Antidiabetic and antioxidative effects of hydro-methanolic extract of sepals of *Salmalia malabarica* in streptozotocin induced diabetic rats

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Summary
Natural products with antidiabetic activities provide important sources for the development of new drugs in the treatment of diabetes mellitus. This present work focuses on the antidiabetic activity of a hydro-methanolic (2:3) extract of the sepals of *Salmalia malabarica* on the blood glucose, the carbohydrate metabolic enzyme, oxidative stress, glycated haemoglobin and transaminase activity in streptozotocin (STZ) induced diabetic rats. Diabetic rats show a significant diminution in the activities of hexokinase, glucose-6-phosphate dehydrogenase and an elevation in the activity of glucose-6-phosphatase in the liver and skeletal muscle. Administration of hydro-methanolic extract of the sepals of *Salmalia malabarica* to diabetic rats resulted in a significant recovery in the parameters concerned. In the liver and kidney, the activities of catalase (CAT) and peroxidase (Px) were decreased significantly and levels of conjugated diene (CD) and thio-barbituric acid reactive substance (TBARS) were increased significantly in diabetic rats which recovered significantly after administration of hydro-methanolic extract of *S. malabarica*. Serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) activities which are increased in diabetes were restored by the extract. Glycated haemoglobin (HbA1C) levels were resettled significantly in the extract treated group compared to the diabetic group. The antidiabetic activity of the extract was supported after a comparison with glibenclamide, a standard antidiabetic drug.

Key words: antidiabetic; carbohydrate metabolic enzyme; oxidative stress; *Salmalia malabarica*; transaminases

INTRODUCTION

Diabetes is the most common of the endocrine disorders and poses a serious challenge to health care worldwide. It is projected that by 2010, at least 239 million people will be affected by diabetes (Mandrup-Poulsen 1998). Diabetes mellitus is becoming the third killer of mankind after cancer and cardiovascular disease due to its high prevalence, morbidity and mortality (Li et al. 2004).

Diabetes has been shown to decrease the activities of enzymes in the glycolytic and pentose phosphate
pathways, while increasing the activities of the glucogenic, glycogenolytic and lipolytic pathways (Basu et al. 2005, Ramis et al. 2006). Oxidative stress induced by chronic hyperglycaemia has been associated with dysfunction and apoptosis of several cell types, including pancreatic β cells (Wu et al. 2004), neurons and glial cells (Russel et al. 2002, Vincent et al. 2004). Oxidative stress results from overproduction of reactive oxygen species coupled with insufficient antioxidant capacity. The oral hypoglycaemic drugs have many side effects such as nausea, vomiting, cholestic jaundice, agranulocytosis, aplastic and haemolytic anaemia, generalized hypersensitivity reactions, dermatological reactions and lactic acidosis (Murad et al. 2009).

Several drugs such as biguanides, glibenclamide and sulfonylureas are presently available to reduce hyperglycaemia in diabetes mellitus but these drugs also have side effects and thus searching for a new class of compounds is essential to overcome these problems (Kamaeswara et al. 2001).

Diabetes mellitus was known in ancient times, and some medicinal plants have been used for its control in traditional medicine (Andrade-Cetto and Heinrich 2005, Mukherjee et al. 2006). The efficacy of plants for the management of diabetes requires confirmation and the WHO (World Health Organization 1980) has recommended the assessment of traditional plant remedies. Some of the work in this field, including our own previous work, has noted the remarkable antidiabetic effects of some plant parts (Maiti et al. 2005, Mallick et al. 2006, Deshmukh et al. 2008, Mandal et al. 2008, Senthilkumar and Subramanian 2008).

Salmalia malabarica is a large and tall deciduous tree belonging to the family of ‘Malvaceae’. It is beneficial in acne and skin eruptions (Kumar et al. 2008), and also possesses antibacterial and antifungal properties (Sharma and Patel 2009). From an ethnobotanical survey we came to know that the sepals of this flower are also used as an antidiabetic medicine in tribal communities, especially in our country, but there are no scientific reports on the antidiabetic activity of S. malabarica and the present investigation was conducted to explore the possibilities. Its efficacy was compared with that of glibenclamide, a standard antidiabetic drug.

MATERIALS AND METHODS

Plant materials
The sepals of S. malabarica of the family “Malvaceae” were collected from Midnapur, District, Paschim, Midnapur, West Bengal, India, in the months of January to April, 2008. The materials were identified by a taxonomist in the Botany Department, of Vidyasagar University, Midnapur and the voucher specimen marked BIO-MED-S.M-01, was deposited in the Department of Botany, Vidyasagar University.

Preparation of hydro-methanolic (2:3) extract of sepals of Salmalia malabarica
Fresh sepals were dried in an incubator for 2 days at 37 °C, crushed separately in an electric grinder and then pulverized. From this powder, 50 g was suspended in 80 ml of water and 120 ml methanol (2:3) and kept in an incubator at 37 °C for 72 h. The slurry was stirred intermittently for 2 h and left overnight. The mixture was then filtered, and the filtrate was dried by a low pressure rotary evaporator. The residue was collected, suspended in water in a fixed dose and used for treatment.

Chemicals
Streptozotocin (STZ) was obtained from Spectrochem Pvt. Ltd (India). All other chemicals used were of analytical grade obtained from E. Merck, Mumbai or HIMEDIA, Mumbai, India or purchased from Sigma-Aldrich Diagnostic Ltd. USA. Kits for the various enzyme assays were purchased from Crest Biosystems, Goa, India.

Selection of animal and animal care
Twenty six matured normoglycaemic wistar strain male albino rats, 3 months of age weighing about 120 ± 10 g were chosen for this experiment. The animals were acclimatised to our laboratory conditions for a period of 15 days prior to the experiment. They were housed at an ambient temperature of 25 ± 2 °C with a 12 h light : 12 h dark cycle. They had free access to standard food and water, and the principles of laboratory animal care and particular instructions given by our institutional ethical committee were followed throughout the experiment, which lasted for 28 days.

Induction of diabetes mellitus
Twenty rats, fasted for 12 hours were subjected to a single intramuscular injection of streptozotocin (STZ) at a dose of 4 mg/0.1 ml of citrate buffer/100 g body weight (b.w.)/rat; after 24 h of STZ injection this produced type 1 diabetes (i.e. having a fasting blood glucose level of more than 250 mg/dl but less than 350 mg/dl). This level of fasting blood glucose has been selected here as it represented a moderate diabetic state (Joussen et al. 2001). Subsequently, six days were allowed to stabilize the diabetes and after that eighteen of the rats meeting the above criteria
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were selected for this experiment. Six normoglycaemic rats were included in the control group and rats of this group were subjected to a single intramuscular injection of citrate buffer only to keep all the animals in same condition in relation to the injection process.

Measurement of fasting blood glucose level
At the time of grouping of the animals, their fasting blood glucose (FBG) level was measured. After every six days of treatment (on every 7th day), FBG was further recorded in all the animals of all groups. Blood was collected from the tail vein or from an orbital puncture and the FBG level was measured by a single touch glucometer.

Animal treatment
Twenty-four rats were divided into the following four equal groups. The experiment took place over a period of 28 days.

Group I (Control group): normoglycaemic rats of this group were subjected to forced feeding of 0.5 ml distilled water/100 g b. w./rat/day for 21 days.

Group II (Diabetic group): forced feeding of 0.5 ml distilled water/100 g b. w./rat/day for 21 days by gavage.

Group III (Diabetic + S. malabarica extract): diabetic rats were forcefully fed by gavage with hydro-methanolic (2:3) extract of sepals of S. malabarica at a dose of 20 mg/0.5 ml distilled water/100 g b. w./rat/day from the 7th day of streptozotocin injection for the next 21 days in a fasting state.

Group IV (Diabetic + glibenclamide): rats of this group were administered by gavage with commercial glibenclamide at a dose of 0.06 mg/0.5 ml water/100 gm b. w./rat/day from the 7th day of streptozotocin injection for next 21 days in a fasting state.

The extract was administered to the animals of group III and glibenclamide to the animals of group-IV early in the morning and in a fasting state i.e. 11–12 hrs after feed delivery. Starting from day 1 of extract administration to the diabetic rats, the fasting blood glucose levels in all groups were measured by a single touch glucometer at six day intervals. On the 29th day after the start of the experiment (considering the day of STZ injection as the first day of experiment), all the animals were sacrificed by decapitation after recording the final body weight. Blood was collected from the dorsal aorta by a syringe. A portion of the blood was used for the separation of serum by centrifugation at 3000 g for 5 min for the estimation of serum toxicity study. The liver, kidney and skeletal muscle were dissected out and stored at –20 °C for biochemical analysis. Blood was used for the quantification of haemoglobin and glycated haemoglobin.

Biochemical estimations
The activities of carbohydrate metabolic enzymes such as glucose-6-phosphate dehydrogenase, hexokinase, and glucose-6-phosphatase were assayed by the methods of Langdon (1966), Chou and Wilson (1975) and Swanson (1955) respectively. The liver and skeletal muscle glycogen levels were measured bio-chemically according to Sadasivam and Manickam (1996). An estimation of lipid peroxidation was performed from a concentration of thiobarbituric acid reactive substance (TBARS) and conjugated diene (CD) according to Okhawa et al. (1979) and Slater (1984). A biochemical estimation of antioxidative enzyme activities such as catalase (CAT) and peroxidase (Px) were measured according to Beers and Sizer (1952), Sadasivam and Manickam (1996) respectively. Haemoglobin and glycated haemoglobin levels were measured according to Balasubramaniam and Malathi (1992), and Chandalia et al. (1980) respectively. The activities of these enzymes were expressed as relative units (Henry et al. 1960).

Statistical analysis
Data were presented as mean ± S.E.M. All the data were evaluated statistically using one-way analysis of variance (ANOVA) followed by a multiple comparison two tail ‘t’ test by using the Origin Lab (Ver. 6.0) software at the significance level 2α = 0.05.

RESULTS
Blood glucose level
Diabetes induced by STZ resulted in a significant elevation in blood glucose in comparison to the control group. After the administration of hydro-methanolic (2:3) extract of sepals of S. malabarica or glibenclamide to the diabetic animals for 21 days, a significant recovery of blood glucose level was noted at a level close to the control level. There was an insignificant difference in the level of fasting blood glucose between the extract treated group and the glibenclamide treated group (Table 1).
Table 1. Effect of hydro-methanol (2:3) extract of *S. malabarica* on fasting blood glucose level in streptozotocin induced diabetic male albino rat. Data are expressed as Mean S.E.M; *n* = 6, ANOVA followed by multiple comparisons two tail ‘t’ test. Values with superscripts like a, b, c in each vertical column differ from each other significantly.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fasting blood glucose level (mg/dl)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1st day (The day of STZ injection)</td>
</tr>
<tr>
<td>Control</td>
<td>73.21 ± 4.48&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic</td>
<td>76.58 ± 5.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + <em>S. malabarica</em> extract</td>
<td>78.36 ± 4.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + glibenclamide</td>
<td>76.87 ± 3.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Glycogen level in tissue**

The quantity of glycogen both in liver and skeletal muscle was decreased significantly in the diabetic group compared with the control group. Administration of hydro-methanolic extract of sepals of *S. malabarica* or glibenclamide to the diabetic animals for 21 days resulted in a significant elevation in the levels of glycogen in the liver and skeletal muscle towards the control level. The level of this parameter showed no significant difference between the extract treated group and the glibenclamide treated group (Fig. 1).

![Glycogen level in tissue](image)

**Fig. 1.** Resettlement in the levels of glycogen in liver and skeletal muscle after administration of hydro-methanolic extract of sepals of *S. malabarica* in STZ-induced diabetic male albino rat. Data are expressed as Mean ± S.E.M; *n* = 6, ANOVA followed by multiple comparison two-tail ‘t’-test. Bars with different superscripts like a, b, c differ from each other significantly.
Carbohydrate metabolic enzymes

The streptozotocin induced diabetic animal displayed a significant elevation in glucose-6-phosphatase activity along with a diminution in the activities of glucose-6-phosphate dehydrogenase and hexokinase in the liver and skeletal muscle in comparison to the control group. Administration of the plant extract or glibenclamide to the diabetic animals resulted in significant protection and the levels of these parameters were resettled towards the control group. There was no significant difference in the levels of these parameters between the extract treated group and the glibenclamide treated group (Figs. 2–4).

**Fig. 2.** Correction in the activity of glucose-6-phosphatase in liver and skeletal muscle after administration of hydro-methanolic extract of sepals of *S. malabarica* in STZ-induced diabetic male albino rat. Statistics and symbols as in Fig. 1.

**Fig. 3.** Modulation in the activities of glucose-6-phosphate dehydrogenase in liver and skeletal muscle after administration of hydro-methanolic extract of sepals of *S. malabarica* in STZ-induced diabetic male albino rat. Statistics and symbols as in Fig. 1.

**Fig. 4.** Recovery in the activities of hexokinase in liver and skeletal muscle after administration of hydro-methanolic extract of sepals of *S. malabarica* in STZ-induced diabetic male albino rat. Statistics and symbols as in Fig. 1.
Activities of CAT and Px
The activities of CAT and Px in the liver and kidney were decreased significantly in the diabetic group in comparison with the control group. After the administration of hydro-methanolic (2:3) extract of sepals of *S. malabarica* or glibenclamide to STZ-treated diabetic rat, the activities of the above enzyme were restored towards the control level. Activities of both the said enzymes did not differ significantly between the extract treated group and the glibenclamide treated group (Figs. 5–6).

![Catalase](image1)

**Fig. 5.** Correction in the activities of catalase in liver and kidney after administration of hydro-methanolic extract of sepals of *S. malabarica* in STZ-induced diabetic rat. Statistics and symbols as in Fig. 1.

![Peroxidase](image2)

**Fig. 6.** Remedial effect in the activities of peroxidase in liver and kidney after administration of hydro-methanolic extract of sepals of *S. malabarica* in STZ-induced diabetic rat. Statistics and symbols as in Fig. 1.

Levels of CD and TBARS
The levels of CD and TBARS in the liver and kidney were increased significantly in the diabetic group when compared to the control group. Significant recovery was noted in the levels of the above parameters after administration of the plant part extract or glibenclamide to the diabetic rats. The levels of these parameters differed insignificantly between the extract treated group and glibenclamide treated group (Figs. 7–8).
Fig. 7. Resettlement in the levels of CD in liver and kidney after administration of hydro-methanolic extract of sepals of *S. malabarica* in STZ-induced diabetic rat. Statistics and symbols as in Fig. 1.

Fig. 8. Significant recovery in the levels of TBARS in liver and kidney after administration of hydro-methanolic extract of sepals of *S. malabarica* in STZ-induced diabetic rat. Statistics and symbols as in Fig. 1.

Fig. 9. Significant alteration in the levels of hemoglobin and glycated hemoglobin in blood after administration of hydro-methanolic extract of sepals of *S. malabarica* in STZ-induced diabetic rat. Statistics and symbols as in Fig. 1.

**Levels of hemoglobin and glycated hemoglobin**

The haemoglobin level was decreased significantly and the glycated haemoglobin (HbA1c) level was increased significantly in the diabetic group in comparison with the control group. After treatment with the plant extract or glibenclamide, the levels of haemoglobin and glycated haemoglobin were resettled towards the control group. The haemoglobin level differed significantly whereas the glycated haemoglobin level differed insignificantly between the extract treated and glibenclamide treated groups (Fig. 9).
Activities of GOT and GPT in Serum
The activities of GOT and GPT in serum were increased in the diabetic group compared to the control group. After administration of the plant extract or glibenclamide there was a significant recovery in the levels of these parameters which showed no significant difference between the extract treated and glibenclamide treated groups (Fig. 10).

![Bar chart of SGOT and SGPT activities](image)

**Fig. 10.** Effect of hydro-methanolic extract of sepals of *S. malabarica* on SGOT and SGPT activities in STZ-induced diabetic rat. Statistics and symbols as in Fig. 1.

**DISCUSSION**

The present study was conducted to investigate the antihyperglycaemic and the antioxidative activities of the hydro-methanolic (2:3) extract of sepals of *S. malabarica* in STZ-induced diabetic male albino rats. The streptozotocin-induced diabetic rat was selected as an experimental model because it is the best model for study of the effect of the antidiabetogenic agent (Veeramani et al. 2008); it is also in continuity with our previous work (Maiti et al. 2005, Mallick et al. 2006, 2007a, b, 2009, Mandal et al. 2008).

Catalase and peroxidase play an important role in preventing the cells from oxidative injury. Enzymatic antioxidants like CAT and Px catalyze the reduction of hydrogen peroxides and protect the tissues against reactive hydroxyl radicals (Bukan et al. 2003). A decrease in the activities of the above enzymes in STZ-induced diabetic rats in vital metabolic tissues such as liver and kidney was as reported by others (Kyuichi and Takuji 2006, Kaviarasan et al. 2008) and our own previous work (Mallick et al. 2006, 2007a, b, 2009, Mandal et al. 2008).

Administration of this plant extract or glibenclamide to diabetic rats increased the activities of CAT and Px, supported here by the quantification of the production of free radicals, a marker of oxidative stress (Phillips et al. 2004).

For the assessment of the antidiabetic potency of the plant extract, we measured the activity of hepatic glucose-6-phosphatase, an important enzyme for glycogenolysis (Aiston et al. 2003). In a similar way, glucose-6-phosphate dehydrogenase and hexokinase are two enzymes under the positive control of insulin (Kruszynska et al. 1998). In the diabetic state the activities of these three enzymes were altered as reported by others (Rajasekaran et al. 2004) and by our own publication (Mallick et al. 2006). The plant extract is able to recover this enzymatic biosensor significantly compared to glibenclamide which may be due to the recovery of insulin. Another possibility is the correction of oxidative injury by the plant extract as free radicals have detrimental effects on the enzyme system (Zama et al. 2007).

A low level of glycogen in the liver and skeletal muscle is another parameter indicating diabetes (Luis et al. 2001). The recovery in the glycogen level in the
plant extract group may be due to the correction in plasma insulin, as proposed by others using other plant materials (Shirwaikar et al. 2006).

The antidiabetic activity of *S. malabarica* has been further supported here by the measurement of the glycated haemoglobin level as the diabetic state elevates this level; as reported by others (Denise et al. 2008) and by our own previous work (Mallick et al. 2007b, 2009). The correction of glycated haemoglobin may be due to a correction in the glucose level through plasma insulin.

There was no toxicity of this extract proved by SGOT and SGPT activities, as these enzymes are themselves important sensors for toxicity assessment (Ghosh and Suryawanshi 2001).

From the above results, the antidiabetic efficacy of the plant extract may be explained in two ways. One is the indirect pathway through which the phytomolecule may stimulate the existing β cell or regenerate the β cell for the recovery in serum insulin along with protection of oxidative injury. Another is the direct way where the phytoingredients present there may inhibit enzymes such as α glucosidase that may interfere with the glucose production in the gastro intestinal tract from complex carbohydrates. The actual mechanism may be reported after future research.

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