ABSTRACT

Introduction: Trigonella foenum graecum (fenugreek) seeds have been suggested to have potential anti-diabetic and anti-oxidative effects. Aim: Study the effect of aqueous extract of Trigonella foenum graecum seeds on glycemic status, insulin resistance, HOMA, antioxidant superoxide dismutase (SOD), catalase, glutathione reductase (GRX), glutathione peroxidase (GPX) and oxidative stress MDA, nitric oxide (NO), carbonyl protein, in Type 2 Diabetes Mellitus (T2DM) [EF-2.4.4] subjects and its correlation. Methods and Materials: Blind Randomized case control study consists of healthy control (n=30), T2DM control (n=30) and T2DM cases with adjunct therapy (n=30). T2DM patients with adjunct therapy were given 1.4gm aqueous extract of fenugreek seeds for 3 months. Baseline and 3 months follow up study was done. Blood parameters were analysed using chemistry auto-analysser, Chemiluminescence, and spectrophotometer. Results: Fasting blood sugar, Hb1C, MDA parameters were significantly lowered after three months in the group receiving adjunct therapy. Anthropometric, antioxidant statuses were seen to be improved and oxidative stress decreased. Insulin resistance were improved in the fenugreek group, based on Homeostatic model assessment (HOMA). Conclusion: Aqueous extract of fenugreek is effective and safe to control hyperglycemia by stabilizing glucose homeostasis, glycemic status, antioxidant and reduced oxidative stress in T2DM.

Key words: Fenugreek, Trigonella Foenum-Graecum, Oxidative stress, T2DM.
INTRODUCTION

As per American diabetes association expert committee Diabetes Mellitus [EF-2.4.4] is defined as a group of metabolic disorders characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins resulting from defective insulin secretion, insulin action or both [1, 2]. It is a predominant public health concern, substantially causing morbidity, mortality, and long-term complications and remains an important risk factor for macro and micro vascular diseases like retinopathy, nephropathy, neuropathy, cardiovascular diseases etc. [3]. The most frequent diabetes forms are Type 1 diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM). In India, approximately 31.7 million people suffered from diabetes in 2000 and it is estimated that about 79.4 million people will be diabetic by 2030. According to the estimates of International Diabetes Federation (IDF) in 2013, India alone has 65.1 million people living with diabetes; this places India second to China [1, 4, 5].

Insulin resistance (IR) is a crucially important metabolic abnormality in T2DM, and overt diabetes is thought to be preceded by a long period of insulin resistance, during which blood glucose is maintained near normal levels by compensatory hyperinsulinemia. When β cells are no longer able to compensate for insulin resistance by adequately increasing insulin production, impaired glucose tolerance (IGT) appears. This condition is characterized by an excessive blood glucose concentration in the postprandial phase, with fasting glucose being in the normal range. Persistence of imbalance between caloric intake and expenditure eventually leads to overt diabetes, characterized by hyperglycemia in any condition whether fasting or postprandial [6, 7, 8].

“Oxidative stress” can be defined as any disturbance in the balance of antioxidants and pro-oxidants or excess formation and insufficient removal of highly reactive molecules such as reactive nitrogen species (RNS) and reactive oxygen species (ROS). Damage to DNA, proteins, and other macromolecules due to oxidation has been implicated in the pathogenesis of a wide variety of diseases. ROS level elevation in diabetes may be due to decrease in destruction or/and increase in the production by catalase (CAT—enzymatic/non-enzymatic), superoxide dismutase (SOD) and glutathione peroxidase (GPX) antioxidants. The variation in the levels of these enzymes makes the tissues susceptible to oxidative stress leading to the development of diabetic complications [9].

Diabetes induces alterations in activity of enzymes glutathione peroxidase and
glutathione reductase (GRX). These enzymes are found in cells that metabolize peroxide to water and converting glutathione disulfide back into glutathione. Any alteration in their levels will make the cells prone to oxidative stress and hence cell injury. Catalase is regulator of hydrogen peroxide ($H_2O_2$) metabolism that can, in excess, cause serious damage to lipids, RNA and DNA. CAT converts $H_2O_2$ catalytically into water and oxygen and thus neutralizes it. SOD provides first line defense against ROS mediated cell injury by catalyzing the proportion of superoxide, the primary ROS in oxygen metabolism, to molecular oxygen and peroxide $[10, 11]$. 

Diabetes mellitus has been known since ages and the sweetness of diabetic urine has been mentioned in Ayurveda by Sushruta. Its pharmacotherapy however is over 80 years old. Since ancient times, a number of herbal medicines have been used in the treatment of this disease. Drug-induced liver injury (DILI) is one of the most common causes of discontinued use of drug medications. Hence, there is increasing demand by patients to use the natural products with antidiabetic activity $[12, 13]$. 

Fenugreek ($Trigonella foenum-graecum$) and other traditional plants are currently being investigated for their potential as a source of new hypoglycaemic compounds for the treatment of diabetes. Fenugreek, one of the oldest medicinal plants, is of Mediterranean origin and cultivated worldwide $[5, 14, 15]$. 

Fenugreek is an annual leguminous plant, the seeds of which contain 6-10% lipid, 44-59% carbohydrate and 20-30% protein. In comparison with other legumes, fenugreek seeds contain higher ratios of minerals (i.e., Ca, P, Fe, Zn and Mn). The seeds also contain some aromatic components such as n-alkanes, terpenes, nonalactone and saponins. Also, these seeds are known to be rich in polyphenolic flavonoids, which have antioxidant activities and protect the cellular structures from oxidative damage $[5]$. 

Aqueous extracts of seeds and leaves of fenugreek have been widely used in diabetes treatment and shown to possess hypoglycaemic, anti-lipidimic and antioxidant properties activity $[7, 16, 17]$. 

Our study was designed to determine the protective effects of Fenugreek in Type 2 diabetic patients on oxidant and antioxidant factors using antidiabetic adjunct Fenugreek therapy along with regular diabetic medications. In addition to hypoglycemic (Fasting glucose and HbA1c), antioxidant properties by SOD, Carbonyl Protein, MDA, NO, Catalase, GPX and GRX were also analyzed. We have studied it’s mechanism of action by levels of serum insulin, C-peptide levels and ‘HOMA Insulin Resistance 2’ a computer model $[18, 19]$. 


MATERIAL AND METHODS

Study design: Randomised Case-Control study.

Study population: The study population comprised of Asian Indians residing near and in Mumbai metropolitan city. They were diagnosed for Type 2 diabetes as per American Diabetes Association criteria \(^2\) with age-sex matched between 30 and 60 years. Subjects were from different socioeconomic status.

Study sample: Blood serum samples of the subjects were collecting in fasting state.

Sample size: 30 healthy control, 30 T2DM control and 30 T2DM cases with adjunct fenugreek therapy samples were recruited in the study.

Sample size calculation: Sample size was calculated using the following formula:

\[ n = \frac{4pq}{L^2} \]

Where, \( n \) = Sample size, \( p \) = Prevalence of variable under study, \( q = 100-p \), \( L \) = Allowable error in % of \( p \).

Study settings: Study was undertaken in Department of Biochemistry, Grant medical college and Sir J. J. groups of Hospitals, Mumbai. Approval of the ethical committee of the institute was taken for the study along with written informed consent from the subjects. The diabetic subjects were attending OPD in Sir J. J. group of hospitals, and other private clinics, where as healthy non-diabetic subjects were recruited randomly from our residential area.

Diagnostic criteria: Subjects were classified as per ADA 2004 criterion as diabetic and non-diabetic.

1) Inclusion criteria: Subjects were included in the study based on following criteria -
- Age group of 30 to 60 years
- Either sex
- Subjects willing to give written informed consent for participation in the study.

2) Exclusion criteria: Subjects were excluded from the study based on following criteria -
- Patient with type 2 diabetes who are difficult to control with respect to glucose homeostasis (non-stable patients)
- Severe addicts for alcohol, drugs, tobacco and smoking (more than 10 cigarettes/day).
- Known hyper sensitivity to fenugreek.
- Recent participation in clinical trials.
- Recent use of any herb or anti diabetic drug.
- Patients diagnosed with hepatic, renal impairment, hypertensive, cardiovascular co-morbidities, psychiatric disorders, stroke, human immuno-deficiency virus infection,
pregnancy.

3) **Grouping:** The subjects were grouped into control (n=30), T2DM (n=30) on anti-diabetic conventional treatment and T2DM patients with anti-diabetic treatment and 1.32gm adjunct fenugreek seeds extract tablet therapy (n=30).

**Intervention:**

1) **Drug:** Aqua soluble extract of fenugreek tablets were supplied by FDA approved Ayurvedic manufacturer Sheetal Medicare. Fenugreek seeds purchased from local market were certified by botanist. Material was tested and certified as per Agmark standards for pesticides, fertilizers, toxins and heavy metals residues. Fenugreek Aqueous extract of fenugreek seed powder in 1:10 ratio. 1000gm of fenugreek seed powder with 9000ml of distilled water. Decoction was made with standard procedure, reduced up to 200ml and filtered. Filtration marc was again diluted 9 times with water and reduced by boiling to one forth. Both these extracts were mixed, filtered and evaporated. To this reduced extract, gum acacia was added as filler. Granules were formed and dispensed in tablet form by standard method in text book of Indian medicine, ‘Sharangdhar Samhita’ [20]. 350 mg each tablet consisted of final composition of 330mg fenugreek extract and 20mg gum acacia. All active components were soluble in water.

2) **Dose:** 4 tablets per day = 1.4gm extract = 14gm fenugreek seed powder
   1\(^{st}\) week 1 tablet/day, 5-10 minutes before breakfast.
   2\(^{nd}\) week 2 tablets/day, 5-10 minutes before breakfast and dinner each.
   3\(^{rd}\) week onwards 4 tablets/day, 5-10 minutes before breakfast (2 tablets) and dinner (2 tablets).

3) **Duration:** The duration of the study was of 3 months.

**Assessment criteria:** Evaluation of serum fasting blood sugar, insulin, C-peptide, HbA1c, MDA, NO, Carbonyl protein, SOD, Catalase, Glutathione Reductase and Glutathione peroxidase was done using Chemiluminescence (Immulate 1000), fully auto Chemistry analyzer (Olympus AU-400) and spectrophotometer Jasco-V670.

Homeostatic model (HOMA 2) is a method for assessing β – cell function and insulin resistance (IR) from basal (fasting) glucose and insulin or C-peptide concentrations. HOMA2, the correctly solved
computer model, has nonlinear solutions [18]. Statistical analysis was done using software Mini-tab 17 with 95% confidence interval used and two-sided correlation among groups.

**Follow up:** The analysis of study parameters were repeated on 3 months follow-up of the subject’s treatment response.

## RESULTS

**Table 1: Anthropometric parameters in Healthy Control, T2DM (DM) and Adjunct T2DM (ADJ DM).**

<table>
<thead>
<tr>
<th>ANTHROPOMETRIC PARAMETERS</th>
<th>Control</th>
<th>DM</th>
<th>ADJ DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>Age (Years) Baseline</td>
<td>42.43 ± 7.15</td>
<td>53.7 ± 7.28</td>
<td>48.6 ± 10.52</td>
</tr>
<tr>
<td>Height (cm) Baseline</td>
<td>167.33 ± 9.3</td>
<td>163.2 ± 10.23</td>
<td>165.13 ± 5.26</td>
</tr>
<tr>
<td>Weight (kg) Baseline</td>
<td>64.8 ± 9.82</td>
<td>69.86 ± 13.58</td>
<td>64.43 ± 10.6</td>
</tr>
<tr>
<td>BMI Baseline</td>
<td>21.63 ± 3.02</td>
<td>26.25 ± 3.78</td>
<td>23.56 ± 3.71</td>
</tr>
<tr>
<td>W/H Ratio Baseline</td>
<td>0.99 ± 0.16</td>
<td>0.89 ± 0.06</td>
<td>0.91 ± 0.08</td>
</tr>
</tbody>
</table>

**Table 2: Values of different parameters in Healthy control, T2DM and Adjunct T2DM groups at baseline and after 3 Months.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>DM</th>
<th>ADJ DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>Hb% Baseline</td>
<td>13.75 ± 1.59</td>
<td>11.63 ± 1.18</td>
<td>12.4 ± 1.8</td>
</tr>
<tr>
<td>Hb% after 3 Months</td>
<td>--------</td>
<td>11.91 ± 1.12</td>
<td>12.77 ± 1.79</td>
</tr>
<tr>
<td>FBS (mg/dl) Baseline</td>
<td>82.3 ± 11.712</td>
<td>271.7 ± 57.47</td>
<td>257 ± 42.19</td>
</tr>
<tr>
<td>FBS (mg/dl) after 3 Months</td>
<td>----</td>
<td>266.5 ± 52.6</td>
<td>197 ± 38.83</td>
</tr>
<tr>
<td>Insulin (U/ml) Baseline</td>
<td>5.07 ± 0.91</td>
<td>9.66 ± 5.53</td>
<td>18.6 ± 6.17</td>
</tr>
<tr>
<td>Insulin (U/ml) after 3 Months</td>
<td>----</td>
<td>9.85 ± 5.31</td>
<td>14.96 ± 4.13</td>
</tr>
<tr>
<td>C-peptide (ng/ml) Baseline</td>
<td>5.33 ± 1.17</td>
<td>1.93 ± 1.59</td>
<td>3.98 ± 1.12</td>
</tr>
<tr>
<td>C-peptide (ng/ml) after 3 Months</td>
<td>----</td>
<td>2.09 ± 1.44</td>
<td>3.46 ± 0.93</td>
</tr>
<tr>
<td>HbA1C (%) Baseline</td>
<td>4.35 ± 0.46</td>
<td>10.35 ± 1.04</td>
<td>9.96 ± 0.85</td>
</tr>
<tr>
<td>HbA1C (%) after 3 Months</td>
<td>----</td>
<td>7.8 ± 1.04</td>
<td>5.86 ± 0.41</td>
</tr>
<tr>
<td>HOMA Baseline</td>
<td>0.96 ± 0.13</td>
<td>3.52 ± 1.12</td>
<td>3.36 ± 1.35</td>
</tr>
<tr>
<td>HOMA after 3 Months</td>
<td>----</td>
<td>3.53 ± 1.11</td>
<td>2.32 ± 0.69</td>
</tr>
<tr>
<td>MDA(nmol/ml) Baseline</td>
<td>4.28 ± 0.49</td>
<td>7.11 ± 1.28</td>
<td>6.98 ± 0.9</td>
</tr>
</tbody>
</table>
Table 3: Correlation between Control, T2DM and Adjunct therapy T2DM groups

<table>
<thead>
<tr>
<th>Parameters after 3 months</th>
<th>Control/DM</th>
<th>Control/ADJDM</th>
<th>DM/ADJDM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r- Value</td>
<td>P- Value</td>
<td>r- Value</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>0.026</td>
<td>0.890</td>
<td>0.393</td>
</tr>
<tr>
<td>NO (U/gHb)</td>
<td>-0.044</td>
<td>0.818</td>
<td>0.011</td>
</tr>
<tr>
<td>Carbonyl Protein (nmol/mg)</td>
<td>0.103</td>
<td>0.588</td>
<td>-0.174</td>
</tr>
<tr>
<td>SOD (U/gHb)</td>
<td>0.209</td>
<td>0.269</td>
<td>-0.167</td>
</tr>
<tr>
<td>Catalase (U/GHb)</td>
<td>-0.042</td>
<td>0.824</td>
<td>0.339</td>
</tr>
<tr>
<td>Glutathione Reductase (U/gHb)</td>
<td>0.230</td>
<td>0.222</td>
<td>0.174</td>
</tr>
<tr>
<td>Glutathione Peroxidase (U/gHb)</td>
<td>-0.076</td>
<td>0.688</td>
<td>-0.338</td>
</tr>
</tbody>
</table>

Graph 1: Correlation between (a) MDA, (b) NO, (c) SOD, (d) Catalase, (e) GPX and (f) GRX of DM
DISCUSSION

Oxidative stress plays an important role in the onset and progression of diabetic vascular complications. The common chronic damage in diabetes patients is closely related to elevate oxidative/inflammatory activities with a continuum of tissue insults leading to more severe cardio-metabolic and interrelated complications. Many studies have reported the role of free radicals in the pathogenesis of DM where oxidative stress coexists along with decrease in antioxidant status. SOD catalyses the reaction in which superoxide anion is converted to hydrogen peroxide and oxygen while CAT is a haem-containing ubiquities enzyme that detoxifies $\text{H}_2\text{O}_2$ into water and oxygen. The reductions observed in the activities of SOD and CAT in the diabetic
control group suggest their excessive utilization in attenuating free radicals generated during the metabolism of alloxan \textsuperscript{[21, 22]}. The decrease in levels of antioxidants SOD, Catalase, Glutathione Reductase and Glutathione Peroxidase in diabetic patients can be seen at baseline when compared with the control group. Also, the increased levels of free radicals i.e. MDA, NO and Carbonyl proteins can be observed in the diabetic group (Table 2).

Increase in antioxidant activities is an indication of their ability to scavenge ROS, thus contributing to the protective effect against oxidative stress and preventing further damage \textsuperscript{[22]}. A group analysis had showed a significant increase in superoxide dismutase (SOD) activity ($p = 0.001$) among the fenugreek group without any changes in the control group \textsuperscript{[23]}. In our 3 months follow-up study, the antioxidant levels were increased significantly in the ADJDM group receiving fenugreek adjunct treatment as compared to the DM group which did not take fenugreek treatment. Similarly, beneficial effects of the therapy was seen in ADJDM group by reduction in the levels of MDA, NO and Carbonyl proteins (Table 2 and 3). The results observed in ADJDM group after 3 months clearly showed that oxidative stress in the patients receiving fenugreek therapy was controlled, almost equivalent to normal healthy individuals. Thus, the ratios between the respective parameters in Control and ADJDM groups were insignificant while those between DM and ADJDM group were found to be highly significant (Table 3 and Graph 1).

Substrates of fenugreek were shown to significantly increase phenolic antioxidants that might be useful for the prevention of diabetes pathogenesis and its complications. Fenugreek is therefore reported to have antidiabetic, antiglycemic, stimulating/regenerating effect on beta cells, antilithogenic potential, antioxidant, and neuroprotective effects \textsuperscript{[21, 22, 23]}. 

**CONCLUSION**

The potency of herbal drugs is significant compared to the synthetic anti-diabetic drugs. Medicinal plants like fenugreek provide better alternatives as they are generally less toxic, affordable, non-genotoxic, and have a wide safety margin. Isolation and identification of active constituents from fenugreek, preparation of standardized dose & dosage regimen can play a significant role in improving hypoglycaemic action.

The present study indicates some promising properties for fenugreek seeds on biomarkers of inflammation and oxidative stress in T2DM patients. A scientific investigation of fenugreek applications for diabetes may provide valuable leads for the development of alternative drug and
therapeutic strategies. However, appropriate nutritional management is essential for restoring and maintaining a normal metabolic state. Therefore, diet remains a cornerstone in diabetic management.

REFERENCES


18. HOMA calculator available on https://www.dtu.ox.ac.uk/homacalculator/


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Conflict of interest: None Declared