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Combination treatment of vildagliptin and bay leaf (*Syzygium polyanthum*) extract increased pancreatic beta cells number but have no effect toward glycated albumin levels in diabetic male Wistar rats (*Rattus norvegicus*)

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ABSTRACT

Introduction: Diabetes mellitus is a complex and progressive disease which often lead to several debilitating complications that partly caused by by free radicals which can be overcome with antioxidants. Bay leaves contain essential oils, tannins, flavonoids and terpenoids which have considerable antioxidant properties. Therefore, this study aims to determine the effect combination treatment of vildagliptin and bay leaf (*Syzygium polyanthum*) extract toward pancreatic beta cells density and glycated albumin levels in diabetic male Wistar rats (*Rattus norvegicus*).

Methods: An experimental posttest only control group study was conducted using 36 albino male rats, aged 2-3 months, weighing 180-200 grams. The rats were divided into 2 groups (n= 18) with control group treated with 1.8mg/200g body weight vildagliptin and 2cc placebo (aquabidest) while the treatment group received 1.8mg/200g BW vildagliptin and 250 mg/200g BW bay leaves extract. All treatment lasted for 21 days.

Results: The results showed that pancreatic beta cell counts in the treatment group was significantly higher than the control group (109.07 ± 20.47 cells/field of view vs 90.87 ± 13.91 cells/field of view; p<0.01). However, the levels of Glycated albumin between two groups were not significantly different (treatment vs control: 17.33 ± 4.51 vs 20.18 ± 4.57; p=0.068).

Conclusion: This study suggested that combination treatment of vildagliptin and bay leaf extract increased pancreatic beta cells but did not reduce glycated albumin levels in diabetic male Wistar rats.

Keywords: bay leaf, pancreatic β cell, Glycated albumin, diabetes mellitus

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INTRODUCTION

Aging will be experienced by every human being but the process is vary among individuals. Premature aging is an accelerated aging process due to various factors, both internal and external. Diabetes is often considered as one of the biological models of the premature aging process.[1]

Diabetes mellitus (DM) is a group of metabolic diseases with hyperglycemia as the main symptoms due to impaired insulin secretion, insulin action, or damage to pancreatic beta cells. Type 2 DM is a disease characterized by a progressive decrease in pancreatic β cell function and chronic insulin resistance. On the pancreatic islet of type 2 DM patients, amyloid deposits are found derived from the islet amyloid peptide protein (IAPP). The IAAP causes β-cells apoptosis. Hyperglycemia itself could also induce β cell apoptosis.[2]

Other factors that also contributed to β-cells damage include genetic factors, infection, nutritional factors, diabetogenic substances like Streptozotocin and free radicals (oxidative stress).[3] Streptozotocin is widely used to induce diabetes in animal models, because it causes pancreatic beta cells damage and is able to maintain a long time hyperglycemia to facilitate observation of the pathophysiology and complications of DM.[4] The animal models used to induce diabetogenic substances are male Wistar rats because it gives more stable research results and not affected by the menstrual cycle and pregnancy.[4] Chronic complications of DM comprised of microvascular and macrovascular complications that are closely related with glycemic status. Therefore, in order to prevent the complications, glycemic control is the main goal of treatment and often monitored via glycemic markers that represent long-term glycemic status. Currently, glycated albumin is proposed as marker to assess glycemic status in 2-3 weeks period. Glycated albumin is

ketoamine formed from the bond of albumin and glucose by non-enzymatic oxidation reaction as an index of glycemic control. It is not affected by abnormalities of hemoglobin metabolism. Therefore glycated albumin is expected to be able to monitor therapy and anticipate the onset of DM complications.[5]

Vildagliptin is a new class of dipeptidyl peptidase 4 (DPP-4) inhibitors that is currently being studied. Dipeptidyl peptidase IV (DPP4) is an enzyme that contributes to the inactivation of the hormone glucagon like peptide-1 (GLP-1). GLP-1 stimulates insulin production and slows gastric emptying, inhibits glucagon secretion, and decreases appetite.[6] Inhibition of DPP4 by vildagliptin results in elevated levels of active GLP-1. It has been shown to be an effective treatment for type 2 DM. But in addition to its good pharmacological effects as an antidibetic agent, vildagliptin has some side effects including headache, nasopharyngitis, coughing, constipation, dizziness, and sweating.[7]

Bay leaf (*Syzygium polyanthum*) is a traditional medicinal herb plant in Indonesia.[8] Phytochemical analysis performed at the laboratory of agriculture Technology Udayana University showed that it contains tannins 490mg/100g TAE, flavonoids 730mg/100g QE, antioxidants 260mg/100g GAEAC, Fenols 821mg/100g GAE and vitamin C 253mg/100g. Flavonoids are known to have hypoglycemic activity, antioxidant, and anti-inflammatory properties.[9,10] Because the close relationship between DM complications with free radicals, therefore, it can theoretically be countered by antioxidants derived from bay leaf. Bay leaves are quite abundant, however due to low public awareness of the various benefits of this plant, this plant is more often used as a cooking spice. Therefore, more in-depth research to prove antidiabetic effect of this herbs is necessary.

METHODS

An experimental posttest only control group study was conducted using 36 albino male rats, aged 2-3 months, weighing 200 grams. The sampels were divided into 2 groups (n= 18). The control group was treated with 1.8mg/200g BW Vildagliptin and 2cc placebo for 21 days while the treatment group was treated with 1.8mg/200g BW Vildagliptin and 250 mg/200g BW bay leaves extract for 21 day.

After 21 days of treatment, blood samples were collected for glycated albumin measurement. Glycated albumin level was assessed by the ELISA technique. Rats were then sacrificed and euthanized using inhaled ether and the pancreas was taken to be processed for histological examination of β-cells density stained by regular H&E staining and observed by electric microscope with 400x magnification in 3 field of view.

RESULTS

Histological examination and glycated albumin assessment were conudcted simultaneously to evaluate the efficacy of the extract. HE staining showed that the mean number of pancreatic beta cells in the control group was 90.87 ± 13.91 cells/field of view (Figure 1A), whereas in the treatment group was 109.07±20.47 cells/field of view (Figure 1B). Statistical evaluation showed that the difference was statistically significant (p <0.05).

In contrast with histological examination, the extract seemed to have no effect toward the mean level of glycated albumin. The level of glycated albumin in the control group was 20.18 ± 4.57 ng/ml while in the treatment group was 17.33±4.51 ng/ml (p >0.05). Therefore, this study suggested that combination treatment of vildagliptin and bay leaf (*Syzygium polyanthum*) extract significantly increased pancreatic beta cells but have no effect on glycated albumin levels in diabetic male Wistar rats (Table 1).

DISCUSSION

The Effect of bay leaf (Syzygium polyanthum) on Pancreatic Beta Cells

Theoretically, as a potent selective and reversible antidiabetic agent, Vildagliptin prolongs GLP-1 half-life and enhance insulin secretion while simultaneously suppresses glucagon secretion, resulting in better blood glucose control (Ahren *et al.*, 2011). Previous study proved that DPP-4 inhibitors improve insulin sensitivity and reduce glucose toxicity which underlies its application as one of therapeutic choice of type 2 diabetes mellitus. DPP-4 inhibitors improve the amount of endogenous GLP-1 so that GLP-1 can stimulate pancreatic β-cells. GLP-1 also prevent apoptosis in has anti-apoptotic effects on pancreatic β-cells mainly due to amiloid accumulation type 2 diabetes mellitus. This function was proved by Duttaroy *et al.*, (2011) that reported that DPP-4 inhibitors administration increased the number of pancreatic beta cells, the formation of new pancreatic beta cells (neogenesis), and preventing apoptosis.[11] Animal study also showed similar result and it also implied that Vildagliptin restore the damaged pancreatic β-cells function.[12]

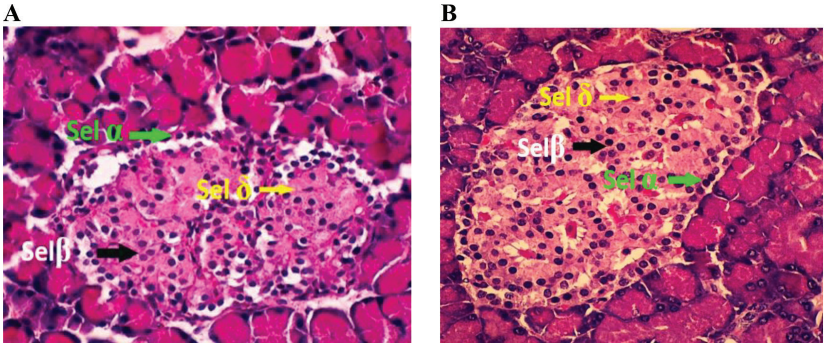

Figure 1. **Histopathological comparison of the number of pancreatic beta cells between: (A) control group, and (B) treatment group. (HE staining, 400x magnification)**

Table 1. **Comparison of the Pancreatic Beta Cells Number and the Glycated Albumin Level between the Control Group and Treatment Group**

Variable	Group		*T*	*P*
	Control (Mean±SD)	Treatment (Mean±SD)		
Pancreatic Beta Cells (cells/field of view)	90.87 ± 13.91	109.07 ± 20.47	-3.121	0.004
Glycated Albumin (ng/ml)	20.18 ± 4.57	17.33 ± 4.51	1.882	0.068

In addition to the direct effect of Vildagliptin in increasing the number of pancreatic beta cells, studies have shown that vildagliptin maintains the number of β cells in diabetic rats through reducing oxidative stress and cell apoptosis related to endoplasmic reticulum stress. Vildagliptin enhance the level of superoxid dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), reduce DNA-damage-inducible transcript 3 (Ddit3) and X-box binding protein 1 (Xbp-1).[13] It is known that hyperglycemia not only causes oxidative stress but also lead to endoplasmic reticulum stress which causes further damage to pancreatic beta cells.

Therefore, the result of this study was consistent with previous ones. Here, it was showed that supplementation of 250mg/200gBW bay leaf extract on Vildagliptin treated rats significantly enhanced the effect of Vildagliptin in restoring the number of pancreatic β-cells. Bay leaf extract contains active compounds which might play important role in inducing its effect. Phytochemical analysis showed that it contains tannin, flavonoid, phenol, vitamin C and antioxidant which might support the efficacy of Vildagliptin.

Research showed that tannin is a direct radical scavenger which can neutralize free radicals directly and can also act as chain-breaking antioxidants.[15] Flavonoids and phenols have long been known to have activity as chain-breaking antioxidants and can neutralize antioxidants directly.[16] In addition, it also contains Vitamin C which is also regarded as strong antioxidant due to its nature as a very strong electron donor without changing its own molecular stability.[17] Tannin, flavonoids, phenol and vitamin C might support the Vildagliptin to prevent further damage on pancreatic β-cells due to oxidative stress induced by hyperglycemia with mutually supportive mechanisms. Bioactive compounds in bay leaf extract directly neutralize free radicals and Vildagliptin activates endogenous antioxidants.[13,18] In addition, to prevent further pancreatic beta cell damage, the active compound in bay leaf extract could also act in concert with Vildagliptin to stimulate β-cells neogenesis. Research showed that tannins, flavonoids, phenols and alkaloids can induce pancreatic beta cell regeneration.[19] Flavonoids activate the cAMP-protein kinase A (PKA) pathway and phosphorylate Extracellular Signal-Regulated Kinase (ERK1/2) in pancreatic beta cells. This pathway increases the level of cyclin D1 which induces pancreatic beta cell proliferation.[20]

The Effect of bay leaf (Syzygium polyanthum) on Glycated Albumin

Several studies have shown the potential of bay leaf extract as a hypoglycemic. Hikmah *et al.* showed that bay leaf extract significantly potentiates glibenclamide in reducing blood glucose levels with effective combination dose of 0.65 mg/kg BW glibenclamid and 250 mg/kg BW bay leaf extract.[21] It was proposed that flavonoids in bay leaves induce this effect by means of alpha glucosidase inhibition which plays a role in the conversion of carbohydrates to glucose.[22] Another study showed that bay leaf extract infusion at as dose 825 mg/150gr in rats could reduce blood glucose levels in alloxan-induced diabetic rats.[23]

Chronic complications of DM could affect all organs as microvascular and macrovascular complications that are closely related with glycemic status. In order to prevent the complications of DM, glycemic control markers is necessary. Glycated albumin is a ketoamine complex which is formed by glucose binding to a albumin molecule via non enzymatic oxidation reaction. It considered as the best index of glycemic control because it is not affected by hemoglobin metabolic abnormalities which affects glycated hemoglobin. Glycated albumin is a form of bonding between glucose molecules with albumin. Lower glucose levels will produce lower glycated

albumin levels because glucose is a precursor to the glycation process. Glycated albumin examination described glycemic status in the previous 2-3 weeks. Therefore glycated albumin is expected to be able to monitor the therapeutic outcomes and anticipate the onset of diabetes complications.[5]

In this study, examination of glycated albumin was carried out after 21 days (3 weeks) of treatment, so that the levels of glycated albumin in this study described glycemic status before treatment (day 0) up to 1 week after treatment. The levels of glycated albumin that rather similar between the control and the treatment group could be due to the fact that bay leaf extract was still working and did not have a significant effect on blood glucose levels in the first week, so the levels of glycated albumin on the 21st day also did not affected. This assumption is supported by Hikmah *et al.* (2016) who found that the administration of bay leaf extract reduced blood glucose levels starting from day 15 (week 2) and day 22 (week 3), while assessment at day 8 (week 1) showed no difference between group with glibenclamide alone and group treated with glibenclamide + bay leaf extract. So it indicates that longer period of observation is needed.

Nevertheless, it appeared that the level of glycated albumin in the treatment group was slightly lower than the control group although it was not statistically significant. It could be a hint that 21 day treatment is rather too short and longer period of observation as well as higher dose of extract is needed.

CONCLUSIONS

This study suggested that combination treatment of vildagliptin and bay leaf (*Syzygium polyanthum*) extract significantly increased pancreatic beta cells regeneration but did not reduce glycated albumin levels in diabetic male wistar rats (*Rattus norvegicus*).

CONFLICT OF INTEREST

All authors declared that there is no conflict of interest regarding this publication

AUTHOR CONTRIBUTION

All authors contributed equally in the writing of this article

FUNDING

This study was self-funded without any contribution from third party.

ETHIC APPROVAL

This study had been ethically approved by ethical commission of Faculty of Medicine Udayana University with approval letter number 402/KE-PH-Lit-2/VII/2018

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