Antihyperglycemic and antioxidative effects of the hydro-methanolic extract of the seeds of *Caesalpinia bonduc* on streptozotocin-induced diabetes in male albino rats

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ABSTRACT

Background: No satisfactory effective treatment is available yet to cure diabetes mellitus. Though, synthetic drugs are used but there are several drawbacks. The attributed antihyperglycemic effects of many traditional plants are due to their ability for the management of diabetes mellitus. **Materials and Methods:** A hydromethanolic extract was administered orally at a dose of 250 mg/kg of body weight per day for 21 days. Its effects on the fasting blood glucose (FBG) level, activities of key carbohydrate metabolic enzymes like hexokinase, glucose-6-phosphatase, and glucose-6-phosphate dehydrogenase, and antioxidant enzymes like catalase and superoxide dismutase along with the effect on the lipid peroxidation level in hepatic tissues were measured. Glycogen levels were also assessed in hepatic and skeletal muscles and some toxicity parameters, such as serum glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, and alkaline phosphates activities were measured. **Results:** Treatment of the hydromethanolic extract of the seeds of *Caesalpinia bonduc* resulted in a significant ($P < 0.05$) recovery in the activities of carbohydrate metabolic enzymes along with correction in FBG and glycogen levels as compared with the untreated diabetic group. The extract also resulted in a significant ($P < 0.05$) recovery in the activities of toxicity assessment enzyme parameters. Activities of antioxidant enzymes like catalase and superoxide dismutase along with the lipid peroxidation levels were also recovered significantly ($P < 0.05$) after the treatment of the extract. The corrective effects produced by the extract were compared with the standard antidiabetic drug, glibenclamide. **Conclusion:** Our findings provide that the extract shows possible antihyperglycemic and antioxidative activities.

Key words: Antihyperglycemic, antioxidative, *C. bonduc*, streptozotocin

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INTRODUCTION

Diabetes mellitus (DM) is a syndrome characterized by hyperglycemia and changed metabolism of carbohydrates, lipids, and proteins.[1] Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications disturbing eyes, kidneys, nerves, and arteries.[2] Increasing evidences from both experimental and clinical studies propose that oxidative stress plays a major role in the pathogenesis of DM.[3] Free radicals are formed suspiciously in diabetes by glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative deficiency of glycated proteins.[4] An abnormally high level of free radicals and the simultaneous decline in antioxidant defense mechanisms may lead to the damage of cellular organelles and enzymes, increased lipid peroxidation, and the development of insulin resistance.[5] Several synthetic drugs used for the treatment of diabetes have various side effects and hence there is a need for alternative improved oral hypoglycemic agents.[6,7] In the indigenous system of medicine, Ayurveda, medicines have been made using a number of plants for controlling diabetes but only a few have been scientifically evaluated with the active principles isolated.[8,9] Antihyperglycemic effects of various plants are credited to their ability to restore the function of pancreatic tissues by causing an

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increase in the insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin-dependent processes.[10,11] More than 400 plant species having a hypoglycemic activity are mentioned in the literature.[12,13] Most of plants which contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., are frequently implicated as having an antidiabetic effect.[14,15]

Caesalpinia bonduc (*C. bonduc*) is a medicinal plant belonging to the family of "Fabeaceae." It is a large, straggling, very thorny shrub; the branches are armed with hooks and straight, hard yellow prickles. In Indian traditional plant medicine, it has been considered an important remedy for the treatment of filarial infection, tumor, asthma, and diabetes.[16] Data on the antidiabetic activity study of this plant through scientific manners are scanty.[17] Hence, the present study was conducted to explore the antihyperglycemic and antioxidative effect of the hydromethanolic extract of the seeds of *C. bonduc* on streptozotocin (STZ)-induced diabetes in rats.

MATERIALS AND METHODS

Collection of plant materials

The dried seeds of *C. bonduc* were collected from the village area of Paschim Medinipur, West Bengal, India, and the plant was identified by a taxonomist in Department of Botany and Forestry, Vidyasagar University, Midnapore, West Bengal, and the voucher specimen was deposited (reference no. Bio-Med/V.U/C.B/24/10).

Preparation of the hydromethanolic extract of seeds of *C. bonduc*

The hydromethanolic extract of seeds of *C. bonduc* was prepared as per the standard method.[18] In brief, fresh seeds of *C. bonduc* were dried in an incubator for 2 days at 40°C, then crushed in an electric grinder, and pulverized. From this powder, 50 g was suspended in the mixture solvent consisting of 80 ml of water and 120 ml methanol in a container for 48 h at room temperature and then the supernatant was filtered through Whatman grade no. 3 filter paper. The filtrate was concentrated and the collected residue was preserved in a refrigerator at 2–8°C for use in the experiments.

Chemicals

Sigma (USA)-made STZ was used here. All other used chemicals were analytical grade purchased from Sigma-Aldrich Diagnostic Ltd., USA. For the assessment of toxicity parameters, reagents were purchased from Merck Diagnostic Ltd., India (24-26) following a spectrometric method.

Selection of animals and animal care

Wistar strain matured male albino rats 3 months of age, weighing about 120 ± 10 g with normoglycemic level (having a FBG level of 80–90 mg/dl) were used for this experiment. All the animals were acclimated for a period of 15 days under laboratory conditions prior to the experiment. Rats were kept at an ambient temperature of 25 ± 2°C with a 12-h light:12-h dark cycle. Rats were provided rich standard feed and water *ad libitum*. The principles of laboratory animal care and instructions given by our Institutional Ethical Committee (IEC), which are in compliance with the guidelines of Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), were followed throughout the experiment.

Induction of diabetes mellitus

Diabetes was induced by a single intramuscular injection of STZ (Sigma-Aldrich) at a dose of 40 mg/kg of body weight. STZ was dissolved in a 0.1 M citrate buffer (pH 4.5). Diabetes was confirmed 7 days after STZ injection by the FBG concentration. Only animals with FBG levels of 300–350 mg/dl were considered for the experiment.[19]

Measurement of fasting blood glucose level

After grouping of all the animals, the FBG level was measured. On every seventh day of treatment, FBG was further recorded for all the animals of all groups. Blood was collected from the tip of the tail vein and the FBG level was measured by a single touch glucometer (Bayer's Ascensia Entrust, Bayer, Germany).

Experimental design

Before the induction of diabetes the rats were divided into four groups of six animals each:

- *Control group*: Rats of this group received a single intramuscular injection of the citrate buffer (1 ml/kg body weight).
- *Diabetic group*: Rats of this group were made diabetic by a single intramuscular injection of STZ at a dose of 40 mg/kg body weight.
- *Extract-treated group*: Diabetic rats of this group were treated with the hydromethanolic (2:3) extract of the seeds of *C. bonduc* at a dose of 250 mg/kg of body weight per day for 21 days in a fasting state.
- *Glibenclamide-treated group*: Diabetic rats of this group were treated with glibenclamide at a dose of 0.6 mg/kg body weight per day.

The dose of the extract used here is taken from a dose-dependent pilot study and the dose of the glibenclamide was used as per our previous report[10] and another study.[20] The extract and glibenclamide were administered orally in the respective groups of animals by an intragastric tube daily for 21 days. The FBG level was measured every seventh day of extract treatment. On day 22 of extract

treatment (day 29 of STZ injection), all animals were sacrificed by cervical decapitation. Blood was collected from the dorsal aorta by a syringe and serum was separated at 3000 rpm for 10 min for the assessment of the activity of serum alkaline phosphatase (ALP) and transaminase enzymes. Livers and skeletal muscles were dissected out and stored at −20°C for biochemical analysis of the activities of enzymes and glycogen content in the respective tissue sample.

Evaluation of carbohydrate metabolic enzyme markers

Activities of carbohydrate metabolic key enzymes, i.e., hepatic hexokinase,[21] glucose-6-phosphate dehydrogenase,[22] and glucose-6-phosphatase[23] were measured biochemically by recording the optical density by a spectrophotometer.

Assay of glycogen

The levels of glycogen in liver and skeletal muscles were measured biochemically[24] with a slight modification.[25]

Toxicity assessment

Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and ALP levels were measured using kits from Merck Diagnostic Ltd. (24-26) following a spectrometric method. The values were expressed in mg/dl in all the cases.

Biochemical estimation of the tissue homogenate

The liver and kidney were removed from the dissected animal, freed from adhering tissues, and washed with an ice-cold normal saline solution (0.9%). Weights of all the organs were taken only after drying the tissue using a blotting paper; 1 g tissue was homogenized in 10 ml of 0.2 M Tris-HCl with the help of a homogenizer. The homogenate was filtered and then centrifuged at 10,000 rpm for 20 min at 4C. The supernatant was used for the estimation of the activities of superoxide dismutase (SOD)[26] and catalase (CAT).[27] The quantification of thiobarbituric acid reactive substances (TBARS) was performed in the same tissue sample.[28]

Statistical analysis

Analysis of variance (ANOVA) followed by a multiple comparison two-tail *t*-test was used for statistical analysis of collected data.[29] Differences were considered significant at $P < 0.05$. All the values have been indicated by mean ± SEM.

RESULTS

Fasting blood glucose level

A significant elevation was noted in FBG levels in STZ-injected diabetic rats as compared to the control group. The administration of the hydromethanolic extract for 21 days resulted in a significant decrease in the FBG level in the diabetic animals toward control levels with respect to the untreated diabetic rats. There was no significant difference in the levels of FBG between glibenclamide-treated and hydromethanolic extract-treated groups. The percentage of recovery in the extract-treated group was 73.73%, where glibenclamide showed 74.05% recovery in FBG levels with respect to the untreated diabetic group [Table 1].

Activities of carbohydrate metabolic enzymes

Hexokinase and glucose-6-phosphate dehydrogenase activities in the liver were significantly decreased and the activity of glucose-6-phosphatase was significantly increased in the STZ-injected diabetic group with respect to the control group. After the administration of the hydromethanolic extract to the diabetic rats, a significant recovery was noted in the activities of the said enzymes. No significant difference was noted in the activities of the said enzymes between extract-treated and glibenclamide-treated groups. The percentage of recovery in the activities of hexokinase and glucose-6-phosphate dehydrogenase in the liver was 16.32% and 52.09%, respectively, in the hydromethanolic extract-treated group whereas the recovery of the said parameters was 15.54% and 53.37%, respectively, in the glibenclamide-treated group when compared with the untreated diabetic group. The activity of glucose-6-phosphate in the liver was recovered by 25.95% in the hydromethanolic extract-treated group whereas 28.77% recovery was noted in the glibanclamide-treated group when compared to the diabetic group [Figure 1].

Glycogen levels in tissues

Glycogen levels in the liver and skeletal muscles were

Table 1: Fasting blood glucose levels in control and different experimental groups

Groups	Fasting blood glucose level (mg/dl)				
	Day 1	Day 8	Day 15	Day 22	Day 29
Control	84.83 ± 4.8^a	86.83 ± 3.3^a	81.83 ± 4.7^a	80.50 ± 4.1^a	86.32 ± 4.7^a
Diabetic	88.66 ± 4.7^a	347.60 ± 8.2^b	353.33 ± 4.1^b	345.83 ± 2.2^b	341.33 ± 2.6^b
Extract treated	84.16 ± 6.5^a	335.33 ± 6.4^b	246.00 ± 2.6^c	120.81 ± 2.9^c	89.00 ± 3.8^a
Glibenclamide treated	86.66 ± 4.6^a	325.66 ± 5.0^b	241.50 ± 3.6^c	123.33 ± 3.2^c	88.80 ± 4.1^a

Data are expressed as mean ± SEM ($n = 6$). ANOVA was followed by a multiple comparison two-tail *t*-test. Values with different superscripts (a, b, and c) in each column differ from each other significantly, $P < 0.05$.

significantly decreased in the diabetic group compared with the control group. After the administration of the hydromethanolic extract of the seeds of *C. bonduc* or glibencalmide to diabetic rats, a significant recovery was noted in the levels of glycogen in the liver and skeletal muscles toward the control level. After the administration of the extract to diabetic rats, the percentage of recovery in the levels of glycogen in the liver and skeletal muscles was 72.10% and 77.68%, respectively, whereas it was 65.58% and 71.55%, respectively, in the glibenclamide-treated diabetic group. The level of this parameter showed no significant difference between the extract-treated and glibenclamide-treated groups [Figure 2].

Activities of antioxidant enzymes, and lipid peroxidation level in the hepatic tissue

The activities of hepatic antioxidant enzymes, i.e. CAT and SOD, were decreased and quantity of TBARS was increased significantly in STZ-injected diabetic rats with respect to the control group. The administration of the hydromethanolic extract resulted in a significant recovery in the levels of these parameters with respect to the diabetic group. No significant difference was noted in the activities of CAT, SOD, and the quantity of the TBARS level between the glibenclamide-treated and hydromethanolic extract-treated groups. The percentage of recoveries in the levels of CAT and SOD was 51.08% and 44.24%, respectively, after the treatment with the hydromethanolic extract, whereas it was 54.89% and 51.76%, respectively, after the administration of glibenclamide to diabetic rats with respect to the untreated diabetic group. The percentage of recovery in the level of TBARS was 29.54% and 34.29% in the extract-treated and glibenclamide-treated diabetic groups, respectively [Figure 3].

Activities of GOT, GPT, and ALP in serum

Activities of GOT, GPT, and ALP in serum increased significantly in the diabetic group compared with the

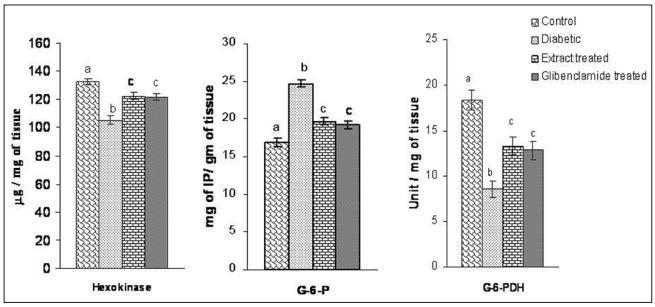

Figure 1: Activity of hepatic hexokinase, glucose-6-phosphatase (G-6-P), and glucose-6-phosphate dehydrogenase (G-6-PDH) after the administration of the hydromethanolic extract of the seeds of *C. bonduc* in the streptozotocin-injected diabetic group. Bars are expressed as mean ± SEM ($n = 6$). ANOVA was followed by a multiple comparison two-tail *t*-test. Values with different superscripts like a, b, and c on bars differ from each other significantly, $P < 0.05$

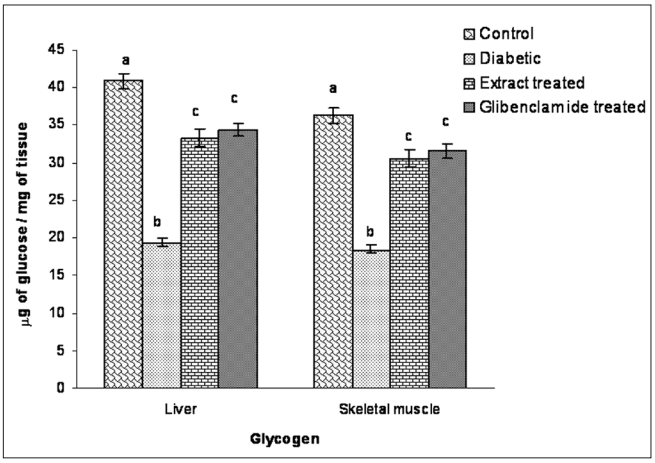

Figure 2: Levels of glycogen in the liver and skeletal muscles in control and different experimental groups of rats. Bars are expressed as mean ± SEM ($n = 6$). ANOVA was followed by a multiple comparison two-tail *t*-test. Values with different superscripts like a, b, and c on bars differ from each other significantly, $P < 0.05$

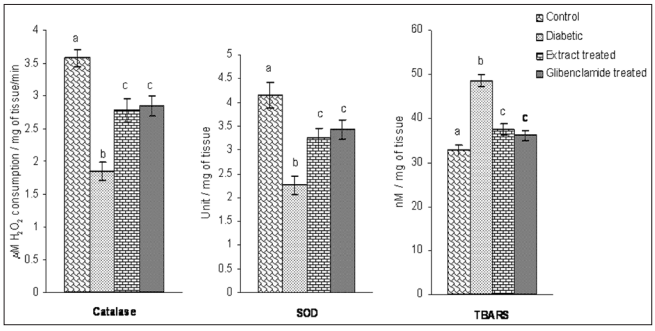

Figure 3: Activities of hepatic catalase (CAT) and superoxidase dismutase (SOD), and quantity of thiobarbituric acid reactive substances (TBARS) after the administration of the hydromethanolic extract of the seeds of *C. bonduc* in streptozotocin-injected diabetic rats. Bars are expressed as mean ± SEM ($n = 6$). ANOVA was followed by a multiple comparison two-tail *t*-test. Values with different superscripts like a, b, and c on bars differ from each other significantly, $P < 0.05$

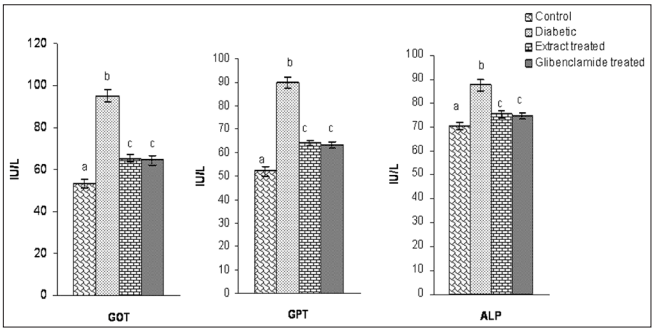

Figure 4: Activities of serum GOT, GPT, and ALP in different experimental groups compared to the control group of rats. Bars are given as mean ± SEM ($n = 6$). ANOVA was followed by a multiple comparison two-tail *t*-test. Values with different superscripts like a, b, and c on bars differ from each other significantly, $P < 0.05$

control group. The administration of the hydromethanolic extract to diabetic animals resulted in a significant recovery in the activities of these three enzymes toward the control level. No significant difference was noted in the activities of serum GOT, GPT, and ALP between the glibenclamide-treated and extract-treated groups. The percentage of recoveries in the levels of serum GOT, GPT, and ALP was 30.90%, 28.75%, and 12.84%, respectively, after the administration of the plant extract and 32.04%, 29.72%, and 14.92% recoveries were noted after the administration of glibenclamide to diabetic rats with respect to the untreated diabetic group [Figure 4].

DISCUSSION

Diabetes was induced by a single intramuscular injection of STZ, which is toxic to β-cells.[30] In the present study, the administration of the hydromethanolic extract of *C. bonduc* effectively reduced the blood glucose level in STZ-injected diabetic rats. Since the hydromethanolic extract of *C. bonduc* reduced the FBG level, it may be implicit that the extract may result in stimulations of the remaining β-cells of the pancreas in diabetic rats for insulin secretion or it may help in the regeneration of pancreatic β-cells which is in agreement with other reports[31] as well as our previous reports on this aspect using other plant parts.[32]

Another view for the regenerative activities of pancreatic β-cells of this extract in STZ-induced diabetes is the improvement of activities of hexokinase, glucose-6-phosphate dehydrogenase, and glucose-6-phosphatase in the liver as well as the improvement in the levels of glycogen in the liver and skeletal muscles.[33] As these biomarkers are under the positive control of insulin[25] so, it may be predicted that the extract may progress the pancreatic insulin synthesis or secretion through β-cell regeneration. In our previous work, this type of observation has been noted using other plants for this purpose.[34]

Another view for the antidiabetic effect of the said plant extract is the antioxidative effect. The decreased activities of CAT and SOD in the diabetic group may be a response to the increased production of H_2O_2 and O_2 by the auto-oxidation of glucose and nonenzymatic glycation.[35] Hepatic SOD and CAT activities were reduced during diabetes and this may result in a number of harmful effects due to the accumulation of hydrogen peroxides and superoxide radicals.[36] The administration of the *C. bonduc* extract showed decreased lipid peroxidation, which is associated with an increased activity of SOD and CAT which indicates that the extract can reduce reactive oxygen free radicals and improve the activities of the hepatic antioxidant enzymes.[37] The antioxidative activity of the said extract has been strengthened here from the quantification of TBARS in hepatic tissues as there is an inverse relationship between the activities of antioxidant enzymes and the quantity of free radicals, which is in agreement with previous reports.[19,38]

In diabetes, serum GOT, GPT, and ALP activities were increased,[39] which may be due to the cellular damage.[40] The plant extract was shown to normalize the levels of these enzymes, which indicates that it has a promising antidiabetic effect without inducing toxicity.

CONCLUSION

On the basis of the aforementioned results, it may be concluded that the *C. bonduc* seed extract has significant antihyperglycemic and antioxidative effects in diabetic experimental rats. Therefore, it may be useful for alternative and early treatment of diabetic disorders. Further studies are in progress to isolate, identify, and characterize the active principles.

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