AMPK-mediatedhy Poglycemic Effect of Banana Stem Juice on Type 2 Diabetes

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Musa paradisiaca L. (Musaceae) or banana is a common plant in the tropics. Its stem juice has been long used as a traditional cure for diabetic people in several tropical countries. Its hypoglycemiceffect has been reported on some experimental hyperglycemic models. However, there has not been any study revealing the hypoglycemic effect and mechanism of banana stem juice in type 2 diabetes. The current study aimed atdiscovering its effects by a glucose tolerant test on experimental type 2 diabetic rats and an *in vitro* test on adenosine monophosphate - activated protein kinase (AMPK), a key enzyme in metabolic regulation.The glucose tolerance test was done after 2 weeks of banana stem juice treatment. The glucose AUC of the treated rats was significantly lower (p<0.05%) compared with that of the controls.At the concentration of $50 \mu g/ml$, the banana sample significantly increased the quantity of phosphorylated AMPK,the active form of AMPK, in C2C12 skeleton cells(p<0.001). the glucose tolerance enhancing effect of the banana stem juice was explained by the activation of AMPK which plays anessential role in metabolic homeostasis.

Keywords: *Musa paradisiaca, stem juice, type 2 diabetes, AMPK, glucose tolerance.*

Musa paradisiaca (M. paradisiaca) L. (Musaceae) or banana is a common plant in the tropics. Stem juice of *M.paradisiaca* has been long used as a traditional cure for diabetic people in several tropical countries, including Vietnam. It was not until recently, in the global trend of using natural antidiabetic productswhich are safe, effective and at reasonable costs, banana became the focus of scientists[1-3]. In our preliminary studies, banana had significant hypoglycemic effects on both streptozocine (STZ)-induced hyperglycemic mice and high fat (HF) diet-induced type 2 diabetes(T2D) mice (data not shown). Similarly, in the study of Eleazu et al, STZ-injected rats fed with *M. paradisiaca* had notably lower fasting blood glucose (FBG) level compared to those fed with standard diet[1]. Moreover, Ajiboye reported that *M. paradisiaca*feeding significantly reduced FBG level, with remarkable elevation in insulin and glycogen levels in alloxan-induced hyperglycemic rats[2]. However, there have not been any study revealing the hypoglycemic effect and mechanism of banana stem juice in type 2 diabetes.

Adenosine monophosphate - activated protein kinase (AMPK)emerges as an important target for type 2 diabetes treatment due to its function as anessential mediator of energy metabolism anda regulatingcenter of mitochondrial

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dynamics[4].AMPK activation in skeletal muscle enhances glucose disposal and lowered plasma glucose level in diabetic mice [5].A number of antidiabetic agents, including metformin, the most important drug in T2D, have AMPK-dependent mechanisms[6].

The aims of this study isdiscoveringthe antidiabeticeffectsof *Musa paradisiaca*stem juice by a glucose tolerant test on experimental T2D rats and an *in vitro* test on AMPK activation.

METHODS

Sampling: Stems of *Musa paradisiaca* L. were collected from Bacninh province, Vietnam after being authenticated by the Department of Botany, Hanoi University of Pharmacy, Hanoi, Vietnam with voucher number HNIP18149/16. The samples were then crushed and pressed to obtain the juice. The filtered juice was evaporated to residue at reduced pressure(yield was 1.6%). The residue was suspended in a 0.5% NaCMC solution to a 50 mg residue/ml suspension for the *in vivo* test. The residue was suspended in 1% DMSO for the *in vitro* test.

Animals:Healthy adult male *Wistar albino* rats weighing 80±20g were used. The rats were kept under standard conditions (25 ± 5°C;12-h light and 12-h dark cycle; 35-60% humidity) and were providedwith water *ad libitum*.

Glucose tolerant test: T2D was induced by the method previously described bySrinivasan[7]with a combination of HFdiet and low dose of streptozocine (STZ). The T2D rats were divided into 4 groups (n= 8). Group 1 includednormal rats administered with vehicle;group 2wasT2D rats administered with vehicle;group 3wasT2D ratstreated with the banana samplesuspension at the dose of 10 ml/kg (equivalent to 500 mg residue/kg), group 4 was T2D rats treated with metformin (120 mg/kg). After 15 days of treatment, anoral glucosetolerance test (OGTT) was done according to the method described by Kwon[8]. FBG levels was determined using GOD method (Accu-check Active, Roche) at 0; 30; 60; 90 and 120 minutes after the glucose loading. The increment in blood glucose level (FBG) after the glucose loading was expressed byarea under the curve (AUC).The*in vivo*research was complied with all the relevant national and institutional regulations for the care

and use of animals and was approved by the IACUC committee of the HaiduongCentral College of Pharmacy.

Cell Culture and Differentiation: Mouse C2C12 skeletal myoblasts (Clone CRL-1772) (ATCC) were cultured in DMEM with a supplement of100 u/ml penicillin; 100 mg/ml streptomycin, 10% fetal bovine serum (Invitrogen) at 37°C in an incubator with humidified atmosphere containing 5% CO_2. The cells were reseeded in six-well plates for a further 24h incubation and then the medium was switched to the differentiation medium (5% horse serum added DMEM) for 72h. Before experiments,the samples were added into the wells for 1h incubation.

Western Blot (WB): The C2C12 cells were washed twice with ice-cold PBS and then lysed with ice-cold ECB (50 mMTris-Cl, pH 7.4, 120 mMNaCl, 1mM EDTA, 0.5% Nonidet P-40, 50 mMNaF) for 30 min. The lysates were then centrifuged at 12,000xg for 15 min at 4°C for supernatants. 30 mg of the total proteins were resolved by 12% SDS-PAGE gel before transferringonto PVDF membranes (Millipore). The membranes were incubated with 5% non-fat dry milk and then were probed overnight with phosphorylated-AMPK(p-AMPKá Thr172) antibody(Cell Signaling Technologies)at 4°C, followed by incubation with horse radish anti-rabbit IgG, HRP-linked secondary antibody (Cell Signaling Technologies) for 2 h. Immunoreactive bands were exposed on X-ray film using the enhanced chemiluminescenceWB detection system (GE Healthcare). The band density was quantified using ImageJ software. The level of each protein was normalized to â-actin.

Statistical Analysis: Data were presented as means ±SE. Two-tailed Student's *t*-test was used to compare values between two groups using the SPSS 16.0 software. p-values $p < 0.05$ were considered as statistically significant.

RESULTS

Effect of banana stem juice extract on glucose tolerance of type 2 diabetes rats

After the OGTT, the glucose retention in blood, expressed by AUC, of the T2D rats increased significantly compared with that of the normal control ($p < 0.001$).Treatment with metformin

(group 3) and the sample (group 4) remarkably lowered the AUC of the T2D rats(p<0.05% and p<0.01%, respectively) **(Figure 1)**.

Effect of banana stem juice extract on AMPK of the C2C12 cells

The activation of AMPK was evaluated by the quantity of itsactive form (phosphorylated-AMPK).Different concentrations of the banana samples were incubated with the C2C12 cells. Aicar (5-aminoimidazole-4-carboxamide-1-D-ribonucleoside) was used at the concentration of 1mM as positive control. The p-AMPK quantity of
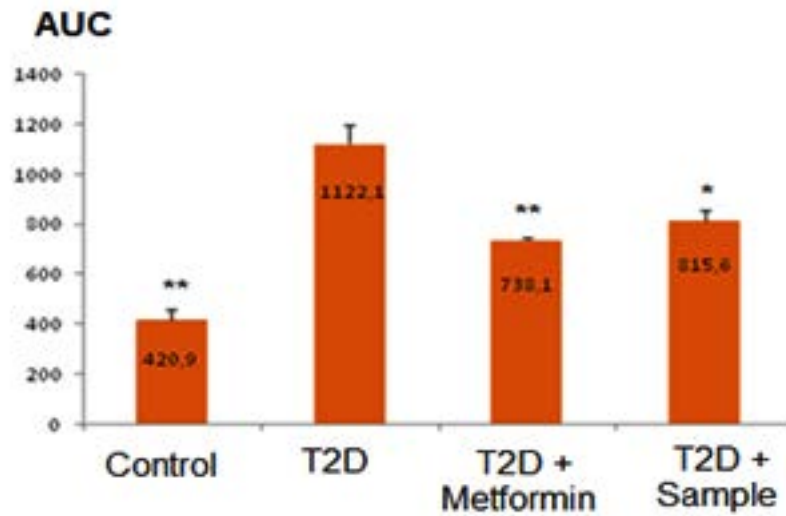
(*): p<0.05; (**) p<0.01
AUC of the rats in group 3 (metformin treated) and group 4 (sample treated) was significantly lower (p<0.01% and p<0.05%, respectively) compared with that of the group 2 (untreated rats).
Fig. 1. AUC of glucose at 120 minutes after glucose loading
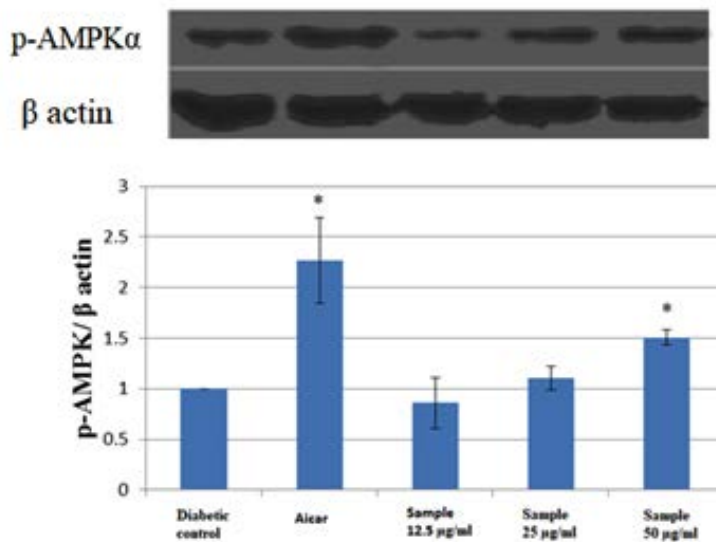
(*): p<0.001. The quantity of AMPK active form (phosphorylated-AMPK) significantly increased in the cells treated with either Aicaror the sample at concentration of 50 μg/ml (p<0.001)
Fig. 2. Activation of AMPK in C2C12 cells

the aicar treated cells doubled that of the untreated cells (p<0.001). There was a gradual increment of p-AMPK following the increment of sample concentration. However only at the concentration of 50 µg/ml,the banana sample significantly increased the quantity of p-AMPK (p<0.001) **(Figure 2)**.

DISCUSSIONS

There have been a number of studies on the effect of various parts of *M. paradisiaca* on glucose tolerance of STZ-induced hyperglycemic rats[9, 10]. However, this is the first study on *M. paradisiaca* effects on glucose tolerance of HF diet–induced T2D rats.This model which was established by a complex of insulin resistance due to obesity and impaired pancreas function due to a low dose of STZ are more stable and more resemble to human type 2 diabetes pathology[11, 12].In the current study, there was a significant increase in the glucose AUC after oral glucose loading in the T2D rats compared with the non-diabetic rats. This effect was also reported by various studies with HF diet-induced type 2 diabetes[7, 13].On such T2D rats with impaired glucose tolerance, both the banana extract sample and metformin significantly reduced the glucose retention at 120 minutes after the glucose load.The benefits of banana stem juice on glucose tolerance was also reported on STZ-induced hyperglycemic rats[10]. The amelioration of glucose tolerance may be due to the enhancement of glucose utilization in the body tissues of diabetes rats. In fact, metformin is well knownfor glucose homeostasis effectsmediated by AMPK activation[6, 14]. That's why we continued to evaluate the *in vitro* effect of the banana extract on AMPK.

AMPK plays a central role in metabolic regulation, includingthe metabolism of protein, lipid glucose and autophagy/ mitochondrial homeostasis[6].Once activated, AMPK phosphorylates and activatesglucose transporters, thereby increasing glucose uptake into cells and on the other hand, inhibits expression of the gluconeogenic enzymes[4]. Therefore, AMPK has emerged as a target for diabetes and other metabolic syndrome[15].Notably, AMPK is among the most essential targets in T2D treatmentbecause its function as an energy sensor of the cell and its activation modulate a number of downstreamproteins which are also T2D targets such asglucose transporter 4 (GLUT4),Acetyl-coA Carboxylase (ACC),glucose-6-phosphatase (G6Pase)phosphoenol pyruvate carboxykinase (PEPCK)[16]…One of the most important mechanism of metformin, the first line T2D medicine is AMPK activation[6]. A number of antidiabetic herbal medicinesor natural compounds such as resveratrol, epigallocatechin gallate, berberin,*curcumin,* ginsenosides from *Panax ginseng*have shown *in vitro*AMPK activation effect[17].AMPK activity is stimulated more than 100folds by phosphorylation of Thr[172] residue[18, 19].Therefore, in order to evaluate the effect of the banana extract on AMPK, we used a specific p-AMPK Thr172 antibody for the immunoblotting quantification of AMPK activation in C2C12 muscle cells.Aicar, the firstly discovered pharmacological activator of AMPK which mimics all effects of AMP on the AMPK system[20]was chosen as the positive control. Both Aicar and the banana extract sample at the concentration of 50 µg/ml remarkably increased the p-AMPK quantity (p<0.001). The ability of *M. paradisiaca* to activate AMPK in the muscle cells help to explain its beneficial effect on glucose tolerance of the type 2 diabetes in the current study and its anti-hyperglycemic effects on various experimental models in general.

It should be noted that banana stem which is a by-product of banana fruit harvestingisvery easy to be collected and used. Therefore, the revealing of antidiabetic effects and mechanisms of banana stemwould open a perspective of utilizing such a cheap and eco-friendly material for diabetes treatment, especially in low and middle income countries.

CONCLUSION

M. paradisiaca stem juice improved glucose tolerance in HF diet-induced T2D rats. The benefit of *M. paradisiaca* stem juice was explained partly by the activation of AMPK which plays a key role in metabolic homeostasis in muscle cells. These results explain the benefits of banana stem juice used as a folklore remedyin T2D people. There should be further studies on the active ingredients involved in its hypoglycemic effects.

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