Anti-Diabetic Activity of Fruits of *Terminalia chebula* on Streptozotocin Induced Diabetic Rats

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The present study was aimed to evaluate the anti-diabetic potential of *Terminalia chebula* (*T. chebula*) fruits on streptozotocin (STZ)-induced experimental diabetes in rats. Oral administration of ethanolic extract of the fruits (200 mg/kg body weight/rat/day) for 30 days significantly reduced the levels of blood glucose and glycosylated hemoglobin in diabetic rats. Determination of plasma insulin levels revealed the insulin stimulating action of the fruit extract. Also, the alterations observed in the activities of carbohydrate and glycogen metabolising enzymes were reverted back to near normal after 30 days of treatment with the extract. Electron microscopic studies showed significant morphological changes in the mitochondria and endoplasmic reticulum of pancreatic β cells of STZ-induced diabetic rats. Also, a decrease in the number of secretory granules of β-cells was observed in the STZ-induced diabetic rats and a these pathological abnormalities were normalized after treatment with *T. chebula* extract. The efficacy of the fruit extract was comparable with glibenclamide, a well known hypoglycemic drug.

Key words —— diabetes, *Terminalia chebula*, ethanolic extract, carbohydrate metabolism, electron microscope

INTRODUCTION

Diabetes mellitus (DM) is considered as one of the five leading causes of death in the world. About 150 million people are suffering from diabetes worldwide, which is almost five times more than the estimates ten years ago and this may double by the year 2030. India leads the way with its largest number of diabetic subjects in any given country. It has been estimated the number of diabetes in India is expected to increase 57.2 million by the year 2025.[1)] Diabetes is a complex multisystemic disorder characterized by a relative or absolute insufficiency of insulin secretion insulin dependent diabetes mellitus (IDDM) or concomitant resistance of the metabolic action of insulin on target tissues[2)] non insulin dependent diabetes mellitus (NIDDM).

Insulin therapy affords glycemic control in IDDM yet its short comings include ineffectiveness on oral administration, short shelf life, need for preservation in refrigeration, fatal hypoglycemia in the event of excess dosage, reluctance to take injection and above all, the resistance due to prolonged administration, limits its usage. Similarly treatment of NIDDM patients with sulfonylureas and biguanides is always associated with side effects.[3)] Hence, search for a drug with low cost, more potentials, and without adverse side effects is being pursued in several laboratories around the world.

Throughout the world many traditional plants have been found successful for antidiabetic activity. Further, most of the marketed medicines are distillations, combinations, reproductions or variations of substances that are found in nature. Our forefathers recommended some of the substances, which are abundantly found in nature long before their value was demonstrated and understood by scientific methods. However, few have received scientific or medical scrutiny and the World Health Organization (WHO) has recommended the traditional plant treatments for diabetes warrant further evaluation.[4)] Moreover, today it is necessary to provide scientific proof as to whether it is justified to use a plant or its active principles for treatment.[5)]

Terminalia chebula (*T. chebula*) Retz (Combretaceae), a native plant in India and Southeast Asia, is extensively cultivated in Taiwan. Its dried ripe fruit, has traditionally been used to treat various ailments in Asia.[6)] It is a popular folk medi-

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cine and has been studied for its homeostatic laxative, diuretic and cardiotonic activities.[7,8]

T. chebula has been reported to exhibit a variety of biological activities, including antidiabetic,[9] anticancer,[10] antimutagenic[11,12] and antiviral[13] activity. However, no systematic work on its anti-diabetogenic activity has been reported in the literature. Hence, the present study was aimed to evaluate the pharmacological effect of ethanolic extract of *T. chebula* on carbohydrate and glycogen metabolism in both normal and streptozotocin (STZ)-induced diabetic rats. The effects of *T. chebula* are compared to glibenclamide that is often used as a standard drug.

MATERIALS AND METHODS

Chemicals —— Streptozotocin was purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Radioimmunoassay kit for insulin assay was obtained from Linco Research Inc., U.S.A. All the other chemicals used were of analytical grade.

Plant Material —— Fresh mature *T. chebula* fruits were collected from a tree in Kolli Hills, Namakkal District, Tamil Nadu, India. The plant was identified and authenticated by Dr. K. Kaviyarasan, CAS in Botany, University of Madras, and a voucher specimen was deposited at the herbarium of Botany.

Preparation of *T. chebula* Fruit Extract —— Dried fruits were powdered in an electrical grinder and stored at 5°C until further use. 100 g of the powder was extracted with petroleum ether (60–80°C) to remove lipids. It was then filtered and the filtrate was discarded. The residue was extracted with 95% ethanol by Soxhlet extraction. The ethanol was evaporated in a rotary evaporator at 40–50°C under reduced pressure. The yield of the extract was 8.5 g/100 g.

Animals —— Adult male albino rats of Wistar strain weighing approximately 150 to 180 g were procured from Tamil Nadu Veterinary and Animal Sciences University, Chennai, India. They were acclamatized to animal house conditions, fed with standard rat feed supplied by Hindustan Lever Ltd., Bangalore, India. All the animal experiments were conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee guidelines (Approval No. 01/030/04).

Toxicity Studies —— To study any possible toxic effects and/or changes in behavioural pattern, rats were treated with graded dose of *T. chebula* extract (100–500 mg/kg body weight/rat/day) and kept under close observation for 8 hr daily for 30 days. All symptoms including changes in awareness, mood, motor activity, posture, motor-co-ordination, muscle tone and reflexes were recorded for 30 days.

Induction of Experimental Diabetes —— The animals were fasted overnight and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of Streptozotocin (55 mg/kg body weight) in 0.1 M cold citrate buffer (pH 4.5).[14] The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. Control rats were injected with citrate buffer alone. After a week time for the development of diabetes, the rats with moderate diabetes having glycosuria and hyperglycemia (blood glucose range above 250 mg/dl) were considered as diabetic and used for the drug treatment. The fruit extract in aqueous solution was administered orally through a gavage at a concentration of 200 mg/kg body weight/rat/day for 30 days.

Experimental Design —— The animals were divided into two sets, one for the evaluation of a glucose tolerance test and a second one for the analysis of biochemical parameters. Each set was further divided into four groups; each comprising a minimum of six animals in each group as detailed below:

Group I: Normal control rats.

Group II: Diabetic control rats.

Group III: Diabetic rats given *T. chebula* fruit extract (200 mg/kg body weight/day/rat) in aqueous solution orally for 30 days.

Group IV: Diabetic rats administered with glibenclamide (600 μg/kg body weight/day/rat) in aqueous solution orally for 30 days.[15]

The body weight gain and fasting blood glucose levels of all the rats were recorded at regular intervals during the experimental period.

Glucose Tolerance Test —— After 30 days of treatment, a fasting blood sample was collected from all the groups in heparinized tubes. Blood samples were also collected at the time intervals of 30, 60, 90 and 120 min after administration of glucose at a concentration of 2 g/kg of body weight.[16]

Biochemical Assays —— After 30 days of treatment, the fasted rats of various groups were sacrificed by cervical decapitation. Fasting blood glucose was estimated by the O-toluidine method of Sasaki *et al.*[17] The levels of hemoglobin and glycosylated hemoglobin were estimated according to methods of Drabkin *et al.*[18] and Nayak *et al.*[19] respectively. Plasma insulin was estimated by using a radioim-

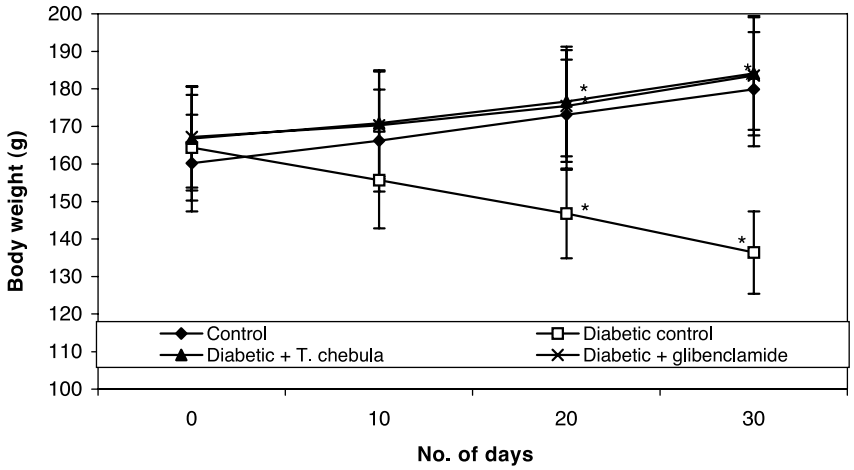

Fig. 1. Changes in Body Weight of Control and Experimental Groups of Rats

Values are given as mean + S.D. for groups of six rats each. Values are statistically significant at $*p < 0.05$, Diabetic control rats were compared with control rats. Diabetic + *T. chebula* and diabetic + glibenclamide treated rats were compared with diabetic control rats.

munoassay kit.

A portion of the liver tissue was dissected, washed with ice cold saline and homogenized in 0.1 M Tris–HCl buffer, pH 7.4. The supernatant was used for the assay of enzyme activity. The hexokinase activity was assayed by the method of Brandstrup *et al*.[20] The activities of glucose-6-phosphatase and fructose-1,6-bisphosphatase were assayed according to the method of Koide and Oda[21] and Gancedo and Gancedo,[22] respectively. The King[23] method was adopted for the assay of lactate dehydrogenase (LDH) activity. Glycogen synthase and phosphorylase activities were assayed by the method of Leloir and Goldenberg[24] and Cornblath *et al*.,[25] respectively. Another portion of wet liver tissue was used for the estimation of glycogen by the method of Morales *et al*.[26]

Electron Microscopy Studies —— For electron microscopic examination of pancreas, primer fixation was made in 3% glutaraldehyde in sodium phosphate buffer (200 mM, pH 7.4) for 3 hr at 4°C. Materials were washed with same buffer and postfixed in 1% osmium tetroxide and in sodium phosphate buffer (pH 7.4) for 1 hr at 4°C. Tissue samples were washed with same buffer for 3 hr at 4°C, and were dehydrated in graded ethanol series and were embedded in Araldite. 60–90 nm sections (60–90 nm) were cut on an LKBUM4 ultramicrotome using a diamond knife and sections were mounted on a copper grid and stained with uranyl acetate and Reynolds lead citrate.[27] The grids were examined under a Phillips electron microscope model 201C (EM201C) transmission electron microscope.

Statistical Analysis —— All the grouped data were statistically evaluated with statistical package for social sciences (SPSS)/10 software. Hypothesis testing methods included one-way analysis of variance (ANOVA) followed by least significant difference (LSD) test; P values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as the mean ± standard deviation (S.D.) for six animals in each group.

RESULTS

Acute toxicity studies conducted by us (data not shown) revealed that the administration of graded doses of *T. chebula* fruit extract (up to a dosage of 500 mg/kg body weight/day) for 30 days produced no effect on the general behaviour or appearance of the animals and all the rats survived the test period. There were no signs and symptoms such as restlessness, respiratory distress, diarrhea, convulsions, coma. Assay of pathophysiological enzymes such as alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT) in plasma revealed the nontoxic nature of fruit extract.

Figure 1 shows the change in body weight gain of control and experimental groups of rats. There was a significant decrease in the body weight of diabetic rats compared with control rats. Upon treatment with *T. chebula* and glibenclamide, the body weight gain was improved but the effect was more pronounced in *T. chebula* treated rats than glibenclamide.

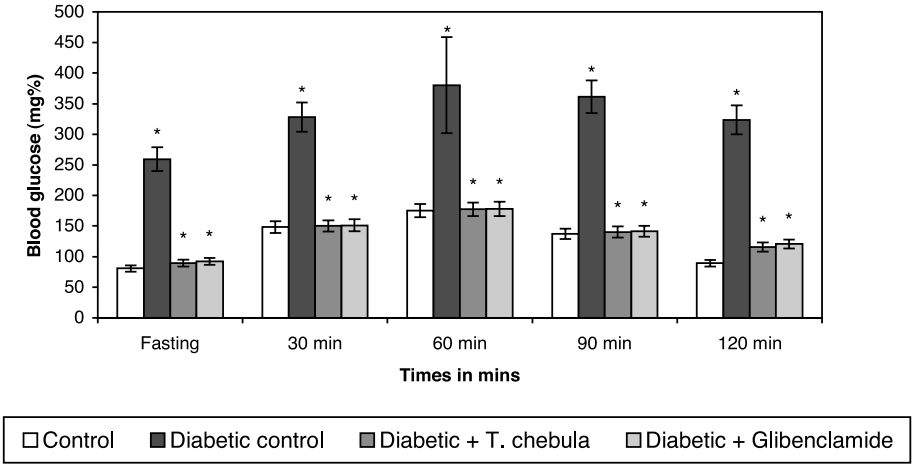

Fig. 2. Glucose Tolerance Test Curve of Control and Experimental Groups of Rats

Blood sample collected at 0, 30, 60, 90 and 120 min intervals after administration of glucose (2 kg/body weight) were assayed for glucose content. Values are given as mean ± S.D. for groups of six animals each; Values are statistically significant at $*p < 0.05$; Diabetic control rats were compared with control rats; diabetic + *T. chebula* and diabetic + glibenclamide treated rats were compared with diabetic control rats.

Table 1. Changes in the Level of Blood Glucose, Plasma Insulin, Hemoglobin, Glycosylated Hemoglobin and Urine Sugar in Control and Experimental Groups of Rats

Groups	Blood glucose milligram/deciliter (mg/dl)	Plasma insulin microunit/milliliter (μU/ml)	Hemoglobin (g/dl)	Glycosylated hemoglobin (% HbA_1c)	Urine sugar
Control	85.43 ± 5.72	16.54 ± 1.07	13.52 ± 0.81	6.24 ± 0.38	Nil
Diabetic control	265.08 ± 20.14*	5.27 ± 0.76*	9.25 ± 0.67*	12.36 ± 0.91*	+++
Diabetic + *T. chebula*	92.30 ± 6.09*	15.26 ± 0.71*	12.93 ± 0.82*	6.72 ± 0.42*	Nil
Diabetic + Glibenclamide	102.40 ± 6.45*	13.86 ± 0.62*	12.46 ± 0.77*	6.95 ± 0.42*	+

Values are given as mean ± S.D. for groups of six animals in each group. Values are statistically significant at $*p < 0.05$. Diabetic control rats were compared with control rats. Diabetic + *T. chebula* and diabetic + glibenclamide treated rats were compared with diabetic control rats. (+) indicate 0.25% sugar and (+++) indicates more than 2% sugar.

The levels of blood glucose in control and experimental groups of rats after oral administration of glucose is shown in Fig. 2. The blood glucose value in the control rats rose to a peak value 60 min after glucose load and decreased to near normal levels at 120 min. In diabetic control rats, the peak increase in blood glucose concentration was observed after 60 min and remained high over the next 60 min. *T. chebula* and glibenclamide treated diabetic rats showed significant decrease in blood glucose concentration at 60 and 120 min compared with diabetic group of rats.

Table 1 shows the level of blood glucose, plasma insulin, total hemoglobin, glycosylated hemoglobin and urine sugar in normal and experimental groups of rats. There was a significant elevation in blood glucose, urine sugar and glycosylated hemoglobin, while the level of plasma insulin and total hemoglobin decreased during diabetes when compared to control group. Administration of *T. chebula* brought back to near normal values as that of standard drug glibenclamide treatment.

Table 2 depicts a significant decrease in the activity of hepatic hexokinase, a significant increase in the activities of lactate dehydrogenase, glucose-6-phosphatase and fructose-1,6-bisphosphatase in STZ-induced diabetic rats when compared to control rats. Treatment with *T. chebula* extracts (group III) and glibenclamide (group IV) significantly controlled the alterations and restored the altered levels to near normalcy. *T. chebula* treatment exerted more effect than glibenclamide in diabetic rats.

Table 3 presents the changes in hepatic glycogen content and in the activities of glycogen synthase and glycogen phosphorylase in the hepatic tissue of control and experimental group of rats. A sig-

Table 2. Changes in the Activities of Hepatic Hexokinase, Lactate Dehydrogenase, Glucose-6-phosphatase and Fructose 1,6-Bisphosphatase of Control and Experimental Groups of Rats

Groups	Hexokinase (micromole Glucose-6-phosphate formed hr/mg protein)	Lactate dehydrogenase (micromole pyruvate formed/hr/mg	Glucose-6-phosphatase (micromole phosphate liberated/hr/mg protein) protein)	Fructose 1,6-bisphosphatase (micromole phosphate liberated/ hr/mg protein)
Control	273.6 ± 16.68	248.23 ± 15.88	1042 ± 84.40	482 ± 29.88
Diabetic control	139.3 ± 10.58*	356.45 ± 27.09*	1968 ± 184.99*	749 ± 55.42*
Diabetic + *T. chebula*	270.1 ± 17.28*	253.92 ± 16.00*	1061 ± 88.06*	502 ± 31.62*
Diabetic + Glibenclamide	257.4 ± 17.50*	268.37 ± 16.90*	1216 ± 103.36*	531 ± 33.98*

Values are given as mean ± S.D. for groups of six animals in each group. Values are statistically significant at $*p < 0.05$. Diabetic control rats were compared with control rats. Diabetic + *T. chebula* and diabetic + glibenclamide treated rats were compared with diabetic control rats.

Table 3. Level of Glycogen, Activities of Glycogen Synthase and Glycogen Phosphorylase in the Liver Tissue of Control and Experimental Groups of Rats

Groups	Glycogen (mg of glucose/g of wet tissue)	Glycogen synthase (micromole of uridine diphosphate formed/hr/mg protein)	Glycogen phosphorylase (micromole of phosphate liberate/hr/mg protein)
Control	58.23 ± 3.55	845.62 ± 69.34	612.18 ± 50.19
Diabetic control	26.80 ± 1.95*	567.43 ± 50.50*	870.64 ± 80.09*
Diabetic + *T. chebula*	56.28 ± 3.54*	812.12 ± 68.21*	653.23 ± 54.87*
Diabetic + Glibenclamide	50.95 ± 3.20*	786.56 ± 66.85*	713.51 ± 57.79*

Values are given as mean ± S.D. for groups of six animals in each group. Values are statistically significant at $*p < 0.05$. Diabetic control rats were compared with control rats. Diabetic + *T. chebula* and diabetic + glibenclamide treated rats were compared with diabetic control rats.

nificant decrease in liver glycogen content and glycogen synthase activity and concomitant increase in the activity of glycogen phosphorylase was observed in the diabetic group of rats and it was normalized after treatment.

A decrease in the number of secretory granules of β-cells was observed in diabetic group (Fig. 3) when compared to the control group (Fig. 4). Also severely decreased secretory granules, severe destruction of nuclear membrane and degenerative changes in the core of islet cells and nucleus were observed in STZ-induced diabetic rats. However, diabetic rats treated with *T. chebula* extract showed apparently normal cell architecture (Fig. 5). It similar observations have also been observed in diabetics rats treated with glibenclamide (Fig. 6).

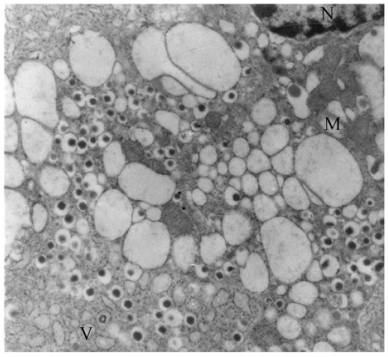

Fig. 3. Swelling of Mitochondria (M), Decreased Secretory Granules (G), Clear Vesicles (→) in Electron Micrograph from of the Diabetic Rat
Magnification: × 15000.

DISCUSSION

Streptozotocin is well known for its selective pancreatic islet β-cell cytotoxicity and has been extensively used to induce Type-1 diabetes in experimental rat model. It interferes with cellular metabolic oxidative mechanisms.[28] Increasing evidence in both experimental and clinical studies suggests that oxidative stress plays a major role in the development and progression of both types of diabetes mellitus. Free radicals are formed disproportionately in diabetes by glucose oxidation, non enzymatic glycation of proteins and subsequent oxidative degradation of glycation proteins. Diabetes is usually accompanied by impaired antioxidant defenses.

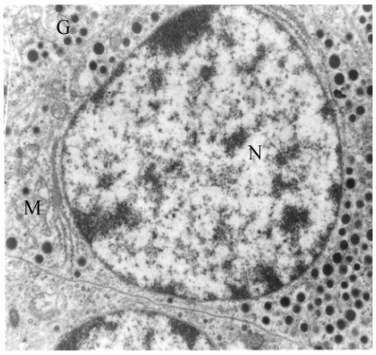

Fig. 4. Electron Micrograph of a Normal β-Cell in the Control Rat
Magnification: × 15000.

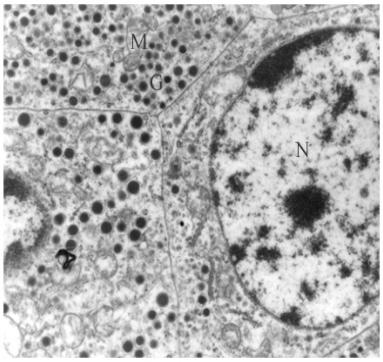

Fig. 5. Apparently Normal Mitochondria (M), Normal Nucleus (N) and Increased Secretory Granules (G) in the Diabetic Group Given *T. chebula*
Magnification: × 15000.

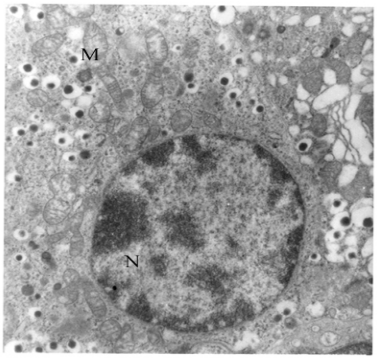

Fig. 6. Normal Mitochondria (M), Normal Nucleus (N) and Increased Secretory Granules (G) in the Diabetic Group Given Glibenclamide
Magnification: × 15000.

Glibenclamide is often used as a standard antidiabetic drug in STZ-induced moderate diabetes to compare the efficacy of variety of hypoglycemic compounds.[29] The present study was conducted to assess the hypoglycemic activity *T. chebula* fruits in STZ-induced diabetic rats. The ability of *T. chebula* fruit extract in significantly increasing the body weight and effectively controlling the increase in blood glucose levels in diabetic group of rats may be attributed to its antihyperglycemic effects. Further, the antihyperglycemic activity of *T. chebula* was associated with an increase in plasma insulin level, suggesting an insulinogenic activity of the fruit extract. The observed increase in the level of plasma insulin indicates that *T. chebula* fruit extract stimulates insulin secretion from the remnant β-cells or from regenerated β-cells. In this context, a number of other plants have also been reported to exert hypoglycemic activity through insulin release stimulatory effect.[30,31]

The observed increase in the levels of glycosylated hemoglobin (HbA_1c) in diabetic control group of rats is due to the presence of excessive amounts of blood glucose. During diabetes the excess of glucose present in blood react with hemoglobin to form glycosylated haemoglobin.[32,33] Mechanisms by which increased oxidative stress is involved in the diabetic complications are partially known, including activation of transcription factors, advanced glycated end products (AGEs), and protein kinase C. Glycosylated hemoglobin has been found to be increased over a long period of time in the diabetic mellitus.[34] There is an evidence that glycation may itself induce the generation of oxygen-derived free radicals in diabetic condition.[35] Treatment with *T. chebula* extract showed a decrease in the glycosylated hemoglobin with a concomitant increase in the level of total hemoglobin in the diabetic rats standard drug glibenclamide also showed the same results.

Liver plays an important role in the maintenance of blood glucose level by regulating its metabolism. Hexokinase, which brings about the first phosphorylation step of glucose metabolism, is reduced significantly in the diabetic group of rats.[36] This may be the reason for the diminished consumption of glucose in the system and increased blood sugar level. In STZ-induced diabetic rats, the hexokinase synthesis is decreased due to low levels of mRNA coding for the hexokinase and insulin administration stimulated transcription of hexokinase mRNA synthesis and thus enhanced the rate of synthesis and

activity of the enzyme.[37] The mechanism played by *T. chebula* extract in enhancing the hexokinase activity could be due to the activation of mRNA coding for hexokinase in diabetic rats.

Lactate dehydrogenase in anaerobic glycolysis, catalyses the conversion of pyruvate to lactate which subsequently is converted to glucose in gluconeogenic flux. In diabetic condition, an increased activity of lactate dehydrogenase was observed.[38,39] The LDH system reflects the Nicotinamide adenine dinucleotide/reduced nicotinamide adenine dinucleotide (NAD^+/NADH) ratio indicated by the lactate/pyruvate ratio of hepatocyte cytosol.[40] In *T. chebula* extract and glibenclamide treated group of rats, the reduction in the LDH activity is probably due to the regulation of NAD^+/NADH ratio by oxidation of NADH.

The hepatic gluconeogenic enzymes, glucose-6-phosphatase and fructose-1,6-bisphosphatase were increased significantly in diabetic rats. The increased activities of these two gluconeogenic enzymes in liver may be due to the activation or increased synthesis of the enzymes contributing to the increased glucose production during diabetes by the liver.[41] The therapeutic role of *T. chebula* and glibenclamide may be due to its primarily modulating and regulating δ activities of the two gluconeogenic enzymes, either through the regulation by 3′,5′-cyclic adenosine monophosphate (cyclic AMP) and any other metabolic activation or inhibition of glycolysis and gluconeogenesis.

The conversion of glucose to glycogen in the liver cells is dependent on the extracellular glucose concentration and on the availability of insulin which stimulates glycogen synthesis over a wide range of glucose concentration.[42] The regulation of glycogen metabolism *in vivo* occurs by the multifunctional enzyme glycogen synthase and glycogen phosphorylase that play a major role in the glycogen metabolism.[43] The reduced glycogen store in diabetic rats has been attributed to reduced activity of glycogen synthase[44] and increased activity of glycogen phosphorylase.[45] In the present study the experimental diabetic rats treated with *T. chebula* extract and glibenclamide treated groups restored the level of hepatic glycogen by means of decreasing the activity of glycogen phosphorylase and increasing the activity of glycogen synthase. This coincides with the previous work in our laboratory.[46]

In the diabetic group of rats treated with *T. chebula* extract, an increase in the number of β-cells in the islets shows that they were regenerated. Also, the increase in secretory granules in the cells indicates that the cells were stimulated for insulin synthesis. A decrease in the number of secretory granules, nuclear shrinkage and pycnosis, swelling of mitochondria and endoplasmic reticulum, round-shaped mitochondria, hypertrophied cytoplasmic organelles such as golgi and endoplasmic reticulum have been reported in the β cells of STZ-induced diabetic rats.[47,48] Our results are also inline with the previous report.

In conclusion, the present study shows that the ethanolic extract of *T. chebula* fruit has potential hypoglycemic action in STZ-induced diabetic rats and the effect was found to be more effective than glibenclamide Further, studies are in progress at molecular level to explicitly explain more about the mechanism of the antidiabetic activity of *T. chebula* and compounds responsible for its antidiabetic effect.

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