# Antidiabetic Activity of *Terfeziaclaveryi*; 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 *Terfeziaclaveryi* 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 ($IC_{50}$) of *Terfeziaclaveryi* methanol extract (38.7µg/ml) is stronger when compared withpositive control (Acarbose $IC_{50}$ value of 45.3 µg/ml).The inhibition of glycosylation of hemoglobin of *Terfeziaclaveryi* methanol extract showed almost the same $IC_{50}$(33.1µg/ml)when compared the positive control, alpha-tocopherol ( 35.4µg/ml ). *In vivo* antidiabetic study revealed that *Terfeziaclaveryi* 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 studyindicated that *Terfeziaclaveryi* methanol extract exhibited considerable *in vitro* and *in vivo* antidiabetic activities.These possible activities could be useful to consider *Terfeziaclaveryi* 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 [1].Natural products have aided humans since long ages. They are considered assources of important activeingredients. In comparison with synthetic drugs, synthetic one may cause many drawbacks such as vomiting, diarrhea, fluid retention, allergic reaction[2]. Recently, the International Diabetes Federation (IDF) 7th edition of the Diabetes Atlas specified that 415 million people worldwide is diabetics[3]. T2DM represents about 90-95% of all cases of diabetes[4]. T2DM is considered one of the main international health concerns. T2DM affects around 422 million people all over the world[5]. 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[6]. The treatment of T2DM is currentlyachieved through the usage of conventional drugs that are effective in treatment

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of diabetes but to some extents still accompanied by some undesirable effects[7]. 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 [8]. 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*[9-11]. *Terfeziaboudieri* ethanol extract showed anti-hyperglycemic effect on streptozotocin (STZ) induced-diabetic rats[8]. Currently, there are no research studies were conducted to investigate the *in vitroand in vivo* antidiabetic potential of *Terfeziaclaveryiý*. The previously mentioned data provoked us to assess the á-amylase inhibitory activityand 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 marketin 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. MamdouhShokry, 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 3lof 70% MeOH/$H_2O$ 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$^o$C for the next steps[12].

### Animals

Male Wistar albino rats having a weight of 150 – 210 g were kept in quarantine for 2 weeks under standard husbandry conditions (27$^o$, Relative humidity 65±10%) for 12 h in dark and light cycle, respectively, and were given standard food and water *ad libitum*[13]. All of the experiments were done in this study according to the Animal Ethics Committeeof 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) ýtogether withdifferent plant extracts in the range of concentrations20-100ìg/ml.Then, it was incubated for 10 min at room temperature followed by the addition of 200ìl of 1% starch suspension to all the tubes containing reaction mixture. The reaction was later terminated bythe 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 ofextracts 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 inhibitionof activity using the following formula:,

$$Inhibition\ activity\ (\%) = \frac{Abs(control) - Abs\ (extract)}{Abs\ (control)} X100$$

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[14,15].The $IC_{50}$ 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 rangedfrom 20 to100 µg/ml[16]. 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 (20-100µg/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 520nm where the percentage of inhibition was calculated applyingthis formula:,

$$Percentage\ of\ inhibition = \frac{Abs(control) - Abs\ (extract)}{Abs\ (control)} X100$$

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 $IC_{50}$ 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[14-16]. 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, were studied 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[17].

**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, fasted 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[18].

**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 extractsadministration[3, 17, 18].

**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-hocTukey 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. clavery* total methanol extracthas potential of α-amylase inhibitory possessions. α-amylase inhibitory activitieswere compared based on the calculated $IC_{50}$ values (Table 1). The observed α-amylase inhibitory activity of *T. claveryi* total methanol extract was (38.7µg/ml). Acarbose was used as the positive standard. It showed $IC_{50}$ 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 $IC_{50}$ (33.1µg/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**

The effect of *T. claveryi* total methanol extract on fasting blood glucose levels of diabetic ratswas presented in table3.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[19-22]. *T. claveryi* total methanol extract ýshowed promising result in α-amylase inhibition assay, suggesting that *T. claveryi* might be effective in slowing down hydrolysis of starch to minimized glucose availability.

*Invitro*non-enzymatic glycosylation of hemoglobin method is one of important assays to judge the control of diabetes. The hemoglobin present in RBCs has anaffinity 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 staysfor1-2 months (the life-span of red blood corpuscles) [22, 23].Consequently presence of higher concentration of glycosylated hemoglobin is a sure guide to the higher concentration of glucose in the blood. Normally, the percentage of glycated hemoglobin should not be exceeding 12%. The current study demonstrated good activity of *T. claveryi* total methanol (almost the same that of positive control, alpha-tocopherol)in preventing such binding of glucose to surface proteins of erythrocytes.

The fundamental mechanism underlying elevated blood sugar in diabetes mellitus involves over-production and decreased utilization of glucose by the tissues. In the current study, the difference observed between the initial and final fasting plasma glucose levels of different groups under investigation, revealed a significant elevation in blood glucose in the diabetic control group as compared to normal animals, at the end of the twelve-day experimental period. When *T. claveryi* total methanol was administered to diabetic rats, a decrease in plasma glucose level was observed after 12 days. *T. claveryi* total methanol

**Table 1.** α-amylase inhibitory effect of *T. claveryi* total methanol extract

| conc. μg/ml | Percentage of inhibition *T.claveryi* methanol extract | standard (Acarbose) |
|---|---|---|
| 20 | 17.1±0.9 | 32.2±1.1 |
| 40 | 28.0±1.1 | 43.8±1.3 |
| 60 | 54.2±1.3 | 64.9±2.3 |
| 80 | 59.5±1.1 | 75.5±1.4 |
| 100 | 68.4±1.7 | 81.1±1.3 |
| $IC_{50}$ μg/ml | 38.7 | 45.3 |

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

**Table 2.** Non-enzymatic glycosylation of hemoglobin effect by *T. claveryi* total methanol extract

| conc. μg/ml | Percentage of inhibition leaves methanol extract | standard (alpha-Tocopherol) |
|---|---|---|
| 20 | 24.4±1.2 | 38.8±0.5 |
| 40 | 28.5±0.3 | 49.3±0.6 |
| 60 | 34.6±1.3 | 71.6±0.6 |
| 80 | 44.2±1.5 | 81.0±1.0 |
| 100 | 50.5±0.5 | 82.7±1.6 |
| $IC_{50}$ μg/ml | 33.1 | 35.4 |

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

| Fasting plasma glucose concentration (mg/dl) Day12 | Day5 | Day 1 | Groups |
|---|---|---|---|
| 81.6±1.1 | 80.9±0.8 | 79.9±1.2 | I- Normal control |
| 200.3±2.5 | 198.18±1.6 | 196.8±2.4 | II- Diabetic control (streptozotocin) (55 mg/kg) |
| 122.1±3.0* | 138.6±1.6* | 197.9±1.9 | III- Diabetic + leaves methanol extract (200 mg/kg) |
| 79.4±1.4* | 91.38±1.1* | 196.5±1.5 | IV- Diabetic + standard glibenclamide (0.25 mg/kg) |

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.

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