The Hypoglycemic Effects of Camellia assamica var. kucha Extract

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Kucha (Camellia assamica var. kucha) is an endemic tea that grows in cloudy and foggy highlands in southwest China. Its hypoglycemic effects were studied here. The postprandial blood glucose levels in mice after sucrose and soluble starch loading were significantly reduced by Kucha administration. The glycosidase inhibitory activities were effective both in vitro and in vivo. It is concluded that the hypoglycemic effects of Kucha might be due to inhibition of disaccharidases activities.

Key words: Camellia assamica var. kucha; carbohydrate tolerance; disaccharidase

Camellia assamica var. kucha (Kucha) is a wild species of Camellia sinensis L. in Jinping, China.1,2 The leaves of Kucha are consumed as a healthy beverage, and the local people have drunk it for a long time. Recently, epidemiological research revealed that there were significantly lower incidences of hyperglycemia and diabetes mellitus patients in these areas.3,4 However, experimental research on the hypoglycemic action of Kucha has not yet been reported. In this study, we examined the effects of Kucha on blood glucose levels and the inhibitory activities of related glycosidases.

Kucha leaves were obtained from plants growing in the wild in the Jinping highlands of Yunnan Province, southwest China, in April 15, 2007. Dry leaves were treated 3 times with 30 parts of hot water for 45 min at 80 °C. After filtration and evaporation of water, the residue was powdered under freezing-decompression conditions. The recovery ratio of Kucha was 21.3%, and the extract was immediately dissolved in water before the experiments. For quality control of Kucha, the chemical pattern of Kucha extract was obtained by RT-HPLC analysis under the conditions used by Yang et al.51 (Fig. 1).

Seven-week-old male ICR mice were purchased from Guangdong Medical Laboratory Animals Center (Guangzhou, China). The animals were housed in groups inside plastic cages and kept in a dedicated pathogen-free animal room at 23 ± 1 °C at a humidity of 70% under a 12 h light-dark cycle, lights on from 6:00 to 18:00. They were provided standard laboratory chow (GB 14924-2001, Guangdong Medical Laboratory Animals Center, China) and water. The animals were acclimated for 1 week before the experiments. The care and treatment of animals conformed to the “Guide for Care and Use of the Laboratory Animals” published by the U.S. NIH (publication no. 85–23, revised 1996). The care and treatment of animals conformed to the ‘Guide for Care and Use of the Laboratory Animals’' published by the U.S. NIH (publication no. 85–23, revised 1996). The care and treatment of animals conformed to the ‘Guide for Care and Use of the Laboratory Animals’' published by the U.S. NIH (publication no. 85–23, revised 1996). The care and treatment of animals conformed to the ‘Guide for Care and Use of the Laboratory Animals’' published by the U.S. NIH (publication no. 85–23, revised 1996).

The effects of Kucha on postprandial glucose levels are shown in Fig. 2. There was no statistically significant difference in initial basal blood glucose levels between the groups studied. The peak time for blood glucose concentration was 15 min for glucose and sucrose tolerance, but 30 min for starch tolerance. As shown in Fig. 2A, no statistically significant difference was observed in the blood glucose concentrations of the different groups after glucose loading. However, the blood glucose concentrations of 6.25 mg/kg of acarbose, 125 mg/kg of Kucha, and 500 mg/kg of Kucha to the mice, which had been starved overnight for 18 h (15:00–9:00). Data are presented as mean ± S.E.M. Two-way analysis of variance (ANOVA) was applied to analyze the differences in biochemical parameters among the experimental groups, followed by Dennett’s test for pair-wise multiple comparisons. Differences were considered statistically significant at p < 0.05.

As shown in Fig. 1, many tea polyphenols and methyl xanthine alkaloids, which might be the active components of Kucha, were separated. These results indicate that the Kucha decoction caused significant decreases in the hyperglycemic peaks during the sucrose and starch tolerance tests, like the drug acarbose, but not on the glucose tolerance test. Hence, glycosidase inhibitory activities both in vitro and in vivo were evaluated for hypoglycemic effects.

Abbreviations: Caff, caffeine; Tb, theobromine; Tp, theophylline; Tc, theacrine; GA, gallic acid; C, (-)-catechin; EC, (-)-epicatechin; GC, (+)-gallocatechin; ECG, (-)-epicatechin gallate; GCG, (-)-gallocatechin gallate; EGCG, (+)-epigallocatechin gallate; CG, (-)-catechin gallate; EGCG, (+)-epigallocatechin; PNPG, p-nitrophenyl-α-D-glucopyranoside
The intestinal mucosa glycosidase inhibitory activities of \( \alpha \)-glucosidase, sucrase, \( \alpha \)-amylase were measured by the method of Matsumoto et al.\(^{11}\) As shown in Fig. 3, compared to the control group, both 125 and 500 mg/kg Kucha significantly inhibited small intestine mucous membrane glycosidase activities in a dose-dependent manner \((p < 0.01)\). The inhibitory activities of acarbose showed similar potency. On the other hand, the inhibitory activities on glycosidases \( \text{in vitro} \) were assayed by the chromogenic method.\(^{12}\) The IC\(_{50}\) values for acarbose on \( \alpha \)-glucosidase, sucrase, and \( \alpha \)-amylase were 1.13, 0.36, and 2.53 mg/ml respectively. The IC\(_{50}\) values for Kucha were 1.95, 0.21, and 2.57 mg/ml, which indicates that Kucha had glycosidase inhibitory effects. The inhibitory activities of Kucha on glycosidases \( \text{in vitro} \) and \( \text{in vivo} \) were similar to acarbose, while the dosage of Kucha was much higher than acarbose \( \text{in vivo} \). This might be due to the complexity and diversity of Kucha’s components and activities.

As is well known, \( \alpha \)-glucosidase plays a major role in degradation of starch and oligosaccharides to monosaccharides before absorbance. It is proposed that suppression of the activities of such digestive enzymes delays the degradation of starch and oligosaccharides. Furthermore, the inhibitor of \( \alpha \)-amylase delayed carbohydrate digestion and prolonged the overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting of the postprandial plasma glucose level. A report of Matsumoto indicated that \( \alpha \)-amylase activity can be depressed by tea polyphenols,\(^{13}\) and the mechanism is probably inhibition of the hydrolysis of \( \alpha \)-amylase and reduction of the glucose transporter of intestinal epithelial cells by the ECG and EGCG of tea polyphenols.\(^{14}\) In addition, sucrase might also have been strongly inhibited by the tea polyphenols in Kucha. Since sucrase is a characterized small-intestinal disaccharidase, it played important roles in carbohydrate digestion by forming a complex enzyme (SI complex) on the brush border membranes. It has been reported that the activity of sucrase, as well as other disaccharidases, is abnormally high in diabetic animals and human diabetes mellitus patients.\(^{15}\)

In conclusion, our aim was to study the hypoglycemic activity of Kucha and to provide an approach to the evaluation and validation of the properties of this plant in diabetes prevention. To the best of our knowledge, this is the first study to investigate and analyze the hypoglycemic activity of Kucha. The mechanism of the hypoglycemic effect of Kucha might be related to inhibition of glycosidases activities. The hypoglycemic effects were the results of delayed hydrolysis of polysaccharides and glucose absorption in the intestinal mucosa.

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**Fig. 1.** Chemical Fingerprint of Kucha Analyzed by HPLC.
A. Thirteen standard compounds, and B. Kucha extract. The analysis was performed with a Cosmosil 5PE-MS column (4.6 mm × 150 mm) at 40 °C. Compounds were eluted (solvent A, 5:95:0.05 v/v/v acetonitrile:H\(_2\)O:85% H\(_3\)PO\(_4\); solvent B, 50:50:0.05 v/v/v acetonitrile:H\(_2\)O:85% H\(_3\)PO\(_4\)) at a flow rate of 1 ml/min using a gradient program (10% solvent B content at 0 min, 10% at 5 min, 30% at 8 min, 30% at 10 min, 80% at 15 min, and 80% at 23 min), and detected at a wavelength of 231 nm.
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References


Fig. 2. Effects of Kucha on Carbohydrate Tolerance Tests. A, Glucose tolerance test; B, sucrose tolerance test; C, starch tolerance test. Oral administration of 125 mg/kg of Kucha, or 500 mg/kg of Kucha, 6.25 mg/kg of acarbose, or water was performed on mice that had been deprived of food for 18 h. Solutions of 20% glucose, 40% sucrose, and 60% soluble starch were given 30 min after drug administration at 10 ml/kg of body weight respectively. Blood glucose levels were analyzed chronologically, and the results were represented as mean ± S.E.M. of the blood glucose levels obtained from seven animals each time. The significance of difference from the normal control group was represented as *p < 0.05, **p < 0.01.

Fig. 3. Effects of Kucha on Glycosidase of the Small Intestine Mucous Membrane. The activities of the small intestine mucous membrane were measured 30 min after administration of 125 mg/kg of Kucha, or 500 mg/kg of Kucha, 6.25 mg/kg of acarbose, or water. The results were represented as the mean ± S.E.M. from the various experiments. Each experiment was performed with seven mice in each group, and significance of difference was compared with the control group at *p < 0.05, **p < 0.01, and ***p < 0.001.