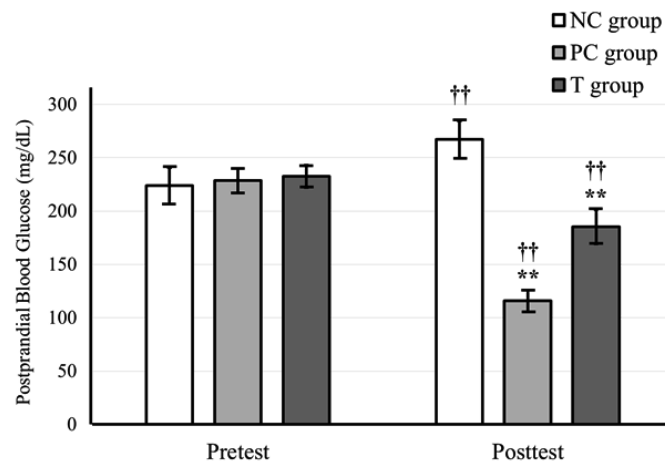


Fig. 1. Effect of glibenclamide and ethanolic extract of *Caesalpinia bonducella* F. seed on the postprandial blood glucose (PPBG) level of diabetic rats during 14 days treatment. All the values are expressed as the mean \pm SD. **indicates the significant difference when compared to the NC group of the same measurement time ($p < 0.01$; one way ANOVA with LSD post hoc test). ††indicates the significant difference when compared to pretest counterparts of the same group ($p < 0.01$; paired t -test)

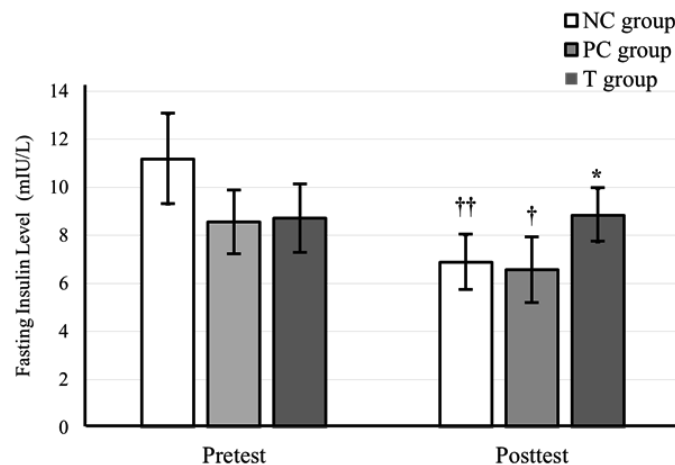


Fig. 2. Effect of glibenclamide and ethanolic extract of *Caesalpinia bonducella* F. seed on the fasting insulin level of diabetic rats during 14 days treatment. All the values are expressed as the mean \pm SD. **indicates the significant difference when compared to the NC group of the same measurement time ($p < 0.01$; one way ANOVA with LSD post hoc test). ††indicates the significant difference when compared to pretest counterparts of the same group ($p < 0.01$; paired t -test)

Table 1. Phytochemical screening and DPPH radical-scavenging activities of *Caesalpinia bonducella* ethanolic seed extract

Screening	Test	Presence or Amount
Alkaloids	Dragendorff's Test	(+)
Phenolic	Kokate's method	(+)
Flavonoids	Kokate's method	(+)
Steroids	Salkowski test	(+)
Tannins	Kokate's method	(+)
Saponins	Kokate's method	(+)
Triterpenoid	Salkowski test	(-)
Glycosides	Keller-Killiani test	(+)
Total Phenol	Folin-Ciocalteu colorimetric method	26.99 µg GAE/mg
Total Flavonoid	Aluminum colorimetric method	16.28 µg CE/mg
Antioxidant activity	DPPH radical-scavenging test	78.41 µg/ml

(+) = presence, (-) = absence, GAE = Gallic acid equivalent, CE = Catechin Equivalent

research found that the *Caesalpinia bonducella* seed extract reduces the inflammatory cytokines which are causes insulin resistance in diabetes patient (Shukla *et al.*, 2011).

Fasting Insulin Level

Decreasing fasting insulin level is one of the risk factors for diabetes. The mean fasting insulin level before treatment was not different among groups ($p > 0.05$). Inversely, after the treatment for 14 days, the *Caesalpinia bonducella* seed ethanolic extract (T) group had the highest fasting insulin level compare to both NC and PC groups ($p < 0.05$ for both). The fasting insulin level of PC group was comparable to the NC group ($p > 0.05$). By comparing pretest and posttest value within the group, it was noticed that the fasting insulin level of both NC group and PC group were declined by 38.4 and 23.4% respectively ($p < 0.05$ for both), after 14 days STZ-nicotinamide treatment with or without Glibenclamide. In contrast, the treatment of ethanol extract of *Caesalpinia bonducella* seed in T group prevented the decreasing fasting insulin level caused by 14 days of STZ-nicotinamide ($p > 0.05$) (Fig 2).

This research is also supported by the previous study 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 (Ghosh *et*

al., 2012). Polyphenolic bioactive compounds of *Caesalpinia bonducella* seed can protect pancreatic beta cells from cytokine-, reactive oxygen species- (ROS-), and glucose-induced toxicity under chronic hyperglycemia conditions (Oh, 2015). They are also able to increase pancreatic beta cell mass (Ogata *et al.*, 2004), as well as improve the insulin production and secretion of the pancreatic beta cell (Chang *et al.*, 2013). Flavonoids improve the insulin granule exocytosis of pancreatic beta cells by modulating Ca^{2+} metabolism, improve ATP generation in β -cells and provide an increase in the transcriptional activation of insulin-mediated by cyclic adenosine monophosphate (cAMP) signaling (Dias Soares *et al.*, 2017).

Interestingly, the *Caesalpinia bonducella* seed extract protected the rats from decreasing fasting insulin level caused by STZ-nicotinamide treatment, while the treatment of Glibenclamide failed to do so (Fig 2). This result differs from the fact that as a standard antidiabetic drug, Glibenclamide supposes to increases second-phase insulin secretion at a stimulating blood glucose level (Proks *et al.*, 2002). In spite of decreasing fasting insulin levels by Glibenclamide treatment (Fig 2), the PPBG level was still decreasing (Fig 1). This might be due to the short half-life of sulfonylureas effect on insulin levels, or the sulfonylureas increase the sensitivity of pancreatic beta cells to glucose by decreasing the glucagon (Pfeifer *et al.*, n.d.).

In general, hyperglycemia lead to the increasing number of free radicals through auto-oxidation of glucose, further leading to oxidative stress and oxidative damage of pancreatic beta cells (Ihara *et al.*, 1999). As we previously proved that *Caesalpinia bonducella* seed extract possesses a strong antioxidant activity (Table 2), it is also possible that *Caesalpinia bonducella* seed extract ameliorates diabetes phenotype by preventing the oxidative stress and oxidative damage in pancreatic beta cells thus decreases PPBG level and prevent the decreasing fasting insulin level. In summary, our results suggest that ethanolic extract of *Caesalpinia bonducella* seed possesses antidiabetic activity against experimentally-induced type 2 diabetes.

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