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In vitro Evaluation of Anti-Diabetic Effect of *Trigonella foenum graecum* Leaves in Different Solvent Extracts

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ABSTRACT

Diabetes mellitus is a metabolic disease characterized by high blood glucose level resulting from defects in insulin secretion, insulin action or both. Diabetes is considered as one of the most significant diseases in developed country. There are increasing incidences of diabetes every day and this indicate the need for treatment for it. The present study aims to evaluate the anti-diabetic activity of *Trigonella foenum-graecum* leaves in different solvent extracts in in vitro. The leaves were collected, dried and subjected to ethanol and petroleum ether extraction. The extracts were than subjected for in-vitro anti-diabetic activity assays such as Alpha-amylase inhibition assays, Non-enzymatic glycosylation of haemoglobin, and Glucose uptake in yeast cell and compared with their respective standards acarbose drug, ascorbic acid and metronidazole. The obtained result signifies that higher concentration of extracts possesses high effective anti-diabetic activity. The results of the work indicate that the both extracts of plant possessed considerable in vitro antidiabetic activity by inhibition of α-amylase, ethanol extract of plant shows maximum inhibition (73.4%) of glycosylation of haemoglobin, while extracts of *T. foenum-graecum* provide uptake of glucose by yeast cells which differ with the sample and glucose concentration, maximum increase in 5mM glucose concentration. Hence, from study it is concluded that *Trigonella foenum-graecum* leaves might be considered as herbal remedies for diabetes. However, the effect need to confirm using further anti-diabetic investigation and clinical trials for its effective utilization.

KEY WORDS: ANTI-DIABETIC ACTIVITY, ETHANOL EXTRACT, PETROLEUM ETHER EXTRACT, *Trigonella foenum-graecum.*

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INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by high blood sugar (glucose) levels that result from defects in insulin secretion or action, or both. Hyperglycemia resulting either due to defective production or action of insulin leads to a number of complications; cardiovascular, renal, neurological, ocular etc (Gray et al., 2000). According to International Diabetes Federation it is estimated that 463 million people have diabetes in 2019. Given that half a billion people are living with diabetes and there is an urgent need for developing and implementing multi-sectoral strategies to tackle diabetes. Without urgent and sufficient actions, it is predicted that 578 million people will have diabetes in 2030 and the number will increase by 51% (700 million) in 2045 (Saeedi et al., 2019)

Medicinal plants are used by 80 % of the world population especially in developing countries to cure and improve the general health, principally due to the common belief that plant-derived drugs are without any side effects along with being economical and locally accessible (Gupta et al.,1998). There has been growing interest in the application of natural components as antidiabetic agents (Qi L et al., 2010). A wide range of products claiming to lower blood glucose levels or prevent and treat diabetes complications and comorbidities are marketed to the public, (Geil et al., 2008). Fenugreek is one of the medicinal plants specially its seeds, which is widely used in folk medicine. It has a diuretic, uterine & cardio tonic, hypotensive, hypolipidemic, hypoglycemic, antinociceptive and anti-inflammatory (Al-Khateeb et al 2012).Among the various medicinal plants documented use as a hypoglycemic agent, *Trigonella foenum-graecum* commonly known as fenugreek in English and Methi in various Indian languages is important dietary and medicinal plants (Al-Khateeb et al 2012, Nathiya et al 2014). The *Trigonella foenum-graecum* seeds solution is effective in hyperlipidemia of diabetic patients, (Geberemeskel et al., 2019).

Fenugreek seeds powder have potent hypolipidemic effects when given with atorvastatin. (Hemavardhini et al., 2018).Though several forms of treatments are available in terms of medications and injectable insulin, they are accompanied with side effects. There are many drugs available in market for the treatment of diabetes like sulfonylurea's, biguanides, and alpha glucosidase inhibitors which are more expensive and have various side effects, but natural herbal drugs have been found to have lesser side effects and also provide long term effect for therapy in treating diabetes, (Sundarrajan et al.2019).

In the perspective on the above mentioned studies, there is no evidence of *in vitro* antidiabetic activity of Trigonella foenum graecum leaves. Hence, the present study was coordinated to investigate the antidiabetic action based on the inhibitory action on alpha-amylase, glycosylation of haemoglobin and glucose utilization by glucose uptake by yeast cells method. The aim of present study is to evaluate the antidiabetic potential of different solvent extracts of *Trigonella foenum-graecum* leaves by *in vitro* antidiabetic method.

MATERIAL AND METHODS

Fresh leaves of plant were purchased from market. The leaves were washed, dried and grind into fine powder. Petroleum ether and ethanol extractions of leaves were done using Soxhlet apparatus. After all petroleum ether and ethanol were removed from respective filtrate. The extracts were stored at the refrigerator for further study. The in vitro studies were carried out according to the method of (Joshi et al., 2013) with some modifications. Alpha-Amylase inhibition assay:Alpha amylase inhibitory activity was based on the starch iodine method. In alpha amylase inhibition method 1ml substrate- potato starch (1% w/v), 1 ml of drug solution (Acarbose std drug/ extract) of five different concentration such as 50, 100, 150, 200, and 250 µg/ml, 1ml of alpha amylase enzyme (1% w/v) and 2ml of acetate buffer (0.1 M, 7.2 pH) was added. The above mixture was incubated for 1 hr. Then 0.1 ml Iodine-iodide indicator was added in the mixture. Absorbance was taken at 565 nm in UV-Visible spectroscopy. % inhibition was calculated by formula,(Gupta et al., 2012).

$$\text{Inhibition of alpha- Amylase (\%)} = \frac{\text{Abs sample} - \text{Abs control}}{\text{Abs sample}} \times 100$$

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample), and Abs sample is the absorbance of the test sample. All the experiments were carried out in triplicates.

Non-enzymatic glycosylation of haemoglobin: Glucose (2%), haemoglobin (0.06%) and Gentamycin (0.02%) solutions were prepared in phosphate buffer 0.01 M, pH 7.4. 1 ml each of above solution was mixed. 1 ml of each concentration was added to above mixture. The reaction mixture was incubated in dark at room temperature for 72 hrs and then the degree of glycosylation of haemoglobin was measured colorimetrically at 520 nm. Ascorbic acid was used as a standard drug for assay and percentage inhibition was calculated using the formula

$$\%\text{inhibition} = \frac{\text{Absorbance Sample} - \text{Absorbance Control}}{\text{Absorbance Sample}} \times 100$$

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample.

Glucose uptake in Yeast cells: Yeast suspension was prepared by repeated washing (by centrifugation at 3,000×g; 5 min) in distilled water until the supernatant fluids were clear (Cirillo 1962). A 10% (v/v) suspension was prepared with the supernatant fluid. 1mL of glucose solution (5, 10 and 20 mM) was added to various concentrations of extracts (50,100,150,200 and 250 µg) and incubated for 10 min at 37°C. Reaction was started by adding 100 µl of yeast suspension, vortex and further

incubated at 37°C for 60 min .After 60 min, the reaction mixture was centrifuged (2,500×g, 5 min) and glucose was estimated in the supernatant. Metronidazole was taken as standard drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula

$$\text{Increase in glucose uptake (\%)} = \frac{\text{Abs sample} - \text{Abs control}}{\text{Abs sample}} \times 100$$

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample), and Abs sample is the absorbance of the test sample. All the experiments were carried out in triplicates.

RESULTS AND DISCUSSION

α-Amylase inhibition assay: Ethanolic and petroleum ether extract of *Trigonella foenum graecum* leaves exhibited significant inhibition in alpha-amylase activity. From table no. 1 it is seen that as the concentration increases inhibition activity is also increases Petroleum ether extract of plant shows higher inhibition (61.4%) at 250µg/ml which considerably more than standard (61.1%) and ethanolic extract (58.8%). Inhibition of α-amylase enzyme reduced the high Postprandial (PP) blood glucose peaks in diabetes. These α- amylase inhibitors are also known as starch blockers as they prevent or slow down the absorption of starch into the body mainly by blocking the hydrolysis of 1,4-glycosidic linkages of starch and other oligosaccharides into other simple sugars (Banerjee et al 2017).

The amylase inhibitors act as an anti-nutrient that obstructs the digestion and absorption of carbohydrates. Acarbose is complex oligosaccharides that delay the digestion of carbohydrates. It inhibits the action of pancreatic amylase in breakdown of starch. Synthetic inhibitor causes side effect such as abdominal pain, diarrhoea and soft faeces in the colon. The reaction mechanisms involved in inhibition of α-amylase enzymes by plant protein inhibitors are not clearly understood. But there are some suggestions that the plant protein might cause conformational changes in structure (Medagama and Sinadhira 2015).

Table 1. Alpha Amylase inhibition method

Concentration in µg/ml	STD %inh	Ethanol %inh	PE %inh
50	34.8±0.7571	30.2±0.4163	31.5±0.5487
100	50.8±0.1001	48.1±0.5572	49±0.5507
150	55.5±0.5773	55.4±0.3464	55.8±0.5825
200	58.8±0.60	57.8±0.5507	59.2±0.4333
250	61.1±0.5487	58.8±0.4977	61.4±0.4333

STD-Standard, PE-Petroleum ether, % inh-Percent inhibition. Values are expressed as ±SEM.

Table 2. Alpha Amylase inhibition method

IC_{50} value	STD	Ethanol	P.E
	28.994	150.451	139.886

IC_{50} value-Inhibitory concentration at 50%

Non-enzymatic glycosylation of haemoglobin: The haemoglobin present in the red blood corpuscles has a tendency to get bound to glucose. The inhibitory activity of ethanol and petroleum ether extract of *Trigonella foenum graecum* was found and compared with the standard drug. Results showed that the ethanol extract showed higher inhibitory activity up to 73.3% (Table No. 3) which is higher when compared to petroleum ether and standard. The greater the blood-glucose concentration, the greater is the amount of glucose-bound haemoglobin which leads to the formation of reactive oxygen species (Ogundele et al., 2017). The glucose autooxidation, protein glycosylation, formation of advanced glycation end products, and polyol pathway all are involved in the generation of the oxidative stress, implicated in the origin of type II DM (Kotb and Khaldun 2015). As the concentration increases, formation of the glucose–haemoglobin complex decreases and free haemoglobin increases, this shows the inhibition of glycosylated haemoglobin.

Table 3. Non-enzymatic glycosylation of haemoglobin method

Concentration in µg/ml	STD %inh	Ethanol %inh	PE %inh
50	18±0.4910	25±0.5811	7.6±0.4582
100	28±0.6359	50±0.5773	17.2±0.3464
150	33.3±0.6350	61.2±0.2309	25±0.5573
200	36.8±0.5206	69.2±0.2728	29.4±0.36055
250	40.2±0.2309	73.3±0.3711	33.3±0.9643

STD-Standard, PE-Petroleum ether, % inh-Percent inhibition. Values are expressed as ±SEM.

Table 4. Non-enzymatic glycosylation of haemoglobin method

IC_{50} value	STD	Ethanol	P.E
	327.358	125.541	366.194

IC_{50} value-Inhibitory concentration at 50%

Table 5. Effect of Standard drug on glucose uptake by yeast cells

Concentration in µg/ml	20 mM	10mM	5mM
50	44.9±0.6064	45.2±0.2603	46.6±0.2333
100	60.2±0.1201	70.3±0.4910	72±0.1154
150	74.4±0.2886	75.1±0.2027	84.9±0.0881
200	75.4±0.4055	78.2±0.2645	87.2±0.23094
250	77.4±0.4333	81.9±0.4666	90.4±0.4255

STD-Standard, PE-Petroleum ether, % inh-Percent inhibition. Values are expressed as means ±SEM.

Table 6. Effect of Petroleum ether extract of *T.foenum-graecum* on glucose uptake by yeast cells.

Concentration in µg/ml	20 mM	10mM	5mM
50	47.2±0.5238	47.5±0.4041	48.1±0.5658
100	69.1±0.4333	70.6±0.3464	74.9±0.5487
150	72.8±0.46188	76±0.5773	78±0.72188
200	76.6±0.4333	78±0.4163	86.9±0.2403
250	78±0.5487	84±0.3179	90.5±0.5131

STD-Standard, PE-Petroleum ether, % inh-Percent inhibition. Values are expressed as means±SEM.

Table 7. Effect of Ethanol extract of *T.foenum* graecum on glucose uptake by yeast cells

Concentration in µg/ml	20 mM	10mM	5mM
50	46.2±0.5607	47.2±0.4163	48.2±0.3756
100	58±0.0333	69±0.2848	71.3±0.333
150	69.1±0.3527	71.2±0.8717	79.2±0.6936
200	73.1±0.6359	74.4±0.3055	82.9±0.5507
250	75.4±0.3055	76.6±0.3464	86.1±0.5925

STD-Standard, PE-Petroleum ether, % inh-Percent inhibition, Conc.-Concentration Values are expressed as ±SEM.

Glucose uptake by yeast cell: It is stated that the transport of glucose across yeast cell membrane occurs by facilitated diffusion down the concentration gradient. Hence glucose transport occurs only if the intracellular glucose is effectively reduced (utilized) (Vijayalakshmi. et al 2014, Shehzadi et al., 2018).The data obtained clearly suggests that the ethanol and petroleum ether extract of *Trigonella foenum graecum* leaves is capable of effectively enhancing glucose uptake which in turn suggests that it is capable of enhancing the effective glucose utilization, thereby controlling blood glucose level. The extracts of *T.foenum graecum* leaves promoted the uptake of glucose across the plasma membrane of yeast cells (Table No.6 and 7). The highest uptake of glucose was seen in 5mM glucose concentration of petroleum ether extract of *T.foenum graecum*.

Table 8. Glucose uptake in different glucose concentration

	5mM			10mM			20mM		
IC_{50} value	STD	PE	Ethanol	STD	PE	Ethanol	STD	PE	Ethanol
	22.53	17.35	14.90	26.23	18.42	12.34	47.5	14.39	52.31

IC_{50} value-Inhibitory concentration at 50%

CONCLUSION

The results indicate that the extracts of T*rigonella foenum graecum* possess antidiabetic properties. This activity may be due to the strong occurrence of phenolic compounds such as alkaloids, flavanoids, tannins, steroids and phenols. This study gives an idea that the compounds of *Trigonella foenum graecum* can be used as lead compound for designing a potent anti-diabetic drug which can be used for treatment.

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