EFFECT OF TRIGONELLINE: AN ACTIVE COMPOUND FROM TRIGONELLA FOENUMGRAECUM LINN. IN ALLOXAN INDUCED DIABETES IN MICE

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Abstract: Trigonelline was extracted from the seeds of Trigonella foenumgraecum and the hypoglycemic activity was studied in alloxan induced diabetic mice. Trigonelline significantly reduced the blood glucose in treated groups as compared to control diabetic animals. Trigonelline treated group showed islet cells in the vicinity of the pancreatic duct which indicates its beneficial effects on beta cells. Glyburide was used as a standard antidiabetic drug and its effect on pancreatic cell was also studied. The pancreatic beta cells of glyburide treated mice did not show any islets in the vicinity of pancreatic duct. Both trigonelline and glyburide arrested the decrease in body weight and mortality of diabetic mice. \(LD_{50}\) of trigonelline was found to be more than 5000 mg/kg.

Key words: Trigonelline, Diabetic mice, Blood glucose, Acute toxicity.

INTRODUCTION

Diabetes is a chronic and progressive disorder. Over the past several decades diabetes has become the major health problem. Worldwide projections suggest that more than 220 million people will have diabetes by the year 2010 [1] and by the year 2035 India may have the maximum number of diabetic patients in the world. Moreover, diabetes manifests at an early age in India as compared to the Western countries [2]. With increasing duration, diabetes causes tissue damage which is proportional to the levels of circulating glucose [2].

Trigonella foenumgraecum [also called as ‘Fenugreek’ (Vernacular name: Methi) a member of family Leguminosae (Fabaceae)] is a commonly used spice in India, Middle East, Egypt, and North Africa. The seeds of the plant have been used as a traditional remedy for numerous conditions including gastrointestinal disorders, gout, wound healing and inflammation, hyperlipidemia and diabetes [3].

Mishkinsky et al. [4] initially discovered the hypoglycemic effect of trigonelline in alloxan induced diabetic rats. However, no report is available regarding its effect on pancreatic beta cells. The objective of the present study was to investigate the effect of trigonelline on blood glucose and to explore its mechanism of action in alloxan induced diabetes in mice.

MATERIALS AND METHODS

Animals: Swiss Albino mice (25 to 30g) were obtained from National Toxicological Centre, Pune. The animals were housed under standard condition of temperature (25 ± 2°C), 12h/12h light dark cycles and fed with standard pelleted diet (Chakan Oil Mills, Sangli) and water was given ad libitum. Animal handling was performed as per Good Laboratory Practice. The research proposal was approved by “The Institutional Animal Ethical Committee (IAEC)” of Poona College of Pharmacy.

Drugs and chemicals: Fenugreek seeds purchased
locally were flaked, loaded in an extractor and water: alcohol (30:70) was circulated in the extractor at room temperature for 8 h. The extract was drained and distilled to remove alcohol. The marc was extracted with hexane to remove oil and other lipids. The oil free water extract was passed through strong acid cation exchange resin in gel form where all the zwitterions were bound on the column (amino acids and trigonelline). The resin was thoroughly washed with de-ionized water to remove adhering sugars and other contaminants. The column was eluted with 10% aqueous ammonia in which all the amino acids and trigonelline was eluted and appeared in ammonia solution. The solution was concentrated to remove ammonia and water. The resulting powder contained about 40% amino acids, 30% trigonelline and remaining 30% soluble sugars (low molecular weight galactomannans). The powder was dissolved in 20 volumes of isopropyl alcohol and treated with activated charcoal to remove coloring impurities. The filtered solution was then cooled to 0°C and slowly 20% hydrochloride gas in isopropyl alcohol was added in stoichiometric quantity and stirred for 6 h at 0°C, where trigonelline was precipitated as trigonelline hydrochloride. It was filtered and washed with isopropyl alcohol, trigonelline hydrochloride was redissolved in water and the free trigonelline was released by adjusting pH to 5 – 5.5 using ammonia. The clear solution was distilled under vacuum to a concentration of 40% solids and the ammonium chloride salt was removed by adsorbent resin (Amberlite XAD 1180) treatment. The eluent from adsorbent resin column was concentrated under vacuum to get pure trigonelline. Glyburide, the oral antidiabetic drug, was obtained as gift sample from Ranbaxy Pvt. Ltd., New Delhi. Appropriate dilutions were made for preparation of the suitable dose and administered orally as a dose of 0.1 ml/10g of body weight of mice. Alloxan monohydrate was purchased from Spectrochem, India

**Induction of diabetes and estimation of Blood glucose:** Diabetes was induced by intravenous administration of 70 mg/kg alloxan monohydrate solution through tail vein. Blood was withdrawn by retro orbital plexus technique (ROP) and blood glucose was estimated by glucose oxidase peroxidase (GOD/POD) method using the kit purchased from Accurex Biomedicals Pvt. Ltd., Mumbai.

**Experimental design:** Normal mice were made diabetic by giving alloxan monohydrate (70 mg/kg) solution intravenously as per the method described by Dunn and Letchie [5]. After 48 hours blood was withdrawn and blood glucose level was estimated. The animals showing blood glucose more than 200 mg/dl were called ‘diabetic’ and were selected for the study. The animals were divided into 4 groups each containing 6 animals. (Group I - control (non diabetic), Group II - only alloxan (70 mg/kg), Group III - only glyburide (10 mg/kg), Group IV - trigonelline (75 mg/kg)). Initially trigonelline was administered at three dose levels (37.5, 75 and 150 mg/kg), but only the dose of 75 mg/kg was selected in the present study as the lower dose (37.5 mg/kg) did not decrease blood glucose significantly and higher dose (150 mg/kg) had a ceiling effect.

**Acute study:** Animals were fasted overnight before commencing the experiment. Blood glucose was estimated before giving any drug and considered as 0 h reading. Drugs were administered to respective groups orally and blood glucose was estimated at 2h, 4h, 6h and 24h for acute study [6]. Acute oral toxicity and LD$_{50}$ determination were carried out as OECD guidelines using AOT425 software.

**Sub acute study:** The drugs were administered daily for 28 days at a prefixed time [6]. Blood glucose was estimated per week. At the end of 28 days the drug administration was stopped and a rest period of 7 days was given to the animals to study the regenerative ability of pancreas and blood glucose was estimated after 7 days.

**Histology of pancreas:** After seven days rest period, pancreas was isolated and histological examination was carried out using Haematoxylin and Eosin staining of sections.

**Body weight and mortality:** During the study period of 35 days, animals were weighed daily and their body weight was expressed as mean weight ± S.E.M. Death of animals was noted and percentage mortality was calculated.

**Statistical analysis:** Data was expressed as mean ± S.E.M and statistical analysis was carried out by One Way ANOVA with Tukey’s post was performed using GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego California USA, www.graphpad.com. [7]

**RESULTS**

Glucose levels measured in normal and alloxan indu-
ced diabetes in mice are shown in Table 1. Alloxan (70 mg/kg) treated animals showed significant (P<0.001) increase in blood glucose as compared to the normal animals. The onset of blood glucose reduction in trigonelline (75 mg/kg) group was tardy i.e. less than 6 h. Glyburide on the other hand showed rapid onset i.e. less than 2 h. The peak reduction in blood glucose appeared to be at 24 h in case of trigonelline (234.73), whereas glyburide showed maximum reduction at 6 h (163.06) (Table 1).

The pancreatic sections of non diabetic (Group I) showed normal population of islets in the vicinity of the duct (Fig. A). The diabetic animals (Group II) showed occasional islets which were negligible in the vicinity of the duct (Fig. B). It also showed depleted population of islets which were scanty and smaller in size. The pancreatic section of glyburide (Group III) treated animals showed no islets (Fig. C). Trigonelline treated animals (Group IV) pancreatic section showed a few islets in the vicinity of the duct (Fig. D). This indicated that trigonelline treatment enhanced further decrease was observed during the period of withdrawal of drug (270.09). The blood glucose reduction was sustained during further trigonelline and glyburide administration as well as during the withdrawal period (Table 2).

### Table 1: Effect of trigonelline and glyburide in alloxan induced diabetic mice. (Acute study)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose in mg/dl at</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
</tr>
<tr>
<td>Control</td>
<td>116 ± 6.9</td>
</tr>
<tr>
<td>Only alloxan (70 mg/kg)</td>
<td>431.79 ± 10.96</td>
</tr>
<tr>
<td>Glyburide (10 mg/kg)</td>
<td>442.51 ± 6.99</td>
</tr>
<tr>
<td>Trigonelline (75 mg/kg)</td>
<td>458.78 ± 64.4</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M, n=6 in each group; Statistical analysis by one – way ANOVA followed by Tukey’s test using Graphpad Instat software; P values < 0.05, P < 0.001 compared to only alloxan group and P<0.001, as compared to glyburide group. Drugs were administered orally.

### Table 2: Effect of trigonelline and glyburide in alloxan induced diabetic mice. (Sub acute Study)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose in mg/dl at</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
</tr>
<tr>
<td>Control</td>
<td>116 ± 6.9</td>
</tr>
<tr>
<td>Only alloxan (70 mg/kg)</td>
<td>431.79 ± 10.96</td>
</tr>
<tr>
<td>Glyburide (10 mg/kg)</td>
<td>442.51 ± 6.99</td>
</tr>
<tr>
<td>Trigonelline (75 mg/kg)</td>
<td>368.55 ± 43.10</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M, n=6 in each group; Statistical analysis by one – way ANOVA followed by Tukey’s test using Graphpad Instat software; P values < 0.05, P < 0.001 compared to only alloxan group and P<0.001, as compared to Glyburide group. Drugs were administered orally.

### Table 3: Effect of Trigonelline on body weight of diabetic mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight in g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
</tr>
<tr>
<td>Control</td>
<td>29.0 ± 0.44</td>
</tr>
<tr>
<td>Only alloxan (70 mg/kg)</td>
<td>30.17 ± 0.47</td>
</tr>
<tr>
<td>Glyburide (10 mg/kg)</td>
<td>30.33 ± 0.95</td>
</tr>
<tr>
<td>Trigonelline (75 mg/kg)</td>
<td>28.5 ± 0.56</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M, n=6 in each group; Statistical analysis by one – way ANOVA followed by Tukey’s test using Graphpad Instat software; P values < 0.05, P < 0.01, P < 0.001 compared to only alloxan group.
Alloxan gradually decreased the body weight of mice during the period of 28 days. However, after administration of both glyburide and trigonelline the body weight of the animals did not decrease further. Rather, the animals gained body weight as compared to only alloxan treated group indicating that both glyburide and trigonelline were effective in preventing further loss of body weight (Table 3).

Administration of alloxan resulted in death of 57% of the total animals during 28 days study period. Administration of both glyburide and trigonelline reduced the mortality rate which declined to 45% and 29% respectively. \(LD_{50}\) of trigonelline was found to be greater than 5000mg/kg.

**DISCUSSION**

The hypoglycemic effect of trigonella seeds and their major alkaloids, trigonelline was described by Mishkinsky [4] and Nadakarni [8]). Therefore, the seeds are widely recommended for non insulin dependent diabetes mellitus patients. Fenugreek is, thought to delay gastric emptying, slow carbohydrate absorption, and inhibits glucose transport. It has been shown to increase erythocyte insulin receptors and improve peripheral glucose utilization, thus showing potential pancreatic as well as extra pancreatic effects. Some studies showed that trigonelline may exert hypoglycemic effect in healthy non diabetic volunteers [3], while others reveal no effect on fasting or postprandial blood glucose in non diabetic patients [9]. The addition of trigonella seeds to the diet of diabetic patients was practiced by many Yemenites.
in Israel, who called it as “chilbe” [4]. Mishkinsky et al. [4] proved that trigonelline had some hypoglycemic effect in both alloxan induced diabetic rats and diabetic patients.

Glyburide is a potent, second-generation, oral sulfonylurea antidiabetic agent used as an adjunct to diet to lower blood glucose levels in patients with diabetes mellitus. The hypoglycemic action of glyburide is due to stimulation of pancreatic islet cells, which results in an increase in insulin secretion. The effects of sulphonylureas are initiated by binding to and blocking on ATP – sensitive K+ channel, which have been cloned. The drugs thus resemble physiological secretagogues (e.g. glucose, leucine) which also lower the conductance of this channel. Reduced K+ conductance causes membrane depolarization and influx of Ca2+ through voltage sensitive Ca2+ channel. Prolonged administration of glyburide also produces extrapancreatic effects that contribute to its hypoglycemic activity [10]. The results of both acute and sub-acute study hypothesized that the late onset of action and prolonged duration of action of trigonelline may result from improved pancreatic cytoarchitecture. Further evidence to support this hypothesis was obtained from histological examination which showed that in trigonelline treated pancreatic sections few islets in the vicinity of the duct were observed. On the other hand only alloxan treated animals showed occasional islets with less islets in the vicinity of pancreatic duct. This study thus indicated that trigonelline treatment enhanced the pancreatic regeneration.

Reduction in the body weight in diabetic animals including humans is a well known effect. In the present investigation, both glyburide and trigonelline treated animals, the onset of action occurs within 2 hours and a maximal decrease in serum glucose occurred within 3 to 4 hours. Trigonelline thus differs from glyburide in respect to mechanism of action and pharmacodynamic properties. Improvement in the body weights indicated better the antidiabetic action would be useful in preventing further weight loss. More increase in the body weight of trigonelline group compared to glyburide group might be due to increased insulin secretion and better glycemic control. The increase in body weights of animals is well comparable with other anti diabetic plants such as Ficus bengalensis and Momordica cymbalaria [11,12].

Administration of trigonelline showed less mortality (29%) as compared to untreated diabetic animals (57%) or glyburide (45%). It appears that hypoglycemic effect of trigonelline may be contributing to reduction in mortality. The acute oral toxicity indicated trigonelline has good safety profile as the LD50 was greater than 5000 mg/kg. It is thus apparent that, trigonelline (75 mg/kg, p.o) possesses significant (P<0.001) hypoglycemic activity which may be due to enhanced pancreatic regeneration. Thus the oral administration of trigonelline (75 mg/kg) causes hypoglycemic effect probably by formation of new beta cells, therefore, it can be a useful drug for the diabetic patients.

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[7] One-way ANOVA with Tukey’s post test was performed using GraphPad InStat version 3.01 for Windows 95, GraphPad Software Inc., 5755 Oberlin drive, #110, San Diego California 92121, USA, www.graphpad.com. 
