

Evaluation of anti-hyperglycemic effect of *Actinidia kolomikta* (Maxim. et Rur.) Maxim. root extract

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Abstract: This study aimed to evaluate the anti-hyperglycemic effect of ethanol extract from *Actinidia kolomikta* (Maxim. et Rur.) Maxim. root (AKE). An *in vitro* evaluation was performed by using rat intestinal α -glucosidase (maltase and sucrase), the key enzymes linked with type 2 diabetes. And an *in vivo* evaluation was also performed by loading maltose, sucrose, glucose to normal rats. As a result, AKE showed concentration-dependent inhibition effects on rat intestinal maltase and rat intestinal sucrase with IC₅₀ values of 1.83 and 1.03mg/mL, respectively. In normal rats, after loaded with maltose, sucrose and glucose, administration of AKE significantly reduced postprandial hyperglycemia, which is similar to acarbose used as an anti-diabetic drug. High contents of total phenolics (80.49±0.05mg GAE/g extract) and total flavonoids (430.69±0.91mg RE/g extract) were detected in AKE. In conclusion, AKE possessed anti-hyperglycemic effects and the possible mechanisms were associated with its inhibition on α -glucosidase and the improvement on insulin release and/or insulin sensitivity as well. The anti-hyperglycemic activity possessed by AKE may be attributable to its high contents of phenolic and flavonoid compounds.

Keywords: *Actinidia kolomikta* (Maxim. et Rupr.) Maxim., Diabetes, Anti-hyperglycemic, Blood glucose, Insulin.

INTRODUCTION

Diabetes, a metabolic disease, which can cause many serious complications, is the fifth leading cause of death worldwide (Chandramohan *et al.*, 2008). Diabetes can be generally classified into type 1 and type 2, over 90% of which are type 2 diabetics (Attele *et al.*, 2002) caused by insulin resistance and β -cell hypofunction (Laakso, 2001; Gispensand Biessels, 2000). All diabetes are characterized by hyperglycaemia. Hence, it's crucial for the diabetic patients to effectively control their blood glucose levels.

In recent years, the number and proportion of diabetics is growing rapidly. International Diabetes Federation (IDF) has estimated that the number of diabetics will increase up to 439 million in 2030 (Shaw *et al.*, 2010), and the cost of treating diabetes and associated complications will exceed 490 billion US dollar (Javanbakht *et al.*, 2011). With the significant economic burden, diabetes mellitus has become a global health problem. Therefore, there is an urgent demand for new curative approaches to diabetes mellitus.

Reducing postprandial hyperglycemia is one of the therapies for the treatment of diabetes mellitus (Stratmann and Tschoepe, 2009). Previous research has shown that α -glucosidase inhibitors could decrease postprandial hyperglycemia by inhibiting carbohydrate hydrolysis enzymes (Mohamed *et al.*, 2012; Bhandari *et al.*, 2008). As anti-hyperglycemic drugs, α -glucosidase

inhibitors have been widely used to treat diabetes in clinical trials, such as acarbose, miglitol and voglibose (Trapero and Llebaria, 2012). However, several studies have demonstrated that anti-hyperglycemic drugs have side effects and high cost for patients (Grover *et al.*, 2002; Bhatnagar, 1998; May *et al.*, 2002). Thus, more researchers are focusing their research on alternative therapies for treatment of diabetes. On the other hand, it has been affirmed fully by World Health Organization to utilize traditional plants for diabetes because they possess high security and efficiency (Stephen *et al.*, 2010). It has become a hotspot in diabetes treatment research to develop safe and effective anti-hyperglycemic drugs from traditional medicinal plants, and many traditional medicinal plants have been shown to have anti-hyperglycemic effects (Bailey and Day, 1989). In Laos, the root of *Actinidia kolomikta* (Maxim. et Rupr.) Maxim. has been traditionally used to treat diabetes mellitus. Our previous work reported the *in vitro* α -glucosidase inhibitory activity of the aqueous extract from *Actinidia kolomikta* (Maxim. et Rupr.) Maxim. root (Hu *et al.*, 2013). Up to now, however, the effect of ethanol extract of *Actinidia kolomikta* (Maxim. et Rupr.) Maxim. root on hyperglycemic activity has not been assessed yet.

This study aimed to evaluate the anti-hyperglycemic effect of ethanol extract from *Actinidia kolomikta* (Maxim. et Rupr.) Maxim. root (AKE). And the mechanisms involved were also discussed. The anti-hyperglycemic effects of AKE were assessed by using rat

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intestinal α -glucosidase and normal rats. The inhibitory activities of AKE on rat intestinal maltase and rat intestinal sucrase were measured, and oral maltose, sucrose and glucose tolerance tests of AKE were carried out in normal rats.

MATERIALS AND METHODS

Plant material and extraction

Actinidia kolomikta (Maxim. et Rupr.) Maxim. roots were collected from Laos and washed thoroughly with distilled water. Then they were dried in the shade (avoiding sunshine) and crushed into powder. The powder (500 g) was soaked in 70% ethanol (5 L) at 25°C for three days. Then the extract was centrifuged and filtered, and the filtrate was concentrated and lyophilized to get powder (yield: 10.8%, w/w). The obtained 70% ethanol extract from *Actinidia kolomikta* (Maxim. et Rupr.) Maxim. root (namely, AKE) was stored at -20°C till further use.

Assay for rat intestinal maltase inhibitory activity

In vitro rat intestinal maltase inhibitory activity was determined according to Hu *et al.* (2013). The inhibitory activity was calculated according to the following equation:

$$\text{Maltase inhibitory activity (\%)} = \left[1 - \frac{A_{SB} - A_S}{A_{CB} - A_C} \right] \times 100 (\%)$$

where A_{CB} is the absorbance with rat intestinal maltase and without the sample, A_C is the absorbance without rat intestinal maltase and the sample, A_{SB} is the absorbance with rat intestinal maltase and the sample, and A_S is the absorbance with the sample but without rat intestinal maltase.

Assay for rat intestinal sucrase inhibitory activity

In vitro rat intestinal sucrase inhibitory activity was determined according to Hu *et al.* (2013) with slight modifications, i.e. the supernatant was diluted 2 times and the concentration of sucrose substrate solution was adjusted to 2% (w/v) with phosphate buffer. The inhibitory activity was calculated according to the following equation:

$$\text{Sucrase inhibitory activity (\%)} = \left[1 - \frac{A_{SB} - A_S}{A_{CB} - A_C} \right] \times 100 (\%)$$

where A_{CB} is the absorbance with rat intestinal sucrase and without the sample, A_C is the absorbance without rat intestinal sucrase and the sample, A_{SB} is the absorbance with rat intestinal sucrase and the sample, and A_S is the absorbance with the sample but without rat intestinal sucrase.

Animals

Healthy adult male Sprague-Dawley rats (180-220 g) were obtained from Japan SLC, Inc (Shizuoka, Japan) and maintained in the Laboratory Animal Resource Center, University of Tsukuba. The rats were kept in cages with 3

rats in each cage under controlled conditions: temperature ($23 \pm 1^\circ\text{C}$), humidity ($55 \pm 5\%$) and 12 h (7:00 - 19:00) light/dark cycle. The rats had free access to tap water and food before the experiments. All the animal experiments were based on the guideline of the maintenance and use of laboratory animals at the Laboratory Animal Resource Center, University of Tsukuba and were approved by the Animal Experiments Committee, University of Tsukuba (Approval number 13-305).

Oral sucrose tolerance test in normal rats (OSTT)

OSTT was carried out according to Honma *et al.* (2010) with slight modifications. The rats were randomly divided into three groups with 6 rats in each group, after an overnight fast, they were administered with distilled water, AKE (300 mg/kg) and acarbose (5 mg/kg), respectively. 30 min later, sucrose was given (2 g/kg) to each group. Blood samples were obtained from the tail vein at 0, 30, 60, 90 and 120 min after sucrose administration, and the glucose levels were estimated using a glucometer (Sanwa Kagaku Kenkyusho, Japan). The area under the curves (AUC) during the OSTT was also calculated using the trapezoidal rule.

Oral maltose tolerance test in normal rats (OMTT)

OMTT was carried out using the same procedure of OSTT and the only difference was to replace sucrose by maltose.

Oral glucose tolerance test in normal rats (OGTT)

OGTT was carried out according to Sundaram *et al.* (2012) with slight modifications. The rats were randomly divided into four groups with 6 rats in each group, after an overnight fast, they were administered with distilled water, AKE (100 mg/kg), AKE (200 mg/kg) and AKE (400 mg/kg), respectively. 30 min later, glucose was given (2 g/kg) to each group. Blood samples were obtained from the tail vein at 0, 30, 60, 90 and 120 min after glucose administration, and the glucose levels were estimated using a glucometer (Sanwa Kagaku Kenkyusho, Japan). And the area under the curves (AUC) during the OGTT was also calculated using the trapezoidal rule.

Total phenolic content

The total phenolic content was determined with the Folin-Ciocalteu method (Kim *et al.*, 2003). The total phenolic content of AKE was calculated based on the standard curve obtained with gallic acid and was expressed as gallic acid equivalent (GAE) in mg/g dry sample.

Total flavonoid content

The total flavonoid content was measured by using the aluminium chloride colorimetric method (Yao *et al.*, 2013). The total flavonoid content of AKE was calculated based on the standard curve obtained with rutin and was expressed as rutin equivalent (RE) in mg/g dry sample.

STATISTICAL ANALYSIS

All the data were expressed as mean \pm standard deviation (SD), and ANOVA followed by Duncan's multiple range test (DMRT) was used for statistical analysis by using SPSS software (version 17.0). Significance was assumed if $p < 0.05$.

RESULTS

Assay for rat intestinal maltase inhibitory activity

The rat intestinal maltase inhibitory activity of AKE was depicted in Fig. 1. AKE showed a concentration-dependent effect on the rat intestinal maltase inhibitory activity and 53.7% inhibition was detected at 2 mg/mL ($IC_{50} = 1.83$ mg/mL). However, the inhibitory activity of AKE was lower than acarbose, which had 96.2% of rat intestinal maltase inhibitory activity even at 0.125 mg/mL.

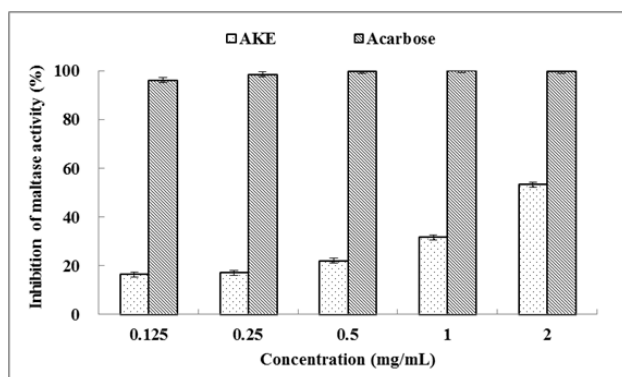


Fig. 1: Inhibitory effect of AKE on rat intestinal maltase activity.

Assay for rat intestinal sucrase inhibitory activity

Seen from Fig. 2, AKE dose-dependently inhibited rat intestinal sucrase ($IC_{50} = 1.03$ mg/mL). AKE inhibited 69.3% of rat intestinal sucrase at 2 mg/mL while acarbose had 98.5% of rat intestinal sucrase inhibitory activity at the same concentration.

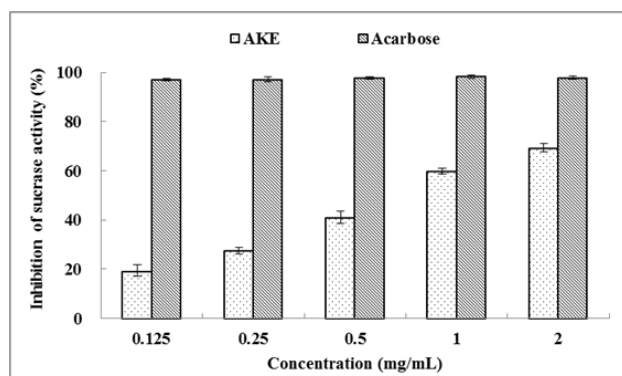


Fig. 2: Inhibitory effect of AKE on rat intestinal sucrase activity.

Oral maltose tolerance test in normal rats (OMTT)

The effect of AKE on OMTT was shown in Fig. 3. As seen from Fig. 3A, after maltose loaded for 30 and 60 min, the blood glucose levels of control group were averagely 116 ± 5 and 144 ± 5 mg/dL, respectively. The blood glucose levels of AKE (300 mg/kg) treatment group were 104 ± 1 and 106 ± 1 mg/dL, respectively. AKE (300 mg/kg) significantly decreased the blood glucose levels after administration of maltose for 30 min ($p < 0.05$) and 60 min ($p < 0.01$) in the normal rats in comparison to the control group. As illustrated in Fig. 3B, compared to the control group, the area under the blood glucose-time curve (AUC) for the AKE (300 mg/kg) treatment group was significantly decreased ($p < 0.05$). Result from this assay also showed the better reduction effect of AKE (300 mg/kg) on blood glucose than that of acarbose (10 mg/kg).

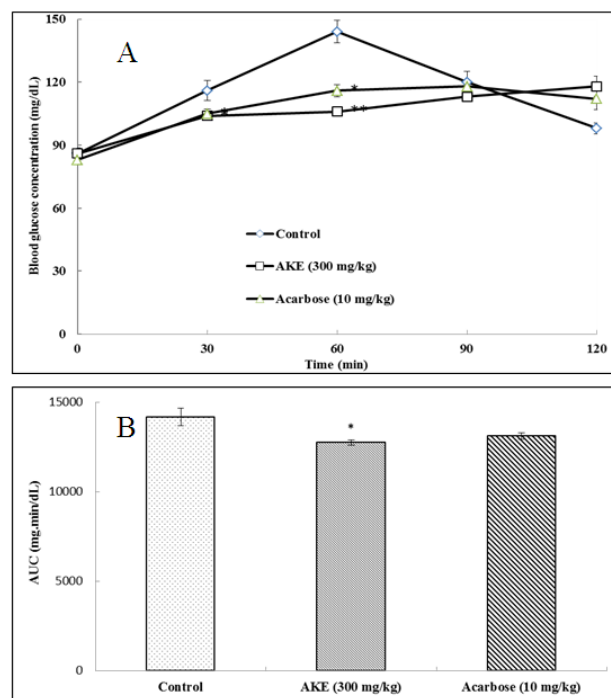


Fig. 3: Effect of AKE on maltose tolerance in normal rats. Blood glucose curve in OMTT (A), AUC values in OMTT (B). Results are mean \pm SD ($n = 6$). Significant difference from control: * $p < 0.05$, ** $p < 0.01$.

Oral sucrose tolerance test in normal rats (OSTT)

The effect of AKE on OSTT was shown in Fig. 4. As shown in Fig. 4A, after sucrose loaded for 30, 60 and 90 min, the blood glucose levels of control group were averagely 152 ± 1 , 138 ± 3 and 134 ± 2 mg/dL, respectively. The blood glucose levels of AKE (300 mg/kg) treatment group were 124 ± 7 , 121 ± 5 and 119 ± 4 mg/dL, respectively. AKE (300 mg/kg) significantly decreased the blood glucose levels after maltose administration for 30 min ($p < 0.05$) in the normal rats in comparison to the control group. As seen from Fig. 4B,

compared to the control group, the area under the blood glucose-time curve (AUC) in the AKE (300 mg/kg) treatment group was much lower, but still a little higher than that of acarbose (10 mg/kg) treatment group,

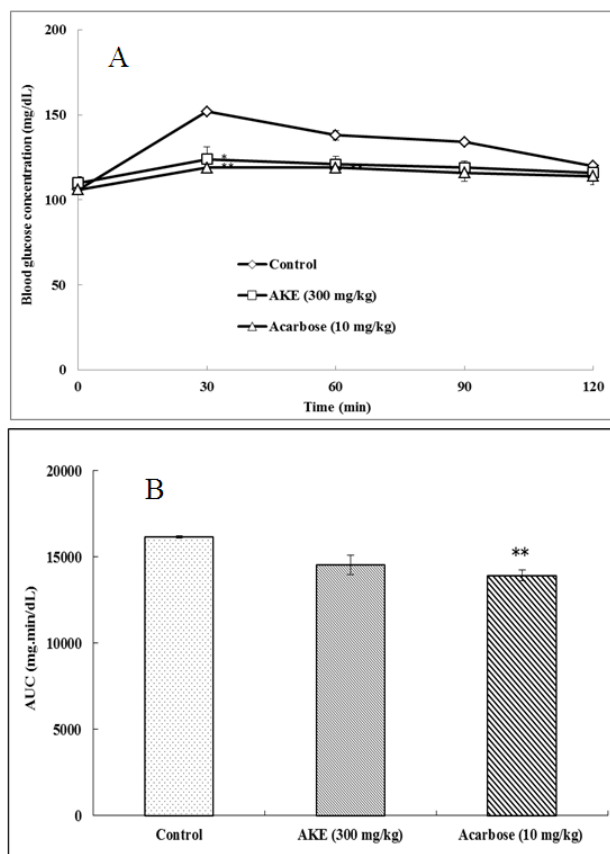


Fig. 4: Effect of AKE on sucrose tolerance in normal rats. Blood glucose curve in OSTT (A), AUC values in OSTT (B). Results are mean \pm SD (n = 6). Significant difference from control: *p < 0.05, **p < 0.01.

Oral glucose tolerance test in normal rats (OGTT)

The blood glucose levels of AKE treatment groups trended to decrease in a dose-dependent manner during OGTT (Fig. 5A). AKE produced a significant decrease in blood glucose levels after glucose loaded for 30 min in the normal rats when compared to the control group (p < 0.05). After glucose loaded for 60 and 90 min, the blood glucose levels of control group were 133 \pm 5 and 129 \pm 3 mg/dL, respectively. The blood glucose levels of AKE (400 mg/kg) treatment group were 113 \pm 1 and 105 \pm 1 mg/dL, respectively. AKE (400 mg/kg) administration caused a significant reduction in blood glucose levels after glucose loaded for 60 min (p < 0.05) and 90 min (p < 0.01) in the normal rats in comparison to the control group. Further more, compared to the control group, the areas under the blood glucose-time curve (AUC) for the AKE (200 mg/kg) treatment group (p < 0.05) and AKE (400 mg/kg) treatment group (p < 0.01) were significantly decreased (Fig. 5B).

Total phenolic and total flavonoid contents

As summarized in table 1, the total phenolic content of AKE was 80.49 \pm 0.05 mg GAE/g and the total flavonoid content of AKE was 430.69 \pm 0.91 mg RE/g.

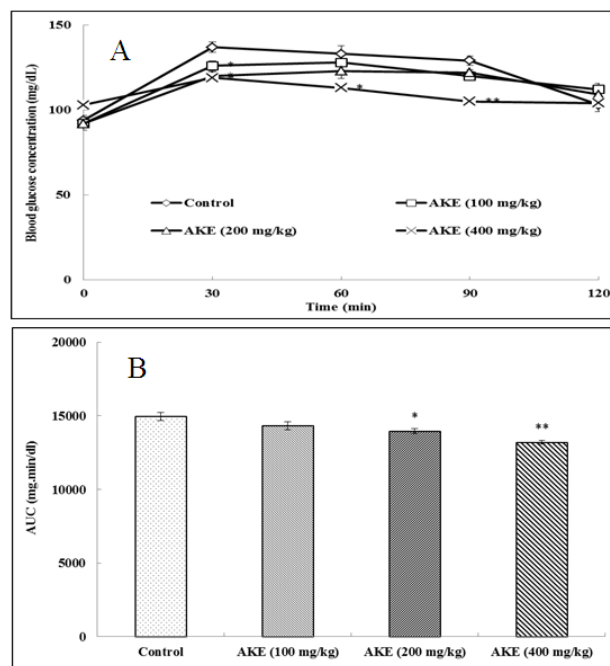


Fig. 5: Effect of AKE on glucose tolerance in normal rats. Blood glucose curve in OGTT (A), AUC values in OGTT (B). Results are mean \pm SD (n = 6). Significant difference from control: *p < 0.05, **p < 0.01.

Table 1: Total polyphenol and total flavonoid contents of AKE. The values are presented as means \pm SD.

Active component	Total polyphenol content (mg GAE/g)	Total flavonoid content (mg RE/g)
Value	80.49 \pm 0.05	430.69 \pm 0.91

DISCUSSION

In recent years, yeast α -glucosidase is widely used to seek for α -glucosidase inhibitors, but the results do not always agree with those obtained in mammals (Karato *et al.*, 2006; Nimal *et al.*, 2013). In order to make the experiments more close to mammals, intestinal acetone powder from rats was used in this study to evaluate the α -glucosidase inhibitory activity of AKE.

In this study, the anti-hyperglycemic effect of AKE was evaluated *in vitro* and *in vivo* for the first time. As a result, AKE could not only inhibit rat intestinal maltase activity and rat intestinal sucrase activity *in vitro*, but also decrease postprandial hyperglycemia *in vivo*. The results indicated that AKE possessed significant anti-hyperglycemic effect. Results from this work provide a

scientific evidence for using *Actinidia kolomikta* (Maxim. et Rupr.) Maxim. root to treat diabetes.

Although the present results suggest that AKE possesses significant anti-hyperglycemic effect, the mechanism of anti-hyperglycemic effect of AKE has not been disclosed. Therefore, we further tried to investigate the possible mechanisms involved in the anti-hyperglycemic effect of AKE. There are three major mechanisms to reduce hyperglycemia: decreasing postprandial hyperglycemia, increasing insulin secretion and decreasing insulin resistance (Lebovitz, 1999). Accordingly, this work was designed to evaluate the anti-hyperglycemic effect of AKE through α -glucosidase (rat intestinal maltase and rat intestinal sucrase) inhibitory activity assay and OSTT, OMTT in normal rats. OGTT was also performed *in vivo* to find the mechanisms relating with insulin release and insulin resistance.

From the results of OMTT and OSTT, AKE showed significant anti-hyperglycemic effect. This is the first time to investigate the anti-hyperglycemic effect of AKE *in vivo*, and these results are in consistence with the results from α -glucosidase inhibitory activity assay *in vitro*. Therefore, it can be concluded that AKE exert its anti-hyperglycemic effect by inhibiting α -glucosidase from intestine such as sucrase, maltase to decrease postprandial hyperglycemia, in which the mechanism is similar to acarbose.

OGTT is not only used to diagnose diabetes mellitus, but also used to explore insulin release and insulin resistance (Stumvoll *et al.*, 2000; Rijkelijkhuizen *et al.*, 2009). The results of OGTT showed that AKE decreased the blood glucose level in a dose-dependent manner. More specifically, AKE (400 mg/kg) administration caused a significant decrease in blood glucose levels after glucose loaded. It's well known that insulin is the only hormone, which can reduce blood glucose level, and the latter can be decreased by promoting insulin release or improving insulin sensitivity. Therefore, it is conjectured that AKE might exert its anti-hyperglycemic effect by improving insulin release and/or insulin sensitivity. Further experiments are necessary to clarify its real mechanisms involved.

Previous studies showed that polyphenols play an important role in decreasing postprandial hyperglycemia (McDougall *et al.*, 2005; Matsui *et al.*, 2001). Flavonoids, as the largest family of polyphenolic compounds, have anti-diabetic activity (Ahmad *et al.*, 2000; Li *et al.*, 2009; Orhan *et al.*, 2013). The results from this study indicate that AKE has higher phenolic content and flavonoid content than those of mulberry leaves which have been reported to possess anti-hyperglycemic activities (Wanyo *et al.*, 2011). Therefore, the high phenolic contents and flavonoid contents of AKE may be the reason for its anti-

hyperglycemic effects. Further attempts should be focused on the isolation and identification of the active ingredients in AKE.

CONCLUSION

In conclusion, this study for the first time confirms that the ethanol extract of *Actinidia kolomikta* (Maxim. et Rupr.) Maxim. root has anti-hyperglycemic effects and the possible mechanisms involved are not only the inhibition to α -glucosidase, but also the improvement on insulin release and/or insulin sensitivity.

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