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INVOLVEMENT OF PANCREATIC ISLET PROTECTION AND AMPK ACTIVATION IN ANTIHYPERGLYCEMIC ACTIVITY OF *ENSETE GLAUCUM* SEED EXTRACT

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Summary

Involvement of Pancreatic Islet Protection and AMPK Activation in Antihyperglycemic Activity of *Ensete glaucum* Seed Extract

The purpose of this study was to evaluate the protective effects of pancreatic islet cells and hepatic AMPK activation of the *Ensete glaucum* seed extract. The protective effect of pancreatic islet cells was evaluated on isolated islets exposed to streptozotocin (STZ) toxicity, while the effect of hepatic AMPK activation was evaluated in the STZ-induced mouse model using Western blot. The results showed that the extract (100 µg/mL) had an *in vitro* protective effect on pancreatic islet cells against the STZ (5 mM)-induced apoptosis by significantly reducing the expression of Bax and PARP proteins, slightly reducing the expression of Bax/Bcl-2 ratio and cleaved caspase-3 protein. Regarding the *in vivo* effect, the extract at the doses of 25 and 50 mg/kg (*p.o.*) affected hepatic AMPK activation after 7-day treatment in a hyperglycemic mouse model caused by STZ (170 mg/kg). Taken together, the extract both has the protective effect on pancreatic islet cells and hepatic AMPK activation, which may be related to the antihyperglycemic effect of the extract. This study suggests that the *E. glaucum* seed extract is a potential candidate to study the chemical components responsible for the antihyperglycemic activity of *E. glaucum* seeds as well as advanced research on its products.

Keywords: *Ensete glaucum seed extract, Pancreatic islet isolation, Pancreatic islet protection, AMPK activation, Hyperglycemic mouse model.*

1. Introduction

The number of people with diabetes is expected to increase worldwide. Of these, type 2 diabetes accounts for more than 90%, the disease is closely related to obesity and characterized by insulin resistance. Pancreatic islet cell failure, namely partial loss of pancreatic islet cell mass (islet cell death) or islet cell dysfunction is observed in patients with type 2 diabetes [1]. Lipotoxicity, glucotoxicity, and oxidative stress may be some of the factors leading to islet cell dysfunction or cell death. Therefore, islet cell protection is recognized as an effective strategy that should be investigated for the treatment of type 2 diabetes [1],[2].

Another approach, targeting AMPK activation is currently a strategy in the treatment of metabolic syndrome. AMPK (Adenosine monophosphate-activated protein kinase) is considered a cell intracellular energy sensor of the cells, it is activated when intracellular energy is low and regulates cellular processes. This kinase inhibits insulin-stimulated anabolic processes such as *de novo* lipogenesis and glycogen synthesis. AMPK activation also supports whole-body glucose homeostasis and improves insulin sensitivity by promoting processes such as glucose uptake and energy expenditure [3],[4]. Metformin is a drug used as first-line treatment for type 2 diabetes that primarily activates AMPK in targets including liver, muscle, and adipose tissues. This drug is effective as monotherapy and in combination with other hypoglycemic agents. However, cases of poor tolerance to metformin or the need to use metformin at high doses could cause many inconveniences and side effects, especially, caution should be emphasized in patients with kidney or liver diseases due to the increased risk of lactic acidosis [5]. Currently, the effects of the combination regimen between metformin and sulfonylureas are also proven and applied clinically. This regimen combines the insulin resistance-reducing effect of metformin and the insulin-stimulating effect of sulfonylureas [6]. However, caution is also needed regarding side effects and contraindications to this regimen. Therefore, candidates with synergistic effects such as protecting pancreatic islet β cells, stimulating insulin secretion, increasing insulin sensitivity in target tissue cells, and protecting the liver and kidneys, are receiving increasing research attention. Plant extracts are one of the important possible sources for this approach due

to the existence of many primary metabolites in them that could produce synergistic effects [7].

Our previous study demonstrated the antihyperglycemic, insulin-stimulating, pancreatic, hepatic, and renal protective effects of *Ensete glaucum* seed extract on streptozotocin-induced hyperglycemic mouse model [8]. In order to add further data, the *in vitro* protective effects of pancreatic islet cells and *in vivo* hepatic AMPK activation of *E. glaucum* seed extract, which contribute to its antihyperglycemic effect, were demonstrated via protein expression by western blot in this study.

2. Materials and methods

2.1. Plant materials

Ensete glaucum (Roxb.) Cheesman fruits were collected in April 2020 from Bac Ai District, Ninh Thuan Province, Vietnam. Seeds were separated from ripe fruits, dried, and ground into powder for study. The *E. glaucum* seed extract was derived from our previous study [8].

2.2. Animals

Swiss albino male mice (3-4 weeks old, 18-22 g) were provided by the Institute of Vaccines and Medical Biologicals Nha Trang City. The mice were housed in the standard laboratory (relative humidity 50 - 60%, temperature 25°C, and 12 hours of light-dark cycles). They were acclimatized for 7 days in the animal house before the experiment. They were provided with adequate tap water *ad libitum* and standard food. The protocols of this study were approved by the institutional animal ethical committee of University of Medicine and Pharmacy at Ho Chi Minh City, Viet Nam (Ref: 591/GCN-HĐĐĐNCTĐV).

2.3. Pancreatic islet isolation and culture

The protocol was performed as described previously with some adjustments to suit laboratory conditions [9]. Briefly, mouse pancreas was collected and treated with 200 U/mL collagenase II (Gibco, US) in sterile Hank's balanced salt solution-HBSS (Gibco, US) at 37 °C for 30 min. The mixture was rapidly cooled and 3 mM HBSS/$CaCl_2$ was added with vigorous shaking 40 times for 10 sec. The sample was centrifuged at 290×g for 30 sec at 4°C. The precipitate was suspended in 3 mM HBSS/$CaCl_2$ solution and passed through a 0.419 mm sieve. The sample was centrifuged at 290×g for 30 sec at 4°C. The precipitate was suspended in Ficoll 1077/RPMI 1640 (1:1 *v/v*). The sample was continuously centrifuged at 900×g for 20 min at 20°C. Pancreatic islets from the middle layer of Ficoll/medium were collected in RPMI 1640 containing FBS 10%, penicillin 100 U/mL, and streptomycin 100 µg/mL and centrifuged at 290×g for 5 min at 4°C. Filter the pancreatic islets through a 70 nm filter in the culture medium. Quality isolated islets were handpicked using a 200-µL micropipette under the microscope and grown in RPMI 1640 medium at 37 °C, 5% CO_2 for 24 hours.

2.4. Evaluation of islet specificity

Islet specificity was then examined by dithizone staining [10]. Briefly, 10 µL of 10 mg/mL dithizone solution (Sigma-Aldrich) was added to islets suspended in 1 mL Krebs-Ringer bicarbonate (KRB) buffer (pH 7.4) and incubated at 37 °C for 10 min. The stained islets appeared as bright red under the inverted microscope (Nikon Eclipse Ts2R-FL, China).

2.5. Streptozotocin (STZ)-induced pancreatic β-cell-specific cytotoxicity

Islets were cultured in RPMI 1640 medium and treated without or with the 100 µg/mL extract or 10 µM glimepiride (Stellapharm J.V. Co., Ltd.) in the presence of 5 mM STZ at 37 °C, 5% CO_2 for 24 hours [11]. Accordingly, four groups in this experiment included: (I) Normal control - Islets + 0.2% DMSO + 0.5 mM sodium citrate; (II) STZ control - Islets + 0.2% DMSO + 5 mM STZ; (III) Extract - Islets + 100 µg/mL extract + 5 mM STZ; (IV) Positive control - Islets + 10 µM glimepiride + 5 mM STZ. Islets were then collected to examine protein expression by western blot.

2.6. STZ-induced hyperglycemic mouse model and treatment

Normoglycemic mice were intraperitoneally injected with a single dose of 170 mg/kg streptozotocin (Sigma-Aldrich) in sodium citrate (0.1 M, pH = 4.5) after overnight fasting [12]. Fasting plasma glucose (FPG) of mice was determined on the 7^{th} day after STZ administration and those with FPG ≥ 200 mg/dL [13] were classified as hyperglycemia. Accordingly, five groups in this experiment included: (I) Normal control - Normoglycemic mice + distilled water; (II) STZ control - Hyperglycemic mice + distilled water; (III) Extract 25 - Hyperglycemic mice + 25 mg/kg extract; (IV) Extract 50 - Hyperglycemic mice + 50 mg/kg extract; (V) Reference drug - Hyperglycemic mice + 5 mg/kg glibenclamide (Medical Import Export Joint Stock Company DOMESCO, Vietnam). Mice were administered with or without extract and glibenclamide for

seven consecutive days by oral gavage. Finally, mice were euthanized and necropsied using CO_2 asphyxiation. Liver tissues were collected and washed with physiological saline to examine protein expression by western blot.

2.7. Western blot analysis

Islets were washed in ice-cold PBS and lysed on ice for 2 hours in RIPA lysis buffer containing 50 mM Tris-HCl (pH 8.0), 0.1% Triton X100, 150 mM sodium chloride, 0.5% sodium deoxycholate, 0.1% SDS, 1 M sodium orthovanadate, 1 mM sodium fluoride, 1 mM PMSF, and phosphatase inhibitor II, III (Sigma-Aldrich). For liver tissues, they were homogenized in RIPA lysis buffer and kept on ice similar to pancreatic islets. The cell/tissue lysate was centrifuged at 16,000×g for 20 min at 4°C to obtain supernatant using Centrifuge 5430R (Eppendorf, US). Protein content was estimated by Bradford protein assay using the Pierce Bovine serum albumin standard (Thermo Scientific). Briefly, 4 µL cell lysate was mixed with 96 µL distilled water and 1 mL Coomassie Brilliant Blue G-250. The mixture was mixed and absorbance was recorded at 595 nm. The content of protein (µg/mL) was calculated by the linear regression equation of the BSA standard. Aliquots of the lysates (40 µg of protein) were boiled for 5 min and electrophoresed on 10% sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE). Proteins in the gels were transferred onto nitrocellulose membranes and which were then incubated with primary antibodies including Bax 1: 500 (633801, BioLegend), Bcl-2 1: 500 (633501, BioLegend), and Cleaved-caspase 3 1: 1000 (9664S, Cell Signaling Technology-CST) for pancreatic islets while p-AMPK 1: 1000 (2535S, CST) for liver tissues, and α-tubulin 1: 1000 (2144S, CST) was used as a housekeeping protein for both. The membranes were further incubated with horseradish peroxidase-conjugated secondary anti-mouse or anti-rabbit antibody (anti-rabbit IgG, HRP-linked Antibody 1: 2000 (7074S, CST) and anti-mouse IgG, HRP-linked Antibody 1: 2000 (7076, CST)). Finally, protein bands were detected using an enhanced chemiluminescence western blotting detection kit (Thermo Scientific) [14]. For chemiluminescence detection and image analysis, the ImageQuant LAS 500 system and ImageQuant TL 1D v8.2.0 (Cytiva, GE Healthcare Life Sciences, US) were used.

2.8. Statistical analysis

Data were expressed in terms of mean ± standard deviation (Mean ± SD) and analyzed by GraphPad Prism software (version 8.0.2, Inc., La Jolla, CA, USA) using t-test and one-way analysis of variance (ANOVA) followed by Tukey's test. $p < 0.05$ is considered statistically significant.

3. Results

3.1. Pancreatic islet cell protection of E. glaucum seed extract

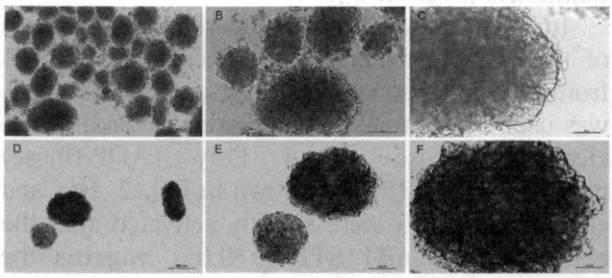

Fig.1. Representative images of isolated pancreatic islets and their specificity result.
(A-C) Islet images at different objectives (scale bar A = 200 µm, B = 100 µm, C = 50 µm), (D-F) Islet specificity was demonstrated through dithizone staining (scale bar D = 200 µm, E = 100 µm, F = 50 µm).

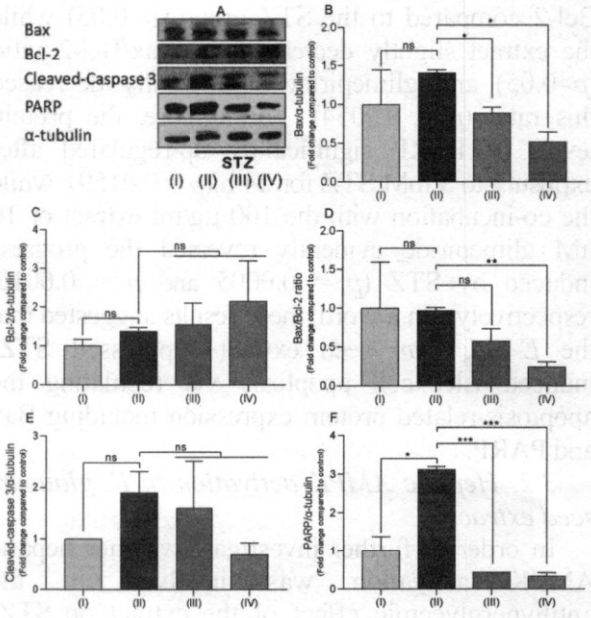

Fig.2. Effect of *E. glaucum* seed extract on the expression of apoptosis-related proteins in pancreatic islet cells. (A) Western blot of Bax, Bcl-2, cleaved caspase-3, PARP, and α-tubulin; (B-F) Average relative quantitative expression of (B) Bax, (C) Bcl-2, (D) Bax/Bcl-2 ratio, (E) cleaved caspase-3, (F) PARP ($n = 2$); $^{ns}p > 0.05$: no statistically significant difference, $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$: statistically significant difference compared to group (II) using t-test. (I) Normal control, (II) STZ control, (III) STZ+extract (100 µg/mL), and (IV) STZ+glimepiride (10 µM).

This study isolated good pancreatic islets for research. **Fig.** 1 shows that isolated pancreatic

islets have smooth borders, uniform boundaries, and no darkening at the center (Fig. 1A-C). Pancreatic islet specificity was assessed by dithizone staining since dithizone is a compound that easily forms complexes with zinc in zinc-rich cells such as islets. Stained islets were all pink to crimson and no other cell types were observed with islets (Fig. 1D-F).

In order to evaluate the underlying mechanism of the extract protecting the pancreatic islet cells from STZ-induced apoptosis, western blot analysis was used to examine the expression of Bax, Bcl-2, cleaved caspase-3, and Poly (ADP-ribose) polymerase (PARP). As shown in **Fig. 2**, Bax and cleaved caspase-3 were slightly activated after the exposure to 5 mM STZ (p>0.05), whereas the treatment with the 100 μg/mL extract or 10 μM glimepiride markedly suppressed the expression of Bax (p = 0.0142 and p = 0.0112, respectively). However, they only slightly reduced the expression of cleaved caspase-3 (p>0.05). Exposure to 5 mM STZ for 24 h did not down-regulate Bcl-2 expression, resulting in no change in the Bax/Bcl-2 ratio. The extract and glimepiride slightly increased Bcl-2 compared to the STZ group (p>0.05) while the extract slightly decreased the Bax/Bcl-2 ratio (p>0.05) and glimepiride significantly decreased this ratio (p = 0.0054). Furthermore, the protein levels of PARP significantly up-regulated after exposure to 5 mM STZ for 24 h (p = 0.0159), while the co-incubation with the 100 μg/ml extract or 10 μM glimepiride evidently reversed the progress induced by STZ (p = 0.0005 and p = 0.0010, respectively). In a word, these results suggested that the *E. glaucum* seed extract suppressed STZ-induced islet cell apoptosis via regulating the apoptosis-related protein expression including Bax and PARP.

3.2. Hepatic AMPK activation of E. glaucum seed extract

In order to further investigate whether hepatic AMPK activation was involved in the antihyperglycemic effect of the extract on STZ-induced mice, western blot analysis was also used to determine the level of p-AMPK in the liver. The results shown in **Fig. 3** indicated that the level of p-AMPK expression was significantly declined by STZ (170 mg/kg, *i.p.*) in *Swiss albino* male mice (p = 0.0180), while the expression of this protein induced by STZ was significantly increased by 7-day treatment of the extract (25 and 50 mg/kg, *p.o.*) or glibenclamide (5 mg/kg, *p.o.*). On the other hand, the hepatic AMPK activation effect of the extract at the dose of 50 mg/kg was equivalent to that of glibenclamide at the dose of 5 mg/kg while the extract at the dose of 25 mg/kg showed better this effect than the 50 mg/kg extract and 5 mg/kg glibenclamide (p = 0.0104 and p = 0.0059, respectively). The result suggested that the *E. glaucum* seed extract activated AMPK in mouse hepatic tissue against hyperglycemia caused by STZ.

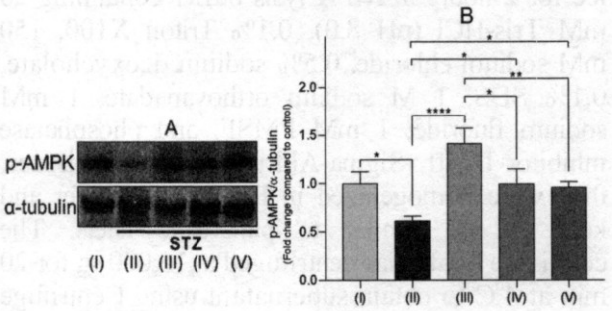

Fig.3. Effect of *E. glaucum* seed extract on the expression of Phospho-AMPKα (Thr172) protein in liver tissue. (A) Western blot of p-AMPK and α-tubulin; (B) Average relative quantitative expression of p-AMPK (n = 3); $^{*}p$ < 0.05, $^{**}p$ < 0.01, and $^{****}p$ < 0.0001: statistically significant difference using Tukey's test. (I) Normal control, (II) STZ control, (III) STZ+extract (25 mg/kg), (IV) STZ+extract (50 mg/kg), and (V) STZ+glibenclamide (5 mg/kg).

4. Discussion

Pancreatic islet cells play an important role in regulating blood glucose levels within the normal range. In particular, β cells have the function of producing and secreting insulin in response to hyperglycemia. β-cell deficiency and functional defects play an essential role in the development and progression of diabetes. Increased β-cell depletion due to apoptosis leading to reduced pancreatic mass is observed in patients with type 2 diabetes, especially when β-cells adapt to insulin resistance and their secretion increases, ultimately leading to exhaustion and reduction in β-cell mass [15],[16]. Therefore, preserving β cells is a necessary issue and a reasonable strategy to treat and prevent type 2 diabetes. However, restoring β-cell mass remains a challenge in diabetes treatment research. In this study, streptozotocin, a common chemical used to induce diabetes models in animals, was used to induce apoptosis of mouse isolated pancreatic islet β cells and the protective effect of the *E. glaucum* seed extract against STZ-induced apoptosis was evaluated. Experimental evidence showed that STZ at the concentration of 5 mM inhibited islet insulin secretion [11], concentration of 3 mM induced INS-1 β-cell apoptosis and insulin secretion reduction via increasing Bax, caspase-3, p-p38, p-JNK, ROS, and MDA, while decreasing Bcl-2, insulin 1, insulin 2,

and SOD [17]. STZ at the concentration of 0.3 mM also induced INS-1 β-cell apoptosis and insulin secretion inhibition through increasing the expression of NF-κB, Bax, cleaved caspase-3, p-p38, p-JNK and decreasing the expression of Bcl-2 and Pdx-1 [18]. In the present study, STZ at the concentration of 5 mM could lead to pancreatic islet cell apoptosis through increased expression of Bax, cleaved caspase-3, and PARP. Interestingly, the *E. glaucum* seed extract and glimepiride were able to protect pancreatic islet cells against the apoptotic toxicity of STZ by reversing the expression of these proteins, notably Bax and PARP. These results are similar to our previous publication, the extract also had protective effects on the pancreas in an STZ-induced mouse model [8]. Several previous studies also showed that plant extracts also had the effect of stimulating insulin secretion and protect β cells against apoptosis [7],[17],[18]. These confirm the potential of medicinal herbs to improve β-cell function and preservation, a vital requirement in diabetes treatment. The ability of the *E. glaucum* seed extract to protect pancreatic islet cells may be due to the existence of active components in it. Flavonoids and polyphenols are present in *E. glaucum* seeds [8], these are phytochemicals that reportedly have the effects to preserve pancreatic β cell survival and function [19],[20]. Afzelechin (a flavonoid) and coniferaldehyde (a phenolic aldehyde) might be relevant components contributing to the anti-hyperglycemic effect of *E. glaucum* seeds [8]. These compounds had the effect of protecting and stimulating insulin secretion from isolated mouse pancreatic islet β cells (Data not published in this publication). Importantly, from the results of this study, a proposed mechanism for the pancreatic islet cell protective effect is shown in **Fig. 4**. Briefly, STZ enters β cells via GLUT2 (Glucose transporter 2), and its degradation in the cell cytosol produces DNA alkylating agents and leads to DNA strand breaks. Next, poly (ADP-ribose) polymerase (PARP) is activated to participate in the repair of damaged DNA. However, persistent and excessive activities of PARP lead to NAD^+ deficiency and reduced ATP synthesis, resulting in inhibition of insulin synthesis and secretion, and β-cell apoptosis. Besides, the metabolism of STZ in cells leads to an increase in ROS, consequent increase in Bax expression and enhancement of the caspase-dependent apoptosis pathway. Increased cleaved caspase-3 stimulates PARP cleavage and DNA fragmentation and causes cell death. Furthermore, the nitrosourea structure of STZ is responsible for increased NO production in cells which causes DNA damage leading to PARP overactivity. The results showed that the *E. glaucum* seed extract downregulated the expression of Bax and PARP, which significantly contributed to the protective effect of β cells against STZ-induced apoptosis.

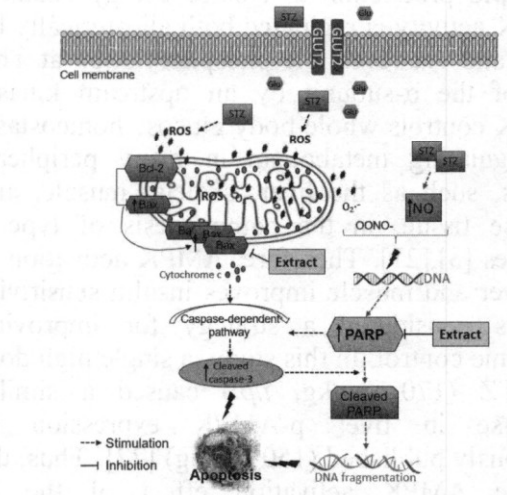

Fig. 4. A proposed schematic representation of the protective mechanism of *E. glaucum* seed extract against STZ-induced pancreatic islet cell apoptosis. STZ, streptozotocin; Glu, glucose; GLUT2, glucose transporter 2; ROS, reactive oxygen species; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein; NO, nitric oxide; PARP, Poly (ADP-ribose) polymerase.

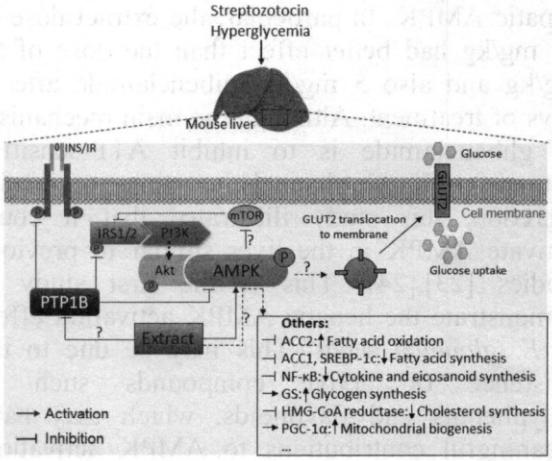

Fig. 5. Proposed possible impacts of *E. glaucum* seed extract-induced AMPK activation involved in its antihyperglycemic effect. AMPK, 5′ adenosine monophosphate-activated protein kinase; INS, insulin; IR, insulin receptor; IRS, insulin receptor substrate; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; PTP1B, protein tyrosine phosphatase 1B; mTOR, mammalian target of rapamycin; GLUT2, glucose transporter 2; ACC, acetyl-CoA carboxylase; SREBP-1c, sterol regulatory element-binding protein 1c; NF-κB, nuclear factor kappa-B; GS, glycogen synthase; HMG-CoA reductase, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha.

On the other hand, AMPK is a heterotrimeric serine/threonine protein kinase that acts as a metabolic sensor related to cellular energy. AMPK activation induces glucose uptake and lipid oxidation to produce energy while inhibiting energy consuming processes including glucose and lipid production to restore energy balance. AMPK activity is regulated both allosterically by AMP and via reversible phosphorylation at Thr-172 of the α-subunit by an upstream kinase. AMPK controls whole-body glucose homeostasis by regulating metabolism in many peripheral tissues, such as the liver, skeletal muscle, and adipose tissue, in the pathogenesis of type 2 diabetes [3],[21]. Therefore, AMPK activation in the liver and muscle improves insulin sensitivity and is considered a strategy for improving glycemic control. In this study, a single high dose of STZ (170 mg/kg, *i.p.*) caused a similar decrease in liver p-AMPK expression as previously published (150 mg/kg) [22]. Thus, the hepatic AMPK activation effect of the *E. glaucum* seed extract was evaluated in an STZ-induced mouse model. The results showed that STZ reduced the expression of p-AMPK protein in the liver while the extract and glibenclamide reversed this condition, showing that the extract and glibenclamide had the effect of activating hepatic AMPK. In particular, the extract dose of 25 mg/kg had better effect than the dose of 50 mg/kg and also 5 mg/kg glibenclamide after 7 days of treatment. Although the main mechanism of glibenclamide is to inhibit ATP-sensitive potassium (K_{ATP}) channels to stimulate insulin secretion, this study illustrated that it could activate AMPK in the liver similar to previous studies [23],[24]. This is the first study to demonstrate the hepatic AMPK activating effect of *E. glaucum* seeds. This may be due to the existence of active compounds such as polyphenols and flavonoids, which may have meaningful contributions to AMPK activation. Additionally, coniferaldehyde isolated from *E. glaucum* seeds demonstrated the effect of improving lipid and glucose metabolism in palmitic acid-induced HepG2 cells via the LKB1/AMPK signaling pathway [25]. Plant extracts and natural compounds have the effect of activating AMPK, making an important contribution to the treatment of diabetes [21]. In the present study, *E. glaucum* seed extract also activated AMPK in the liver, it may have some possible beneficial effects in the diabetic state (**Fig. 5**) such as increased glucose absorption due to stimulation of GLUT translocation to the cell membrane, fatty acid oxidation, and glycogen synthesis, reduced glucose synthesis, fatty acid and cholesterol synthesis,... thereby improving insulin sensitivity.

5. Conclusion

From the results obtained experimentally, it can be concluded: (1) Ethanol extract of *E. glaucum* seeds can protect *in vitro* mouse pancreatic islet β cells from apoptosis in the model of cytotoxicity by STZ, (2) The mechanism of *in vitro* protection of mouse pancreatic islet β cells by *E. glaucum* seed ethanol extract on the STZ-induced apoptosis model may be partly through down-regulation of Bax and PARP expression, and (3) *E. glaucum* seed ethanol extract can activate liver AMPK *in vivo*, which is one of the potential and important signals when researching antidiabetic drugs. These findings can also serve as a basis to explain the mechanism of action of *E. glaucum* seed extract against pancreatic islet β cell damage in the experimental mouse diabetes model and the antihyperglycemic effect of the extract. At the same time, it suggests that *E. glaucum* seeds need to be studied further as a potential agent on the path to finding natural active ingredients, to supplement the ability to control diabetes in humans.

Declare: *There are no conflicts of interest.*

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CORN STEEP LIQUOR PREPARATION FOR USE IN THE FERMENTATION PROCESS

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Summary

Corn Steep Liquor Preparation for Use in the Fermentation Process

The primary goal of this work was to develop a laboratory-scale process for producing corn-steep liquor from locally available materials to replace imported commercial products used in microbe culturing media. Additionally, the study aimed to compare the quality of our locally-made corn-steep liquor with commercial products. The findings indicated that corn sourced from Hai Duong Province had more advantages as a starting material for production. The corn-steep liquor was obtained using the process of hot water extraction, wherein 2 kilograms of corn kernel were soaked in water at a temperature of 50°C for 48 hours (water-to-corn ratio of 1.5:1). The resulting liquid was separated and condensed after the extraction process. The locally-produced corn-steep liquor meets the required moisture, pH, and total amino acid content standards, surpassing the imported products in protein concentration. The obtained corn-steep liquor was used for calcium lactate and antibiotics fermentation process, exhibiting a comparable overall yield to commercially available products. The potential use of our corn-steep liquor to substitute imported products in industrial fermentation applications is worth considering.

Keywords: *Corn-steep liquor, Extraction, Amino acid, Total nitrogen, Microorganism media.*