The present study evaluated antidiabetic activity of hydroalcoholic extract of Sansevieria roxburghiana rhizome (HASR) in streptozotocin-induced diabetic rats. Hyperglycemia was induced in rats by streptozotocin (STZ, 65 mg/kg body weight). Three days after STZ induction, diabetic rats received HASR orally at 50 and 100 mg/kg body weight daily for 15 days. Glibenclamide (0.5 mg/kg, orally) served as reference. Blood glucose levels were measured on every 5th day during 15 days. Serum biochemical parameters viz. SGPT, SGOT, SALP, cholesterol, triglycerides, and total protein were estimated. Hepatic and renal lipid peroxidation, reduced glutathione (GSH) and catalase (CAT) were also assessed. HASR significantly (P<0.001) and dose dependently normalized blood glucose levels, serum biochemical parameters; decreased lipid peroxidation and recovered GSH and CAT as compared to those of STZ controls. Therefore, S. roxburghiana rhizome demonstrated remarkable antidiabetic activity in STZ-induced diabetic rats. The potential antidiabetic action is plausibly due to its modulation of endogenous antioxidant status.

INTRODUCTION

Diabetes mellitus (DM) is still not completely curable by current antidiabetic drugs. Insulin therapy is the only satisfactory approach in diabetes mellitus, even though it has several drawbacks like insulin resistance, anorexia, brain atrophy and fatty liver in chronic treatment (1). Treatment of type 2 diabetes mellitus (T2DM) patients with sulfonylureas and biguanides is always associated with side effects (2). So, herbal drugs are gaining popularity in the treatment of diabetes mellitus. The major merits of herbal medicines seem to be their efficacy, low incidence of side effects, and low cost.

T2DM is more common because of increasing obesity and reduced activity levels, environmental and psychosocial stress factors, as well as malnutrition and increased xenobiotic intakes. In the past few decades, T2DM has rapidly increased in the world. It has been estimated that the number of diabetic patients will more than double within 15 years (3). T2DM is mainly characterized by the increased morbidity and mortality.
from cardiovascular diseases (4). DM is also associated with a high risk of atherosclerosis and renal, nervous system and ocular complications (5).

Oxidative stress is the imbalance between the generation of reactive oxygen species (ROS) and the body defense mechanisms. Environmental pollutants, toxic habits (drugs, smoking, and/or alcohol), inadequate nutrition, excess solar radiation, large exposure to toxic substances (fertilizers and pesticides), drug metabolism (side effects), and a high physical or psychical stress are the most common exogenous factors originating ROS in the human body (6). Oxidative stress has also been implicated in the pathogenesis of diabetes, liver damage, nephrotoxicity, inflammation, cancer, cardiovascular disorders, neurologic disorders, as well as in the process of aging (7).

Sansevieria roxburghiana Schult. & Schult. f. (Agavaceae), called Murva in Sanskrit and Hindi, Indian bowstring hemp in English is a herbaceous perennial plant with short fleshy stem and stout rootstock, occurring in eastern coastal region of India, also in Sri Lanka, Indonesia and tropical Africa (8,9). In India, this plant has been traditionally used for several medicinal purposes. The whole plant is traditionally used as a cardiotonic, expectorant, febrifuge, purgative, tonic, in glandular enlargement and rheumatism (10-12). The rhizomes are mucilaginous and used in consumptive complaints, long lasting chronic persistent coughs, for quick relief of common cough and cold, in ear pain, etc. (9,12-14). The juice of tender shoots is administered to children for clearing viscid phlegm from throats. The roots are used as febrifuge in snake bite and hemorrhoids (13,14). Recently, the authors have reported antitumor activity of S. roxburghiana rhizome against Ehrlich ascites carcinoma in mice (15). No other pharmacological study on S. roxburghiana has been reported. The present study was therefore aimed to investigate the antidiabetic effects of hydroalcoholic extract of S. roxburghiana (HASR) against streptozotocin (STZ)-induced diabetes in Wistar rats.

**METHODS**

**Plant material**

The rhizome of S. roxburghiana was collected during October 2008 from the forest region of Cuttack, Orissa, India. The specimen was identified by Dr. M. S. Mondal, taxonomist at Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India and the voucher specimen (CNH/I-I/(280)/2008/Tech.II/322) was preserved in our research laboratory for future reference. Just after collection, the rhizomes were washed thoroughly with water, cut into small pieces, shade dried at room temperature (24-26 °C) and ground mechanically into a coarse powder.

**Drugs and chemicals**

Bovine serum albumin was obtained from Sigma Chemical Co., St. Louis, Mo, USA; trichloroacetic acid (TCA) from Merck Ltd., Mumbai, India; thiobarbituric acid (TBA), streptozotocin (STZ), 5,5’-dithio bis-2-nitro benzoic acid (DTNB), phenazonium methosulfate (PMS), nicotinamide adenine dinucleotide (NADH) and reduced glutathione (GSH) from SISCO Research Laboratory, Mumbai, India; potassium dichromate, glacial acetic acid from Ranbaxy, Mumbai; and glibenclamide from Hoechst, India. All the other reagents used were of analytical reagent grade obtained commercially.

**Preparation of extract**

The powdered plant material (450 g) was macerated at room temperature (24-26 °C) with 60% aqueous ethanol (750 mL) for 4 days with occasional shaking, followed by re-maceration with the same solvent similarly for 3 days. The macerates were combined, filtered and evaporated to dryness in vacuo (at 35 °C and 0.8 MPa) in a Buchi evaporator, R-114. The dry extract (HASR, yield 9.35% w/w) was kept in a vacuum desiccator until use. Preliminary phytochemical analysis and chromatographic studies of HASR revealed the presence of alkaloids, triterpenes, steroids, flavonoids, saponins and mucilage (16).
Animals

Adult male Wistar albino rats weighing 170-200 g, obtained from Laboratory Animal Centre, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India were used in the present study. They were housed in a clean polypropylene cage with not more than four animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2 ºC with 12/12 h dark/light cycle). They were fed standard pellet diet (Hindustan Lever, Kolkata, India) and water ad libitum. The animals were acclimatized to laboratory conditions for one week prior to the experiment. All procedures described were reviewed and approved by the University Animal Ethics Committee, Jadavpur University.

Acute toxicity

The acute oral toxicity of HASR in male Swiss albino mice was studied as per OECD guideline 425 (17). The median lethal dose (LD₅₀) value was determined using the method of maximum likelihood.

Oral glucose tolerance test

The oral glucose tolerance test was performed in overnight fasted normal rats. Rats were divided into three groups (n=6). Group I served as normal control and received distilled water (5 mL/kg b.w. p.o), and groups II and III received HASR at doses of 50 and 100 mg/kg b.w., respectively. After these treatments, all groups received glucose (2 g/kg b.w.) orally. Blood was withdrawn from the tail vein just prior to and 30, 60, 120 and 240 min after oral glucose administration (18). Blood glucose levels were measured using single touch glucometer (Accu-check, Roche Diagnostics, USA).

Induction of diabetes

Diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal STZ injection (65 mg/kg b.w.) (19). After 3 days, fasting blood glucose levels were measured and the animals showing blood glucose level ≥225 mg/dL were used for the study (20).

Treatment schedule and estimation of fasting blood glucose (FBG) level

The rats were divided into five groups (n=6). Except for group I, which served as normal non-diabetic control, all other groups were comprised of diabetic rats. Group II served as diabetic (STZ) control. Groups III and IV received HASR (50 and 100 mg/kg b.w., p.o., respectively), and group V received reference drug glibenclamide (0.5 mg/kg b.w., p.o.) daily for 15 days. Fasting blood glucose was measured on days 0, 5, 10 and 15 by using a one-touch glucometer (Accu-check®). At 24 h of the last dose, blood was collected from overnight fasted rats from each group by cardiac puncture for estimation of serum biochemical parameters. Then the rats were sacrificed by cervical dislocation for the study of liver and kidney biochemical parameters.

Body weight

Body weight of rats from each group was measured on days 1, 7 and 15 of HASR treatment.

Estimation of serum biochemical parameters

Collected blood was used for the estimation of serum biochemical parameters viz. serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), total cholesterol, serum triglycerides and total protein (21-25).

Estimation of liver and kidney biochemical parameters

Lipid peroxidation, i.e. thiobarbituric acid reactive substances (TBARS) was estimated and expressed as mM/100 g of tissue (26). Reduced glutathione (GSH) was determined and expressed as mg/100 g of tissue (27). Catalase (CAT) activity was assayed and expressed as μmoles of H₂O₂ decomposed/min/mg of tissue (28).
Statistical analysis

Experimental data were expressed as mean ± standard error of mean (SEM). Statistical significance was analyzed by one-way ANOVA followed by Dunnett’s post hoc test of significance. *P values of <0.01 were considered as statistically significant.

RESULTS

Acute toxicity

The oral LD<sub>50</sub> value of HASR in mice was 500 mg/kg body weight.

Oral glucose tolerance

Glucose loading to normal rats increased serum glucose levels from 63.78±2.3 to 186.54±4.6 at 60 min and returned to normal at 240 min. HASR administration improved glucose tolerance significantly (*P<0.01) in a concentration dependent manner at 60 min (Table 1). The effect of HASR on glucose tolerance remained statistically significant (*P<0.01) at 120 min with the higher dose (100 mg/kg).

Fasting blood glucose (FBG) levels

The fasting blood glucose levels of normal, diabetic and treated rats are summarized in Table 2. STZ at a dose of 65 mg/kg produced marked hyperglycemia as
evident from significant \((P<0.001)\) elevation in FBG level in STZ control group as compared to normal control group. The administration of HASR in STZ-induced diabetic rats at doses of 100 and 200 mg/kg produced significant \((P<0.001)\) and dose dependent fall in blood glucose levels when compared with the STZ-control group. The FBG reducing effect of HASR at a dose of 200 mg/kg was found to be comparable to that of the reference drug glibenclamide \((0.5 \text{ mg/kg})\).

**Effect on body weight**

Vehicle control animals were found to be stable in their body weight but diabetic rats showed significant reduction in body weight during 15 days (Table 3). Streptozotocin caused body weight reduction, which was significantly reversed by HASR after 7 days of treatment.

**Serum biochemical parameters**

Biochemical parameters like SGOT, SGPT, SALP, total cholesterol and triglycerides in STZ control group were significantly \((P<0.001)\) elevated as compared to normal control group. Treatment with HASR at a dose of 50 and 100 mg/kg significantly \((P<0.001)\) brought their levels towards normal values in a dose dependent manner. Total protein was found to be significantly and dose dependently \((P<0.001)\) elevated towards normal level upon administration of HASR as compared with STZ control group (Table 4).

**Liver and kidney biochemical parameters**

The levels of TBARS were significantly \((P<0.001)\) increased in STZ control animals as compared to normal control group. Treatment with HASR at 50 and 100 mg/kg significantly \((P<0.001)\) reduced TBARS levels when compared with STZ control animals in dose related manner. The level of reduced glutathione (GSH) was significantly \((P<0.001)\) depleted in STZ control group as compared with normal control group. Reduced GSH level was found to be significantly and dose dependently \((P<0.001)\) elevated towards normal level upon administration of HASR as compared with STZ control group. There was significant \((P<0.001)\)

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**Table 4. Effects of hydroalcoholic extract of *S. roxburghiana* (HASR) on serum biochemical parameters in streptozotocin (STZ)-induced diabetic rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>SALP (U/L)</th>
<th>Total protein (g/dL)</th>
<th>Total cholesterol (mg/dL)</th>
<th>Triglycerides (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Normal saline)</td>
<td>5 mL/kg</td>
<td>23.1±4.9</td>
<td>25.8±3.8</td>
<td>170.1±13.2</td>
<td>8.1±1.1</td>
<td>121.6±9.6</td>
<td>81.50±8.1</td>
</tr>
<tr>
<td>II (STZ)</td>
<td>65 mg/kg</td>
<td>40.4±5.5*</td>
<td>42.5±4.5*</td>
<td>238.5±11.8*</td>
<td>4.4±0.5*</td>
<td>208.6±13.8*</td>
<td>240.09±12.4*</td>
</tr>
<tr>
<td>III (STZ+HASR)</td>
<td>50 mg/kg</td>
<td>29.9±3.6**</td>
<td>31.6±3.7**</td>
<td>220.9±13.3**</td>
<td>6.5±2.9**</td>
<td>181.8±12.8</td>
<td>191.45±5.9**</td>
</tr>
<tr>
<td>IV (STZ+HASR)</td>
<td>100 mg/kg</td>
<td>26.3±3.3**</td>
<td>27.8±3.3**</td>
<td>198.6±11.6**</td>
<td>6.9±3.2**</td>
<td>166.5±11.2</td>
<td>138.56±3.7**</td>
</tr>
<tr>
<td>V (STZ+Gliben)</td>
<td>0.5 mg/kg</td>
<td>22.5±2.9**</td>
<td>24.9±3.7**</td>
<td>191.7±9.8**</td>
<td>7.4±3.3**</td>
<td>149.6±10.5**</td>
<td>102.42±9.2**</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM \((n=6)\); *P<0.001 compared with normal saline control; **P<0.001 and #P<0.01 compared to STZ control group. Gliben: glibenclamide.

**Table 5. Effect of hydroalcoholic extract of *S. roxburghiana* (HASR) on thiobarbituric acid reactive substances (TBARS) levels in streptozotocin (STZ)-induced diabetic rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>TBARS (mM/100 g of wet liver tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>I (Normal saline)</td>
<td>5 mL/kg</td>
<td>1.05±0.09</td>
</tr>
<tr>
<td>II (STZ)</td>
<td>65 mg/kg</td>
<td>1.87±0.07*</td>
</tr>
<tr>
<td>III (STZ+HASR)</td>
<td>50 mg/kg</td>
<td>1.49±0.09**</td>
</tr>
<tr>
<td>IV (STZ+HASR)</td>
<td>100 mg/kg</td>
<td>1.24±0.08**</td>
</tr>
<tr>
<td>V (STZ+Gliben)</td>
<td>0.5 mg/kg</td>
<td>1.17±0.09**</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM \((n = 6)\); *P<0.001 compared with normal saline control and **P<0.001 compared with STZ control group. Gliben: glibenclamide.
reduction in catalase activity in STZ control group compared with normal group. The administration of HASR recovered CAT activity significantly ($P<0.001$) towards normal when compared with STZ control animals (Tables 5, 6 and 7).

DISCUSSION

The present study was aimed to investigate the antihyperglycemic activity of hydroalcoholic extract of *S. roxburghiana* rhizome (HASR) in STZ-induced diabetic rats. The results of the study revealed that HASR at doses of 50 and 100 mg/kg significantly normalized elevated blood glucose level, body weight and restored serum and liver biochemical parameters towards normal values.

Streptozotocin (STZ) is an antibiotic obtained from *Streptomyces achromogenes*. STZ enters pancreatic β cells via the glucose transporter GLUT2 and causes alkylation of deoxyribonucleic acid (DNA). Its toxicity depends on the potent alkylating properties combined with the synergistic action of nitric oxide and reactive oxygen species that continue to DNA fragmentation. As the result of STZ action, pancreatic β cells are destroyed by necrosis (29). STZ is not only damaging to the pancreatic β cells but also to hepatocytes, nephrons and cardiomyocytes.

Hyperglycemia was observed after 3 days of STZ induction. Treatment with HASR in STZ-induced diabetic rats started reducing fasting blood glucose levels in a dose dependent manner after 5 days and made them normoglycemic after 15 days. The antihyperglycemic effect of HASR at a dose of 100 mg/kg was found to be comparable to the effect exerted by the reference drug glibenclamide at a dose of 0.5 mg/kg.

Induction of diabetes with STZ is associated with a characteristic loss of body weight, which is due to increased muscle wasting and loss of tissue proteins (30). Diabetic rats treated with HASR showed significant improvement in body weight as compared to STZ control animals, hence HASR exhibited a marked effect in controlling the loss of body weight of diabetic rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>GSH (mg/100 g of wet liver tissue)</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Normal saline)</td>
<td>5 mL/kg</td>
<td>46.50±1.7</td>
<td>23.58±1.8</td>
<td></td>
</tr>
<tr>
<td>II (STZ)</td>
<td>65 mg/kg</td>
<td>25.48±1.7*</td>
<td>6.88±2.1*</td>
<td></td>
</tr>
<tr>
<td>III (STZ+HASR)</td>
<td>50 mg/kg</td>
<td>31.30±2.1*</td>
<td>15.65±1.4**</td>
<td></td>
</tr>
<tr>
<td>IV (STZ+HASR)</td>
<td>100 mg/kg</td>
<td>40.56±3.2**</td>
<td>17.70±1.0**</td>
<td></td>
</tr>
<tr>
<td>V (STZ+Gliben)</td>
<td>0.5 mg/kg</td>
<td>38.58±1.9**</td>
<td>19.51±1.9**</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM (n=6); *P<0.001 compared with normal saline control; **P<0.001 and #P<0.01 compared to STZ control group. Gliben: glibenclamide.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>CAT (µmoles of H₂O₂ decomposed/min/mg of wet liver tissue)</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Normal saline)</td>
<td>5 mL/kg</td>
<td>85.58±2.2</td>
<td>39.50±2.3</td>
<td></td>
</tr>
<tr>
<td>II (STZ)</td>
<td>65 mg/kg</td>
<td>46.16±2.9*</td>
<td>23.25±1.9**</td>
<td></td>
</tr>
<tr>
<td>III (STZ+HASR)</td>
<td>50 mg/kg</td>
<td>63.33±2.6***</td>
<td>29.91±2.1*</td>
<td></td>
</tr>
<tr>
<td>IV (STZ+HASR)</td>
<td>100 mg/kg</td>
<td>72.75±2.1***</td>
<td>37.16±2.3**</td>
<td></td>
</tr>
<tr>
<td>V (STZ+Gliben)</td>
<td>0.5 mg/kg</td>
<td>77.58±2.3***</td>
<td>35.58±1.8**</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM (n=6); *P<0.001 compared with normal saline control; **P<0.001 and *P<0.01 compared to STZ control group. Gliben: glibenclamide.
Elevation of serum biomarker enzymes such as SGOT, SGPT and SALP was observed in diabetic rats indicating impaired liver function, which is obviously due to hepatocellular necrosis. The decreased total protein content in STZ-induced animals also substantiated the hepatic damage by STZ. Diabetic complications such as increased gluconeogenesis and ketogenesis may be due to elevated transaminase activities (31). The 15-day treatment with HASR restored all the above mentioned hepatic biochemical parameters towards the normal levels in a dose dependent manner.

Hypercholesterolemia and hypertriglyceridemia have been induced in STZ-induced diabetic rats (32). It is well known that in uncontrolled diabetes mellitus, there is an increase in total cholesterol in blood, which may contribute to coronary artery diseases (33). In the present study, the elevated serum total cholesterol and triglyceride levels in diabetic rats were brought down by HASR treatment.

Oxidative stress in diabetes mellitus has been shown to coexist with impairment in the endogenous antioxidant status (34). Our results indicated that HASR strongly restored liver and kidney antioxidant parameters and decreased lipid peroxidation in diabetic animals. The reduction in liver antioxidant status during diabetes may be the result of counteraction against increased formation of lipid peroxides (35). A marked increase in the concentration of TBARS in STZ-induced diabetic rats indicated enhanced lipid peroxidation leading to tissue injury and failure of the endogenous antioxidant defense mechanisms to prevent overproduction of free radicals. Lipid peroxidation is usually measured in terms of TBARS as a biomarker of oxidative stress (36). Treatment with HASR inhibited hepatic and renal lipid peroxidation in diabetic rats as revealed by the reduction of TBARS levels towards normal levels, suggesting that HASR could improve the pathologic condition of diabetes by inhibiting lipid peroxidation in diabetic rats.

Glutathione plays an important role in the endogenous non-enzymatic antioxidant system. It primarily acts as a reducing agent and detoxifies hydrogen peroxide in the presence of the enzyme glutathione peroxidase (37). The depleted reduced glutathione (GSH) may be due to reduction in GSH synthesis or degradation of GSH by oxidative stress in STZ-induced hyperglycemic animals (38). HASR treatment significantly elevated the liver and kidney reduced glutathione levels towards normal in diabetic rats. The results showed that the antihyperglycemic activity of HASR was accompanied by enhancement in non-enzymatic antioxidant protection.

Enzymatic antioxidant mechanisms play an important role in the elimination of free radicals (ROS). Catalase (CAT) is a haem containing enzyme catalyzing detoxification of H₂O₂ to water and oxygen (39). The inhibition of catalase activity as the result of STZ-induced hyperglycemia was reported earlier (40) and similar findings were observed in our present study. HASR treatment significantly recovered the hepatic and renal CAT activities towards normal in a dose dependent manner.

Preliminary phytochemical studies showed the presence of alkaloids, triterpenes, steroids, flavonoids and saponins in HASR. Flavonoids are putative phenolic natural antioxidants, which would be responsible for the antioxidant property of HASR.

In the present study, the administration of HASR to STZ-induced hyperglycemic rats demonstrated prominent reduction in blood sugar level, normalization of serum biochemical profile including lipid contents, as compared to STZ control rats. Also, HASR treatment resulted in significant modulation of lipid peroxidation, endogenous non-enzymatic (GSH) and enzymatic (CAT) antioxidant and detoxification status. Therefore, it can be concluded that the hydroalcoholic extract of Sansevieria roxburghiana rhizome is remarkably effective against streptozotocin-induced diabetes in Wistar rats plausibly by virtue of its augmenting the endogenous antioxidant mechanisms.

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REFERENCES


