ANTIHYPERGLYCAEMIC AND ANTIOXIDANT EFFECTS OF METHANOLIC EXTRACT OF TINOSPORA CORDIFOLIA IN ALLOXAN INDUCED DIABETIC RATS

SHARMA, D. K., VARSHNEYA, C. AND CHAUHAN, S.
Department of Pharmacology and Toxicology, COVAS, CSKHPKVV, Palampur (H.P).
E. mail: dineshkovas79@gmail.com, Cell: 09418477233
Received: January 10, 2013; Accepted: January 28, 2013

Abstract: In the present study, methanolic extracts of Tinospora cordifolia (T. cordifolia) stem were administered orally @ 100 and 200 mg/kg body weight to alloxan induced diabetic rats for 15 days. This resulted in significant reduction (p<0.01) in blood glucose level in diabetic animals. The levels of blood glycosylated hemoglobin (HbA1c), serum AST and ALT significantly (p<0.01) increased in diabetic rats, however these levels returned to normal in T. cordifolia extract treated animals. There was a significant (p<0.01) increase in LPO and reduction in the activities of antioxidant enzymes CAT, GSH & SOD in diabetic rats. The administration of methanolic extracts of T. cordifolia (200) significantly reversed the condition. CAT level also elevated significantly in treated animals. However, glibenclamide restored blood glucose, HbA1c, ALT, AST, LPO, CAT and SOD and the restoration was greater than the treated groups. The study, therefore, indicated that T. cordifolia possesses antidiabetic activities along with good antioxidant properties and can be used as an adjunct therapy in diabetes.

Key words: Diabetes, Alloxan, Tinospora cordifolia

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia, which results from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels [1]. Diabetes mellitus is found worldwide and becoming a serious threat to mankind. It is third killer of human beings after cancer, cardiovascular and cerebrovascular diseases [2]. Antidiabetic allopathic medicines are often overprescribed, found to be dangerous on long term use due to its toxicity and adverse effects in the body. World Health Organization (WHO) has recommended the evaluation of traditional plant treatments for diabetes [3]. Tinospora cordifolia is widely used in Indian Ayurvedic medicine as a tonic, vitalizer and as a remedy for metabolic disorders [4,5]. The plant stem has been considered as an indigenous source of medicines with immunomodulatory [6], hepatoprotective [7] and antipyretic actions [8]. The roots of Tinospora cordifolia exhibit antiulcer [9] and antistress [10] actions. An increase in oxidative stress and the changes in antioxidant capacity are the main participants in the development of diabetic complications [11]. In this study, we have, therefore, investigated the effect of the methanolic extract of Tinospora cordifolia (T. cordifolia) stem (MTCS-Et) on glycemic control and antioxidant status in male wistar rats.

MATERIALS AND METHODS

Animals: Albino male Wistar rats (150 to 200 g body
weight) procured from disease free animal house, Hisar (Haryana, India) were housed in standard environmental conditions (23 ± 1°C, 12-hour light/dark cycles) and fed with standard pellet diet and water ad libitum. The animals were acclimatized in new environment for 2 weeks. All the experiments were performed in accordance with the guidelines of Institutional Animal Ethical Committee.

Plant material: The stems of T. cordifolia were collected from the vicinity of palampur H.P. These were washed with distilled water, shade dried, powdered and stored in an airtight container until for further use. The powder was used for preparation of extracts.

Preparation of methanolic T. cordifolia stem extract (MTCSEt): 100g fine stem powder of T. cordifolia was soaked in 800ml of methanol for 24h with continuous stirring. The mixture was filtered through filter paper. The filtrate was vacuum dried overnight. The powder was used for preparation of extracts.

Induction of experimental diabetes: Overnight fasted male Wistar rats were made diabetic by injecting alloxan monohydrate (SDFCL, Mumbai) @ 120mg/kg body weight intraperitoneally (in ice cold normal saline). Fasting blood glucose (FBG) was measured 72 hour after alloxanization by using glucose oxidase reagent strips with glucometer. All the animals were found diabetic. However rats showing blood glucose level above 200mg/dl were considered as diabetic and were used in the study.

Antidiabetic activity screening in experimentally induced diabetic rats: A total of 30 rats were used for the present work and they were divided into five groups comprising six rats each. Suspension of T. cordifolia stem extract & glibenclamide were prepared in 1% (w/v) Tween-80 solution. Group I and Group II served as normal and diabetic control respectively and received vehicle i.e. 1% Tween-80 solution (1ml/kg,orally). Group III, Group IV received (MTCSEt) @100 and 200mg/kg (p.o) respectively and group V received standard antidiabetic drug glibenclamide (Daonil, Aventis pharma) @5mg/kg (p.o.), once daily for 15 days.

Estimation of blood glucose: Fasting blood glucose levels were estimated before the experiment (Day0) and at the end of 10th and 15th day with the help of glucometer (Bayer’s) using glucose oxidase reagent strips. For estimation of blood glucose level, a drop of blood was obtained by puncturing the tip of tail vein.

Table 1: Effect of daily administration of MTCSEt on blood glucose level in alloxan-induced diabetic rats [mean ± SE].Figures in parenthesis indicates % fall in blood glucose level as compared to Day 0.

<table>
<thead>
<tr>
<th>Group (dose mg/kg, p.o)</th>
<th>Pretreatment</th>
<th>Post treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day0</td>
<td>Day10</td>
</tr>
<tr>
<td>Normal control</td>
<td>75.83 ± 2.48</td>
<td>76.5 ± 2.05</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>326.3 ± 7.14</td>
<td>293.67 ± 13.95 (10%)</td>
</tr>
<tr>
<td>MTCSEt(100)</td>
<td>308.17 ± 8.61</td>
<td>204.83 ± 4.73 (33.5%)</td>
</tr>
<tr>
<td>MTCSEt(200)</td>
<td>298.67 ± 9.51</td>
<td>180.83 ± 5.24 (39.45%)</td>
</tr>
<tr>
<td>Glibenclamide (5)</td>
<td>294.3 ± 10.35</td>
<td>76.3 ± 4.54 (40.9%)</td>
</tr>
</tbody>
</table>

Table 2: Effect of MTCSEt on biochemical parameters in alloxan diabetic rats [mean ± SE]. [n=6]. Means within a column with different superscripts differ at p<0.01 using Dunnet’s test as a post hoc test. 1, 2 (IU/L), 3 nM MDA/g in tissue, 4(nM/g), 5(μmol H2O2 utilized/min/mg of protein), 6(Units/ gm protein)

<table>
<thead>
<tr>
<th>Group (dose mg/kg, p.o)</th>
<th>%GHB Alc</th>
<th>AST 1</th>
<th>ALT 2</th>
<th>LPO 3</th>
<th>GSH 4</th>
<th>CAT 5</th>
<th>SOD 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>4.94 ± 0.109</td>
<td>47.5 ± 2.277</td>
<td>73 ± 2.309</td>
<td>6.34 ± 0.0417</td>
<td>0.2 ± 0.17</td>
<td>76.4 ± 36.22</td>
<td>1.742</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>7.64 ± 0.047</td>
<td>104.3 ± 2.753</td>
<td>139 ± 1.461</td>
<td>10.21 ± 0.672</td>
<td>0.15 ± 0.009</td>
<td>38.87 ± 4.270</td>
<td>10.86 ± 2.111</td>
</tr>
<tr>
<td>MTCSEt(100)</td>
<td>6.98 ± 0.086</td>
<td>80.17 ± 2.428</td>
<td>118 ± 2.582</td>
<td>8.38 ± 0.316</td>
<td>0.15 ± 0.009</td>
<td>46.03 ± 4.603</td>
<td>16.29 ± 0.420</td>
</tr>
<tr>
<td>MTCSEt(200)</td>
<td>6.63 ± 0.050</td>
<td>72.5 ± 2.941</td>
<td>108.83 ± 3.535</td>
<td>6.96 ± 0.561</td>
<td>0.19 ± 0.011</td>
<td>77.60 ± 4.341</td>
<td>23.79 ± 5.415</td>
</tr>
<tr>
<td>Glibenclamide (5)</td>
<td>5.48 ± 0.087</td>
<td>65.5 ± 1.232</td>
<td>81.0 ± 5.260</td>
<td>6.74 ± 0.257</td>
<td>0.2 ± 0.002</td>
<td>63.65 ± 3.127</td>
<td>27.47 ± 3.821</td>
</tr>
</tbody>
</table>

3500
Biochemical determinations: After 15 days of treatment, overnight fasted rats were sacrificed and blood was collected. Glycosylated hemoglobin (HbA1c) was determined in heparanized whole blood [12]. The serum was separated and analyzed for enzymes aspartate amino transferase (AST) and alanine amino transferase (ALT) by using siemens kit. A portion of liver tissue was homogenized & the homogenate was used for the estimation of enzymatic antioxidants; catalase activity (CAT) [13], reduced glutathione (GSH) [14], superoxide dismutase (SOD) [15] and lipid peroxidation (LPO) [16].

Statistical analysis: Statistical analysis was carried out using ANOVA followed by Dunnet’s test. A ‘p’ Value < 0.01 was considered to be significant.

RESULTS AND DISCUSSION

Oral administration of MTCSEt produces significant (p<0.01) and sustained fall in blood glucose level both @ 100 & 200mg/kg on day 10 and 15. However, the fall in blood glucose was greater with MTCSEt @ 200mg/kg but lesser when compared with glibenclamide treated group @ 5mg/kg (p.o) (Table 1). In an earlier study significant reduction in blood glucose in alloxan diabetic rats was observed on oral administration of extract of roots of T. cordifolia for 6 weeks [17]. There was a significant (p<0.01) increase in HbA1c, serum AST, ALT and LPO levels in alloxan-induced diabetic rats as compared to control animals. The administration of MTCSEt (100 & 200mg/kg) caused significant (p<0.01) decrease in the values of HbA1c, serum AST and ALT levels. Significant (p<0.01) decrease in LPO level was observed at MTCSEt (200mg/kg). However, glibenclamide treated rats showed significant (p<0.01) and greater decrease in the values of these parameters (Table 2).

The increase in the glycosylated levels (HbA1c) is due to interaction of haemoglobin with excess of glucose. The rise in glycosylated haemoglobin is a more reliable indicator of the diabetes. A significant (p<0.01) reduction in the activities of antioxidant enzymes CAT and SOD was observed in alloxan induced diabetic rats, indicating a free radical damage in diabetic rats. MTCSEt (200mg/kg) significantly (p<0.01) elevated CAT. However, glibenclamide significantly (p<0.01) restored CAT and SOD (Table 2). Our studies are in agreement with the findings of Prince et al. [18] who observed decreased concentration of GSH, SOD, Catalase and glutathione peroxidase in heart and brain of diabetic rats which was normalized by T. cordifolia root extract when administered orally @ 100mg/kg for 6 weeks.

CONCLUSION

In conclusion, oral administration of methanolic extract of T. cordifolia stem @ 100 & 200 mg/kg to alloxan induced diabetic rats for 15 days, resulted in significant reduction in blood glucose level, HbA1c, serum AST and ALT levels. Significant (p<0.01) decrease in LPO level was observed at MTCSEt (200mg/kg). Significant increase in the level of CAT was observed by T. cordifolia stem @ 200 mg/kg. However, glibenclamide restored blood glucose, HbA1c, ALT, AST, LPO, CAT and SOD and the restoration was greater than the treated groups. Thus, the present study indicated the possible use of T. cordifolia as an adjunct therapy in diabetes.

REFERENCES