

IN VITRO ANTIDIABETIC EFFECTS OF *FERULA ASSA-FOETIDA* EXTRACTS THROUGH DIPEPTIDYL PEPTIDASE IV AND α -GLUCOSIDASE INHIBITORY ACTIVITY

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ABSTRACT

Objective: Diabetes mellitus (DM) causes hyperglycemia, which is one of the most common diseases in the world. One of the strategies for the treatment of diabetes is maintaining postprandial glucose level through inhibition of dipeptidyl peptidase IV (DPP-IV) and α -glucosidase enzymes. The aim of this study was to determine *in vitro* antidiabetic potential of *Ferula assa-foetida* via DPP-IV and α -glucosidase inhibitory activities.

Methods: *F. assa-foetida* seeds were extracted in methanol, ethanol, ethanol-methanol, and water. Inhibitory activity on DPP-IV and α -glucosidase was performed *in vitro* and measured spectrophotometrically at $\lambda=405$ nm.

Results: The result showed that the *F. assa-foetida* seed extract is effective against both enzymes. All fractions had DPP-IV inhibitory activity, but the ethanolic fraction had the highest inhibitory activity on DPP-IV enzyme and significantly decreased DPP-IV activity (24.5%). With respect to α -glucosidase inhibitory activity, the aqueous extract has the highest inhibitory activity (28%).

Conclusion: According to the results of this study, *F. assa-foetida* contains DPP-IV and α -glucosidase inhibitors and could be a potential source for the discovery of active constituents as α -glucosidase and DPP-IV inhibitors to treat Type 2 DM.

Keywords: Diabetes mellitus, Herbal medicine, Dipeptidyl peptidase IV, α -glucosidase.

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INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders in which the body cannot effectively use the insulin, has defects in insulin secretion or both, that lead to high blood glucose. Type 2 DM (T2DM) is the most common type of this disease and accounts for at least 90% of all cases of diabetes [1]. Although in most patients with T2DM, there may be no obvious symptoms for a long period, it still threatens human health. Blurred vision, drowsiness, weight gain, numbness in hands and feet, and gum disease are some symptoms of T2DM in patient [2].

The incidence of T2DM is rising dramatically worldwide. According to the International Diabetes Federation, 425 million people are affected by diabetes worldwide, every 6 seconds one person dies from diabetes with 5 million deaths reported in 2015 and diabetes expenditure reached USD 1.197 billion [3].

At first, T2DM can be managed by lifestyle changes that include diet and exercise, but due to the progressive nature of this disease, exercise and proper diet may not completely solve the problem so oral hypoglycemic drugs are necessary to treat T2DM. Insulin therapy is the most effective treatment, generally used in advanced stages of T2DM when other therapeutic strategies are no longer effective [4-6].

Based on the pathogenic mechanisms of T2DM, various medications have been produced and are prescribed for patients according to symptoms of disease. α -glucosidase inhibition is one of the mechanisms of action of these medications. α -glucosidase plays a role in the conversion of carbohydrates into glucose. By inhibiting α -glucosidase, glucose levels in the blood can be returned to normal limits. Hence, use of pharmaceuticals that inhibit activity of this digestive enzyme could be a good treatment for T2DM. The most recent medications used to treat T2DM are inhibitors of dipeptidyl peptidase IV (DPP-IV). DPP-IV rapidly

destroys the incretin hormones, glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP). These peptides are members of the glucagon peptide superfamily that helps the body produce more insulin when it is needed [1,7-11].

Nowadays, several DPP-IV inhibitors are available that can be taken orally in tablet form. Synthetic oral hypoglycemic drugs are the most common form of treatment for T2DM, but they have undesirable side effects in patients with T2DM [4]. Moreover, these drugs impose a high cost to patients and sometimes, especially in developing countries, the majority of patients cannot afford the high cost of the drugs. Therefore, research on medicinal plants as inexpensive resources containing valuable pharmaceutical metabolites and identification of drugs with high efficiency and lower side effects is essential.

Ferula assa-foetida L. is an important medicinal plant belonging to the Apiaceae family with many medicinal benefits. "Asafoetida" derived from the Persian word "asa" (resin) and Latin foetida that means "odorous," refers to its strong sulfurous odor.

According to the literatures, this herbaceous perennial plant is found in central Asia, Iran to Afghanistan, from which it is exported to the entire world. Traditionally, it is used for the treatment of different diseases, such as stomach ache, asthma, epilepsy, flatulence, weak digestion, intestinal parasites and influenza [12]. Recent pharmacological and biological studies have also shown several activities, such as antioxidant [13], antiviral [14], antimicrobial [15], antispasmodic and hypotensive [16], and antidiabetic [5,17].

Nowadays identification of more efficient pharmaceuticals with less side effects and understanding the mechanism of action of hypoglycemic activity of medicinal plants is an important matter. Since the antidiabetic effects of *F. assa-foetida* L. have been proven in *in vivo*

studies, but the exact mechanism(s) of blood glucose-lowering effect of this herb is not well-known, so this study was carried out to investigate the α -glucosidase and DPP-IV inhibitory activities of *Ferula asafoetida* L. as two of the most important hypoglycemic mechanisms.

MATERIAL AND METHODS

Chemicals

DPP-IV from porcine kidney, Gly-Pro p-nitroanilide p-toluenesulfonate, α -glucosidase from *Saccharomyces cerevisiae*, 4-nitrophenyl α -D-glucopyranoside, and Acarbose purchased from Sigma. All other chemicals used for the experiment were of analytical grade.

Sample preparation

The *F. asafoetida* seeds were collected and then powdered using grinder. 25 g of powdered plant seed were extracted with 50 ml methanol, ethanol, methanol-ethanol and water on an orbital shaker for 24 hrs. The resulting extracts were filtered using Whatman No. 1 filter paper to remove plant debris, and the filtrate was allowed to dry under a stream of air at room temperature. Dried extracts were weighed and dissolved in dimethylsulphoxide to yield a stock solution. The solution was stored at 4°C for subsequent use.

α -glucosidase inhibitory activity assay

The α -glucosidase inhibition was determined using the modified method according to Ooi *et al.* [18]. In brief, the reaction mixture contained 50 mM 4-nitrophenyl α -D-glucopyranoside as substrate, 50 μ L of the extract (0.5 mg/ml) or Acarbose (positive control) and 50 μ L of α -glucosidase (0.05 U/ml) and 50 μ L sodium phosphate buffer (0.1 mM, pH 6.9) were incubated at 30°C for 40 minutes. Then, the reaction was terminated by the addition of 100 μ L of 0.2 M sodium carbonate solution and absorbance was determined at 405 nm using plate reader.

α -glucosidase inhibitory activity was determined as follows:

$$\% \text{ Inhibition} = \frac{\alpha\text{-glucosidase activity with extract}}{\alpha\text{-glucosidase activity without extract}} \times 100$$

DPP-IV inhibitory activity assay

DPP-IV inhibitory activity assay was performed colorimetrically using Gly-Pro p-nitroanilide p-toluenesulfonate as chromogenic substrate in triplicate following the modified method of Matheussen *et al.* [19]. DPP-IV cleaves the chromogenic substrate to release the yellow colored product measured at 405 nm. Briefly, in a 96-well plate, DPP-IV inhibition test was conducted with the following conditions: 50 μ L of various extract (0.5 mg/ml) and 50 μ L of DPP-IV enzyme (0.01 U/ml) were added to each well, pre-incubated at 37°C for 10 minutes, then 25 μ L of the substrate (1.5 mM) was added to the mixture bringing the final volume to 200 μ L with Tris buffer pH=8, incubated at 37°C and the reaction was stopped after 1 hr by adding 100 μ L of sodium acetate pH=4. Absorbance was taken at 405 nm by ELx808LBS Absorbance Plate Reader (Bio-TEK, USA).

The results obtained were compared with the negative control (no inhibitor).

Percentage of inhibition was calculated by the following formula:

$$\% \text{ Inhibition} = \frac{\text{DPP-IV activity with extract}}{\text{DPP-IV activity without extract}} \times 100$$

RESULTS AND DISCUSSION

α -glucosidase inhibitory activity assay

Inhibition of the α -glucosidase that retards the liberation of glucose from dietary complex carbohydrates is one of the important methods to delay the absorption of glucose from the digestive system. Nowadays, one group of drugs that is used to treat T2DM are this kind of inhibitors, but due to the cost of synthetic drugs and their side effects, finding

more efficient and safer drugs is crucial. Therefore, the use of herbs as alternative medicine as well as to find valuable pharmaceutical metabolites has significant value, especially for the treatment of diabetes. In this study, the results of the α -glucosidase inhibitory activity assay showed that aqueous extract of *Asafoetida* seed had strong inhibitory activity (Table 1), and this inhibitory activity was concentration-dependent. So that by increasing the concentration of extract from 0.1 to 1 mg/L, the inhibitory activity increased dramatically from 10% to 40% (Fig. 1).

Although it has long been proven that *assa-foetida* has anti-diabetic activity, the glucose-lowering mechanisms of this plant are not clearly known. When streptozotocin-induced diabetic rats were treated with the *Asafoetida* extract at a dose of 50 mg/kg, the serum glucose concentration decreased significantly in comparison with untreated diabetic rats [20]. Hence, one possible reason for the decreased blood glucose level caused by *Asafoetida* extract in streptozotocin-induced diabetic rats could be due to having inhibitory effect on glucosidase enzyme activity. Because our result showed that *Asafoetida* has inhibitory effect on this enzyme.

In another study to find out the mechanisms of the blood glucose lowering action of *assa-foetida*, the glucose transporter type 4 (GLUT4) was studied in C2C12 cell. Data indicated that *assa-foetida* treatment increases translocation of the GLUT4 to the cell membrane which may be one of the possible mechanisms of antihyperglycemic effect of *assa-foetida* [21].

According to our results, α -glucosidase inhibition is one of the main mechanisms of antihyperglycemic activity of *Asafoetida* and thereby reduces blood glucose.

Several studies have been conducted to evaluate α -glucosidase inhibitory activity of medicinal plants as an important antidiabetic factor. For instance, Hasimun *et al.* evaluated α -glucosidase inhibitory activity of Zingiberaceae family and found that selected members of this family had a varied range of IC50 from 28.4 μ g/ml to 269.2 μ g/ml against α -glucosidase. So concluded that the Zingiberaceae family has good potential for use as an alternative medicine to treat DM [22].

Table 1: Percent reduction in α -glucosidase enzyme activity by different extracts of *F. asafoetida* seed

Extracts	Percentage of α -glucosidase inhibitory activity (%)
Ethanol	14.627±0.707
Methanol	14.860±1.092
Ethanol-methanol	7.40±7.01
Aqueous	28.21±2.63

F. asafoetida: *Ferula asafoetida*

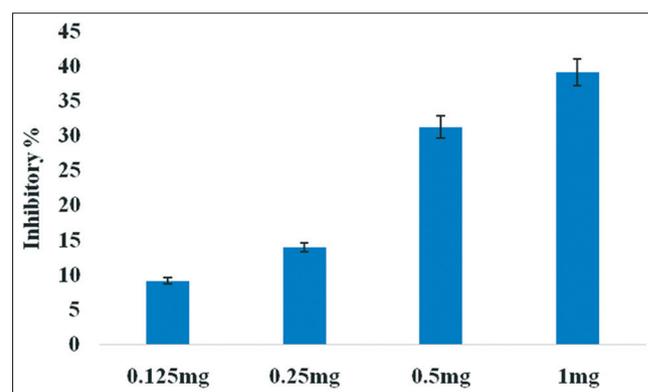


Fig. 1: α -glucosidase inhibitory activity of different concentrations of aqueous extract of *Ferula asafoetida* seed

Furthermore, α -glucosidase inhibitory activity of *Abutilon indicum* was investigated and IC50 of the *A. indicum* methanolic leaf extract on α -glucosidase was 137.61 μ g/ml indicates that this plant has good potential antidiabetic activity [23].

DPP-IV inhibitory activity

This approach is the latest treatment method to deal with T2DM, including inhibition of DPP-IV enzyme that breaks down GLP-1, the insulin secretion stimulating peptides, and reduces its half-life to just under 2 minutes. Due to the disadvantages mentioned on chemical medicines and the benefits of herbs and natural metabolites, *assa-foetida* was studied to identify the possible mechanisms of antidiabetic activity. Results showed that all four fractions of methanolic, ethanolic, methanol-ethanolic and water extract had DPP-IV inhibitory properties, but it is noteworthy that ethanolic and ethanol-methanolic fractions had the highest inhibitory activity and aqueous fraction showed the lowest inhibition activity (Table 2).

According to the results of this study, *assa-foetida* probably reduces blood glucose by inhibiting α -glucosidase and DPP-IV activity, leading to reduce glucose absorption from the digestive system and stimulating the production of insulin by increasing the half-life of GLP-1, respectively. The results of some *in vivo* studies showed that the use of *Asafoetida* increases the amount of insulin in diabetic mice.

Yusufoglu suggested that the antihyperglycemic effects of *assa-foetida* could be due to its insulin-secreting activity. Administration of *F. assa-foetida* (200 and 400 mg/kg) extract to diabetic rats increased the levels of insulin compared to the diabetic control rats [17]. Forasmuch as GLP-1 has a potent effect on insulin secretion so when the GLP-1-degrading enzyme is inhibited (DPP-IV) by an inhibitor, the level of GLP-1 will increase and induces insulin secretion and finally the blood glucose decreases.

The effectiveness of medicinal plants in reducing blood glucose and increasing plasma insulin levels has been proven in many studies.

Feeding the streptozotocin-induced diabetic rats with the powdered *Du-zhong* (*Eucommia ulmoides* Oliv.) leaves and its aqueous extract for 3 weeks based on 1% dried *Du-zhong* leaves, reduced plasma glucose occurred simultaneously with the increase in plasma insulin [24].

To evaluate antidiabetic effect of *Caulerpa lentillifera*, the inhibitory effect of *C. lentillifera* extract on DPP-IV and α -glucosidase activity was measured in a cell-free system. Results showed that *C. lentillifera* extract significantly decreased DPP-IV and α -glucosidase activities and, therefore, *C. lentillifera* could be used as a potential anti-diabetic agent [25].

Methanolic leaf extract of *Mangifera indica* [26], hexane extract of *Annona squamosa* [27], the ethanolic extract of *Urena lobata* [28], and the aqueous leaves extract of *Cistus incanus* L. [29] is some studies that have inhibited DPP-IV enzymatic activity. The level of plasma GLP-1 in rats treated with the polar fraction of *Pueraria tuberosa* was increased that it was due to the inhibitory effects on DPP-IV enzyme [30].

CONCLUSION

T2DM is the most common type of diabetes. Symptoms such as blurred vision, drowsiness, weight gain, numbness in hands and feet, and gum

disease are some symptoms of T2DM in patients [31]. According to the International Diabetes Federation report, 425 million people are affected by diabetes worldwide; every 6 seconds one person dies from diabetes [23].

Up to now various medications have been produced and α -glucosidase inhibition is one of these medications. By inhibiting α -glucosidase, glucose levels in the blood can be returned to normal limits. Hence, the inhibitory activity of this digestive enzyme can be a good treatment for T2DM.

The most recent medications used to treat T2DM are inhibitors of the DPP-IV enzyme. DPP-IV rapidly destroys the incretin hormones, GLP-1 and GIP. These peptides help the body produce more insulin when it is needed.

Synthetic oral hypoglycemic drugs are the most common form of treatment for T2DM, but they have undesirable side effects in patients with T2DM and also these drugs impose a high cost to patients. Therefore, research on medicinal plants as inexpensive resources containing valuable pharmaceutical metabolites is essential. *F. assa-foetida* L. is an important medicinal plant belonging to the Apiaceae, traditionally used for the treatment of different diseases and recent pharmacological and biological studies have shown its anti-diabetic activities.

Considering the importance of identification of more efficient drugs with less side effects and also understanding the mechanism of action of hypoglycemic plants and since the antidiabetic activity of *F. assa-foetida* has not previously been investigated mechanistically, this study was done to investigate the α -glucosidase and DPP-IV inhibitory activities of *F. assa-foetida*. In conclusion, the results of this study suggest that *F. assafoetida* through DPP-IV and α -glucosidase inhibitory activities can be involved in the blood glucose control and could be recommended for the treatment of T2DM.

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Table 2: DPP-IV inhibitory activity of different extractions of *F. assa-foetida* seed

Extracts	Percentage of DPP-IV inhibitory activity (%)
Ethanol	24.533±1.080
Methanol	14.346±1.080
Ethanol-methanol	22.33±1.56
Aqueous	12.322±0.838

DPP-IV: Dipeptidyl peptidase IV, *F. assafoetida*: *Ferula assafoetida*

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