Antidiabetic Activity of *Terfezia claveryi*; An *in vitro* and *in vivo* Study

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The main objective of current study was to investigate the *in vitro* and *in vivo* antidiabetic activity of *Terfezia claveryi* methanol extract. *In vitro* antidiabetic assays such as inhibition of α-amylase enzyme and non-enzymatic glycosylation of hemoglobin were carried out. The results of α-amylase inhibition assay revealed that the inhibitory activity (IC₅₀) of *Terfezia claveryi* methanol extract (38.7µg/ml) is stronger when compared with positive control (Acarbose IC₅₀ value of 45.3 µg/ml). The inhibition of glycosylation of hemoglobin of *Terfezia claveryi* methanol extract showed almost the same IC₅₀(33.1µg/ml) when compared with the positive control, alpha-tocopherol (35.4µg/ml). *In vivo* antidiabetic study revealed that *Terfezia claveryi* methanol extract possessed good activity at a dose of 200 mg/kg through reducing the fasting plasma glucose level (122.1±3.0 mg/dl) when compared with positive control (Glibenclamide of 79.4±1.4mg/dl) (p < 0.001). The results from this study indicated that *Terfezia claveryi* methanol extract exhibited considerable *in vitro* and *in vivo* antidiabetic activities. These possible activities could be useful to consider *Terfezia claveryi* as therapeutic antidiabetic candidate.

**Keywords:** Terfezia, antidiabetic, α-amylase, hemoglobin, streptozotocin.

Diabetes is considered one of the world’s largest endocrine disease, that characterized by an increased blood glucose level (hyperglycemia). Clinically, Diabetes is classified as type-1 (T1DM) characterized by insulin deficiency and type-2 (T2DM) characterized by insulin inefficiency. Uncontrolled diabetes could lead to severe complications to the cardiovascular system. Natural products have aided humans since long ages. They are considered as sources of important active ingredients. In comparison with synthetic drugs, synthetic one may cause many drawbacks such as vomiting, diarrhea, fluid retention, allergic reaction. Recently, the International Diabetes Federation (IDF) 7th edition of the Diabetes Atlas specified that 415 million people worldwide is diabetics. T2DM represents about 90-95% of all cases of diabetes. T2DM is considered one of the main international health concerns. T2DM affects around 422 million people all over the world. Prediabetes and diabetes prevalence and complications are growing in a bothersome way. By year of 2035, it is anticipated that about 592 million people will suffer from DM. The treatment of T2DM is currently achieved through the usage of conventional drugs that are effective in treatment.
of diabetes but to some extents still accompanied by some undesirable effects. The management of diabetes is considered a global problem and the search for a definite therapy is still ongoing. Truffle is a fungus, which grows wildly in desert regions depending on water rainfall. In addition, many researches stated that truffle can be used in many purposes such as source of energy, activation of sex hormones, and as antibiotics against gram positive bacteria including Bacillus subtilis and Staphylococcus aureus. Terfeziaboudieri ethanol extract showed anti-hyperglycemic effect on streptozotocin (STZ) induced-diabetic rats. Currently, there are no research studies were conducted to investigate the in vitro and in vivo antidiabetic potential of Terfeziaclaveryi. The previously mentioned data provoked us to assess the α-amylase inhibitory activity and effect on inhibition of glycosylation of hemoglobin as well as in vivo studies in streptozotocin-induced diabetic rats to evaluate and confirm its potential hypoglycemic effect.

MATERIAL AND METHODS

Plant Material

Terfeziaclaveryi (T. claveryi) was purchased from a local folk market in spring season, Al-Hasa, eastern region of Saudi Arabia. The fungus was subjected to air-drying according to the standard protocols. T. claveryi was kindly identified by Dr. Mamdouh Shokry, director of El-Zohria botanical garden, Giza, Egypt. A voucher specimen was kept in Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University, Al-Hasa, Saudi Arabia (03-17-Apr-TC).

Extraction and fractionation of different plant organs extracts

The air dried powdered material (500.0g) was exhaustively extracted three times at room temperature (for 5 days) using 3l of 70% MeOH/H₂O applying cold maceration technique at room temperature to protect the potential active ingredients from being decreased or destroyed. The solvent mixture was removed through distillation under vacuum using Rota vapor and dried extracts were directly freeze-dried to give the total methanol extract weighting 60.2g that were kept in -20°C for the next steps.

Animals

Male Wistar albino rats having a weight of 150 – 210 g were kept in quarantine for 2 weeks under standard husbandry conditions (27°C, Relative humidity 65±10%) for 12 h in dark and light cycle, respectively, and were given standard food and water ad libitum. All of the experiments were done in this study according to the Animal Ethics Committee of King Faisal University.

Chemicals

Acarbose, glibenclamide, streptozotocin, metformin, gentamycin, α-amylase from porcine pancreas, hemoglobin porcine and alpha-tocopherol were purchased from Sigma Aldrich (ST. Louis, Mo, USA). Solvents used for extraction and assays were all of analytical grade.

In vitro anti-diabetic models

α-Amylase inhibitory activity

The assay mixture was prepared to contain 0.02M sodium phosphate buffer (200 µl), α-amylase enzyme (20 µl, 2 unit/ml) together with different plant extracts in the range of concentrations 20-100µg/ml. Then, it was incubated for 10 min at room temperature followed by the addition of 200 µl of 1% starch suspension to all the tubes containing reaction mixture. The reaction was later terminated by the addition of 400 µl of 3, 5 di-nitro salicylic acid (DNSA) color reagent. Then the tubes were kept in boiling water bath for 5 minutes, and later were kept till being cooled at room temperature and diluted with 15 ml of distilled water. The absorbance of each reaction mixture was measured at 540nm. Control mixture reactions were also prepared accordingly without addition of extracts of plant under investigation and were compared with the test samples containing concentration of different plant extracts (20-100µg/ml) freshly prepared in DMSO. The results were indicated as % of inhibition of activity using the following formula:

\[ \text{Inhibition activity (\%) = } \frac{\text{Abs(control)} - \text{Abs(extract)}}{\text{Abs(control)}} \times 100 \]

where; Abs (control) is the absorbance of the control reaction (containing all reagents except the test sample) and Abs (sample) is the absorbance of different plant extracts. The IC₅₀ values (inhibitory concentration which will produce 50% inhibition of the enzyme activity) of the plant extracts were determined. Acarbose which is a well-known and safe anti-diabetic drug used to
treat T2DM, was applied as a positive control in the concentrations ranging from 20 to 100 µg/ml. Experiments were achieved in triplicates.

**Non-enzymatic glycosylation of hemoglobin assay**

Solutions of glucose (2%), hemoglobin (0.06%), and gentamycin (0.02%), were freshly prepared in phosphate buffer (0.01 M, pH 7.4). One ml of each of above mentioned solution was mixed. One ml of each concentration of different plant extracts (20–100 µg/ml) was added to the prepared mixture. Then, the test tubes containing reaction mixture were incubated in dark place at room temperature for three days. After, the degree of glycosylation of hemoglobin was obtained colorimetrically at 520 nm where the percentage of inhibition was calculated applying this formula:

\[
\text{Percentage of inhibition} = \left( \frac{\text{Abs (control)} - \text{Abs (extract)}}{\text{Abs (control)}} \right) \times 100
\]

where; Abs (control) is the absorbance of the control reaction (containing all reagents except the test sample) and Abs (sample) is the absorbance of different plant extracts. The IC\textsubscript{50} values (inhibitory concentration which will produce 50% inhibition of the enzyme activity) of the plant extracts were determined. Alpha-Tocopherol was used as a standard drug. Experiments were carried out in triplicates.

**In vivo anti-diabetic model**

**Acute toxicity testing**

Acute toxicity testing was performed for T. claveryi total methanol extract, where the rats took ascending oral doses up to 2000 mg/kg of each extract, and signs and symptoms of toxicity were observed for the next 48 h.

**Induction of diabetes**

Diabetes was induced by intraperitoneal (i.p.) injection of streptozotocin (STZ) dissolved in 0.1 M cold citrate buffer (pH=4.4) at a dose of 60 mg/kg body weight. On the third day after STZ injection, fasting blood glucose levels were measured by hand-held glucose monitoring (BAYER Contour). Only rats with serum glucose levels of 190–200 mg/dl were selected and considered diabetic animals.

**Experimental design**

The animals were segregated into five groups of five rats each. Group I served as normal control rats, administered drinking water and 0.1 M cold citrate buffer (pH=4.4) daily for 12 d; Group II had diabetic control rats, administered drinking water daily for 12 days; Group III diabetic rats were administered T. claveryi total methanol extract (200 mg/kg) for 12 d; and Group IV diabetic rats were administered standard drug glibenclamide (0.25 mg/kg) for 12 d. The fasting glucose levels were determined on days 1, 5, and 12 of extract administration.

**Statistical analysis**

Values were expressed as mean±SE (Standard Error). To analyze the differences between groups, statistical analysis was performed by one-way ANOVA followed by post-hoc Tukey using a computer soft program SPSS v.20. Significance was considered at a p value <0.05.

**RESULTS**

**α-Amylase inhibitory activity**

The in vitro α-amylase inhibitory measurements demonstrated that T. claveryi total methanol extract has potential α-amylase inhibitory properties. α-amylase inhibitory activities were compared based on the calculated IC\textsubscript{50} values (Table 1). The observed α-amylase inhibitory activity of T. claveryi total methanol extract was (38.7 µg/ml). Acarbose was used as the positive standard. It showed IC\textsubscript{50} value of 45.3 µg/ml under similar conditions.

**Non-enzymatic glycosylation of hemoglobin assay**

The inhibitory activities of T. claveryi total methanol extracts were recorded (Table 2). T. claveryi total methanol extract showed almost the same value of IC\textsubscript{50} (33.1 µg/ml) to the positive control, alpha-tocopherol (35.4 µg/ml).

**Acute toxicity study**

No toxicity or death was observed in the experimental rats. Hence 200 mg/kg (1/10 of the 2000 mg/kg) was selected as a maximum safety dose.

**In vivo antidiabetic activity**

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**Acute toxicity study**

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**In vivo antidiabetic activity**

The effect of T. claveryi total methanol extract on fasting blood glucose levels of diabetic rats was presented in Table 3. In diabetic rats, as shown in Table 3, T. claveryi total methanol extract and glibenclamide had a significant time dependent hypoglycemic activity, compared with the diabetic control group at each time point (p<0.001).
**DISCUSSION**

α-Amylase enzyme is one of the enzymes responsible for the hydrolysis of α-oriented bond polysaccharides and oligosaccharides such as starch, glycogen and other macromolecules of α-bond linked monosaccharides to disaccharides and finally to glucose. \[ T. claveryi \] total methanol extract showed promising result in α-amylase inhibition assay, suggesting that \([ T. claveryi ]\) might be effective in slowing down hydrolysis of starch to minimized glucose availability.

*Invitronon*-enzymatic glycosylation of hemoglobin method is one of important assays to judge the control of diabetes. The hemoglobin present in RBCs has an affinity to bind to glucose. The greater the glucose level in blood, more amount of glucose-bound (called glycosylated) hemoglobin will be formed. Such glucose hemoglobin association is to some extent stable and stays for 1-2 months (the life-span of red blood corpuscles).

<table>
<thead>
<tr>
<th>Table 1. α-amylase inhibitory effect of ([ T. claveryi ]) total methanol extract</th>
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</thead>
<tbody>
<tr>
<td>Percentage of inhibition</td>
</tr>
<tr>
<td>--------------------------</td>
</tr>
<tr>
<td>conc. µg/ml</td>
</tr>
<tr>
<td>17.1±0.9</td>
</tr>
<tr>
<td>32.2±1.1</td>
</tr>
</tbody>
</table>

Table 2. Non-enzymatic glycosylation of hemoglobin effect by \([ T. claveryi ]\) total methanol extract

<table>
<thead>
<tr>
<th>Percentage of inhibition</th>
<th>([ T. claveryi ]) leaves methanol extract</th>
<th>standard (alpha-Tocopherol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>conc. µg/ml</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>24.4±1.2</td>
<td>28.5±0.3</td>
<td>34.6±1.3</td>
</tr>
<tr>
<td>38.8±0.5</td>
<td>49.3±0.6</td>
<td>71.6±0.6</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SE (Standard Error, n=3 independent experiments)

**Table 3. Results of the in vivo study on STZ-induced diabetic rats by \([ T. claveryi ]\) total methanol extract**

<table>
<thead>
<tr>
<th>Fasting plasma glucose concentration (mg/dl)</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 12</td>
<td>Day 5</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>81.6±1.1</td>
<td>80.9±0.8</td>
</tr>
<tr>
<td>200.3±2.5</td>
<td>198.18±1.6</td>
</tr>
<tr>
<td>122.1±3.0*</td>
<td>138.6±1.6*</td>
</tr>
<tr>
<td>79.4±1.4*</td>
<td>91.38±1.1*</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SE (Standard Error, n=6), *significantly different from diabetic control (p<0.001).
reduced plasma glucose (Table 3). During the study it was found that T. claveryi total methanol significantly controlled the blood glucose level in Streptozotocin-induced diabetic rats as compared to the diabetic control group (Table 3).

CONCLUSION

The above conducted in vitro examinations depict a substantial α-amylase inhibitory and percentage of inhibition glycosylation of hemoglobin of T. claveryi total methanol. Which was further confirmed by in vivo studies that showed T. claveryi total methanol significantly controlled the blood glucose level diabetic rats. It could be therefore conclude from this study that T. claveryi can serve as a therapeutic agent and can be used as a potential source of new antidiabetic product.

REFERENCES


