Antidiabetic Effect of Garcinol on Streptozotocin-induced Diabetic Rats

K. K. MALI*, R. J. DIAS, V. D. HAVALDAR, S. J. YADAV

Department of Pharmaceutics, YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara-415 011, Sr. Research Associate, LifeSan Clinical Research, Centaur House, Santacruz (East), Mumbai-400 055, India

Mali, et al.: Antidiabetic Effect of Garcinol

The objective of the present study was to investigate the antidiabetic effect of garcinol on streptozotocin-induced diabetic rats. Acute toxicity of garcinol was studied according to the guidelines of Organization for Economic Co-operation and Development. Oral glucose tolerance test was used as a preliminary screening method. Diabetes was induced in rats by intraperitoneal injection of streptozotocin (60 mg/kg). After induction of diabetes, rats were treated with garcinol (25, 50 and 100 mg/kg) and metformin (100 mg/kg) for 28 days. Blood samples were collected for estimating biochemical parameters. Administration of garcinol caused significant reduction in elevated levels of blood glucose, glycosyated hemoglobin and lipids in diabetic rats. Histopathological study revealed regeneration of pancreatic β cells in the garcinol-treated groups. These results indicated that garcinol showed significant antidiabetic and lipid-lowering effects in streptozotocin-induced diabetes rats.

Key words: Garcinol, antidiabetic activity, streptozotocin

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycaemia and disturbances in carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both[1]. The number of patients with DM is predicted to increase globally to 200 million within the next few years[2]. Various oral hypoglycaemic agents such as sulfonylureas, biguanides, meglitinides, thiazolidinediones, glucosidase inhibitors and peptide analogues were used alone or in combination to treat diabetes. Some like biguanides and sulfonylureas have the potential to cause serious side effects but these agents are required to be taken throughout life[3]. Extracts of some medicinal plants have shown promising antidiabetic effects due to the presence of antioxidant and free radical scavenging constituents like polyphenols. These polyphenols might also help in regeneration of β-cells previously damaged due to the oxidation caused by high blood glucose level. Polyphenols might also protect pancreatic islets against the cytotoxic effects of streptozotocin (STZ)[4]. Thus, the natural antioxidants might have the potential to be used as effective alternatives to the oral hypoglycaemic agents in the treatment of diabetes.

Garcinol is a polyisoprenylated benzophenone derivative isolated from Garcinia indica (Kokum). Garinol is structurally similar to a well-known antioxidant curcumin, which contained both phenolic hydroxyl groups and a β-diketone moiety[5]. Literature revealed that garcinol exhibited antioxidant, anti-inflammatory, anticancer and hepatoprotective activities[6-9]. Garcinol was also reported to exhibit radical scavenging activity and suppressed protein glycation in vitro[5]. Till date, the antidiabetic activity of garcinol has not been tested in vivo. Hence, the present investigation was undertaken to investigate the effect of garcinol in STZ-induced diabetic rats and its acute toxicity.

Garcinol was obtained from Sami Labs Pvt. Ltd, Bangalore, India. STZ was procured from Chemvenio, LLC, USA. All other chemicals and kits used in the study were of analytical grade. Female Wistar rats (150-200 g) procured from Shri Venkateshware Enterprises, Bengaluru, were used to assess antidiabetic activity[10]. Female Swiss albino mice (20-25 g) procured from Smt. Kashibai Navale College
of Pharmacy, Pune were used for the acute toxicity study. Animals were housed in the animal house, Satara College of Pharmacy, Satara, at a temperature of 25±1° and 45±5% relative humidity with a 12:12 h day:night cycle. The animals were fed with standard laboratory diet and allowed drinking water ad libitum. Studies were carried out in the Pharmacology Department, Satara College of Pharmacy. The experimental protocol was approved by the Institutional Animal Ethics Committee (SCOP/IAEC/022/11-12).

Oral acute toxicity study of garcinol was carried out according to the Organization for Economic Co-operation and Development (OECD)-guidelines 423[11]. Animals were divided into six groups of three animals each. All animals were fasted overnight with access to water ad libitum. Single dose of garcinol (50, 100, 300, 500, 1000 and 2000 mg/kg) was given orally to each animal. The animals were observed individually during the first 30 min after dosing, periodically during the first 24 h and daily thereafter for 14 d.

Oral glucose tolerance test (OGTT) was performed in overnight fasted (18 h) normal rats. Rats were divided into six groups of six animals each. Normal control and diabetic control received 0.1% w/v carboxymethylcellulose (CMC) and standard treatment group received glibenclamide (2.5 mg/kg). Treatment groups I, II and III have received garcinol 10, 20 and 30 mg/kg, respectively. Glucose (3 g/kg) was administered orally 30 min after the administration of garcinol to treatment group. Blood was withdrawn from the retro orbital sinus under mild ether anaesthesia at 0, 30, 60 and 120 min of glucose administration and blood glucose levels were estimated using glucose oxidase-peroxidase method[12].

Rats weighing 150-200 g were selected and fasted for 16 h prior to experiments and allowed an excess ad libitum. Experimental diabetes was induced by single intraperitoneal injection of freshly prepared STZ (60 mg/kg) in 0.1 M citrate buffer (pH 4.5). Seven days after STZ administration blood glucose level of each rat was determined. Rats with blood glucose level above 200 mg/dl were considered diabetic and were included in the study[13].

Wistar rats were divided into six groups each consisting of six animals. CMC in distilled water (0.1% w/v) was used as vehicle. Doses of garcinol at 25, 50 and 100 mg/kg were suspended in the vehicle and administered to treatment groups orally once daily for 28 d. Group I served as control, group II as diabetic control, group III as standard treatment (metformin 100 mg/kg); group IV, V and VI as treatment groups and received garcinol at 25, 50 and 100 mg/kg, respectively. Body weight and blood glucose levels were estimated on 0, 7, 14, 21 and 28 d. Glycosylated haemoglobin and lipid profile were estimated on d 28. Blood glucose levels were estimated by glucose oxidase-peroxidase method.

Colorimetric method based on the phenol-sulphuric acid reaction of carbohydrates for the determination of sugars bound to haemoglobin was used. Glycosylated haemoglobin (HbA1c) was estimated by the method of Nayak and Pattabiraman[14]. Total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL) was estimated according to the procedure of the kits supplied by Merck, Mumbai, India. Low density lipoprotein (LDL) and very low density lipoprotein (VLDL) was calculated by using Friedewald's formula (FF)[15].

On d 28 of treatment, rats were anesthetised by intraperitoneal sodium pentobarbital (150 mg/kg) and euthanasia was confirmed by cervical dislocation. Pancreas was dissected and immediately kept in 10% formalin. The fixed tissues or organs were then dehydrated and embedded in paraffin wax before being sectioned to approximately 5 µm thickness. The sectioned tissues were stained with haematoxylin and eosin (H and E) followed by histopathological examinations[16]. Results were expressed as mean±SEM. Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test using GraphPad Prism. P<0.05 was considered as the minimal level of statistical significance.

In the acute toxicity study, significant signs of toxicity such as respiratory embarrassment, sleep, lethargy and mortality were found at a dose 1000 and 2000 mg/kg. Two deaths were observed at a dose of 2000 mg/kg. According to the OECD 423 guidelines, dose of garcinol was reduced to 300 mg/kg. Repeated administration of 300 mg/kg of garcinol did not show any signs of toxicity or mortality. Hence a dose between 300-2000 mg/kg was selected, and when the study was repeated with 1000 mg/kg, garcinol showed death of one animal. The LD$_{50}$ value of garcinol was thus taken as 1000 mg/kg.

Blood glucose levels increased rapidly above the fasting value and then subsequently decreased at 30 min after glucose administration. No significant difference
between the effects of 10 and 30 mg garcinol/kg could be observed. Garcinol at 30 mg/kg showed maximum fall of 18.2% in blood glucose level at 60 min after glucose administration.

Administration of STZ (60 mg/kg) to groups of animals produced severe loss in body weight as compared to normal control. Oral administration of garcinol showed significant \( P<0.05 \) increase in body weight on 14, 21 and 28 d after initiation of treatment. Administration of STZ (60 mg/kg) to diabetic control group showed increase in blood glucose level as compared to normal control. Administration of STZ (60 mg/kg) resulted in the levels of TC, TG, LDL and VLDL to get elevated significantly and HDL levels were significantly decreased as compared to controls. Administration of garcinol at doses of 25, 50 and 100 mg/kg, showed significant \( P<0.05 \) reduction in TC, TG, LDL and VLDL levels and an increase in HDL levels as compared to diabetic control group (Table 3).

Histopathological study of the pancreas showed normal acini, and normal cellularity in the islets of Langerhans of control pancreas (NC-P, fig. 1). Diabetic control group showed extensive damage to islets of Langerhans and decreased number of islet cells (DC-

### TABLE 1: EFFECT OF GARCINOL ON BLOOD GLUCOSE LEVELS

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Blood glucose levels (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
</tr>
<tr>
<td>Normal control</td>
<td>96.3±8.3</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>213±7.4</td>
</tr>
<tr>
<td>Standard treatment</td>
<td>211±6.5</td>
</tr>
<tr>
<td>Treatment group I</td>
<td>208±4.5</td>
</tr>
<tr>
<td>Treatment group II</td>
<td>202±9.2</td>
</tr>
<tr>
<td>Treatment group III</td>
<td>213±7.3</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM. Number of animals in each group was 6. Statistical evaluation using one way ANOVA followed by Dunnett multiple comparison tests. \( P<0.05 \) was considered statistically significant.

### TABLE 2: EFFECT OF GARCINOL ON GLYCOSLYTED HAEMOGLOBIN (HBA1C)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose</th>
<th>TC (U/l)</th>
<th>TG (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>0.1% CMC</td>
<td>60.6±1.71*</td>
<td>31.2±1.86*</td>
<td>12.5±0.30*</td>
<td>54.2±1.94*</td>
<td>6.23±0.37*</td>
</tr>
<tr>
<td>DC</td>
<td>0.1% CMC</td>
<td>70.5±1.41†</td>
<td>40.1±1.84†</td>
<td>11.1±0.11†</td>
<td>67.5±1.38†</td>
<td>8.63±0.36†</td>
</tr>
<tr>
<td>SC</td>
<td>100 mg/kg metformin</td>
<td>61.2±2.94*</td>
<td>32.4±1.95*</td>
<td>13.0±0.20*</td>
<td>57.6±2.08*</td>
<td>6.82±0.25*</td>
</tr>
<tr>
<td>TG I</td>
<td>25 mg/kg garcinol</td>
<td>63.7±0.95a</td>
<td>33.7±1.26a</td>
<td>12.8±0.13a</td>
<td>58.5±1.29a</td>
<td>6.87±0.20a</td>
</tr>
<tr>
<td>TG II</td>
<td>50 mg/kg garcinol</td>
<td>64.3±1.34</td>
<td>33.0±0.86</td>
<td>13.0±0.46</td>
<td>57.8±2.15</td>
<td>6.38±0.27</td>
</tr>
<tr>
<td>TG III</td>
<td>100 mg/kg garcinol</td>
<td>63.8±1.62a</td>
<td>32.3±0.31a</td>
<td>12.8±0.11</td>
<td>57.5±1.55</td>
<td>6.45±0.06</td>
</tr>
</tbody>
</table>

NC: Normal control, DC: diabetic control, SC: standard control, TG I: treatment group I, TG II: treatment group II, TG III: treatment group III, HbA1c: glycosylated hemoglobin, CMC: carboxymethyl cellulose. Values are expressed as mean±SEM. Number of animals in each group was 6. Statistical test used, one way ANOVA followed by Dunnett multiple comparison tests.

### TABLE 3: EFFECT OF GARCINOL ON LIPID PROFILE

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<thead>
<tr>
<th>Groups</th>
<th>Dose</th>
<th>TC (U/l)</th>
<th>TG (mg/dl)</th>
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NC: Normal control, DC: diabetic control, SC: standard control, TG I: treatment group I, TG II: treatment group II, TG III: treatment group III, TC: total cholesterol, TG: triglyceride, HDL: high density lipoprotein, LDL: low density lipoprotein and VLDL: very low density lipoprotein. CMC: carboxymethyl cellulose. Values are expressed as mean±SEM. Number of animals in each group was 6. Statistical analysis was done by using One way ANOVA followed by Dunnett Multiple Comparison Test. \( P<0.05 \) was considered statistically significant. \( P<0.05 \). *Data compared with diabetic control. †Data compared with normal control.
P, fig. 1). Diabetic animals treated with (25 mg/kg) of garcinol (TG I-P, fig. 1) showed normal acini cells, presence of islet cells and few foci of regeneration of pancreatic β cells.

In this study, diabetes was induced in the rats using 60 mg/kg dose of STZ, a glucosamine derivative of nitrosourea, which selectively destroys pancreatic islets of β-cells and causes development of hyperglycaemia and glycosuria, as seen in type 1 diabetic patients. Metformin hydrochloride, a biguanide provides an effective treatment for patients with diabetes, which promotes glucose uptake by GLUT4 transporters and reduces glucose production in liver. Other than its glucose-lowering properties, metformin is supposed to have an antioxidant property. It is capable of restoring liver antioxidant enzymes, superoxide dismutase (SOD), and catalase (CAT) in STZ-induced diabetic rats.[17]

The increase in blood glucose level might be due to shortage of insulin as a result of destruction of pancreas interceded by STZ action. STZ boosts ATP dephosphorylation, which in turn generates superoxide anions, hydrogen peroxide, and hydroxyl radicals. This leads to an elevation in the intracellular peroxides in pancreatic islets, which may induce damage due to the reactive oxygen species (ROS).[18] Under hyperglycaemic conditions, antioxidants are supposed to regenerate damaged extracellular matrix proteins and promote cell growth.[19] Thus, antioxidants might help in the management of DM.

In acute toxicity study, garcinol was not safe above 1000 mg/kg possibly due to its toxic effect on the organs of the animals. When garcinol was administered to glucose-loaded animals, reduction in blood glucose levels was observed after 60 min. This may be due to prevention of damage to the β-cells of islets of langerhans resulting from the antioxidant effect of garcinol[4]. The phenolic hydroxyl groups coupled with the β-diketone moiety in garcinol may, via formation of resonance stabilized intermediates, help to prevent the free radical species from propagating and limit the further oxidative-stress-related damage.[20] Besides, closing of K+ATP (adenosine triphosphate) channel and release of insulin from the undamaged β-cells may also be responsible for this decrease in glucose level[21,22].
STZ-induced diabetes is characterized by a severe loss in body weight[23]. Oral administration of garcinol showed significant improvement in body weight on 21st and 28th d. The increase in body weight could be due to amelioration of glycemic control and structural proteins synthesis[24]. Administration of STZ (60 mg/kg) to diabetic control group showed increase in blood glucose level as compared to normal control. All garcinol treated groups exhibited significant reduction in blood glucose level, which may be attributed to the regeneration of β-cells due to antioxidant effect of garcinol as discussed above, leading to more insulin release.

Generally, in uncontrolled or poorly controlled diabetes, there is an increased glycosylation of a number of proteins, including HbA1c[25]. The level of HbA1c is a reliable index of glycemic control in DM, which reflects the average blood concentration. In the present study, HbA1c levels were found to be increased in diabetic animals compared to normal rats. There is an evidence that glycation may itself induces the generation of oxygen-derived free radicals in diabetic conditions. Treatment with garcinol showed decrease in the glycosylated haemoglobin, comparable to animals, which received standard drug metformin[26].

Garcinol also showed significant reduction in TC, TG, LDL and VLDL as compared to diabetic control group. The levels of HDL increased significantly as compared to diabetic control group. This may be due to antihyperlipidemic properties of garcinol[21] associated with AMPK activation via LKB1, increasing glucose uptake and GLUT4 translocation to the plasma membrane[24].

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STZ acted as a diabetogenic through destruction of the β cells of the islets of Langerhans[21]. Histopathological investigation supports presence of islets cell and few foci of regeneration of pancreatic β-cells, in garcinol treated animals. This suggests that garcinol assists in generation of insulin producing β-cells.

On the basis of results, garcinol showed significant antidiabetic and lipid lowering effect on STZ-induced diabetic rats due to its antioxidant activity. The present study indicated the usefulness of garcinol for further development as a therapeutic agent for treatment of diabetes. Moreover, further study is required to illustrate the mechanism of action of garcinol at the cellular and molecular level.

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Conflict of interest:

All authors declare no conflict of interests.

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