

Effects of *Kaempferia parviflora* Extract on Glucose Transporters in Human Renal Proximal Tubular Cells

Natechanok Thipboonchoo*, Sunhapas Soodvilai*

Abstract

Kaempferia parviflora Wall. Ex. Baker is the one herb widely used as food supplements and for therapeutic purposes, including antioxidant, anti-inflammation, antiobesity, and antidiabetes. Kidney plays a crucial role in glucose reabsorption which is mainly mediated by the function of glucose transporters named sodium glucose co-transporter 2 (SGLT2) and facilitated glucose transport 2 (GLUT2). Therefore, blocking of SGLT2 is a potential target of anti-diabetes. This study was performed to determine whether *K. parviflora* extract (KPE) and its active compound (5, 7-dimethoxyflavone; DMF) inhibit SGLT2 and GLUT2 in human renal proximal tubular cells (HK-2 cells). The effects of KPE and DMF on SGLT2- and GLUT2-mediated [³H]-2-deoxyglucose (2DG) uptake were measured. The results showed that KPE inhibited SGLT2- and GLUT2-mediated [³H]-2-DG uptake with half maximal inhibition concentrations (IC₅₀) of 124 µg/ml and 62 µg/ml, respectively. In addition, DMF at 50 µM significantly inhibited SGLT2 and GLUT2 transport functions. Treating the cells with KPE for 24 hours inhibited SGLT2 and GLUT2 transports. The inhibitory effects of KPE and DMF were not the results from cytotoxicity as the evidence showed that KPE and DMF did not reduce cell viability. In conclusion, KPE and DMF inhibit SGLT2- and GLUT2-mediated glucose transport in human renal proximal tubule.

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Introduction

Diabetes mellitus (DM) is the major long-term life threatening nowadays because of changing lifestyle and its trend to increase every year.¹ There are two major types of DM, type 1 DM and type 2 DM (T2DM). T2DM is the most common type of DM.² Elevation of blood glucose in DM patients can worsen the whole body by increasing ROS production and causing complications, such as retinopathy, nephropathy, neuropathy, and cardiovascular disease.³ It is important to have DM well-controlled. There are six current oral antihyperglycemic drugs, namely, α glycosidase inhibitor (AGI), glucagon like peptide 1 (GLP 1) analog, dipeptidyl peptidase 4 inhibitor (DPP4i), sulfonyl urea or glinide, biguanide, and peroxisome proliferator-activated receptor γ (PPAR γ) agonist.⁴ However, these drugs cannot prevent the progression of T2DM, overweight, β cells mass and function loss, and hypoglycemic effect.⁵

Kidney plays a role in glucose homeostasis. It reabsorbs all filtered glucose; 90% of filtered glucose is reabsorbed by sodium glucose cotransporter 2 (SGLT2), and the uptaken glucose gets into the plasma via glucose transporter 2 (GLUT2). The remaining 10% is contributed by SGLT1 at S2/3 segment, in concomitant with GLUT1.⁶ In diabetes, SGLT2 is expressed more, resulting in worsening diabetes.⁷ Therefore, SGLT2 inhibition is a good target for reducing blood glucose. Recently, inhibition of SGLT2 has been found to be the new drug target for T2DM treatment.⁸ This new drug is believed to solve previous problems because its action is independent from insulin action and produce no hypoglycemic effect.⁵

Kaempferia parviflora wall. Ex. Baker, a member of Zingiberaceae family, is widely cultivated in Thailand and Laos. Rhizomes extract of *K. parviflora* (KPE) is used as folk medicine. KPE has many physiological effects including antiobesity,⁹ anti-hyperglycemia,^{10,11} and improving vascular function.¹² A previous study showed that KPE and its active compound (5,7-dimethoxyflavone; DMF) improved obesity by reducing many factors for adipogenesis and adipocytes hypertrophy.¹³ Antidiabetic and antiobesity effects of KPE in TSOD mice, a spontaneous type 2 diabetic model, were also reported by Shimada and coworkers.¹⁰ Moreover, KPE reduced hyperglycemia and fat accumulation in mice fed with a high fat, high sucrose diet.¹¹ KPE and DMF might reduce hyperglycemia by activating AMP-activated protein kinase (AMPK) signaling,

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*Department of Physiology, Faculty of Science, Mahidol University, Bangkok, Thailand.

Corresponding author: Sunhapas Soodvilai, PhD
E-mail: sunhapas.soo@mahidol.ac.th

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resulting in increased glucose uptake by increasing glucose transporter 4 (GLUT4) expression, and increased glucose and lipid metabolism through increasing peroxisome proliferator-activated receptor- γ coactivator (PGC)-1 α activity in skeletal muscle cells.¹⁴ Moreover, dichloromethane and ethyl acetate extract of *K. parviflora* was reported to have α -glucosidase inhibitory activity.¹⁵ However, glucose lowering process requires many sites of action in the body, for instance, pancreas, liver, adipose tissue, muscle, and kidney. It is possible that KPE and DMF may inhibit SGLT2/GLUT2 and subsequently reduce the plasma glucose. Thus, it is imperative to examine the effects of KPE and DMF on glucose transport in renal proximal tubular cells.

Materials and Methods

Chemicals

3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) and 5,7 dimethoxy flavones (DMF) were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). Deoxy-D-glucose, 2-[1,2-³H(N)] (20 Ci/mmol) was purchased from Perkin Elmer (Boston, MA, USA). Rhizomes of *K. parviflora* were purchased from a fresh market named "Talat Thai." The ethanol extract of *K. parviflora* was kindly provided by Prof. Pratoomratana Tuchinda from Department of Chemistry, Faculty of Science, Mahidol University, Thailand. All other chemicals used are analytical grade and purchased from commercial sources.

Cell culture

The human renal proximal tubular cell (HK-2) line was obtained from ATCC (Manassas, VA, USA) cultured in DMEM-low glucose medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin G, and 100 μ g/ml streptomycin. HK-2 cells were maintained in a humidified atmosphere at 5% CO₂ and 37°C.

Cell viability assay

Cell viability was evaluated by MTT assay. HK-2 cells (4×10^5 cells/well) were seeded into 96-well plates and incubated in the 5% CO₂ incubator at 37 °C. The confluent cell monolayer was treated with several conditions. At the end of incubation periods, the cell monolayers were incubated with 100 μ l MTT reagent (0.5 mg/ml serum free media) for 4 h at 37°C, followed by the addition of 150 μ l of DMSO to dissolve the formazan crystals. MTT-reducing activity of the cells was determined using a microplate reader (Model: Spectra Max-M3) at 540 nm. Cell viability was calculated as percent of control (vehicle-treated groups).

Glucose uptake assays

HK-2 cells were seeded on 24-well plates with 1:4 seeding ratio. After confluence, cells were washed twice with warm glucose transport buffer (GB) [GB:

(in mM): 10 HEPES, 150 NaCl, 5 KCl, 5 MgSO₄, 1.2 KH₂PO₄, and 2.5 CaCl₂, pH 7.4] and incubated for 20 minutes. To measure SGLT2 and GLUT2 functions, the cells were incubated for 30 minutes in GB containing ~10 nM [³H]-deoxy-D-glucose ([³H]-2DG) in the presence of 10 μ M cytochalasin B (an inhibitor of GLUTs) or 200 μ M phlorizin (SGLTs inhibitor) for measurement of SGLT2- and GLUT2-mediated transports, respectively. The reaction was stopped by washing 3 times with cold GB. After that, cells were lysed using 0.4 M NaOH in 10% SDS for at least 6 hours. Cells were normalized using 1 M HCl. Next, cells were harvested and added with scintillation liquid. The radioactivity was measured by using a β counter.

Statistical analysis

The results of all experiments were expressed as mean \pm SEM and analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Test. Data were plotted using GraphPad Prism 5.0 for Window XP. The level of significance for statistical tests was accepted at *P* value < 0.05.

Results

Inhibitory effect of KPE and DMF on SGLT2 transport function

The inhibitory effect of KPE and DMF was determined. Confluent cells were incubated with GB

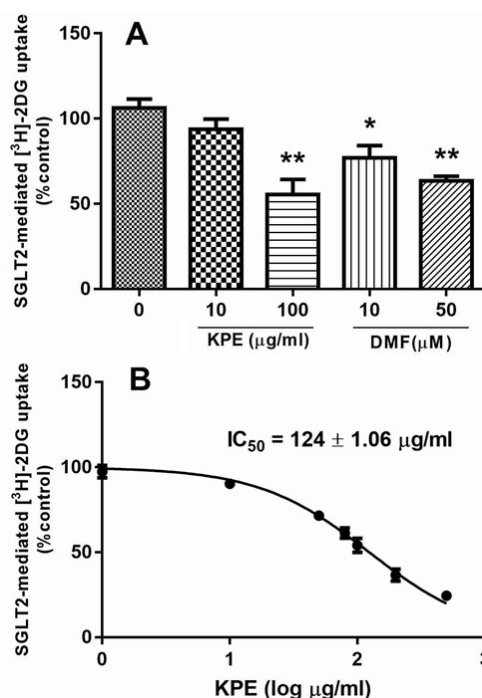


Figure 1 Effect of KPE and DMF on SGLT2-mediated [³H]-2DG uptake. (A) HK-2 cells were incubated with [³H]-2DG alone, in the presence of KPE (10 and 100 μ g/ml), or DMF (10 and 50 μ M), for 30 minutes. (B) HK-2 cells were incubated with [³H]-2DG with KPE (1-500 μ g/ml) for 30 minutes. The data are mean \pm SEM from three different experiments. **P* < 0.05, ** *P* < 0.01.

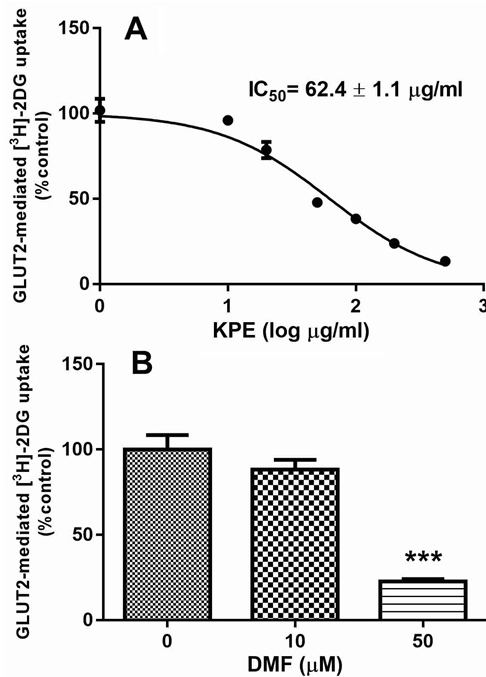


Figure 2 Effect of KPE and DMF on GLUT2-mediated [3H]-2DG uptake. SGLTs and GLUTs activities were elicited by adding 200 µM phlorizin and 10 µM cytochalasin B, respectively. (A) HK-2 cells were incubated with [3H]-2DG alone or with various concentration of KPE (1-500 µg/ml) for 30 minutes. (B) HK-2 cells were treated with [3H]-2DG in the present of vehicle or DMF (10 and 50 µM) for 30 minutes. The data are mean ± SEM from three different experiments. *** $P < 0.001$.

containing [3H]-2DG alone, 100 µg/ml KPE, or DMF (10 and 50 µM) for 30 minutes. The results showed that (Figure 1A) 100 µg/ml KPE and DMF significantly reduced SGLT2-mediated [3H]-2DG uptake ($P < 0.05$). KPE inhibited SGLT2-mediated [3H]-2DG uptake with a half maximal inhibition concentration (IC_{50}) of 124 µg/ml (Figure 1B). The IC_{50} of DMF for SGLT2 inhibition could not be analyzed due to the limited solubility.

Inhibitory effect of KPE and DMF on GLUT2 transport function

Effect of KPE and DMF on GLUT2 was examined by glucose uptake assay. Confluent cells were incubated with GB, various dose of KPE (1-500 µg/ml), or DMF (10 and 50 µM) for 30 minutes. The results showed that KPE inhibited GLUT2-mediated [3H]-2DG uptake with an IC_{50} of 62 µg/ml (Figure 2A). DMF significantly inhibited GLUT2-mediated [3H]-2DG uptake at a dose of 50 µM ($P < 0.001$) (Figure 2B).

Treatment of KPE for 24 hours inhibited SGLT2 and GLUT2 activities

The cells were incubated with vehicle or KPE (10 and 50 µg/ml) for 24 hours, followed by measurements of SGLT2- and GLUT2-mediated [3H]-2DG uptake. As shown in Figure 3, KPE significantly inhibited SGLT2 and GLUT2 transport function ($P < 0.01$).

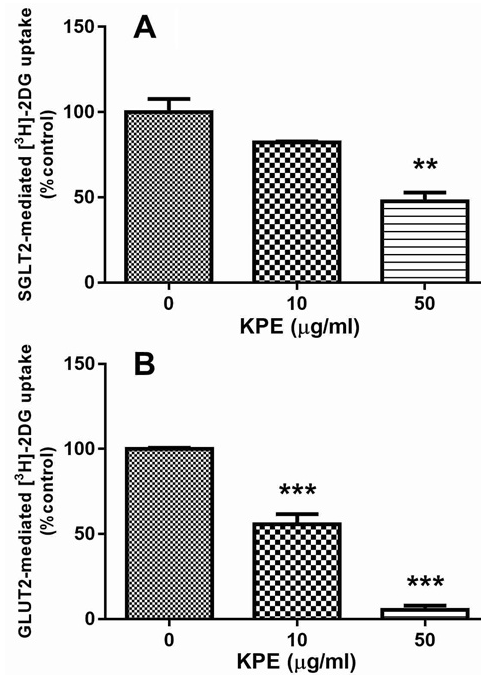


Figure 3 Effect of a 24-hour treatment of KPE on SGLT2 and GLUT2 activities. HK-2 cells were treated with KPE (10 and 100 µg/ml) for 24 hours, followed by measurements of (A) SGLT2- and (B) GLUT2-mediated [3H]-2DG uptake. The data are mean ± SEM from three different experiments. ** $P < 0.01$, *** $P < 0.001$.

Effect of KPE and DMF on cell viability

HK-2 cells were treated with KPE (10-50 µg/ml) or DMF (10-50 µM) for 24 hours, followed by a measurement of cell viability using MTT assay. As shown in Figure 4, KPE and DMF did not reduce the viability of HK-2 cells.

Discussion

T2DM is worldwide life threatening disease. Although, there are many types of drugs for diabetic treatment, they cannot prevent progression of the disease.⁵ SGLT2 inhibitor, a new type of antidiabetic

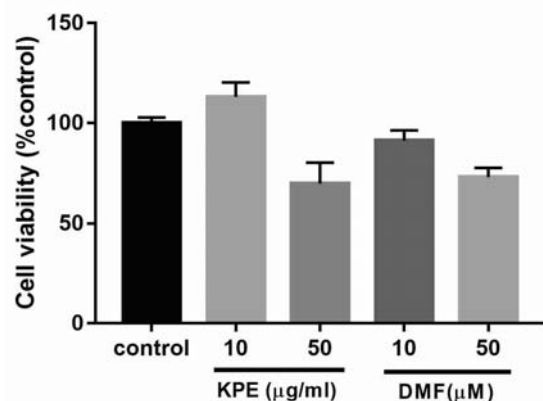


Figure 4 Effect of KPE and DMF on cell viability. Confluent HK-2 cells were treated with KPE (10-50 µg/ml) or DMF (10-50 µM) for 24 hours. The data are mean ± SEM from three different experiments.

drug, is believed to solve this problem. KPE has been shown to reduce plasma glucose of diabetic rats,^{10,11} however, the underlying mechanism of KPE on diabetes is unknown. Here, the effects of KPE and DMF on renal glucose transporters were investigated. We first determined whether KPE inhibited SGLT2-mediated [³H]-2DG uptake in renal proximal tubular cells that express both SGLT2¹⁶ and GLUT2.¹⁷ Incubating HK-2 cells with an ethanol extract of *K. parviflora* significantly inhibited SGLT2-mediated glucose transport. These data indicated that the glucose lowering effect of KPE might be mediated by inhibition of renal glucose reabsorption. KPE is composed of two major compounds, 5,7-dimethoxyflavone (DMF) and 5,7,4'-trimethoxyflavone (TMF).¹⁸ We tested whether DMF, a commercially available compound, inhibited SGLT2 function. Data showed that DMF also inhibited SGLT2-mediated glucose transport indicating that DMF found in KPE exhibited an inhibitory effect on SGLT2, although we could not rule out that other compounds in the composition of KPE may inhibit SGLT2. The inhibitory effects of KPE and DMF on SGLT2 were not mediated by their toxicity, as our evidence showed that KPE and DMF did not reduce HK-2 cell viability. Renal glucose reabsorption depends on a sequential transport of glucose across apical membrane (via SGLT2) and basolateral membrane (via GLUT2).⁵ Thereby, we further determined the effect of KPE and DMF on GLUT2-mediated glucose transport. KPE and DMF significantly inhibited GLUT2 transport function, as found with SGLT2. These data indicated that KPE and DMF inhibited both steps of renal glucose reabsorption. Next, we also determined whether prolonged KPE and DMF exposure can produce their inhibitory effect. It was found that HK-2 cell incubation with KPE and DMF for 24 hours also significantly reduced the transport function of SGLT2 and GLUT2.

In summary, KPE and DMF inhibited SGLT2- and GLUT2-mediated [³H]-2DG uptake in renal proximal tubular cells. To prove the potential application of KPE as anti-hyperglycemic agent, the effect of the compounds need to be studied in animal models of diabetes.

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Conflict of Interest

None to declare.

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