Oral administration Of Gedi Leaves Extract (*Abelmoschus Manihot*) Increased Glp-1 Serum and Decreased Fasting Glucose Level on Hyperglycaemia Male White Rat (*Rattus Novergicus*)

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Abstract

Objectives: Aging is related to organ decreases including pancreas. Pancreas dysfunctions affect the insulin secretion and lead to hyperglycemia condition. Gedi leaves contains of flavonoid and tannin which are known as substances to improve hyperglycemia through the mechanism like DPP-4 Inhibitor agent. This study aimed to prove that Gedi leaves extract effective to lower fasting blood glucose and increase GLP-1 serum comparable to the effectiveness of DPP-4 inhibitor.
Methods: This research was an experimental study with randomized pretest-posttest control group design conducted for 21 days using 30 white male rats induced to hyperglycemia state using Streptozotocin and Nicotinamide. Samples were randomly divided into 3 groups; the negative control group (10 rats) treated with distilled water, the treatment group (10 rats) treated with gedi leaves extract 300 mg/ kgBW and positive control group (10 rats) treated with Dpp-4 inhibitor agent. Fasting blood glucose and GLP-1 serum were measured before and after treatment. The data obtained were analyzed using *SPSS Version 2.0 for windows*
Results: This research provided the significant improvement of GLP-1 serum in treatment group (692.7 mg to 890.6 mg, $p< 0.01$) which similar to the increase in positive control group (772.3 mg to 945.5 mg, $p<0.01$). The results also provided significant decline of fasting blood glucose after treated with Gedi leaves extract (316,11 mg/dl to 195,44 mg/dl; $p= 0.01$). Furthermore, the comparison between Gedi leaves extract and DPP-4 inhibitor agent did not show significant difference in reducing blood glucose level ($p = 0.071$) and increasing GLP-1 serum ($p =0.589$).
Conclusion: Oral administration of Gedi leaves extract effective to lower fasting blood glucose and improve GLP-1 serum on Hyperglycemic male white rat and has been shown to be equivalent to DPP-4 inhibitor in its effectiveness. Future study will be required to examine the toxic dose and the side effect of long-term treatment using Gedi leaves extract.

Keywords: *Abelmoschus manihot;* GLP-1 serum; Fasting blood glucose; DPP-4 inhibitor

1. Introduction

Aging is a natural process which affects the ability of organs function including pancreas. Researchers found a decrease of pancreas mass after age 40. Research using isolated elderly islet cell showed the decreased of glucose dependent insulin secretion as well as the decreased of total islet in pancreas (1–3).

Several hormones play a role in insulin synthesis and secretion. One of them is Incretin which is secreted by the intestine within minutes after ingestion and has potent effect (about 50%) in insulin secretion after meal. Incretin hormone consists of 2 types of peptides, namely glucagon-like-peptide 1 (GLP-1) and glucagon-dependent *insulinotropic polypeptide* (GIP). Both peptides are superfamilies of glucagon with significant character (4,5). Glucagon-like-peptide 1 is secreted from the post translational process of proglucagon contained in L-cell intestine and will enter the circulation. The biological process of GLP-1 depends on the presence of two N-Terminal amino acids. By binding with G-protein receptors in beta cells, Camp intercellular levels will increase and protein kinase, Epac 1 and Epac 2 will be activated which leads to increased insulin secretion. Furthermore, GLP-1 plays a role in multiplying beta cell mass by mediating proliferation and differentiation and inhibiting beta cell apoptosis (4,5).

On contrary, *Dipeptidyl peptidase-4* (*DPP-4*) is protease enzyme with wide distribution within circulation such as liver, intestine, endotel capillary, and kidney. *DPP4* has a role to separate amino acid from alanine or proline contained peptide in second position N-terminal peptide. *DPP4*'s activity, affinity and wide distribution caused GLP-1 to lose its half-life in circulation. Furthermore *DPP-4* inhibitor is agent with ability to stimulate insulin secretion through the inhibition of *DPP-4* (4,5).

Gedi (A. manihot) is an endemic plant in east Europe and Asia. In Indonesia, Gedi can be easily found in north Sulawesi (6). *Abelmoschus manihot* has been long known as herbal medicine to lower the blood glucose (6). Research for its active compound proved that gedi contains of flavonoid and tannin with abilities to increase insulin stimulation and improve glucose regulation. The derivatives of flavonoid have been proven to have a similar effect to DPP-4 inhibitor agent (7). Another research found ten substrates from flavonoid which showed similar effect to DPP4 inhibitor agent such as *hibiscetin, gossypentin, gossypetin -3-glucoside, myricetin, myricetin 3-glucoside, alpha spinasterol, quercetin, syringaresinol, stigmasterol,* and *isoquercetin* (8–10). This study aimed to prove the effect of oral Gedi leaves extract in increase GLP-1 serum and decrease Fasting Blood Glucose (FBG) level compared to DPP-4 inhibitor in hyperglycemia male rat.

2. Materials and Methods

2.1. Study design

We used an experimental study with a randomized pretest-posttest control group design using 30 white rats (*Rattus Novergicus)* aged 3 months with a body weight of +/-200 gram which administering Streptozotocin and Nicotinamide injections to obtain higher fasting blood glucose levels. The sample was randomly divided into 3 groups, namely the negative control group (distilled water), the treatment group (Gedi extract at a dose of 300 mg/kgBB) and the positive control group (20mg/kgBW sitagliptin). Fasting blood glucose (FBG) level and GLP-1 serum were examined before and after treatment for 21 days. FBG levels were checked using a glucometer, and GLP-1 serum was examined using ELISA with Rat Glucagonlike peptide 1 receptor kit no E0918Ra.

2.2. Preparation of extract

Gedi leaf samples were taken from Manado, North Sulawesi. Gedi leaf was cleaned by distilled water and dried for 3 days until there is no remaining water content. Then a dry sorting process is carried out to separate the remaining dirt or foreign matter. The dried Gedi leaf samples were then crushed using a blender to become simplicia powder. The extraction method uses maceration technique. Gedi leaves were weighed for 1000 grams of powder, then the sample was put in a closed container and soaked in 96% ethanol for 3x24 hours. After that, the sample was filtered using filter paper. The maceration results of red Gedi leaves obtained were concentrated with a rotary evaporator at 50^0C and 80^0C until a thick extract of Gedi leaves was obtained and weighing was carried out. The resulting extract is stored in the refrigerator at 4^0C for long term use.

2.3. Data Analysis

The data obtained were analyzed and processed using the *SPSS Version 2.0 for windows*. Data analysis went through the stages of descriptive analysis, normality test, comparison test and treatment effect test.

3. Results

Table 1. GLP-1 Serum

	Average (s.d)	Difference (s.d)	CI 95%		*p* value
			Lower	upper	
GLP-1 before distilled water treatment	672,6 (+/-68,22)	141,7 (66,6)	90,6	193,0	<0,001
GLP-1 after distilled water treatment	530,8 (8,87)				
GLP-1 before Gedi Leaves extract treatment	692,7 (69,5)	197,82 (67,34)	146,1	249,6	<0,001
GLP-1 after Gedi Leaves extract treatment	890,6 (10,7)				
GLP-1 before Sitagliptin treatment	772,3 (27,9)	173,17 (49,17)	138,0	208,3	<0,001
GLP-1 after Sitagliptin treatment	945,5 (28,3)				

In table 1, GLP-1 Serum Results on Gedi extract group and Sitagliptin group experienced significant increase after 3 weeks of treatment compared to data before treatment ($p<0.001$). The average GLP-1 before Gedi extract administration was 692.7 mg to 890.6 mg after treatment for 3 weeks. Furthermore, the average GLP-1 before the administration of sitagliptin was 772.3 to 945.5 after treatment. These data prove that the administration of Gedi leaves and Sitagliptin have positive effect on increasing GLP-1 serum in hyperglycemia male Wistar rat. On contrary, GLP-1 Serum results on distilled water group showed significant decrease for 141.7 mg in differences ($p< 0.001$).

Table 2. FBG levels

	Average (s.d)	Difference (s.d)	CI 95%		*p* value
			Lower	upper	
FBG before distilled water treatment	323,78 (165,33)	115,67 (203,47)	-272,06	40,73	0,27
FBG after distilled water treatment	439,44 (193,81)				
FBG before Gedi Leaves extract treatment	316,11 (122,16)	120,67 (68,34)	68,13	173,19	0,01
FBG after Gedi Leaves extract treatment	195,44 (139,86)				
FBG before Sitagliptin treatment	288,30 (129,26)	101,60 (135,83)	4,43	198,76	0,042
FBG after Sitagliptin treatment	186,70 (165,56)				

Based on table 2, Comparison of FBG levels in distilled water showed no significant difference before and after treatment ($p = 0.27$). It can be concluded that the administration of distilled water has no effect on

improving diabetic condition of the study subjects. Besides, Comparison of FBG levels in groups subjects given Gedi and Sitagliptin extract before and after treatment shows a meaningful difference (p= 0.01 and p= 0.042). Average FBG in subject groups before treatment was 316.11 mg to 195.44 mg after oral treatment with Gedi leaves extract. FBG levels was 288.30 mg to 186.70 mg after sitagliptin treatment. This indicates a positive effect in the improvement of hyperglycemic conditions in Wistar male rats with administration of both Gedi extract and Sitagliptin for 3 weeks.

Table 3. Independent sample Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
GLP-1	Equal variances assumed	2,434	,136	-,558	18	,584	-37,3887	67,0337	-178,221	103,443
	Equal variances not assumed			-,558	10,02	,589	-37,3887	67,0337	-186,708	111,93
FBG	Equal variances assumed	1,021	,326	,379	17	,709	19,0666	50,2589	-86,9704	125,103
	Equal variances not assumed			,392	13,56	,701	19,0666	48,6195	-85,5245	123,657

We decided to compare the effectiveness between Gedi leaves extract and sitagliptin. Table 3 showed no significant difference between Gedi leaves extract and sitagliptin (p= 0,584) which proves that Gedi leaves extract has similar effect to lower FBG and increase GLP-1 compared to sitagliptin.

4. Discussion

This study proved significant improvement from pretest and posttest results both in the Gedi leaf extract column and the sitagliptin column on Serum GLP-1 levels. Unexpected results were also obtained from the reduction in fasting blood glucose (FBG) after Gedi leaf extract treatment.

Further tests were carried out to compare the effectiveness of Gedi and Sitagliptin leaf extracts. The results of the independent T-Test showed that Gedi leaf extract had similar effectiveness to sitagliptin in reducing FBG levels and increasing serum GLP-1. The ability of Gedi leaf extract to increase GLP-1 levels is related to the various compounds contained in the Gedi extract.

Based on examination of Gedi leaf extract, it was found that the active content in the form of flavonoids was 264.19 mg QE/100 g, and tannins were 57.06 mg TAE/100 g. Gedi leaf extract also affects the increase in serum GLP-1. GLP-1 is an endogenous peptide released by intestinal epithelial endocrine L cells. The effects of GLP-1 include increasing insulin secretion and decreasing glucagon activity, inhibiting gastric emptying, stimulating beta cell regeneration and inhibiting beta cell apoptosis. The content of Gedi leaves, especially

flavonoids, are known to suppress blood glucose levels through various mechanisms including by increasing GLP-1 serum.

Flavonoid has been studied to increase GLP-1 through binding to TGR5. TGR5 plays a role to stimulate mitochondrial oxidative phosphorylation which affects the increase of ATP/ADP ratio, closing the potassium channel and affect the opening of calcium channel which cause the increase of GLP-1 secretion. Besides, *Isoquerectin* is flavonoid derivative and plays a role in blood glucose regulation. The study by Zhang et al, compared the effects of *isoquercetin*, sitagliptin and placebo. An eight-week study showed medium and high doses of *isoquercetin* had an antagonistic effect on increasing blood glucose like the effect of sitaglipitin. It is estimated that DPP-4 is the target mechanism of action of *isoquercetin* in increasing insulin sensitivity. *Isoquercetin* competitively inhibits DPP-4 activity in vitro by blocking the active site of DPP-4 (7,11).

Flavonoids are also known to play an active role in improving blood glucose levels. The mechanism of flavonoids in reducing glucose levels is associated with the inhibitory effect of alpha glucosidase and alpha amylase enzymes. This inhibition depends on the interaction of hydrogen bonds between the hydroxyl groups in flavonoids and the enzyme catalytic residues leading to decreased digestion of postprandial carbohydrates and glucose. Besides, another mechanism is through interactions with starch and forming complexes that are difficult to digest. (12–14) Interaction of flavonoids with starch through covalent bonds has been reported in different studies. This interaction causes the formation of resistant starch which is more difficult to digest. The ability to form complexes between flavonoids and starch depends on the position of the hydroxy group, so that different flavonoid derivatives will show different binding capacities. (12,13)

Flavonoids also affect glucose metabolism through glucose transporters. One of the flavonoid derivatives known to play a role in lowering blood glucose concentrations is quercetin. Quercetin works through the inhibition of glucose absorption in intestinal cells by direct inhibition of the glucose transporters SGLT1 and GLUT2. In addition, flavonoids are known to increase GLUT4 expression which causes an increase in glucose usage by cells and lowers blood glucose levels (15–17). Besides, the tannin compounds contained in Gedi leaf extract play a role in suppressing blood glucose levels through an inhibitory effect on the alpha-glucosidase enzyme. In addition, tannins also capable to increase fat metabolism and indirectly affects preventing insulin resistance through conditions of lipotoxicity (13,18).

Interesting results were found in the significant differences from the group of mice given distilled water before and after treatment. The data showed a significant decrease in GLP-1 but otherwise, there was no significant difference in the results of FBG before and after administration of distilled water. GLP-1 is an incretin peptide hormone produced by intestinal L cells. GLP-1 has an important role in stimulating insulin release and mediating the proliferation and suppression of apoptosis of pancreatic beta cells. GLP-1 secretion is affected by food ingestion, and it is thought that glucose is an important stimulator of the secretion process. On the other hand, there are several compounds with ability to inhibit the action and secretion of GLP-1, such as DPP-4 and Somatostatin (19). Somatostatin is known to be a regulatory hormone with many target organs. Somatostatin produced from delta cells plays a role in digestive processes in various ways, such as inhibiting the absorption of nutrients including calcium, galactose, glucose, glycerol, fructose, amino acids and triglycerides, inhibiting the secretion of the hormones gastrin, secretin, cholecystokinin, motilin, and glucagon (19,20).

In conditions of insulin deficiency, such as in rat models with streptozotocin induction, will affect the increase of somatostatin secretion. Increased secretion of Somatostatin with receptors that are widespread in the body, mostly in the intestine, will slow down intestinal absorption of nutrients, including glucose absorption. It is estimated that with an increase in somatostatin secretion, GLP-1 whose secretion depends on nutrition and glucose will then decrease, this might explain the significant decrease in GLP-1 from the distilled water rat group model. To prove this hypothesis further research is needed (19–21). This study proves that Gedi leaf extract can reduce blood glucose levels and increase GLP-1 levels with the same

significance as the standardized drug Sitagliptin ($p < 0.01$). The advantages of this extract are that Gedi is easier to obtain, can be easily grown, and is sold at a low price in the market compared to standard treatments such as Sitagliptin. Gedi, in this study is also easy to process and is a natural medicine taken from nature without using pro-oxidant chemicals.

Several studies have discussed the side effects of using DPP-4 inhibitors in the treatment of diabetes. The effects that are often reported include the discovery of a higher prevalence of pneumonia and infection compared to the use of other anti-diabetic preparations (22). Flavonoid contained in Gedi extract has high antioxidant effect which can provide protective effect from infection, so it is expected to avoid side effects of increasing the risk of infection similar to DPP-4 inhibitors (23).

In addition, cases of myalgia and changes in pain threshold were also reported in patients taking DPP-4 inhibitors. DPP-4 inhibitors are widely distributed in striated muscles with an effect on increasing substance P levels which results in a decrease in the pain threshold (22). The use of Gedi leaves has been widely practiced by eastern people as a special food, and cases of myalgia have never been reported in the surrounding community who regularly consume Gedi leaves, but this needs further investigation. Gedi leaves with a mechanism similar to DPP-4 inhibitors are expected to be used as a treatment option to improve pancreatic function through in vivo research in human at a more affordable price, provide ease of processing and reduce side effects resulting from the use of DPP-4 inhibitor drugs.

5. Conclusion

From this research it can be concluded through the mechanisms discussed that:

- Oral administration of Gedi leaf extract (*Abelmoschus manihot*) effectively lowers fasting blood glucose levels in hyperglycemic male Wistar rats.
- Oral administration of Gedi leaf extract (*Abelmoschus manihot*) is as effective as DPP-4 inhibitor agents in reducing fasting blood glucose levels in hyperglycemic Wistar strain male rats.
- Oral administration of Gedi leaf extract (*Abelmoschus manihot*) was effective in increasing GLP-1 levels in hyperglycemic male Wistar rats.
- Oral administration of Gedi leaf extract (*Abelmoschus manihot*) is as effective as DPP-4 inhibitor agents in increasing GLP-1 levels in hyperglycemic Wistar male rats.

It is necessary to carry out further research regarding the maximum dose of Gedi and to determine the level of toxicity from administering Gedi. Further research is also needed regarding the GLP-1 cycle and the significant decrease in GLP-1 serum that occurred in the negative controls of this study, especially related to somatostatin levels in mice with insulin deficiency.

Acknowledgements

Author would like to thank to Faculty medicine of Udayana University to facilitate this research. Also thanks to all parties involved in this research**.**

Conflicts of interest

No conflict of interest was declared by the authors.

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