International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2019): 7.583

Administration of Butterfly Pea (*Clitoria ternatea*) Flower Etanol Extract Oral Increased the Number of Pancreatic Beta Cells and Decrease the Level of Glycated Hemoglobin (HbA1C) of Diabetic Male Wistar Rats (*Rattus norvegicus*)

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Abstract: ***Background and purpose: Type 2 Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia that occurs due to abnormalities in insulin secretion, insulin action or both. Chronic hyperglycemia causes a cumulation of glucose and hemoglobin bonds and an increase in the level of HbA1c. Glimepiride is an oral anti-hyperglycemia drug in the third generation sulfonylurea class which works to stimulate insulin secretion in the pancreas gland, making it effective in people with diabetes whose pancreatic beta cells are still functioning properly. Giving Butterfly pea (Clitoria ternatea) has been studied to increase the number of pancreatic beta cells and reduce HbA1c levels in diabetic rats (Rattus norvegicus) Wistar male induced by streptozotocin. Methods: Experimental research was conducted with a randomized posttest only control group design. Subjects were while male Wistar rats (Rattus norvegicus). 36 animals treatment group were divided into two group (n=18 individuals). The control group was given aquabidest and glimepiride (0,036mg/200gr body weight), and the treatment group was given butterfly pea flower part extract (80mg/200gr body weight) and glimepiride (0,036mg/200gr body weight) via sonde once per day for 60 days. After 60 days of treatment, the immunohistochemical examination of pancreatic beta cells and HbA1c levels with the Rat Hemoglobin HbA1c Elisa kit. Analysis of pancreatic beta cells is the average number of pancreatic beta cells from all Langerhans islands in a three field preparation of 400x magnification by means of zigzag, the number of pancreatic beta cells is summed then averaged to get the mean number of pancreatic beta cells per large field of view. Result: There was a significant difference in the number of pancreatic beta cells in the treatment group (19.54±8.11cells/field) of view compared to the control group (8.84±5.32cells/field of view) (p<0.001). Meanwhile, in the HbA1c level there was a significant difference in the treatment group (28.91±8.73ng/mL) when compared to the control group (35.78 ± 8.96ng/mL) (p 0.013). Conclusion: Based on the results of this study it can be concluded that the combination of butterfly pea flower part extract and glimepiride increased the number of pancreatic beta cells and decreased the levels of HbA1c in diabetic male Wistar rats.***

Keywords: Butterfly pea, DM, HbA1c, Pancreatic beta cells

1. Introduction

The aging process is a natural process that can occur to anyone where the body has decreased function due to the inability of the tissues to repair itself from the damage that has occurred.

There are many factors that play a role in the aging process. Basically, the causes of aging are grouped into internal and external factors. Some of the internal factors are free radicals, changes in hormone levels, glycosylation processes, methylation, apoptosis, decreased immune system and genetics. The main external factors are unhealthy lifestyles, unhealthy diets, wrong habits, environmental pollution, stress and poverty [13].

One of the factors causing the aging process associated with degenerative diseases is Diabetes Mellitus.DM is a group of metabolic diseases characterized by hyperglycemia that occurs due to abnormalities in insulin secretion, insulin action or both. People with T2DM are particularly affected by AGE cross-linking. In the elderly, there are many proteins that have cross-linkages with AGE and slowly accumulated, whereas in people with DM, they have a very high amount of glucose which causes high glycate protein [13]-[14].

Insulin resistance in muscles and liver and pancreatic beta cell failure have been recognized as the pathophysiology of central damage from type 2 Diabetes Mellitus. DM cannot be cured but controlling blood sugar levels through diet, exercise, and drugs can prevent chronic complications [14].

Clitoria ternatea is a type of plant used to lower blood sugar levels and can be found in Indonesia, India, China, the Philippines, and Madagascar. *Clitorea ternatea* is a plant from the Fabaceae family, or better known as Butterfly pea [2]. The phytochemicals of Butterfly pea contains tannins, flobatins, carbohydrates, saponins, triterpenoids, flavonoid phenols, flavanol glycosides, proteins, routine, alkaloids, anthraquinones, anthocyanins, stigmatic 4-ene-3,6 diones,

volatile oils and steroids. The composition of fatty acids includes palmitic, stearate, linoleic and linoleic oleic acids [11]. Butterfly pea antioxidants such as phenolics, flavonoids, anthocyanins, flavonol glycosides, kaempferol glycosides, quercetin glycosides, myristetin glycosides, terpenoids, flavonoids, tannins and steroids have been studied to reduce blood sugar levels [2].

Research on the ethanol extract of *Clitoria ternatea L.* aerial parts on pancreatic regeneration has been carried out before. Evaluation was carried out to determine the antidiabetic and antihyperlipidemic effects in diabetic rats given streptozotocin injection and the relationship with antioxidant activity in vivo and in vitro. Administration of extracts was given at a dose of 100-200 mg/kg and evaluated for regeneration of pancreatic beta cells on antioxidant and antihyperlipidemic activity. The most significant regeneration of pancreatic beta cells was shown in ethanol extract and butanol solution at a dose of 200 mg /kg, after 21 days treatment found that the average number of pancreatic beta cells increased in ethanol extract and n-butanol extract of Butterfly pea by 8.20 and 7.20 times (0.87 ± 0.02 cells and 7.12 ± 0.02 cells) obtained the average area of beta cells of Langerhans Island in the extract treatment group. Butterfly pea Ethanol and n-butanol extract were 38.55% and the area of normal control islands was 31.06%. It can be seen that the Butterfly pea extract can make the regeneration process of pancreatic beta cells that have been damaged [17].

Previous research by Daisy and Rajathi, using extra flowers and leaves of Butterfly pea at a dose of 400 mg / kgBB for 84 days orally in diabetic rats induced with alloxan showed that there was a significant decrease in HbA1c levels ($p<0,05$) [6].

Base on this, Butterfly pea flower ethanol extract was selected as an ingredient in this study with given a flower extract of Butterfly pea (*Clitoria ternatea L.*) as much as 80mg/200mg body weight and glimepiride 0.036 mg/200mg of body weight for 60 days by oral administration to diabetic rats. The purpose of this study was conducted to look at the effect of the Butterfly pea flower extract (*Clitoria ternatea*) in decreasing HbA1c levels and increasing the number of pancreatic beta cells in male Wistar rats injected by Streptozotocin.

2. Method

Thirty-six white male Wistar rats (*Rattus norvegicus*) were enrolled in this study. Animal Ethics Committee of the Faculty Veterimary Science, University of Udayana had approved this study. The subjects were selected to undergo the initial screening for inclusion criteria : healthy male white rats (*Rattus norvegicus*), age: 8,5-12,5 weeks, weight: 200-250g.

Rats were induced to become diabetes by injecting streptozotozin 12 mg/200 mg body weight and nicotinamide 46 mg/200 mg body weight as much as once and waited for 3 days. Subjects were divided into two groups, the control group was given aquabidest and glimepiride (0,036mg/200gr body weight), and the treatment group was given butterly pea flower part extract (80mg/200gr body weight) and glimepiride (0,036mg/200gr body weight) via sonde once a day for 60 days.

After 60 days of treatment, the immunohistochemical examination of pancreatic beta cells and HbA1c levels with the Rat Hemoglobin HbA1c Elisa kit. The blood sample that examined was serum blood collected in a vacutainer tube without EDTA. Analysis of Examination on immunohistochemistry staining will reveal round cells with brownish cytoplasm and bluish cell nuclei. The count by counting the nuclei of pancreatic cells using a 400x magnification microscope with an Olympus CX21 light microscope. Observation of pancreatic beta cells is counting from left to right then sigsag direction, which is calculated from the three view fields of Langerhans Island per preparation then averaged. Obtained pancreatic beta cells per large field of view. Langerhans Island from pancreatic tissue in the photo with *Optilab Viewer 2.2*, computed with *Image Raster*.

The data obtained then analyzed with independent T-Test by using *SPSS Version 25.0 for Window*.

3. Result

The statistical analysis results showed that the average number of pancreatic beta cells in treatment group was significantly increased (19,54±8,11 cell/field of view) compared to control group (8,84±5,32 cell/field of view). The independent T-test showed $p<0,001$ ($p<0,05$), there was significant difference in the average number of pancreatic beta cells between two groups (Fig 1).

Meanwhile, the average percentage level of glycated hemoglobin (HbA1c) in treatment group (28,91±8,73 ng/mL) was lower than the control group (35,78±8,96 ng/mL). Independent sample T test showed p 0,013 ($p<0,05$), which means that there was significant difference in the average percentage level of glycated hemoglobin (Fig 2).

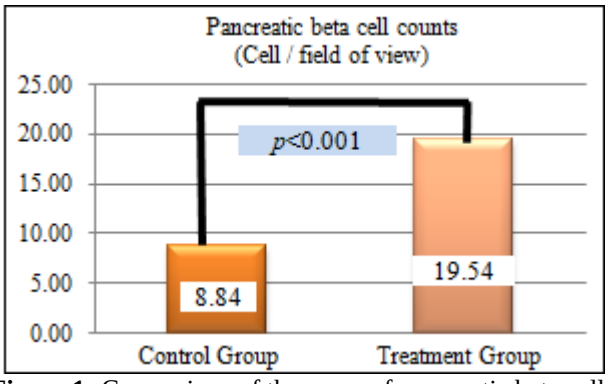

Figure 1: Comparison of the mean of pancreatic beta cells between groups after given treatment

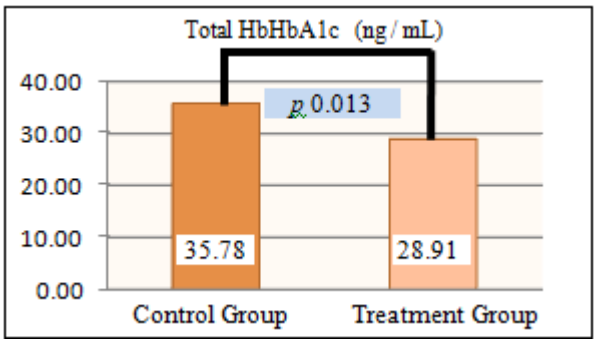

Figure 2: Comparison of the mean of HbA1c between groups after given treatment

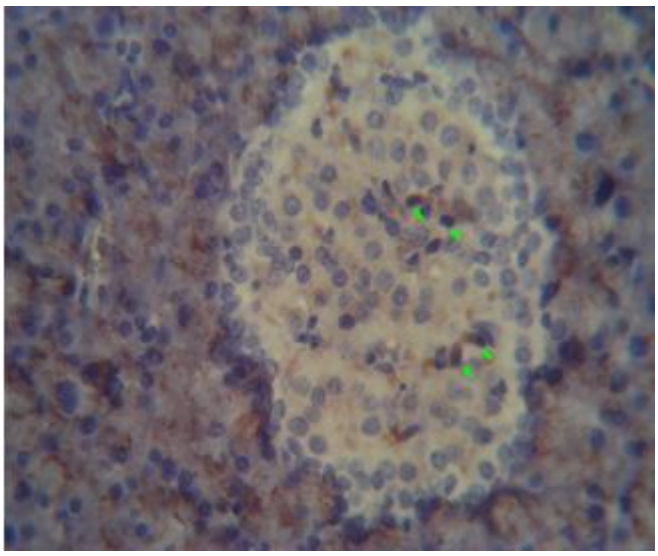

Figure 3: The results of CPI staining were also Langerhans pancreas 400x enlargement of rats; Green arrows obtained less pancreatic beta cells in the control group

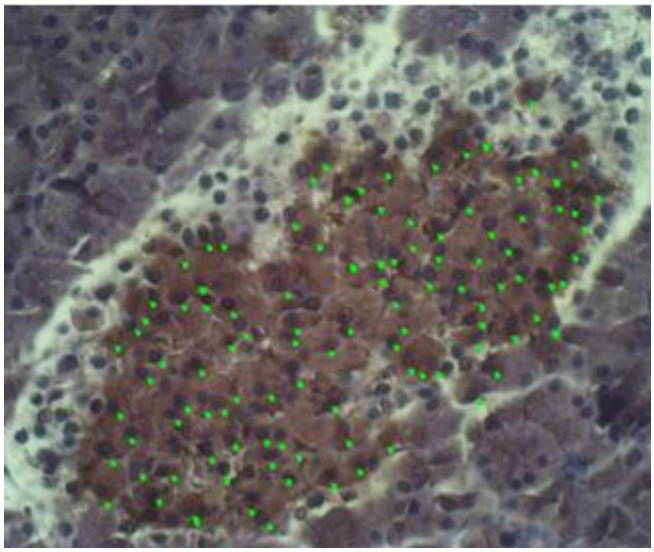

Figure 4: The results of CPI staining were also Langerhans pancreas 400x enlargement of rats; Green arrows obtained more pancreatic beta cells in the treatment group

4. Discussion

The prevalence of glucose intolerance (pre-diabetes and T2DM) increases with age. Aging causes a decrease in insulin sensitivity and changes or a lack of compensation for beta cell function in the face of increased insulin resistance [14].Langerhans Island is a multihormonal endocrine microorgan in the pancreas. These islands appear as groups of round structures with the cells buried evenly in the exocrine network of the pancreas but are more common in the cauda [3]. Decreased beta cell proliferative capacity and increased sensitivity to apoptosis are conditions associated with aging. Studies by Szoke show that the first and second phases of insulin secretion usually decrease at a rate of about 0.7% per year with aging, this reduction in beta cell function is accelerated about twofold in people with impaired glucose tolerance [16].

In T2DM, there is glycosylation where glucose combines with proteins that are dehydrated and cause systemic disorders related to complications in diabetes mellitus [13]. HbA1c describes glycemic control for 3 months in humans according to the age of erythrocytes where irreversible Hemoglobin and glucose bonds occur for 120 days while the erythrocyte age in mice is shorter, namely 45-50 days. So that the HbA1c examination on the 50 day after treatment should be able to describe glycemic control in mice [4].

In diabetic micethat high extracellular glucose concentrations increase the endocytosis of GLUT2, which leads to excessive secretion of GLUT2 in beta cells. However, the internalized protein GLUT2 is rapidly degraded due to chronic high glucose stimulation, suggesting that hyperglycemia directly affects pancreatic beta cell function [8]. It was found that there was a decrease in blood sugar levels and an increase in insulin levels after giving Butterfly pea leaf extract for 8 weeks, it was found that blood sugar levels in mice began to return to normal [9]. The flavonoid content in the form of Kaempferol, delphinin and kuercetin contained in Butterfly pea provides an antioxidant effect by intercepting the generation of ROS, directly capturing ROS or indirectly increasing enzymes, and as anti-cancer.

In the phytochemical examination of the ethanol extract of Butterfly pea leaves, the flavonoid levels were 20.48 ± 0.96 mg RE / g extract, tannin 78.75 ± 2.09 mg TAE / g extract, total phenol 245.14 ± 6.97 mg TAE / g extract [10]. While the phytochemical examination of the Butterfly pea flower extract the total phenol was 105.40 ± 2.47 mg GAE / g extract, flavonoids 72.21 ± 0.05 mg CE / g extract, IC50 of 327 µg. / mL [12]. IC_{50} checks for Flower Butterfly pea have also been carried out in Indonesia, especially in Denpasar using 80% ethanol, it was obtained at 87.86 ppm [5] while in this study the IC_{50} was 73.79 ppm. This states that Butterfly pea is included in the moderate antioxidant category.

In vitro results showed that kaempferol increased viability, inhibited cellular apoptosis, and reduced caspase-3 activity in islet beta Langerhans cells. This protective effect is associated with increased cAMP signaling, expression of the anti-apoptotic Akt and Bcl-2 proteins, and insulin secretion and synthesis in pancreatic beta cells. Kaempferol increases antioxidants in the body and reduces IL-1β, TNF-α, lipid peroxidation and nitrite. Kaempferol also decreases the expression of PPAR-γ and SREBP-1c. Anti-obesity and anti-diabetic effects of kaempferol are mediated by SREBP-

1c and PPAR-γ via AMPK activation. Indirectly, Kaempferol also reduces fasting blood glucose, HbA1c serum levels and increases insulin resistance [18].

Several studies have reported the mechanism of action of quercetin in diabetes, such as decreased lipid peroxidation, increased antioxidant enzymes (such as SOD, GPX, and CAT), inhibition of insulin-dependent activation of PI3K, and reduced glucose absorption by inhibiting GLUT2. Kuercetin stimulates translocation of GLUT4 and expression in skeletal muscle by mechanisms associated with AMPK activation. The study showed that quercetin improved the expression of markers of oxidative stress and inflammation such as Nrf2, heme oxygenase-1, and NFκB. This suggests that the anti-inflammatory effect of kuercetin on adipose tissue can be associated with weight loss as well as in the pancreas showing the restoration of cell proliferation in diabetic rats [18].

Flavonoids can also act outside the pancreas by stimulating peripheral glucose utilization and increasing glycolytic and glycogenic pathways, simultaneously suppressing glycogenolysis and gluconeogenesis pathways. Prevention of the formation of ROS by flavonoids can be done in several ways, namely by inhibiting the action of the enzymes xanthine oxidase and Nicotinamide Adenine Dinucleotide Phospate (NADPH), as well as chelating metals ($Fe2^+$ and $Cu2^+$). Flavonoids can also directly capture superoxide and peroxynitrite. Through superoxide capture, flavonoids increase the bioavailability of NO and inhibit the formation of peroxynitrite, it is expected that free radicals will be more stable [1].

Apart from flavonoids, repair of pancreatic damage is thought to be caused by the activity of active compounds in the ethyl acetate fraction, namely alkaloids. The anti-inflammatory activity of flavonoid compounds, as well as antioxidant activity, can prevent and stop further pancreatic beta cell damage. Meanwhile, alkaloids play a role in cell regeneration, by restoring pancreatic beta cells that have been partially damaged. Repair of pancreatic beta cells is expected to restore its function in producing insulin [15], [18].

Research by Fernandes shows that routine content can improve metabolic status in mice with induced diabetes. Routine works also in the activation of liver enzymes related to gluconeogenesis, lipid metabolism processes and also significantly reduces fasting blood glucose, creatinine, blood urea nitrogen, urine protein, oxidative stress intensity and p-Smad 7. Rutin has been shown to stimulate glucose uptake in mouse muscle via PI3K, a characteristic protein kinase C and protein kinase activated by mitogen pathways. Rutin has been reported to significantly increase body weight, decrease plasma glucose and HbA1c, proinflammatory cytokines (IL-6 and TNF-α), and restore decreased hepatic antioxidant and serum lipid profiles in HFD / STZ-induced diabetic rats [7]. In particular, it has been shown to protect and improve myocardial dysfunction, oxidative stress, apoptosis and inflammation in the heart of diabetic rats [19].

Gluconeogenesis also can reduce fasting glucose levels by increasing insulin secretion and improving pancreatic beta cell mass through the prevention of apoptosis, which indirectly improves insulin levels [19].

From this study, it appears that the control group given aquabidest and Glimepiride had fewer pancreatic beta cells (8.84 ± 5.32 cells/field of view) when compared to the treatment group given Butterfly pea flower extracts and Glimepiride (19.54 ± 8.11 cells/field of view). This proves that giving oral ethanol extract of Butterfly pea flower (*Clitoria ternatea*) increases the number of pancreatic beta cells of mice (*Rattus norvegicus*) Wistar strain male diabetes mellitus.

Examination of HbA1c levels that the control groups given aquabidest and Glimepiride had higher levels of HbA1c (35.78 ± 8.96 ng/mL) when compared to the treatment group given Butterfly pea flower extracts and Glimepiride (28.91 ± 8.73 ng/mL). This proves that giving oral ethanol extract of Butterfly pea flower (*Clitoria Ternatea*) reduces the levels of glycated hemoglobin (HbA1c) of rats (*Rattus Norvegicus*) Wistar strain male diabetes mellitus.

From this research, it has been proven that administration of butterfly pea (*Clitoria ternatea*) flower etanol extract oral increased the number of pancreatic beta cells and decrease the level of glycated hemoglobin (HbA1c) of diabetic male Wistar rats (*Rattus norvegicus*).

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