Therapeutic Efficacy of Acidic and Neutral Fractions of Tinospora cordifolia Stem on Diabetes Induced Animal Model

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The present investigation is aimed at bio-graded fractionation of Tinospora cordifolia stem extract for screening antidiabetic activity in diabetic Wistar rats. T. cordifolia stem was subjected for alcoholic fractions by standard protocol. The Wistar rats were fasted overnight and diabetes was induced by a single intra-peritoneal (i.p.) injection of a freshly prepared solution of Streptozotocin (STZ)-55 mg/kg body weight in 0.1 M citrate buffer (pH 4.5). Rats whose serum glucose level measured in the range of ≥ 287 mg/dl were designated as diabetic and selected for experiment. Acidic fraction (F₄) and neutral fraction (F₃) were orally fed to diabetes induced rats with two different doses of 100 and 200 mg/kg body weight during the tenure of the experiment. Blood glucose, body weights were measured every week whereas glycosylated hemoglobin, triglyceride, cholesterol, blood urea, liver glycogen and insulin were measured at the termination of the experiment. Though both fractions showed significant hypoglycemic effect, F₃ performed better and demonstrated antidiabetic properties by enhancing insulin secretion. F₃ and F₄ contained glycosides, flavones, and phenolic compounds of plant extract which are the common compounds present in both the fractions. Hence it may be concluded that active compounds for antidiabetic activity may be present in these compounds.

Key words: Antidiabetic activity, Fractions of Tinospora cordifolia, Liver glycogen, Wistar rat.

INTRODUCTION

Several medicinal plants are having hypoglycemic effects.[1] The World Health Organization has recommended that the assessment of traditional plant treatments for diabetes mellitus needs further investigation.[2] Several herbal medicines were described for the care of diabetes mellitus in ancient literature of Ayurveda in India. Many of the plants used as diet were reported to have antidiabetic properties.[3] Ethnopharmacology surveys indicate that more than 1200 plants are used worldwide in traditional medicine for their alleged hypoglycemic activity[4].

Tinospora cordifolia (Menispermacaeae) is a glabrous climbing succulent shrub, commonly found in hedge. It is native to India, thrives easily in the tropical region. It also occurs in Burma and Ceylon. It is widely used in Ayurvedic medicine in India as tonic, vitalizer, and as a remedy for diabetes mellitus and metabolic disorder.[5,6], Grover et al studied aqueous and whole alcoholic extracts of T. cordifolia for antihyperglycemic effect on albino rats and mice. However, they did not mention which part of the plant used for extract.[7] Stanely et al used aqueous root extract of T. cordifolia to test for hypodilicemic and hypoglycemic actions in alloxan induced diabetic rats.[8,9].

The present investigation is aimed at bio-graded fractionation of T. cordifolia stem extract and screening of acidic and neutral fractions for antidiabetic activity in diabetic Wistar rats.

MATERIALS AND METHODS

Extraction and fractionation
Healthy disease free stem of T. cordifolia were collected from our University campus. The authenticity of the plant was confirmed by a taxonomist of the Department of Studies in Botany University of Mysore, Mysore, and preserved in the form of herbarium. Collected stem were shade dried and ground in warring blender and subjected for fractionation. The ground powder was extracted in soxhlet apparatus using ethanol until the final drop of the extract become colorless. The extract was dried using evaporator. The dried extract was further fractionated by dissolving in methanol: water (4:1) evaporated to one tenth the volume and filtered. The residue obtained was further dissolved in ethyl acetate and filtered to obtain two fractions i.e., ethyl acetate soluble fraction (F₂) and ethyl acetate insoluble fraction (F₃). The filtrate was further extracted using acid base extraction. The filtrate was first acidified using 5% H₂SO₄ (pH 2) and extracted twice with chloroform. The remaining aqueous layer was made alkaline using 5% NaOH (pH 10) and later extracted twice using chloroform:methanol (3:1). The remaining aqueous layer was neutralized using 5% H₂SO₄. Thus five fractions were obtained namely, ethyl acetate soluble fraction (F₁), ethyl acetate insoluble fraction (F₂), acidic fraction (F₃), basic fraction (F₄) and neutral fraction (F₅). Phytochemical analysis revealed that they contain following compounds F₁ (Steroids), F₂ (Oils and Fats), F₃ (Steroids, Glycosides, Flavones and Phenolic compounds), F₄ (Glycosides, Flavones and Phenolic compounds) and F₅ (Alkaloids, Glycosides, Saponins, Flavones and Phenolic compounds).[10] Since F₂ contained oils and fats, antidiabetic properties of fractions F₁ and F₄ were reported in our earlier publication,[11] while acidic fraction F₃ and neutral fraction F₅ were not obtained in sufficient quantity, the extraction process was repeated to get the desirable quantity. F₃ and F₅ were chosen to test antidiabetic activity in the present investigation.

Experimental animals
The Wistar rats weighing in the range of 138-216 g were procured from the Animal House of the Department of Zoology. The Animals were maintained in a room with
temperature 22±2°C and 50-70 % Relative Humidity (R.H) and 12:12 h L: D cycle. The animals were fed with standard diet supplied by Ambroth Feeds Pvt Ltd Bangalore and water ad libitum throughout the experiment. The Institutional Animal Ethics Committee (IAEC) approved the experimental protocols of the present study.

**Induction of diabetes in experimental animals**

The animals were fasted overnight and diabetes was induced by a single intraperitoneal (i.p.) injection of freshly prepared solution of Streptozotocin (STZ) - 55 mg/ kg body weight in 0.1 M citrate buffer (pH 4.5). [12] Control rats were injected with citrate buffer alone. On the third day of STZ injection, the rats were fasted for 6 h and blood was taken from tail vein of the rats [13], and Rats whose serum glucose level measured in the range of ≥ 287 mg/dl were designated as diabetic and selected for experiment.

**Experimental Protocols**

The experiment was conducted by dissolving F<sub>3</sub> and F<sub>5</sub> in Tween- 80 (1%) and fed orally with two concentrations of 100 and 200 mg/kg body weight to diabetes induced Wistar rats.

Diabetes induced Wistar rats were randomly divided into following groups with five animals in each.

- Group 1: control rats treated with vehicle alone.
- Group 2: diabetic rats treated with standard drug Glibenclamide (0.5 mg/kg body weight/ day).
- Group 4: diabetic rats treated with F<sub>3</sub> (100 mg/kg body weight).
- Group 5: diabetic rats treated with F<sub>3</sub> (200 mg/kg body weight).
- Group 6: diabetic rats treated with F<sub>5</sub> (100 mg/kg body weight).
- Group 7: diabetic rats treated with F<sub>5</sub> (200 mg/kg body weight).

Since diabetes is a chronic disorder requiring long term therapy, there is a need to assess the effect of different extracts for antidiabetic activity for a longer duration. Hence the experiment was conducted for six weeks.

**Biochemical analysis**

Serum glucose level was estimated using a glucometer (EZ Omnitest) every week to ascertain the status of diabetes in different groups of rats. Similarly body weight was recorded once in a week. After six weeks the experimental rats were allowed to fast overnight with free access to water and autopsied under light ether anesthesia. The blood was collected from the carotid artery at the time of autopsy, [14] and centrifuged at 4°C, at 1000 rpm for 10 minutes; the separated serum was used for various biochemical analyses.

Serum glucose level was estimated by Trinder’s method using GOD/ POD enzymatic kit [15], Glycosylated haemoglobin [16], triglycerides [17], HDL-cholesterol [18], LDL-cholesterol and VLD-cholesterol were estimated [19], Blood urea was estimated by urea-glutamate dehydrogenase (GLDH) method [20]. All the parameters were estimated by using Semi Bioauto Analyser from Swemed Diagnostics with appropriate kits supplied by manufacturer of instrument. Liver glycogen content was determined by method of Carroll [21]. Serum insulin levels were estimated by using ELISA method [22]. All the above parameters were recorded at the termination of experiment.

**Statistical analysis**

Data were statically evaluated by using one way ANOVA. Wherever the ANOVA values were found to be significant Duncan’s new multiple range test (DMRT) was applied (SPSS computer software). The values were considered significant when P<0.05.

**RESULTS**

Body weight: There was significant decrease in body weight in group-2 animals whereas there was a significant increase in body weight of the remaining groups of animals at the termination of the experiment (Table 1).

<table>
<thead>
<tr>
<th>Groups n=5</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control rats</td>
<td>138.2±2.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>192±7.15&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>2. Diabetic rats</td>
<td>140.8±4.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>114.4±3.76&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3. Diabetic rats treated with Glibenclamide</td>
<td>216.6±7.92&lt;sup&gt;c&lt;/sup&gt;</td>
<td>255.2±14.22&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>4. Diabetic rats treated with F&lt;sub&gt;3&lt;/sub&gt; (100 mg/kg b.w)</td>
<td>135.6±3.93&lt;sup&gt;c&lt;/sup&gt;</td>
<td>141.6±6.04&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>5. Diabetic rats treated with F&lt;sub&gt;3&lt;/sub&gt; (200 mg/kg b.w)</td>
<td>134.4±1.72&lt;sup&gt;c&lt;/sup&gt;</td>
<td>147.2±3.13&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>6. Diabetic rats treated with F&lt;sub&gt;5&lt;/sub&gt; (100 mg/kg b.w)</td>
<td>145.2±3.92&lt;sup&gt;c&lt;/sup&gt;</td>
<td>199.6±5.32&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>7. Diabetic rats treated with F&lt;sub&gt;5&lt;/sub&gt; (200 mg/kg b.w)</td>
<td>151.2±4.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>175.4±6.37&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All values of final body weight are significant at P < 0.05 vs diabetic rats.

Serum glucose level: Serum glucose level remained 84.2 mg/dl in group-1 on the day of commencement of experiment and remained in the normal state at the termination of the experiment (106.80 mg/dl) (Figure 1). Group-2 animals exhibited hyperglycemia (369.8 mg/dl) on the day of commencement of experiment and remained in the diabetic state throughout experiment. Group-3 animals exhibited hyperglycemia (287.6 mg/dl) on the day of commencement of experiment but after intra-peritoneal daily injection of Glibenclamide resulted in bringing the serum glucose level to non-diabetic status (86.2 mg/dl). Animals in group-4 and 5 exhibited hyperglycemia with the fasting blood glucose level of 441.2 and 480.8 mg/dl respectively on the day of commencement of the experiment. Serum glucose level significantly decreased to 237.6 and 152.4 mg/dl respectively at the end of experiment (P < 0.05) after oral feeding of F<sub>3</sub> (100 and 200 mg/kg body weight respectively). Similarly group-6 and 7 animals also exhibited hyperglycemia with fasting serum glucose values of 311.4 and 333.4 mg/dl (Figure 1) respectively on the day of commencement of the experiment. After oral feeding of F<sub>3</sub> (100 and 200 mg/kg body weight respectively) serum glucose level reached to non-diabetic state of 93 and 90.2 mg/dl (P < 0.05) respectively at the termination of the experiment (Figure 1). Since the fraction F<sub>3</sub> and F<sub>5</sub> showed promising hypoglycemic activity on diabetic Wistar rats, the preserved serum was further used for biochemical analysis.
Blood glycosylated hemoglobin level: Table 2 shows the level of fasting glycosylated hemoglobin in all the groups. Group-2 showed maximum levels of glycosylated hemoglobin whereas there was significant decrease in fasting glycosylated hemoglobin in Glibenclamide group. Oral administration of F3 and F5 for six weeks at two different doses (100 and 200 mg/kg body weight) resulted in a significant decrease in fasting glycosylated hemoglobin (Table 2).

Table 2: Effect of acidic and neutral fractions of Tinospora cordifolia stem on glycosylated hemoglobin, triglycerides, cholesterol, blood urea, liver glycogen and insulin levels of experimental groups at termination of experiment.

<table>
<thead>
<tr>
<th>Groups n=5</th>
<th>Glycosylated hemoglobin ( %)</th>
<th>Triglycerides (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>Blood urea (mg/dl)</th>
<th>Liver glycogen (mg/g)</th>
<th>Insulin ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control rats</td>
<td>7.15±0.29a</td>
<td>115.09±13.03a</td>
<td>69.78±3.14a</td>
<td>30.3±2.04a</td>
<td>28.31±0.82a</td>
<td>1.69±0.43a</td>
</tr>
<tr>
<td>2. Diabetic rats</td>
<td>10.09±0.42b</td>
<td>141.11±7.54b</td>
<td>80.95±5.75b</td>
<td>101.3±4.2b</td>
<td>5.26±1.99b</td>
<td>0.69±0.05b</td>
</tr>
<tr>
<td>3. Diabetic rats treated with Glibenclamide</td>
<td>6.03±0.42c</td>
<td>18.30±0.32c</td>
<td>58.42±0.38c</td>
<td>25.2±1.43c</td>
<td>17.53±1.4c</td>
<td>1.65±0.02c</td>
</tr>
<tr>
<td>4. Diabetic rats treated with F3 (100 mg/kg b.w)</td>
<td>8.97±1.01d</td>
<td>112.90±6.30a</td>
<td>78.34±2.11bc</td>
<td>76.93±3.06e</td>
<td>12.11±1.1d</td>
<td>1.1±0.18a</td>
</tr>
<tr>
<td>5. Diabetic rats treated with F3 (200 mg/kg b.w)</td>
<td>6.53±0.18cd</td>
<td>86.51±3.90a</td>
<td>69.92±1.59a</td>
<td>59.6±4.6c</td>
<td>17.98±2.4a</td>
<td>1.4±0.06c</td>
</tr>
<tr>
<td>6. Diabetic rats treated with F5 (100 mg/kg b.w)</td>
<td>6.17±0.37cd</td>
<td>82.5±2.21a</td>
<td>71.52±2.21bc</td>
<td>28.6±2.0c</td>
<td>23.3±0.76a</td>
<td>1.54±0.09a</td>
</tr>
<tr>
<td>7. Diabetic rats treated with F5 (200 mg/kg b.w)</td>
<td>6.08±0.33cd</td>
<td>70.17±4.53a</td>
<td>62.37±2.83bc</td>
<td>37.35±3.1a</td>
<td>24.74±1.9a</td>
<td>1.73±1.5a</td>
</tr>
</tbody>
</table>

[Values expressed are mean ±SE from 5 animals in each group. All values are significant at \( P < 0.05 \) vs diabetic rats.]

Blood triglycerides: The triglycerides levels significantly decreased in group-3 to 7 compared to diabetic animals which remained minimum after Glibenclamide treatment (Table 2).

Cholesterol: The cholesterol level significantly decreased in group-1 to 7 compared to diabetic animals (Table 2). Blood urea: The blood urea in diabetic animals showed higher value whereas it remained significantly lower in all the remaining groups of animals (Table 2).

Liver glycogen: Liver glycogen in diabetic group remained least whereas it was significantly higher in the remaining groups of animals (Table 2).

Serum Insulin: Serum Insulin level remained minimum in diabetic group. Though fractions F3 (with 100 and 200 mg/kg b.w) and F5 (with 100 and 200 mg/kg b.w) enhanced insulin secretion compared to diabetic group, F5 (with 200 mg/kg b.w) could secrete insulin better than the Glibenclamide drug in diabetic rats (Table 2).

LDL: LDL values remained higher in group-2 and group-4 animals. There was significant decrease in LDL value in remaining groups of animals (Figure 2).

VLDL: The levels of VLDL in different groups of experimental animals at the termination of experiment remained significantly decreased compared to that of group-2 animals (Figure 2).

HDL: The values of HDL in different groups of experimental animals at the termination of the experiment remained
Currently available drugs regimens for management of diabetes mellitus have certain drawbacks and therefore, there is a need for safer and more effective antidiabetic drugs.[2,23]. This study was undertaken to assess the antidiabetic properties of T.cordifolia alcoholic extracts namely acidic fraction F3 and neutral fraction F5. Diabetic control rats significantly lost the body weight and this is associated with hyperglycemia throughout the experiment (Table 1 and Figure 1). Whereas all the remaining groups gained body weight either with treatment of Glibenclamide (group-3) or with fractions F3 (group- 4 and 5) and F5 (group- 6 and 7). Stanley et al[7], and Grover et al[9], demonstrated characteristic loss of body weight after induction of diabetes with Streptozotocin in sharp contrast to oral administration of crude plant extract of T. cordifolia which caused significant increase in body weight. There was significant reduction in the serum glucose level in group-4 and 5 animals after oral feeding of F3 with 100 and 200 mg/kg body weight respectively. However the serum glucose level did not reach the non-diabetic status. Group-6 and 7 animals after oral feeding of F5 with 100 and 200 mg/kg body weight reduced the serum glucose level to that of control animals (Fig. 2). Grover et al[7], reported maximum antihyperglycemic effect after oral feeding of aqueous extract of T. cordifolia with 400 mg/kg body weight after six weeks. Stanly et al[9], used the T. cordifolia aqueous root extract on alloxan-induced diabetic rats. They used very high concentration of aqueous extract 2.5, 5 and 7.5 gr/kg body weight for 42 days. Only 2.5 and 5 gram crude extracts were antihyperglycemic. A1 Eryani & Naik[28], published that T. cordifolia whole alcoholic extract with 100 and 200 mg/kg body weight had significant hypoglycemic effect in both type-1 and Type-2 diabetic animals. In the present investigation F3 (acidic fraction) though reduced the serum glucose level significantly it failed to reach non diabetic condition, where as F5 (neutral fraction) with both concentrations reached the euaglycemic status. Several investigators have recommended that glycosylated hemoglobin be used as an indicator of metabolic control of diabetes since glycohemoglobin levels approach normal values in diabetic in metabolic control.[28]. The study of Stanly et al[9], was confined to antihyperglycemic, brain lipid, and hexokinase studies. Grover et al[7], deliberated hypoglycemic effect; glycogen content and on the enzymes in carbohydrate metabolism. The present investigation reports on various parameters like glycosylated hemoglobin, triglycerides, cholesterol, blood urea and liver glycogen. It also reports on LDL, VLDL and HDL values. Our previous report demonstrated for the first time that basic fraction of T. cordifolia (F4) enhanced insulin secretion from diabetic pancreas after oral feeding. F3 enhanced insulin secretion on par with control animals and Glibenclamide. In the present investigation both fractions F3 and F5 enhanced insulin secretion from diabetic pancreas after oral feeding. However F5 with 200 mg/kg.b.w oral feeding enhanced insulin secretion better than the standard drug glibenclamide (Table 2). Hyperglycemia leading to damage of β-cells of islets of Langerhans thus producing insufficient insulin or excess hepatic glucose output associated with insulin resistance cause leads to diabetes mellitus. Chougale et al[28], demonstrated alpha glucosidase inhibition by stem extracts of T. cordifolia. The glucosidase inhibitory activity of the fractions F3 and F5 of T. cordifolia cause delay in the digestion of complex carbohydrates thus prevents the rise of postprandial glucose level. Histological studies demonstrated that damaged β-cells in diabetes induced rats could recover after oral feeding of both the fractions. They could enhance insulin secretion; the same was confirmed by insulin estimation by ELISA (Table 2). These two fractions have different insulin secretion activity.

**DISCUSSION**

Fig. 2. Graph showing the effects of acidic and neutral fractions of Tinospora cordifolia stem on LDL, VLDL, HDL (mg/dl). Values expressed in are mean ±SE from 5 animals in each group. All values are significant at P < 0.05 vs diabetic rats.
CONCLUSION

Hence it may be concluded that active compounds for antidiabetic activity may be present in glycosides, flavones, and phenolic compounds of plant extract which are the common compounds present in the both fractions namely F₁ and F₅.

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REFERENCES


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