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Hypoglycemic Effect of Mushroom Extract (SXF) on Type 2 Diabetes Patients and Its Possible Mechanism

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Abstract

It has been long demanded that a better treatment modality for type 2 diabetes mellitus (T2DM) needs to be established, but few suitable regimens have yet been found. We came across the bioactive mushroom extract, SX-fraction (SXF), which appeared to have a hypoglycemic effect. Hence, we investigated if SXF would actually have such an effect, as well as its possible hypoglycemic mechanism. A small-scale clinical study including ten volunteered T2DM patients was conducted. They took a SXF tablet (500 mg) three times a day for 4 weeks, as the fasting blood glucose (FBG) values were measured periodically. The hypoglycemic mechanism of SXF was explored, focusing on the insulin signal transduction (IST) pathway, using skeletal muscle L6 cells *in vitro*. We found that all 10 patients demonstrated the significant decreases in their FBG levels, from an average of 205 mg/dL to 116 mg/dL, in 4 weeks. This ~42% decline in FBG is remarkable and none of participants presented adverse effects. We then found that the glucose-suppressed IST pathway in L6 cells was significantly *activated* with SXF. The three key parameters, insulin receptor (IR), insulin receptor substrate 1 (IRS-1), and protein kinase B (Akt), were all highly phosphorylated and activated. Glucose transporter 4 (GLUT4) was subsequently translocated (to the plasma membrane), and glucose uptake resulted in a ~1.9-fold greater than that of glucose-suppressed cells or 21% higher than that of control (vehicle) cells. In conclusion, SXF demonstrates its hypoglycemic effect on T2DM patients, significantly (~42%) lowering their FBG levels in 4 weeks. Such a hypoglycemic mechanism appears to be associated with *insulin sensitization* through activation of the IST pathway. Thus, SXF could be a natural, safe, and alternative agent for treatment of T2DM patients, improving their diabetic conditions.

Keywords

SX-Fraction, Hypoglycemic Effect, Type 2 Diabetes Mellitus, L6 Cells, Insulin Signal Transduction Pathway, Glucose Uptake

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder of persistent high blood glucose (hyperglycemia), affecting millions of people globally [1]. Nearly 50 million US population might have diabetes (including undiagnosed) this year (2025) and approximately 90% - 95% of diabetes are found to be type 2 diabetes mellitus (T2DM) and the rest (<10%) are type 1 DM [2]. The incidence of T2DM keeps rising every year [3], while the medical and economic burdens are also overwhelmingly increasing annually, going over $400 billion in US [4] [5]. Hence, it is urgently needed to control or stop steadily growing incidence, particularly in *youth* (younger than 20 years).

T2DM is rather a complicated case as no specific or effective treatments are currently available [6], although it can be controlled to a certain extent. It can lead to serious clinical complications, such as retinopathy, neuropathy, nephropathy, etc., resulting in blindness, renal failure, amputation, coma, and even death [7] [8]. The primary problem with T2DM is not insulin deficiency but *insulin resistance*, defined as a condition where the peripheral organs (skeletal muscles and adipose tissue) become "resistant" or "insensitive" to insulin action [6] [9]. As a result, little or no glucose will be taken up by these organs, leading to an accumulation of glucose in the circulation (hyperglycemia). To enhance/improve peripheral insulin sensitivity, some pharmaceuticals have been developed. Sulfonylurea derivatives [10], which primarily stimulate insulin secretion from pancreatic β-cells, have not been as effective as expected. Troglitazone [11] and metformin [12] showed some improvements, but several adverse effects were also reported. In fact, troglitazone has been taken off the US market, due to severe hepatoxicity [11], while metformin has had adverse effects such as lactic acidosis [12]. Thus, establishing alternative means or finding safer and more effective agents has been demanded to overcome insulin resistance.

We have been seeking and working on natural products/agents with hypoglycemic activity, and we came across a mushroom extract known as "SX-fraction (SXF)", isolated from maitake mushroom (*Grifola frondosa*). SXF is a water-soluble bioactive glycoprotein with a ~20,000 Da molecular weight, and a number of scientific/medical studies have been performed on SXF, including its hypoglycemic and anti-diabetic activity on diabetic mice [13]-[15]. For instance, one animal study [13] found that mice fed with SXF for 8 weeks showed significant improvements in the three diabetic parameters, blood glucose (Glc), insulin (INS), and triglyceride (TGL) levels, compared to those in Sham (control) mice fed without SXF (**Figure 1**).

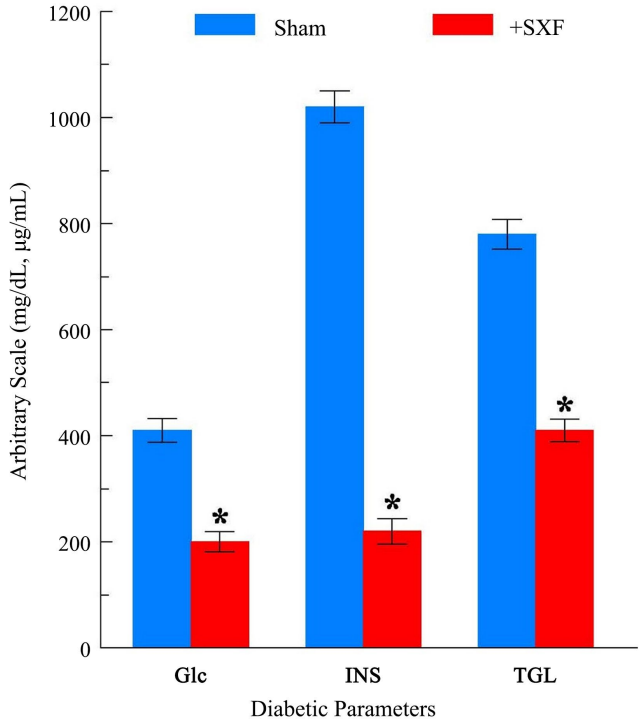

Figure 1. Effects of SXF on three diabetic parameters. Diabetic mice were fed with or without SXF for 8 weeks and the levels of glucose (Glc), insulin (INS), and triglyceride (TGL), were determined (*$p < 0.05$ compared with respective Sham).

These results suggest anti-diabetic effects of SXF in diabetic mice. In addition, how much SXF could be as effective as an anti-diabetic drug clinically used was also examined. Glipizide (Glp), one of oral medications, was chosen to compare its efficacy with SXF. The results showed that SXF more significantly lowered the Glc and Ins levels than Glp in diabetic mice within a week [14], implying that SXF may even have a better efficacy than anti-diabetic drug (Glp).

Nevertheless, it was especially interesting and important to explore the hypoglycemic mechanism of SXF because how it would work had not been fully understood. We hypothesized that SXF might act on the insulin signal transduction (IST) pathway [16], improving *insulin sensitization*. Although it remains unknown how or why insulin-targeted tissues/organs become insulin-resistant, it was apparent that the IST pathway was *not* normally or properly functioning. In particular, the insulin receptor (IR) might play a critical role because it is involved in a committed step (in the IST pathway) and functions through tyrosine kinase (TK) activity [17]. Whether the IR will be activated or inactivated is determined by its *phosphorylation state*—the *phosphorylated* IR is known to be the *active* form [17]. The IST pathway is shown in the simplified diagram (**Figure 2**). Binding of insulin to the IR triggers the IST pathway, by activating (phosphorylating) the IR (seen in Step 1). This IR activation then activates the downstream molecules, such as insulin receptor substrate 1 (IRS-1) (Step 2) and protein kinase B (Akt) (Step 3), subsequently inducing translocation of glucose transporter type 4 (GLUT4) to the plasma membrane (PM) (Step 4). As a result, extracellular glucose will be transported

into or enter the cell through GLUT4 (Step 5). This is how a cascade of signaling events is carried out under a normal condition, but any disruption in the sequence of events would result in the incompletion of the IST pathway (becoming hyperglycemia).

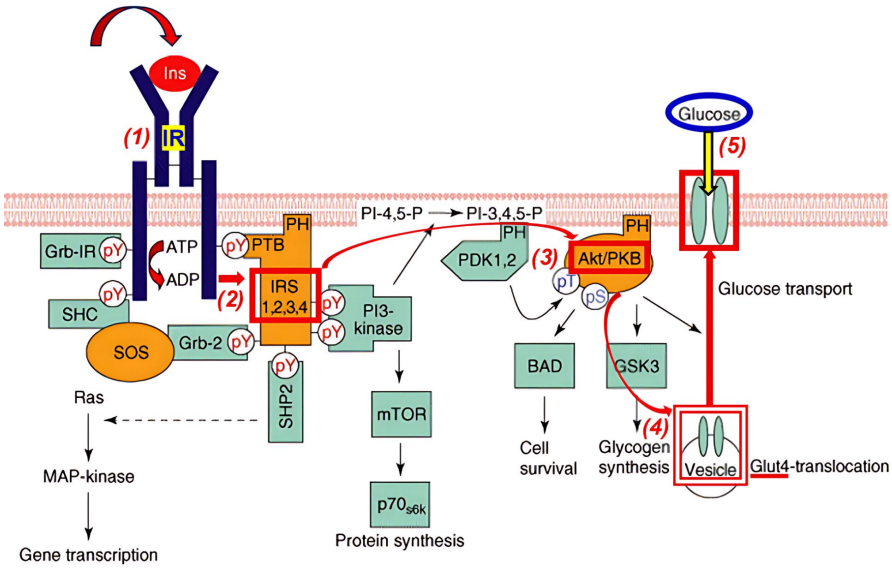

Figure 2. Schematic diagram of insulin signal transduction (IST) pathway, sequentially showing from IR phosphorylation (Step 1) to glucose uptake/influx (Step 5). Courtesy of *Drug Discovery Today*.

Accordingly, we have examined the hypoglycemic effect of SXF on T2DM patients and explored its possible mechanism *in vitro*. Experimental details were described and all 5 steps shown in a diagram (Figure 2) were studied in succession. Additionally, interesting findings were also discussed herein.

2. Materials and Methods

2.1. Hypoglycemic Effect of SXF on Volunteered T2DM Patients

SXF was a kind gift of the manufacturer (Mushroom Wisdom, Inc., East Rutherford, NJ), and ten volunteers with T2DM participated in this study. Actually, it was an "informal" clinical study, which didn't require the approval of Institutional Review Board (IRB). All participants agreed and understood the potential risks involved in the trial. They received a SXF tablet (500 mg) three times a day for 4 weeks. Their FBG levels were measured at a beginning of a SXF trial (Day 0), 2nd week (Day 14), and 4th week (Day 28). What percent (%) of FBG declined with SXF (if any) was also calculated by the differences of FBG values between Day 0 and Day 28. Additionally, a complete glycemic control with SXF during 2 weeks was also presented by one of patients' profiles.

2.2. Cell Culture

For the study of the insulin signal transduction (IST) pathway [16], skeletal muscle

L6 cells (ATCC, Manassas, VA) were used as our *in vitro* model [18]. Cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 μg/mL). They were maintained at 37˚C in a humidified incubator. In experiments, cells were briefly cultured with glucose (Glc), Glc with SXF, or Glc with INS, and activities of three key IST parameters, IR [17], IRS-1 [19], and Akt [20], were assessed as described below.

2.3. Enzyme-Linked Immunosorbent Assay (ELISA) for IR, IRS-1, and Akt

Both IR and Akt assays were performed with some modifications following the manufacturer's protocol (Invitrogen, Carlsbad, CA). First, cell lysates were prepared from control or agent-treated cells by cell lysis using a hypotonic solution. Forty μg of cell lysates was added to the 96-well plate coated with specific antibodies, anti-IR(y) or anti-Akt(s), and incubated for 2 h at room temperature (RT). Antibody detection solution was then added to the plate, followed by 1-h incubation at RT. After discarding the solution, the plate was incubated with antibody conjugate for 30 min, followed by another 30-min incubation with chromogen solution at RT. Stop solution was added to the plate, which was read at 450 nm on a microplate reader.

For IRS-1 assay, all procedures followed the protocol described in the Phospho-IRS-1 (panTyr) Sandwich ELISA Kit (Cell Signaling Technology, Danvers, MA). At the end, all reactions were terminated by adding STOP solution to the plate, and absorbance was taken at 450 nm on a microplate reader. The phosphorylation levels of IR, IRS-1, or Akt were indicated by the intensities of colored products, which were plotted as their OD readings—*the higher color intensity, the greater phosphorylation state*.

2.4. Preparation of Two Cellular Fractions for Western Blot Analysis

To examine the translocation of GLUT4, the plasma membrane (PM) and cytoplasm (CP) fractions were first prepared following the method of Nishiumi and Ashida [21] with some modifications. Briefly, control or agent-treated L6 cells were homogenized in buffer A containing several protease and phosphatase inhibitors. Homogenate was sheared by passing through a 25-gauge needle attached to a 1-mL syringe, followed by centrifugation at 4˚C. The precipitate was resuspended in buffer A and centrifuged again. The precipitate was again resuspended in buffer A and incubated on ice for 1 h. After 1 h, the resuspended precipitate was centrifuged, and the supernatant collected was marked as the PM fraction. For the CP fraction, cells were suspended in lysis (hypotonic) buffer and placed on ice for 1 h with occasional vortexing. They were centrifuged at 4˚C and the supernatant (cell lysates) obtained was referred as the CP fraction. The distribution of GLUT4 proteins in the PM and CP was then analyzed using Western blots. An equal amount (10 μg) of proteins obtained from the PM or CP fraction was first subjected to 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and trans-

ferred to a nitrocellulose membrane. The blot (membrane) was incubated with primary antibody against GLUT4 (Cell Signal Technology) for 90 min, followed by 30-min incubation with secondary antibody conjugates. Specific immunoreactive protein bands (GLUT4) were detected by chemiluminescence following manufacturer's protocol (Seracare, Milford, MA). The expressions of GLUT4 in the PM and CP fractions were seen on autoradiographs.

2.5. Measurement of 2-Deoxyglucose (2-DOG) Uptake

Glucose uptake by L6 cells was performed following the radioligand method of Shrestha *et al.* with some modifications [22]. In this assay, 2-deoxyglucose (2-DOG), instead of glucose, was used because 2-DOG (unlike glucose) cannot be further metabolized once inside the cell. Cells in the 6-well plate were first treated with high Glc (35 mM) for 24 h, and the plate was washed with Krebs-Ringer-Phosphate (KRP) buffer to remove residual Glc. Cells were then exposed to SXF (300 μg/mL) or INS (100 nM) for 15 min, and glucose uptake was initiated by adding a radioactive ligand, 2-deoxy-D-[1-^{3}H]-glucose ([^{3}H]-DOG; specific activity of 10 Ci/mmol), to the plate. After 20-min incubation at 37˚C, cells were solubilized in 0.1 N NaOH. Two hundred μL aliquot of cell lysis was measured for the radioactivity ([^{3}H]-DOG) incorporated into (taken up by) the cells using a scintillation counter. The amount of glucose uptake in radioactive count (cpm) was then expressed by the percent (%) relative to controls (100%).

2.6. Statistical Analysis

All data were calculated as mean ± SD (standard deviation), and statistical differences between groups were assessed with either one-way analysis of variance (ANOVA) or the unpaired Student's *t* test. Values of $p < 0.05$ were considered to indicate statistical significance.

3. Results

3.1. Hypoglycemic Effects of SXF on T2DM Patients

Ten volunteered T2DM patients participated in the study of possible hypoglycemic effect of SXF on their FBG levels. They took a SXF tablet (500 mg) three times a day for 4 weeks, and their FBG levels were measured *before* SXF intake (Day 0), at 2 weeks (Day 14), and *after* SXF trial (Day 28). These results are shown in **Table 1**.

All 10 patients demonstrated a 30% - 63% decrease or an average of ~42% decline in their FBG levels under a SXF regimen in 2 to 4 weeks. In other words, an average FBG of ~205 mg/dL *before* SXF trial has significantly gone down to that of ~116 mg/dL *after* SXF intake for 4 weeks. The actual decreases in the FBG levels were seen in 2 weeks after the first SXF intake, and those values kept declining to 4 weeks. Moreover, none of participants presented palpable ailments or adverse effects related to SXF during the trial, further confirming its safety in human use.

Here is how SXF successfully demonstrated its glycemic control in one of participants.

Table 1. Hypoglycemic effects of SXF on T2DM patients.

Patients	Age (yrs)	Sex	*Before* SXF*	*After* SXF*	% of FBG Declined with SXF
			FBG (mg/dL)		
A	44	M	~260	90 - 100	~63
B	75	F	~200	110 - 130	~40
C	56	F	~220	120 - 130	~43
D	25	F	150 - 180	110 - 120	~30
E	60	M	~210	100 - 130	~45
F	37	M	180 - 200	120 - 140	~32
G	64	F	~220	130 - 150	~37
H	49	M	190 - 200	100 - 120	~41
I	41	M	~210	100 - 110	~50
J	53	F	170 - 190	100 - 110	~42
Mean	50.4	-	**~205**	**~116**	**~42.3**

**Before* SXF and *After* SXF indicate the FBG values measured at Day 0 and Day 28 of a trial, respectively. SXF: SX-fraction; FBG: Fasting blood glucose; M: Male; F: Female.

He was a newly diagnosed T2DM patient with a FBG value of 248 mg/dL and glycosylated hemoglobin A_{1C} (HbA_{1C}) of 11.5%. No retinopathy/neuropathy or other diabetes-related complications were present. He was immediately placed on an oral glyburide (2.5 mg) [10] regimen and his FBG level fell to ~180 mg/dL over the next 2 days. When he also started taking a SXF tablet (500 mg) three times daily with glyburide, his FBG markedly declined to ~100 mg/dL in a couple of days and remained for next 2 weeks (**Figure 3**). This demonstrates the improved glycemic control with SXF added to glyburide.

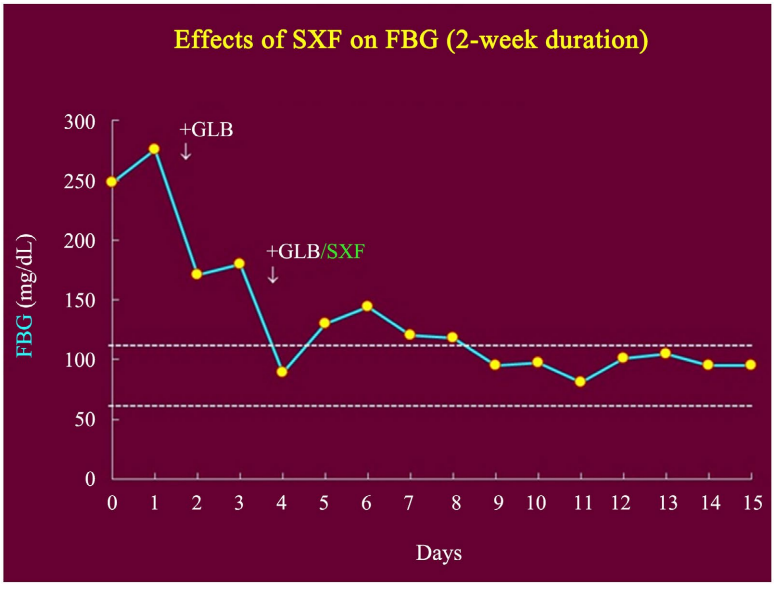

Figure 3. Effects of SXF on FBG in a T2DM patient. The FBG levels of a patient with glyburide (GLB) alone or its combination with SXF were monitored for 2 weeks. The area between two dotted lines is indicative of a normoglycemic status.

3.2. Activation of Insulin Signal Transduction (IST) Pathway with SXF—Phosphorylation of IR and IRS-1

To explore the possible hypoglycemic effect of SXF, the *in vitro* study using skeletal muscle L6 cells was performed. We hypothesized that SXF might be able to *activate* the insulin signal transduction (IST) pathway, overcoming *insulin resistance*, which is believed to be the primary cause of T2DM. Our study focused on the three regulators involved in the pathway, IR, IRS-1, and Akt, whose activation was essential to carry out the consecutive events. We first examined if high Glc (35 mM) would inactivate IR and/or IRS-1, and whether such inactivation could be prevented or reversed with SXF (or INS) was also assessed. L6 cells were treated with high Glc for 24 h, and they were exposed to SXF (300 μg/mL) or INS (100 nM) as a positive control for 15 min. Cell lysates were prepared and subjected to ELISA for IR(y) or IRS-1(y).

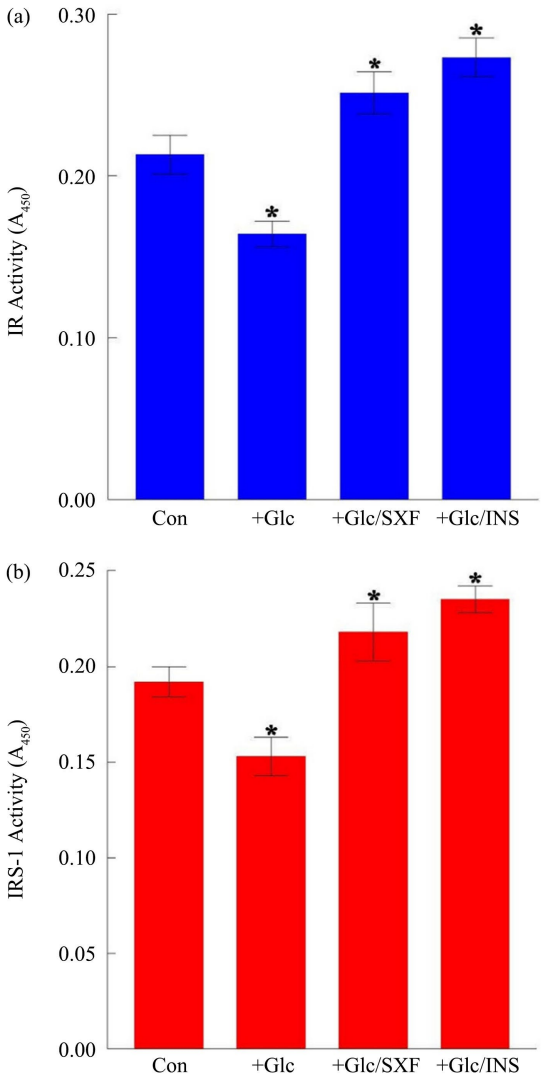

Figure 4. Phosphorylation levels of IR or IRS-1. L6 cells treated with Glc alone or Glc + SXF/INS were subjected to ELISA for phosphorylation of IR (a) or IRS-1 (b). The higher OD reading indicates the greater phosphorylation (activation) level. All data are mean ± SD (standard deviation) from three separate experiments (*$p < 0.05$ compared with control).

As shown in **Figure 4(a)** for the IR(y) assay, the phosphorylation level of tyrosine kinase (TK) in the IR was decreased by ~23% with 24-h Glc treatment, indicating the loss of IR activity. However, SXF was capable of elevating such a reduced IR level to ~18% higher than that of control or ~53% greater than that of Glc-suppressed cells. Similarly, INS raised the IR levels to ~28% and ~67% higher than those of control and Glc-reduced cells, respectively.

For the IRS-1(y) assay, high Glc inactivated IRS-1(y) phosphorylation by ~20% lower in control cells, but SXF raised such a reduced level to ~14% and ~43% higher than those of control and Glc-suppressed cells, respectively (**Figure 4(b)**). INS also elevated the IRS-1 levels significantly higher in control and Glc-reduced cells. Thus, SXF first re-activated Glc-*inactivated* IR, which in turn activated the next molecule, IRS-1.

3.3. Activation of Akt and Translocation of GLUT4 to Plasma Membrane (PM)

To ensure a continuous progress of the signaling cascade, the phosphorylation status of Akt(s) was examined by ELISA on L6 cells treated with Glc, Glc/SXF, or Glc/INS.

The results showed that a ~40% of Akt activity was lost in Glc-treated cells; however, this inactivation was prevented/reversed with SXF, *elevating* it to ~2-fold higher, while the Akt level in control cells was increased by ~20% with SXF (**Figure 5(a)**). INS also raised the Glc-reduced Akt(s) level to ~2.2-fold greater and the Akt level to ~30% higher in control cells.

This activation of Akt is known to be required for executing the final stage of the IST pathway, *i.e.*, translocation of GLUT4 to the plasma membrane (PM) [23]. Such GLUT4 translocation was then examined by analyzing the distribution of GLUT4 in the PM and cytoplasm (CP) on Western blots. The results revealed that GLUT4 (~50 kDa) in control and Glc-treated cells was predominantly seen in the CP (although a basal level of GLUT4 in the PM is seen in control), whereas it was abundantly found in the PM of cells treated with SXF or INS (**Figure 5(b)**). This finding clearly indicates that GLUT4 proteins initially presented in the CP have translocated to the PM.

3.4. Facilitated Glucose Uptake

Once the GLUT4 translocation was accomplished, the glucose transport, the last crucial event, should be facilitated [24]. After a 24-h Glc treatment, cells were exposed to SXF (300 μg/mL) or INS (100 nM) for 15 min and subjected to the glucose uptake assay using 2-deoxy-D-[1-^{3}H]-glucose ([^{3}H]-DOG) as described earlier.

Such study showed that the 37% reduction in glucose uptake with Glc treatment was reversed with SXF, raising it to ~1.9-fold greater or 21% higher than control cells (**Figure 6**). As expected, INS demonstrated the better stimulatory effects of ~2.1-fold and 33% higher glucose uptake than those of Glc-reduced and control cells, respectively. Thus, these results confirm that activation of the IST pathway with SXF triggers the successive signaling events, ultimately resulting in the *increased* glucose uptake by cells (reducing the extracellular glucose level).

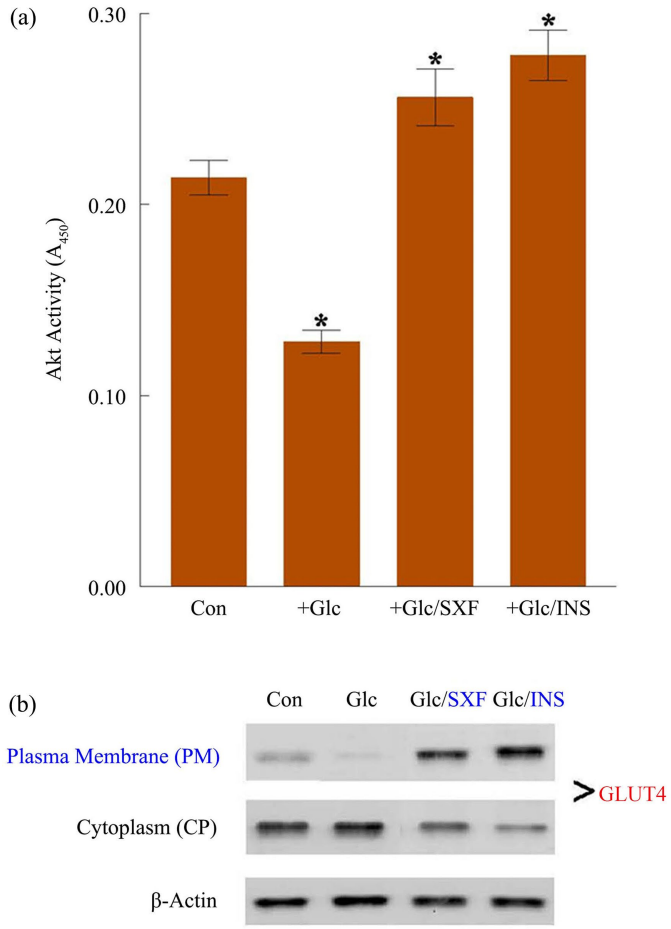

Figure 5. Phosphorylation of Akt and GLUT4 translocation. The phosphorylation levels of Akt were determined by ELISA and expressed by the OD readings (a) (*$p < 0.05$ compared with control). Translocation of GLUT4 to the plasma membrane (PM) was analyzed using Western blots (b). Expressions of GLUT4 proteins in PM or cytoplasm (CP) of Con, Glc-, Glc/SXF-, or Glc/INS-treated cells are shown in autoradiographs. Beta-actin was also run as a protein loading control.

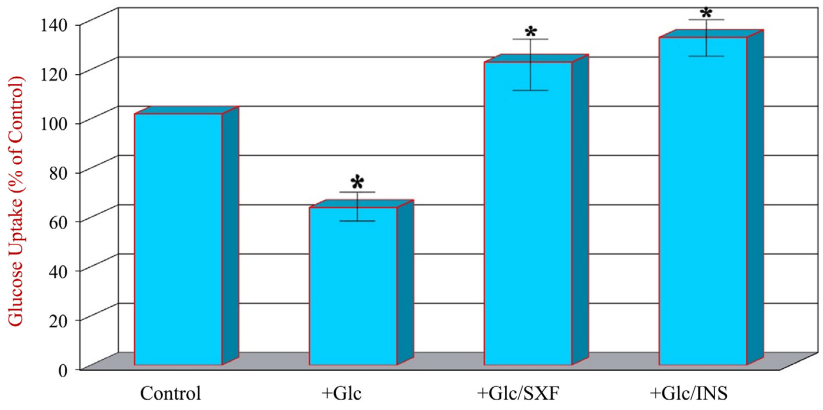

Figure 6. Glucose uptake by cells. The amounts of glucose uptake by Con, Glc-, Glc/SXF-, or Glc/INS-treated cells were measured using radioactive [^{3}H]-DOG. The radioactive count (cpm) was expressed by the % relative to controls (100%). All data are mean ± SD from three separate experiments (*$p < 0.05$ compared with control).

4. Discussion

Unfortunately, it is the fact that there are yet no specific and effective regimens for treatment of T2DM despite many years of intensive and extensive studies. We at least understand that the most effective modality may rely on *how* to overcome *insulin resistance*. We were interested in a safe and effective *natural* product with hypoglycemic effect, which could help control the serum glucose levels in T2DM patients. As SX-fraction (SXF) extracted from maitake mushroom demonstrated its anti-diabetic effect in animal studies [13]-[15], we investigated if it might have any clinically beneficial or positive effects on T2DM patients.

Although it was a small-scale study of 10 volunteered T2DM patients, we found that the FBG levels of all participants had significantly ($p < 0.05$) decreased by ~42% or from ~205 to ~116 mg/dL in 4 weeks of SXF intake (**Table 1**). This finding is indeed encouraging and promising that SXF seems to work well by itself or even with (anti-diabetic) medications, as some of patients took their daily medications with SXF during a 4-week trial. In addition, a chronological progress in glycemic control of a patient is also remarkable (**Figure 3**). His two diabetic parameters (FBG and HbA_{1C}) were well regulated with combination of glyburide and SXF. A follow-up actually showed that his FBG levels stayed nearly 80 - 90 mg/dL for the next 3 months and his HbA_{1C} also fell to normal 5.2% (from initial 11.5%). At the same time, glyburide was cut down to a half (1.25 mg) with only one SXF tablet daily, but his FBG levels yet remained 80 - 90 mg/dL for another 2 months. Consequently, glyburide was completely withdrawn and he was kept on daily SXF only. Nevertheless, his FBG and HbA_{1C} retained ~90 mg/dL and ~5.6% over 6 months, respectively. Eventually, 30 years later, this participant currently remains normoglycemic without glyburide or SXF. It is one successful case, but whether more T2DM patients would respond to SXF to become normoglycemic or at least improve their diabetic conditions in the long term needs to be further confirmed. It should also be noted that no studies have been performed to address whether SXF would work on type 1 DM or insulin-dependent patients at this time. Nevertheless, it is encouraging that at least SXF can be taken safely by T2DM patients without any complications or adverse effects. A larger study with more participants is yet required for further confirmation.

Our attention was then drawn to the hypoglycemic mechanism of SXF—how does it work? Although the exact cause of T2DM still remains uncertain, it is quite plausible that inactivation, impairment, or malfunction in the insulin signal transduction (IST) pathway [16] might consequently lead to *insulin resistance*. Hence, our study focused on this pathway, involving the sequential events regulated by various molecules. Its successful activation with SXF would ultimately facilitate the glucose uptake by insulin-responsive cells, overcoming insulin resistance.

In the IST pathway, the most critical molecule could be the IR with TK activity commanding the committed step [17]. As it triggers and carries on the consecutive signaling events, it is believed that the proper function of the IST pathway will depend on activation (phosphorylation) or inactivation (dephosphorylation) of

IR. In fact, a significantly decreased TK activity in IR has been found in skeletal muscle, adipose tissue, and red blood cells of T2DM patients [25] [26].

We explored the hypoglycemic mechanism of SXF using skeletal muscle L6 cells. High (35 mM) glucose (Glc) was found to significantly ($p < 0.05$) inactivate (dephosphorylate) IR, but such inactivation was significantly ($p < 0.05$) reversed or prevented with SXF (and INS) (**Figure 4(a)**). This IR activation (with SXF) led to activation (phosphorylation) of IRS-1 [19] (**Figure 4(b)**) to carry on the signaling events. The next key molecule, Akt [20], was activated (**Figure 5(a)**), leading to the final step, *i.e.*, translocation of GLUT4 to the PM [23]. GLUT4 molecules are usually localized/distributed in the CP, but they will move/translocate to the PM [23] [27] once they receive a signal from activated Akt. As shown in **Figure 5(b)**, they were exclusively present in the CP of control and Glc-treated cells, whereas they were distinctly found in the PM of cells treated with SXF (or INS). The glucose uptake by these cells was significantly facilitated (**Figure 6**), confirming and owing to successful GLUT4 translocation. Taken together, the hypoglycemic mechanism of SXF primarily involves (re)activation of the IST pathway, from IR activation to GLUT4 translocation (the resulting increase in glucose uptake).

Lastly, while SXF could be used to lower FBG/HbA_{1C} and improve diabetic conditions in T2DM patients, it would be worthwhile mentioning a dietary product as well. All patients are restricted from having any food or beverages containing "sugar", and many sugar substitutes are also commercially available. There is one substitute that any patient might safely take. It is collectively called the Lakanto® (LKT) products manufactured by a Japanese company (Saraya Co., Ltd., Osaka, Japan). They are the extracts of *monk fruit* (*Siraitia grosvenori*) with a high level of sweetness, *i.e.*, 200 - 350 times sweeter than sucrose [28]. Actually, such sweetness comes from its active ingredients known as *mogrosides*, which are *not* sugar but terpenoid glycosides [28]. Hence, LKT products will *not* affect the serum glucose levels and are used at many hospitals and by T2DM patients in Japan as a sugar substitute. Additionally, the US Food and Drug Administration (FDA) has also approved them for Generally Recognized As Safe (GRAS) [29], granting their safety. Furthermore, anti-hyperglycemic (hypoglycemic) effect of monk fruit extract has been reported in diabetic rats [30]. It could be something interesting for T2DM patients who are craving for sweet.

5. Conclusion

In this study, SXF demonstrated its hypoglycemic effect on all ten volunteered T2DM patients, lowering an average of ~42% in their FBG levels at the end of the study (4 weeks). A glycemic profile of a participant also illustrated the steady improvements in his FBG and HbA_{1C} levels. The *in vitro* study (using L6 cells) demonstrated that SXF was capable of (re)activating the insulin signal transduction (IST) pathway *suppressed by high glucose*. Since such inactivation of the IST pathway is considered the primary cause of *insulin resistance*, its activation with SXF may

account for the hypoglycemic effect. Thus, SXF could be a promising agent, lowering the serum glucose levels as well as HbA_{1C} in T2DM patients. However, more detailed studies are yet required for further confirmation.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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