Antihyperglycemic And Antihyperlipidemic Effects Of Hydro-Methanolic Extract Of Seed Of Caesalpinia bonduc In Streptozotocin Induced Diabetic Male Albino Rat

Kishalay Jana¹, Kausik Chatterjee¹, Tushar Kanti Bera¹, ², Soumyajit Maiti¹, Debasis De¹, Kazi Monjur Ali¹, Debidas Ghosh¹*

¹Andrology, Endocrinology and Molecular Medicine Laboratory, Bio-Medical Laboratory Science and Management (U.G.C Innovative Department) Vidyasagar University, Midnapore-721102, West Bengal, India

²Ayurvedic Division, Southern Health Improvement Samity (SHIS), Bhangar, South 24-Pargana, West Bengal, India

*Corres. Author: debidas_ghosh@yahoo.co.in

Abstract: The present study was designed to find out the effect of hydro-methanolic extract of seed of Caesalpinia bonduc L (Fabaceae) on streptozotocin (STZ) induced diabetic rat, was studied by monitoring the fasting blood glucose level, activities of carbohydrate metabolism marker enzymes, levels of serum lipid profile and the activities of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) in serum. Measurement of fasting blood glucose level (FBG), activities of important carbohydrate metabolic enzymes like hexokinase and glucose-6-phosphate dehydrogenase in liver and skeletal muscle as well as hepatic and skeletal muscular glycogen levels, were increased significantly in extract treated diabetic group in respect to untreated diabetic group. Activities of hepatic glucose-6-phosphatase was recovered significantly in extract treated diabetic group when comparison made with untreated diabetic group. To assess the antihyperlipidemic activities of the extract, levels of total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDLc), very low density lipoprotein cholesterol (VLDLc) and high density lipoprotein cholesterol (HDLc) in serum were measured those were significantly recovered by the extract when compared with untreated diabetic group. Resettlement of all the parameters after the treatment of hydro-methanolic extract of seed of Caesalpinia bonduc to diabetic animal was promising which has been reflected here from the comparative study with the antidiabetic drug i.e. glibenclamide. After the monitoring of GOT and GPT activities in serum, it has been noted that the extract significantly correct the activities of these enzymes in STZ-induced diabetic rat. So, the present study enlightened that the seed of Caesalpinia bonduc has a remedial effect on diabetic complications in STZ-induced diabetic animal without any metabolic toxicity induction.

Key Words: Caesalpinia bonduc, Carbohydrate metabolic enzymes, Glycogen, Hyperlipidemia, Transaminases enzymes.
Introduction
Diabetes mellitus is the syndrome of disturbed energy homeostasis, caused by an abnormal metabolism of carbohydrates, proteins and fats. It is the most common endocrine-metabolic disorder of childhood and adolescent with important consequences on physical and emotional development. The number of people suffering from the disease worldwide is increasing at an alarming rate with a projected 366 million peoples likely to be diabetic by the year 2030 as against 191 million estimated in 2000. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs. In diabetic rats, the utilization of impaired carbohydrate leads to accelerated lipolysis, resulting in hyperlipidemia. Diabetes mellitus is a major cause of morbidity such as blindness, kidney failure, lower-extremity amputation, and cardiovascular disease and premature mortality. Despite the presence of known antidiabetic medicine in the pharmaceutical market, diabetes and the related complications continued to be a major medical problem. Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as antidiabetic and anti hyperlipidemic remedies. Antihyperglycemic effects of various plants are attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. More than 800 plant species having hypoglycemic activity have been available in literature. Most of plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., that are frequently implicated as having antidiabetic effect. Caesalpinia bonduc (C. bonduc) is a medicinal plant belonging to the family of ‘fabeaceae’. It is large straggling, very thorny shrub, branches armed with hooks and straight hard yellow prickles tree. In Indian traditional medicine, it has been considered an important remedy for the treatment of filarial infection, tumor, asthma, and diabetes. The antidiabetic activity study of this plant in scientific manner is scanty. Hence, the present study was conducted to explore the antihyperglycemic and antihyperlipidemic effect of hydro-methanolic extract of seed of C. bonduc in streptozotocin-induced diabetic rats and the result is encouraging to continue more work in this concern of the said plant part.

Materials and methods
Collection of plant materials
The seeds of C. bonduc collected from village area of Paschim Medinipur, West Bengal, India and identified by a taxonomist in the Department of Botany, Forestry, Vidyasagar University, Midnapore and the voucher specimen was deposited having the Reference No. Bio-Med / V.U / C.B / 24 / 10.

Preparation of hydro-methanolic (2:3) extract of seeds of C. bonduc
Fresh seeds of C. bonduc were dried in an incubator for 2 days at 40°C, crushed in an electric grinder and then pulverized. Out of this powder, 50 g was suspended in the mixture solvent of hydro-methanol consisting 80 ml of water and 120 ml of methanol and was kept in incubator at 37°C for 36 h. The slurry was stirred intermittently for 2 h and left for overnight. The mixture was then filtered and filtrate was concentrated using rotary evaporator and residue was collected. The residue was suspended in water in a fixed dose and used for treatment.

Chemicals
Streptozotocin (STZ) was obtained from Sigma (USA). All other chemicals used were analytical grade purchased from Sigma-Aldrich Diagnostic Ltd. USA. Kits for assessment of different enzyme activities were purchased from Crest Biosystems, Goa, India.

Selection of animal and animal care
Matured normoglycemic (having fasting blood glucose level 80-90 mg / dl) wistar strain male albino rat, 3 months of age weighting about 120 ± 10 g were used for this experiment. Animals were acclimated for a period of 15 days in our laboratory condition prior to the experiment. Rats were housed at an ambient temperature of 25 ± 2°C with 12 h light: 12 h dark cycle. Rats have free access to standard feed and water ad libitum. The principle of laboratory animal care and instructions given by our Institutional Ethical Committee (IEC) which is 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 at the dose of 40 mg / kg of body weight. STZ was dissolved in 0.1 M citrate buffer (pH 4.5). Diabetes was confirmed 7 days after STZ injection by determining the fasting blood glucose (FBG) concentration. Only animals with FBG levels 300-350 mg / dl were considered for the experiment.

Measurement of fasting blood glucose level
At the time of grouping of the animals, fasting blood glucose (FBG) level was measured. On every 7th day of treatment, FBG was further recorded from all the animals of all groups. Blood was collected from the tip of the tail vein and FBG level was measured by single
touch glucometer (Bayer’s Ascensia Entrust, Bayer, and Germany).

**Experimental design**

After the induction of diabetes the rats were divided into four groups of six animals each.

**Control:** Rats of this group received single intramuscular injection of citrate buffer and given distilled water (vehicle solution) orally for 21 days.

**Diabetic:** Rats of this group were made diabetic by single intramuscular injection of STZ at a dose of 40 mg / kg body weight and given vehicle solution.

**Diabetic + extract:** Diabetic rats of this group were treated with hydro-methanolic (2:3) extract of seed of *C. bonduc* at the dose of 250 mg / kg of body weight / day for 21 days at fasting state.

**Diabetic + glibenclamide:** Diabetic rats of this group were treated with glibenclamide at the dose of 0.6 mg / kg of body weight / day.

The dose of the extract has been selected here from the dose dependent pilot study and the dose of the glibenclamide was used as per our previous report and by other. The extract, glibenclamide and vehicle solution were administered orally in respective group of animals by an intragastric tube daily for 21 days. The fasting blood glucose level was measured in every 7 days interval of extract treatment. On 22nd day of extract treatment (29th day of STZ-injection) all animals were sacrificed by cervical decapitation. Blood was collected from dorsal aorta by syringe and serum was separated at 3000 rpm for 10 min for the assessment of serum lipid profile and the activities of transaminase enzymes. The different tissues were dissected out and stored at -20°C for biochemical analysis of the activities of enzymes and glycogen content in respective tissue sample.

**Measurement of lipid profile**

Serum lipid profile like serum levels of triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDLc), very low density lipoprotein cholesterol (VLDLc) and high density lipoprotein cholesterol (HDLc) were measured biochemically.

**Biochemical assay of GOT, GPT activities**

Activities of serum GOT and GPT were measured according to standard method.

**Statistical analysis**

Analysis of Variance (ANOVA) followed by multiple comparison two-tail ‘t’ test was used for statistical analysis of collected data. Differences were considered significant at p < 0.05. All the values have been indicated by Mean ± SEM.

**Results**

**Levels of FBG**

STZ-induced diabetic animals showed a significant (p < 0.05) elevation in fasting blood glucose (FBG) levels in respect to the control group. Treatment with hydro-methanolic extract of seed of *C. bonduc* to diabetes animal for 21 days resulted a significant reduction of FBG levels towards control in respect to untreated diabetic animals. There was no significant difference between glibenclamide treated group and hydro-methanolic extract treated group. The percentage of recovery in extract treated group was 73.17 %, whereas 75.9 % recovery in FBG levels in respect to their initial hyperglycemic state (Table 1).

**Carbohydrate metabolic enzyme activities**

The activities of hexokinase and glucose-6-phosphate dehydrogenase in liver and skeletal muscle were significantly decreased and the activity of glucose-6-phosphatase was significantly increased in STZ-induced diabetic group with respect to the control group. After treatment with hydro-methanolic extract to diabetic rat, significant recoveries were noted in the activities of the said enzymes. No significant difference was noted in the activities of the concerned enzymes between extract treated group and glibenclamide treated group. Percentage of recovery in the activities of hexokinase in liver and skeletal muscle were 14.65 %, 17.71 % respectively by hydro-methanolic extract of seed of *C. bonduc* whereas 13.94 %, 13.21 % recoveries were noted by glibenclamide when comparison made with the untreated diabetic group (Fig. 1).
In extract treated diabetic group recovery in the activities of glucose-6-phosphate dehydrogenase in liver and skeletal muscle were 64.3 %, 50.48 % respectively, whereas 68.7 %, 67.35 % recoveries were noted in glibenclamide treated diabetic group in respect to untreated diabetic group (Fig. 2). The percentage of recovery in the activities of hepatic and skeletal muscular glucose-6-phosphatase were 33.9 %, 37.3 % respectively where as 36.6 %, 33 % recoveries were noted respectively in the said tissues by the glibenclamide (Fig. 3).

**Glycogen level in tissues**

Levels of glycogen in liver and skeletal muscle were significantly decreased in diabetic group compared to the control group. But after treatment of hydro-methanolic extract of seed of *C. bonduc* or glibenclamide to diabetic rat, a significant recovery were noted in the levels of glycogen in liver and skeletal muscle towards the control level. After the treatment of extract to diabetic rats, percentage of recovery in the levels of glycogen were 72.5 %, 63.07 % in liver and skeletal muscle respectively whereas 77.7 %, 80.8 % recovery were noted in liver and skeletal muscle respectively in glibenclamide treated diabetic group. When comparison was made between glibenclamide and extract treated group, there was no significant variation was observed in the levels of these parameters which focused the remedial effect of this plant extract for the management of diabetes (Fig. 4).

| Table1. Fasting blood glucose levels in extract treated diabetic group and other experimental groups |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Groups          | 1<sup>st</sup> day | 8<sup>th</sup> day | 15<sup>th</sup> day | 22<sup>nd</sup> day |
| Control         | 83.83±4.8<sup>a</sup> | 80.83±3.3<sup>a</sup> | 80.83±4.7<sup>a</sup> | 78.5±4.1<sup>a</sup> |
| Diabetic        | 88.66±4.7<sup>a</sup> | 347.6±8.2<sup>b</sup> | 353.33±4.1<sup>b</sup> | 345.83±2.2<sup>b</sup> |
| Diabetic + extract | 84.16±6.5<sup>a</sup> | 335.33±6.4<sup>b</sup> | 246±2.6<sup>c</sup> | 120.81±2.9<sup>c</sup> |
| Diabetic + glibenclamide | 77.66±4.6<sup>a</sup> | 325.66±5.0<sup>b</sup> | 241.5±3.6<sup>c</sup> | 123.33±3.2<sup>c</sup> |

Data were expressed as Mean ± SEM, n = 6. ANOVA followed by multiple comparisons two tail ‘t’ test. Values with different superscripts (a, b, c) in each column differ from others significantly (p < 0.05).

Figure 1. Activities of hexokinase after administration of hydro-methanolic extract of seed of *C. bonduc* in liver and skeletal muscle of different experimental group. Bars were expressed as Mean ± SEM (n = 6), ANOVA following by multiple comparison two tail ‘t’ test. Bars with different superscript (a, b, c) differ from others significantly at p < 0.05.
Figure 2. Hepatic and skeletal muscular glucose-6-phosphate dehydrogenase activities in control and different experimental group. Data were expressed as Mean ± SEM (n=6), ANOVA following by multiple comparison two tail ‘t’ test. Bars with different superscript (a, b, c) differ from each other significantly at p < 0.05.

Figure 3. Glucose-6-phosphatase activities in liver and skeletal muscle in control and different experimental group. Bars were expressed as Mean ± SEM (n = 6), ANOVA followed by multiple comparison two tail ‘t’ test. Bars with different superscript (a, b, c) differ from others significantly at p < 0.05.
Serum lipid profile

Serum TG and TC levels were increased significantly in STZ-induced diabetic group when compared to the control. After the treatment of glibenclamide and the said plant extract to diabetic rat, a significant recoveries were noted in respect to untreated diabetic group. In the levels of TG and TC 27.26 %, 34.6 % recoveries were noted in extract treated diabetic group respectively where glibenclamide treated diabetic group showed 25.09 %, 38.26 % recoveries in respect to untreated diabetic group. When comparison was made between the glibenclamide treated group and extract treated diabetic group, no significant difference was observed in the levels of serum TG and TC which focused the antihyperlipidemic efficacy of the plant extract (Table 2).

Serum LDLc and VLDLc levels were increased significantly in untreated diabetic group in respect to control. But after the supplementation of hydro-methanolic extract of seeds of C. bonduc or glibenclamide to diabetic animals, the levels of these biomarkers were corrected significantly in respect to untreated diabetic group. Percentage of recoveries in the levels of serum LDLc and VLDLc were 58.96 %, 25.20 % respectively by the treatment of extract and 66.6 %, 29.07 % recoveries were noted after the treatment of glibenclamide to diabetic rats in respect to untreated diabetic group. After compared of the data in this concern between glibenclamide and extract treated diabetic groups, no significant variation was observed which indicate the corrective effect of this extract on hyperlipidemia in experimental diabetic animal. (Table 2).

Serum level of HDLc was decreased significantly in untreated diabetic group when comparison made with the control group. But after treatment of the said plant extract or glibenclamide to diabetic animals, a significant recovery was noted in respect to untreated diabetic group. The recovery of serum HDLc level was 20.13 % by the extract and 37.2 % by glibenclamide in respect to untreated diabetic animals. From the comparative analysis of the data, there was no significant variation was observed in the level of this parameter between glibenclamide treated diabetic group and extract treated diabetic group (Table 2).

Activities of GOT and GPT

GOT and GPT activities in serum were found to be significantly increased in the diabetic group compared to the control group. Treatment with hydro-methanolic extract to diabetic animals resulted in a significant recovery in the levels of these parameters towards the control levels. No significant difference was noted in the activities of serum GOT and GPT between the glibenclamide treated group and hydro-methanolic extract treated group. The percentage of recoveries in the levels of serum GOT and GPT were 26.88 %, 20.29 % respectively by the treatment of extract and 27.72 %, 11.98 % recoveries were noted after the treatment of glibenclamide to diabetic rats in respect to untreated diabetic group. When comparison was made between the glibenclamide treated group and extract treated diabetic group, no significant difference was noted in the activities of serum GOT and GPT between glibenclamide treated diabetic group and extract treated diabetic group (Fig. 5).
Discussion
Streptozotocin is toxic to β cells and has been widely used to induce diabetes in animals\textsuperscript{28}. It is best model to study the effect of the antidiabetogenic agent\textsuperscript{29}. STZ-induced diabetes has been proved here by the significant elevation of FBG level which is consistent with our previous report\textsuperscript{16}. In the present study, administration of the hydro-methanolic extract of seed of \textit{Caesalpinia bonduc} effectively reduced the blood glucose level in STZ-induced diabetic rats. Since the hydro-methanolic extract of \textit{C. bonduc} reduced the FBG level, it may be assumed that the extract may directly results stimulations on the remaining β-cells of pancreas for insulin secretion or it helps the regeneration of pancreatic β-cells which was supported by other worker in the same line\textsuperscript{30}. Another view for the regenerative activities of pancreatic β-cell of this extract is in the recovery of activities of hexokinase, glucose-6-phosphate dehydrogenase in liver and skeletal muscle as well as the levels of glycogen in liver and skeletal muscle as these bio-markers are under positive control of insulin and the diminution in the activity of glucose-6-phosphatase in the said tissue which is regulated...
negatively by insulin\textsuperscript{21}. So, it may predict that the extract may recover the pancreatic insulin synthesis and secretion through \(\beta\)-cell regeneration. The same line of observation has been noted in our previous work using other plants in this purpose\textsuperscript{31}. Diabetes is associated with profound alterations in the serum lipids and lipoprotein profile and with increased risk of coronary heart disease\textsuperscript{32}. Diabetes induced hyperlipidemia may be due to low level of insulin or due to interference in insulin action which is compliance with our previous report\textsuperscript{33} and by other\textsuperscript{34}. Many herbs and plant products have been shown to have hypolipidemic as well as antihyperlipidemic properties\textsuperscript{35}. In the present study the supplementation of \textit{C. bonduc} to STZ-induced diabetic rats mimics insulin which was observed by the recovery of serum lipid profile level in addition to its antihyperglycemic activity.

In diabetes, GOT and GPT activities in serum are increased which may be due to cellular damage of metabolic organs\textsuperscript{36}. From the result of the present study it was observed that the extract has no metabolic toxicity induction which has been focused here from the activities of GOT and GPT in serum as these are the important markers in this concern\textsuperscript{37}. In conclusion, the hydro-methanolic extract of the seed of \textit{C. bonduc} has a promising antihyperglycemic as well as antihyperlipidemic effects on STZ-induced diabetes mellitus in rats which are comparable with the antidiabetic drug glibenclamide. More work is going on in our laboratory for the isolation, purification, and characterization of the bio-active phytomolecule(s) through bio assay guided fractionation.

References