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Antidiabetic Activity Combination of Radish (*Raphanus sativus*L. var. *hortensi* Back.) and Yam Bean (*Pachyrhizuserosus* (L.) Urban) Extract in Alloxan Induced Diabetic Mice

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ABSTRACT

From previous studies, radish and yam bean are known to reduce blood glucose level. The objective of this study was to evaluate the effects of combination of radish extract and yam bean extract in reducing blood glucose level. Diabetic mice was induced by intravenous administration of a single dose of 55 mg/kg alloxan. The mice were divided into seven groups: normal control, diabetic control, gliclazid 5.2 mg/kg, radish 200 mg/kg, yam bean 100 mg/kg, combination with high dose (radish 200 mg/kg and yam bean 100 mg/kg), combination with low dose (radish 100 mg/kg and yam bean 50 mg/kg). The mice were treated with the extracts for 21 days. Blood glucose level was determined every 7 days and at the end of treatment, the β-cell pancreas was observed by Gomori staining. The result showed that radish at dose of 200 mg/kg, combination of high dose and low dose could significantly reduced blood glucose level (45.50%, 34.33%, 50.22%, respectively) compared to diabetic control ($p<0.05$). In conclusion, radish and combination of radish extract and yam bean extract have antidiabetic activity on damage of pancreas.

Key words: Radish, yam bean, diabetes mellitus, alloxan.

ITRODUCTION

Diabetes Mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia. It is associated with abnormalities in carbohydrate, fat, and protein metabolism and results in chronic complications including microvascular, macrovascular, and neuropathic disorders [1]. Several distinct types of DM exist and are caused by a complex interaction of genetics, environmental factors, and life-style choices. Depending on the etiology of the DM, factors contributing to hyperglycemia may include reduced insulin secretion, decreased glucose utilization, and increased glucose production [2].

In 2013, prevalence of diabetes reached 382 million and was expected to increase become 592 million in 2035. Number of patients with type 2 diabetes will continue to increase in every state. Approximately 80% of people with diabetes live in countries with low and middle income [3]. In 2000, prevalence of diabetics in Indonesia reached 8.4 million in 2030 and expected to increase become 21.3 million [4].

Radish and yam bean are known antidiabetic effects. Radish contains alyl isothiocyanate, acid pektat, glakturonat, fitin, pelargonidin, vitamins, minerals and crude fiber. Radish is often prescribed to overcome renal impairment, hypertension and diabetes mellitus [13]. In addition to the antidiabetic, radish also widely studied effect as an antioxidant, anticancer, antimicrobial and anti-inflammatory [7]. Antidiabetic effect of radish juice was report effective reduced blood glucosa level [5]. Yam bean contains pachyrhizon, isoflavones, vitamins, fiber and oligosaccharides. Yam bean can be used to maintain normal blood glucose levels. Dietary fiber contained in yam plays a role in lowering blood glucose levels because it is absorbed slowly and not all converted into glucose [6].

Other research showed of yam bean juice could reduced and maintenence blood glucose level [11]. As both plants has antidiabetic activity, we hypothesized that the combination of both plant could increased its antidiabetic activity.

MATERIALS AND METHODS

Experimental procedure

In this study, 35 male mice weighting 20-30 g were selected. The care and treatment of the animals conformed to the guideline for the Ethical Treatment of Laboratory Animals. They were habituated with standard experimental conditions (temperature of 20-22 ºC, 12 hour period of light and dark and free access to food and water). Diabetes was induced by a single intravenous injection of alloxan (Sigma) at a dose of 55 mg/kg body weight in natrium chloride 0.9%. The animals were kept for the occurrence of diabetes for 7 days. Thirty five diabetic mice were randomly divided into 6 groups, which are positive control group which were receive daqua destillata, reference group (gliclazide 5.2 mg/kg), radish 200 mg/kg, yam bean 100 mg/kg, combination of radish 100 mg/kg and yam bean 50 mg/kg, and combination of radish 200 mg/kg and yam bean 100 mg/kg. Additional negative control group was added. The mice were treated with extracts for 3 weeks. Blood glucose level was determined every 7 days used glucometer *Easy Touch*® and at the end of treatment, the β-cell pancreas was observed by *Gomori* staining.

Histopatological analysis

After 3 weeks of treatment, pancreases of sacrificed mice were removed, and fixed with buffer formaldehyde for 2 weeks. Then the pancreases were processed and stained with *Gomori* staining. Histological evaluation was performed to determine the possible effects of the extract. Histophatological changes of each pancreas were evaluated by calculate the area of Langerhans and calculate the numbers of β cell and α cell. In this system, histpathological evaluation was performed by examination of at least 3 mice from each group.

Obtained data were analyzed using SPSS program and One way analysis of Variance ($p<0.05$).

RESULTAND DISCUSSION

Table 1 shows that alloxan significantly increased the blood glucose level after 7 days compared to negative control ($p < 0.05$). This showed that inducing diabetes successfully. The test results showed radish, gliclazid, combination of radish and yam bean could decrease in blood glucose levels after administration for 21 days. Radish, combination of high dose, low dose and gliclazide showed a decrease in blood glucose levels were significantly different from the positive control.

Table 1. Effect of radish and yam bean on blood glucose level between different groups in alloxan-induced diabetic mice

Group treatment	Blood glucose level on days - (mg/dl)			
	0	7	14	21
Negative control (Normal mice)	122.60 ±18.30	138.60±11.26	103.20±28.21	102.60±23.43
Positive control (Aqua destillata)	569.40 ±8.65‡	539.60±57.85	530.00±40.79	504.80±70.24
Yam bean 100 mg/kg	567.20±38.43‡	430.40± 103.28*#	518.00±100.93	499.00±58.29
Radish 200 mg/kg	538.40 ± 7.52‡	540.20±87.45	536.20±69.88	288.00±96.56*#
Combination of radish 100 mg/kg and yam bean 50 mg/kg	528.80±58.26‡	496.80±96.62	293.00±75.23*#	258.00±69.97*#
Combination of radish 200 mg/kg and yam bean 100 mg/kg	525.80±75.74‡	531.20±55.78	367.80±80.79*#	339.60±7.62*#
Gliclazid 5.2 mg/kg	534.60±1.85‡	340.60 ± 82.24*#	196.40±30.21*#	289.60±64.24*#

The Data are expresses as Mean ±SD.
**p < 0.05 significant difference compared to positive control*
#p < 0.05 significant difference compared to day 0
‡p <0.05 significant difference compared to negative control

Group of yam bean 100 mg/kg could not reduced blood glucose levels after 21 days of treatment. Group of radish 200 mg/kg could reduced blood glucose levels after use for 21 days was significantly different from the positive control. Combination of radish 200 mg/kg with yam bean 100 mg/kg and combination of radish 100 mg/kg with yam bean 50 mg/kg could reduced blood glucose levels after use for 14 days. Gliklazid 5.2 mg/kg showed antidiabetic activity after use for 7 days. Combination of low dose and high dose showed ability to reduced blood glucose levels faster than group of radish.

In used for 7 days, yam bean and gliclazide showed a decrease in blood glucose levels were significantly different from the positive control with successive reduction of 23.35% and 35.13%. In used for 14 days, group the combination of low dose, combination of high dose, and gliclazide showed a decrease in blood glucose levels were significantly different ($p <0.05$) with the positive control with successive reduction of 43.71%, 29.60% and 63.13%. In use for 21 days, group the combination of low dose, combination of high dose, radish and gliclazide showed that blood glucose levels significantly different from the positive control with successive reduction of 50.22%, 34.33%,

45.50% and 44.89%. From these result, it can be concluded that the combination of radish and yam bean extract possess antihyperglycemic effect against alloxan induced diabetic mice.

Alloxan-induced diabetic condition cause morphological changes in β cells pancreas. In the pancreatic beta cell, alloxan reacts with two–SH2 groups in the sugar. Moreover, result of re-oxidized alloxan from dialuric acid establish a redox cycle for reactive oxygen species (ROS) and superoxide radicals. In addition, alloxan mechanism has been reported caused DNA damage[9,10]. To describe the condition of the pancreas after treatment, the organ histology was performed to evaluate the β cells of Langerhans pancreas.

Table 2.Effect of radish and yam bean on area of islet pancreas between different groups in alloxan-induced diabetic mice

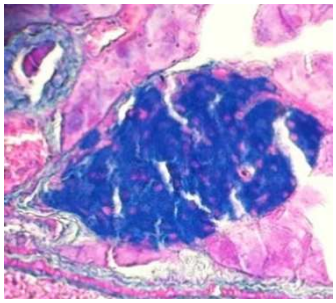
Group treatment	Average area (mm)
Negative control (Normal mice)	0.60 ± 0.26
Positive control (Aqua destillata)	0.07 ± 0.06
Yam bean 100 mg/kg	0.32 ± 0.19
Radish 200 mg/kg	0.57 ± 0.22*
Combination of radish 100 mg/kg and yam bean 50 mg/kg	0.45 ± 0.19*
Combination of radish 200 mg/kg and yam bean 100 mg/kg	0.41 ± 0.28
Gliclazid 5.2mg/kg	0.50 ± 0.26*

***Area of islet pancreas calculated by ImageJ*
**p<0.05significant difference compared to positive control*

Table 3Effect of radish and yam bean on cell of islet pancreas between different groups in alloxan-induced diabetic mice

Group treatment	Average cell/Langerhans	
	Sel β	Sel α
Negative control (Normal mice)	80.00 ± 81.90	47.33 ± 48.64
Positive control (Aqua destillata)	5.00 ± 2.64*	2.33 ± 2.52*
Yam bean 100 mg/kg	13.00 ± 6.08*	1.67 ± 1.53*
Radish 200 mg/kg	33.67 ± 20.98	3.00 ± 2.64*
Combination of radish 100 mg/kg and yam bean 50 mg/kg	28.00 ± 17.09	12.67 ± 10.60
Combination of radish 200 mg/kg and yam bean 100 mg/kg	15.33 ± 14.64*	16.00 ± 17.35
Gliclazid 5.2 mg/kg	33.00 ± 25.94	12.33 ± 5.51

**p < 0.05 significant difference compared to negative control*



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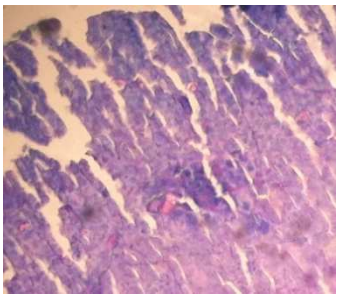

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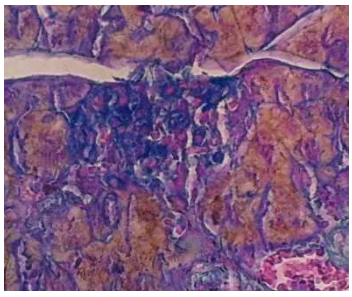

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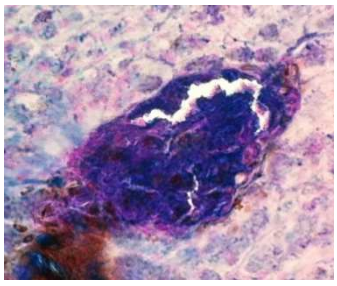

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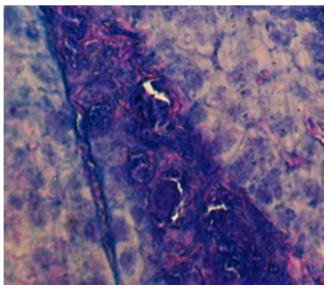

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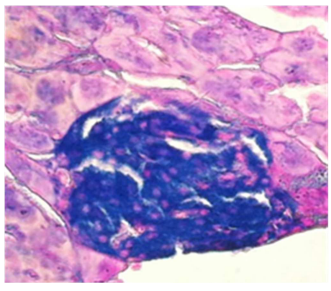

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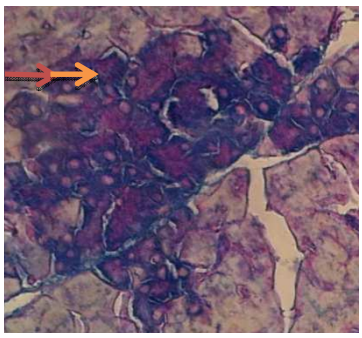

(G)

Figure 1.Histophatological changes of pancreas in various groups. (A) Normal pancreatic island (400×), (B) pancreatic islet in diabetic rats receiving aqua destillata (400×), (C) pancreatic islet in diabetic rats receiving 5,2 mg/kg of gliklazid (400×), (D) pancreatic islet in diabetic rats receiving 100 mg/kg of yam beanextract (100×), (E) pancreatic islet in diabetic rats receiving 200 mg/kg of radish extract (400×), (F) pancreatic islet in diabetic rats receiving combination 100 mg/kg of radish extract and 50 mg/kg of yam bean extract (400×),(G) pancreatic islet in diabetic rats receiving 200 mg/kg of radish extract and 100 mg/kg of yam beanextract (400×), ( ) β cell, ( ) α cell.

CONCLUSION

Radish 200 mg/kg, combination of radish 200 mg/kg with yam bean 100 mg and combination of radish 100 mg/kg with yam bean 50 mg/kg have antidiabetic activity on damage of pancreas in alloxan induced diabetic.

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