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Glucose consumption and α-glucosidase inhibitory activities of aqueous root extract of *Helicteres angustifolia*

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Abstract. – OBJECTIVE: The root of *Helicteres angustifolia* L. (Sterculiaceae) has been used as tea to treat diabetics effectively by local people in Laos. However, no scientific evidence is available for this ethnomedicinal usage. This study was undertaken to explore the hypoglycemic effect of aqueous extract from *Helicteres angustifolia* root.

PATIENTS AND METHODS: The effect of aqueous extract from *Helicteres angustifolia* root on glucose consumption in C2C12 myotubes was investigated at a dose of 25, 50 and 100 μg/mL, respectively. The α-glucosidase inhibitory activity of the extract was evaluated using rat intestinal maltase and sucrose. Moreover, oral sucrose tolerance test (OSTT) in normal and streptozotocin induced diabetic rats was performed. Finally, their cytotoxicity in C2C12 cells and acute oral toxicity in rats was analyzed.

RESULTS: Aqueous root extract of *Helicteres angustifolia* significantly enhanced glucose consumption in C2C12 myotubes. The extract also significantly inhibited rat intestinal maltase (IC_{50} = 1.44 ± 0.24 mg/mL) and sucrase activity (IC_{50} = 0.54 ± 0.12 mg/mL), respectively. The OSTT results showed that the extract significantly suppressed the increase of blood glucose levels in normal and diabetic rats. The extract was also proven to have low acute toxicity (LD_{50} > 5 g/kg) and low cytotoxicity in C2C12 cells (IC_{50} > 0.4 mg/mL).

CONCLUSIONS: The findings from this study indicate that aqueous root extract of *Helicteres angustifolia* possesses significant α-glucosidase inhibitory activity and moderate enhanced glucose consumption activity, while with low cytotoxic and acute toxicity.

Key words:
Helicteres angustifolia, Diabetes, α-Glucosidase, Glucose consumption.

Introduction

Diabetes mellitus, a lifestyle disease, has become a global public health problem. It has been reported that 387 million people were suffering from diabetes mellitus in 2014, and this number is predicted to rise to 592 million by 2035[1]. In order to improve this situation, many researchers are trying to treat diabetes using traditional medicinal plants due to their advantages like lesser side effects and low cost[2]. Up to now, about 800 traditional medicinal plants have been proven to possess antidiabetic activity, and their bioactive compounds are mainly triterpenes, flavonoids, alkaloids, glycans, steroids, etc.[3].

For antidiabetic plants, there are five mechanisms of action against diabetes: to increase insulin secretion at pancreas, to inhibit glucose absorption from intestine, to decrease glucose production at liver, to increase the peripheral uptake of glucose from blood to muscle tissue and to enhance uptake of glucose by adipose[4]. Glucose absorption from intestine can be delayed via inhibiting carbohydrate hydrolysis enzymes such as α-glucosidase[5]. α-glucosidase, located in the brush-border surface membrane of intestinal cells, can catalyze complex polysaccharides into monosaccharides. The liberated monosaccharides are transported into small intestinal epithelial cells, leading to postprandial hyperglycemia. Therefore, α-glucosidase inhibitors can delay the decomposition of complex polysaccharides to monosaccharides through inhibiting α-glucosidase activity, thereby reducing postprandial blood glucose levels and suppressing postpran-

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dial hyperglycemia[6]. Skeletal muscle, the largest insulin target tissue accounting for up to 50% of the whole body weight[7], may be responsible for the disposal of about 80% of total body glucose uptake[8]. Therefore, increasing glucose utilization in skeletal muscle could effectively improve the glucose metabolism of the whole body, which is one of the main therapeutic approaches for diabetes.

Helicteres angustifolia L. (Sterculiaceae) is a traditional medicinal plant in China. It has also been reported to possess anti-bacterial activity, anti-inflammatory activity and analgesic effect[9], anticancer[10], anti-hepatic fibrosis activity[11], and antiviral activity[12]. Our previous work also showed that aqueous and ethanol extracts from *Helicteres angustifolia* root possessed significant antioxidant activity and antitumor efficacy[13]. As for chemical components analysis, Wei et al[14] isolated 14 compounds from this plant. Chen et al[9] isolated 28 compounds from *Helicteres angustifolia,* among which like betulinic acid[15], oleanolic acid[16] and rosmarinic acid[17] have been reported to possess antidiabetic activity. Additionally, *Helicteres isora*, belonging to the same Sterculiaceae family, also exhibits significant antidiabetic activity[18]. In Laos, the root of *Helicteres angustifolia* has been used to effectively treat diabetics by local people. Up to the present, however, there is no scientific evidence associating with its ethnomedicinal use for diabetes treatment.

The purpose of this study was to investigate the hypoglycemic effect of aqueous root extract of *Helicteres angustifolia* by evaluating its glucose consumption and α-glucosidase inhibitory activities. Furthermore, acute oral toxicity in rats and cytotoxicity in C2C12 cells of the extract was evaluated.

Materials and Methods

Chemicals

Fetal bovine serum (FBS) was from Gibco (Grand Island, NJ, USA). Glucose CII test kit was purchased from Wako Pure Chemical Industries (Osaka, Japan). Rat intestinal acetone powder, insulin, 1-dimethylbiguanide hydrochloride (metformin), bovine serum albumin (BSA) and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Sigma Aldrich, Inc. (Saint Louis, MO, USA). All the other chemicals were of analytical grade.

Plant material

The roots of *Helicteres angustifolia* were collected in the rural area near to the city of Vientianei, Laos in June, 2013. It was identified by Dr. Ende Liu from Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China, of which a voucher specimen (KUN-2014-141) was deposited at the Herbarium of the same institute.

Plant extraction

Helicteres angustifolia roots (100 g) were air-dried and extracted three times with distilled water at 100°C for 2 h. Then the extract was centrifuged and filtered with GF/A filter pater (Whatman, UK). After the combined filtrates being concentrated with rotator evaporator (Eyela, Tokyo, Japan), the concentrated extract was further freeze dried. The freeze-dried extract (11 g) was stored at -20°C until further use (yield: 11%, w/w).

Experimental animals

The animal experiments were approved by the Animal Experiments Committee, University of Tsukuba (Approval No. 14-344), and based on the guideline of the maintenance and use of laboratory animals at the Laboratory Animal Resource Center of University of Tsukuba. Healthy adult Sprague-Dawley rats weighting 180-220 g were purchased from Japan SLC, Inc (Shizuoka, Japan), and maintained under controlled conditions of temperature (23 ± 1°C), humidity (55 ± 5%) and 12 h (7:00-19:00) light/dark cycle. Before the experiments, the rats were provided tap water and standard rodent chow ad libitum.

Cell culture and differentiation

C2C12 mouse skeletal muscle cells were purchased from RIKEN Bioresource Center (Tsukuba, Japan). Cells were cultured in DMEM containing 10% FBS and 1% penicillin/streptomycin at 37°C and 5% CO_2 incubator until the cells achieved 80-90% confluence. Differentiation of C2C12 myoblasts to myotubes was performed according to Kimura et al[19] with slight modifications. Concisely, cells were seeded in 24-well plates. When the cells were approximately 80% confluent, the medium was replaced with DMEM containing 2% horse serum and was changed every 2 days until cells were fused to myotubes.

Cytotoxicity assay

Cytotoxicity of aqueous root extract of *Helicteres angustifolia* towards C2C12 cells was measured according to MTT assay[20]. In brief,

C2C12 cells were plated at a density of 5×10^3 cells/well in a 96-well plate and incubated for 24 h, and then incubated with different concentrations of the extract for another 24 h. After incubation, 10 μL MTT (5 mg/mL) was added into each well and further incubated for 4 h, and then the culture medium was removed prior to the addition of 100 μL DMSO. After 10 min of shaking, the absorbance was read at 570 nm using a microplate reader (Bio-Rad, Tokyo, Japan).

Intestinal α-glucosidase inhibitory activity

In vitro rat intestinal α-glucosidase inhibitory activity was determined according to our previous works[21, 22] with slight modifications. In brief, rat intestinal acetone powder was used as the rat small intestinal α-glucosidase (maltase: EC 3.2.1.20, sucrose: EC 3.2.1.48). 2% maltose and sucrose solution were used as substrate. After incubating the mixture of aqueous root extract, substrate and rat small intestinal α-glucosidase, the amount of liberated glucose was determined by glucose oxidase method with glucose CII test kit (Wako, Osaka, Japan).

Glucose consumption in C2C12 myotubes

Glucose consumption in differentiated C2C12 myotubes was measured according to a previously described method[23] with some modifications. Briefly, differentiated C2C12 myotubes were serum-starved for 2 h DMEM containing 0.2% bovine serum albumin (BSA). After incubation, the cells were treated with DMEM containing 0.2% BSA with or without the designated concentrations of test samples for another 4 h. The glucose concentration of the medium was determined at time zero and after 4 h incubation using the glucose CII test kit (Wako, Osaka, Japan). The amount of glucose consumption was calculated by subtracting the remaining glucose concentration at time 4 h from the initial glucose concentration (time zero) in the medium. Sterilized water was utilized as negative while insulin (1 μM) and metformin (1 μM) as positive control.

Induction of diabetes

The rats were kept on fast overnight and then injected intraperitoneally with streptozotocin (dissolved in 0.1 M cold citrate buffer, pH 4.5) at a dose of 50 mg/kg. After 72 h, fasting blood glucose level was measured using one touch select glucometer (Sanwa Kagaku Kenkyusho, Japan) and the rats with blood glucose higher than 250 mg/dL were regarded as diabetic and used for the study[24].

Oral sucrose tolerance test in normal and diabetic rats (OSTT)

Normal and streptozotocin induced diabetic rats were randomly divided into two groups (n=6 rats in each group), respectively. All rats were kept on fast overnight and then administered with distilled water and the aqueous root extract (100 mg/kg), respectively. 30 min later, sucrose (2 g/kg b.w.) was administered orally to each group. Blood glucose levels were determined at 30, 60, 90 and 120 min after sucrose administration using a glucometer (Sanwa Kagaku Kenkyusho, Japan)[21].

Acute oral toxicity study

Acute oral toxicity study of the aqueous root extract of *Helicteres angustifolia* was performed according to OECD 423[25]. The rats were divided into two groups (n = 3) and kept fasting for overnight before being gavaged with distilled water and the aqueous root extract (5 g/kg body weight), respectively. The rats were continuously observed for 24 h after administration. Two weeks later, the rats were dissected and their major organs were examined macroscopically.

Statistical Analysis

All the data were expressed as mean ± SD of three independent experiments, and ANOVA followed by Duncan's multiple range test (DMRT) was used for statistical analysis by using SPSS software (version 22.0). Significant difference was assumed at $p < 0.05$.

Results

Cytotoxicity assay

As summarized in Table I, a low cytotoxicity was observed in C2C12 cells for aqueous root extract of *Helicteres angustifolia*, with IC_{50} values greater than 0.4 mg/mL. This result provided a basis for using the aqueous root extract at a concentration of 25, 50 and 100 μg/mL in the following glucose consumption experiments.

Intestinal α-glucosidase inhibitory activity

As shown in Table I, *Helicteres angustifolia* aqueous root extract exhibited potential α-glucosidase inhibitory activity, with IC_{50} values towards maltase and sucrose activities being 1.44 ± 0.24 and 0.54 ± 0.12 mg/mL, respectively.

Table I. α-glucosidase (maltase and sucrose) inhibitory effect, cytotoxicity and acute oral toxicity of aqueous root extract of *Helicteres angustifolia*.

Sample	IC50 values (mg/mL)			LD50 value (g/kg)
	α-Glucosidase		Cytotoxicity	Acute oral toxicity
	Maltase	Sucrase		
Aqueous root extract of *Helicteres angustifolia*	1.44 ± 0.24	0.54 ± 0.12	> 0.4	> 5

Data were expressed as mean ± SD (n = 3).

Glucose consumption in C2C12 myotubes

Figure 1 shows the effect of aqueous root extract of *Helicteres angustifolia* on glucose consumption in differentiated C2C12 myotubes. At all tested concentrations, the aqueous root extract significantly increased glucose consumption in differentiated C2C12 myotubes, which was greater than the positive controls of insulin (1 μM) and metformin (1 μM).

Oral sucrose tolerance test in normal and diabetic rats (OSTT)

The effects of aqueous root extract of *Helicteres angustifolia* on sucrose tolerance in normal and streptozotocin induced diabetic rats were summarized in Table II. After loaded with sucrose, both normal and diabetic rats showed high blood glucose levels. After the administration of aqueous root extract, the increase of blood glucose levels was suppressed in normal rats to some extent. Specifically, after sucrose loading for 30 min, a significant suppressive effect on blood glucose levels was noticed ($p < 0.05$). At 120 min after sucrose loading into the diabetic rats, the extract was observed to have significant suppressive effect on the increase of blood glucose levels ($p < 0.05$, Table II).

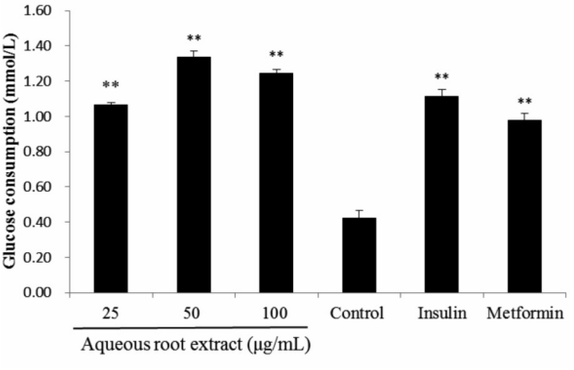

Figure 1. Effect of aqueous root extract of *Helicteres angustifolia* on glucose consumption after 4 h treatment in C2C12 myotubes. Data were presented as the mean ± SD. (n = 3). *$p < 0.05$, **$p < 0.01$, compared to negative control. Insulin (1 μM) and metformin (1 μM) were used as positive controls.

Acute oral toxicity study

During the 14 days' acute oral toxicity experiments, the aqueous root extract of *Helicteres angustifolia* demonstrated no toxic symptoms at a dose of 5 g/kg, and its LD_{50} value was estimated to be higher than 5 g/kg in rats (Table I).

Discussion

α-glucosidase, a key enzyme linked to type 2 diabetes, has been regarded as a therapeutic target to regulate postprandial hyperglycemia[26]. α-glucosidase inhibitors, i.e. acarbose, miglitol and voglibose, have been used as prescription drugs to treat diabetes due to their inhibition to α-glucosidase activity, resulting in delayed glucose absorption, thereby effectively reducing postprandial hyperglycemia. Among them, acarbose has been reported to reduce by 25% diabetes incidence[27]. In this study, significant inhibitory effect was observed for the aqueous root extract of *Helicteres angustifolia* on rat intestinal α-glucosidase activity, with IC_{50} values towards maltase and sucrose activities being 1.44 ± 0.24 and 0.54 ± 0.12 mg/mL, respectively (Table I). The inhibitory effect of *Helicteres angustifolia* from this study is greater than an antidiabetic medicinal plant, *Moringa oleifera* (its IC_{50} values towards maltase and sucrose activities were greater than 5.00 and 0.98 ± 0.21 mg/mL, respectively[26]). This

Table II. Effects of Helicteres angustifolia aqueous root extract on sucrose tolerance in normal and diabetic rats (n=6).

Group	Blood glucose levels (mg/dL)				
	0 min	30 min	60 min	90 min	120 min
NC	108 ± 1	176 ± 5	131 ± 8	118 ± 58	109 ± 6
NC100	109 ± 2	141 ± 7[a]	123 ± 48	111 ± 3	97 ± 4
DC	300 ± 7	> 500	> 500	490 ± 7	400 ± 5
DC100	292 ± 4	> 500	> 500	438 ± 12	359 ± 5[b]

Grouping of the animals: NC, normal control rats treated with distilled water; NC100, normal rats treated with the extract (100 mg/kg); DC, Diabetic control rats treated with distilled water; DC100, diabetic rats treated with the extract (100 mg/kg).
[a]$p < 0.05$ compared with the normal control group at each time point.
[b]$p < 0.05$ compared with the diabetic control group at each time point.

is the first time to prove that *Helicteres angustifolia* root has α-glucosidase inhibitory activity.

To further demonstrate the α-glucosidase inhibitory activity of the extract, we monitored the changes in blood glucose levels in the normal and streptozotocin induced diabetic rats after sucrose loading. The aqueous root extract of *Helicteres angustifolia* showed significant suppressive effect on hyperglycemia in normal and diabetic rats, at 30 min and 120 min after sucrose loading, respectively (Table II). This finding further demonstrates that the aqueous root extract of *Helicteres angustifolia* has inhibitory activity against sucrase, an α-glucosidase hydrolysis of sucrose, thereby improving postprandial hyperglycaemia. We failed to get the blood glucose levels in the diabetic rats at 30 min and 60 min after sucrose loading, due to the fact that their blood glucose levels were too high to use the glucometer used in this study to obtain accurate values.

It is well known that dietary glucose can be reduced under the combined effect of four major organs including the intestines, liver, pancereas and insulin target tissues. As shown in Figure 1, the aqueous root extract of *Helicteres angustifolia* possessed remarkable activity on the enhancement of glucose consumption, which is almost similar to insulin. The latter is usually used to enhance the glucose uptake and glucose utilization in target tissues, e.g. liver, muscle and adipose tissue. Also, this enhancement effect is similar to the antidiabetic drug of metformin, which could activate the AMP-activated protein kinase (AMPK), thereby promoting glucose uptake in muscle and reducing hepatic glucose output[28]. According to the results from glucose consumption assay, it is conjectured that the aqueous root extract of *Helicteres angustifolia* could enhance glucose utilization in insulin target tissues, thereby improving insulin resistance. Further studies are necessary to make clear whether it could activate the AMPK or not.

Helicteres angustifolia root has been used to effectively treat diabetics by local people in Laos for a long time, and some of its isolated chemical compounds have been shown to exhibit antidiabetic activity. For instance, Gao et al[16] declared that oleanolic acid could reduce glucose blood levels and improved oral glucose tolerance. Betulinic acid was confirmed to have α-glucosidase inhibitory activity[15]. Rosmarinic acid was pointed out to have antidiabetic activity via maintaining glycemic control and regulating the key enzymes of carbohydrate metabolism[17]. Moreover, the current study firstly found that the aqueous root extract of *Helicteres angustifolia* could not only inhibit α-glucosidase activity but also enhance the glucose consumption in C2C12 myotubes. Therefore, it is speculated that the glucose consumption and α-glucosidase inhibitory activities of aqueous root extract of *Helicteres angustifolia* could be ascribed to the presence of above mentioned compounds in the extract, which is still under investigation in our followed-up experiments. Further qualitative and quantitative chemical analysis of the extract is also necessary for the exploration and development of effective natural products to treat diabetics.

Conclusions

Helicteres angustifolia aqueous root extract could enhance glucose consumption in skeletal muscle and inhibit α-glucosidase activity, while with low cytotoxic and acute toxicity. This study for the first time disclosed that *Helicteres angustifolia* root exhibited antidiabetic activity, providing a scientific basis for treating diabetics by using this plant in Laos.

Conflict of Interests

The authors declare no conflicts of interest.

Acknowledgment

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