

## ORIGINAL ARTICLE

# Biochemical studies on the effect of *Terminalia chebula* on the levels of glycoproteins in streptozotocin-induced experimental diabetes in rats

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### Summary

This study was designed to evaluate the effect of *Terminalia chebula* fruit extract on the levels of plasma and tissue glycoprotein components in streptozotocin-induced-diabetic rats. Oral administration of *T. chebula* fruit extract at a concentration of 200 mg/kg body weight for 30 days significantly reduced the levels of blood glucose, glycosylated hemoglobin, urea, and creatinine as well as fucose, hexose, hexosamine and sialic acid in the diabetic rats treated with the fruit extract. The observed decrease in the levels of plasma insulin and C-peptide in the diabetic rats was elevated to near normal by *T. chebula* fruit extract treatment. Histological observations made on the pancreatic tissue of control and experimental groups also revealed the beneficial effect of *T. chebula* fruit extract. The efficacy of the fruit extract was comparable with glibenclamide, a known hypoglycaemic drug.

**Keywords:** glycoproteins – *Terminalia chebula* – diabetes mellitus – C-peptide – streptozotocin

### INTRODUCTION

Diabetes mellitus is a metabolic disorder of the endocrine system. The disease occurs worldwide and its incidence is increasing rapidly in most parts of the world. People suffering from diabetes are not able to produce or properly use insulin in the body, so they have a high level of blood glucose. Diabetes

is becoming the third ‘killer’ of mankind, after cancer and cardiovascular diseases, because of its high prevalence, morbidity and mortality (Li et al. 2004).

Glycoproteins are carbohydrate linked protein macromolecules found in the cell surface, which form the principal component of animal cells. Hexose, hexosamine, and sialic acid are the basic components of the glycoproteins. They play an important role in membrane transport, cell differentiation and recognition, the adhesion of macromolecules to the cell surface, and the secretion and absorption of macromolecules (Mittal et al. 1996). Impaired metabolism of glycoproteins plays a major role in the pathogenesis of diabetes mellitus (Knecht et al. 1990).

It has been reported that alterations occur in the concentrations of various glycoproteins in human

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diabetes (Sharma et al. 1987). Raised levels of glycoproteins in diabetics may also be an indicator of angiopathic complications (Konukoglu et al. 1999). Several workers have suggested that elevated levels of glycoproteins in plasma, liver and kidney tissues in the diabetic condition could be a consequence of impaired carbohydrate metabolism. Insulin deficiency and high levels of plasma glucose in the diabetic condition may result in an increased synthesis of glycoproteins (Patti et al. 1999). This increase in plasma glycoproteins has been associated with the severity and duration of diabetes.

Hyperglycaemia in experimental diabetic rats leads to a decreased utilization of glucose by insulin dependent pathways, thereby enhancing the formation of glycoproteins (Youngren et al. 1996). At the cell surface or inside the cells, the glyco-components such as fucose and sialic acid form specific structures, called glycanic chains covalently linked to lipids or proteins. An increase in the biosynthesis and or a decrease in the metabolism of glycoproteins could be related to the deposition of these materials in the basal membrane of pancreatic cells. In recent times, many traditionally important medicinal plants have been tested for their efficacy against impaired glycoprotein levels in diabetes (Ramkumar et al. 2007).

*Terminalia chebula* Retz., an important medicinal plant, is distributed in the sub-Himalayan tracks, and the eastern, western and southern parts of India. Its fruits are extensively used as an adjuvant in medicines for various diseases with special reference to Ayurvedic medicaments. The pericarp of the dried ripe fruit is used in the preparation of many Ayurvedic formulations for infectious diseases (Sharma and Dash 1998).

*T. chebula* is reported to promote digestive power, wound healing, and is curative of ulcers, local swelling, anemia, diabetes, and chronic and recurrent fever. The fruits are astringent, purgative, laxative, gastroprotective and are used to alleviate asthma, piles and coughing (Chatterjee and Pakrasi 2000). *T. chebula* has been reported to exhibit a variety of biological activities, such as anti-diabetic (Sabu et al. 2002), anti-cancer (Saleem et al. 2002), anti-mutagenic (Kaur et al. 2002) and anti-viral (Ahn et al. 2002) activity. Recently, we have reported the anti-diabetic activity of *T. chebula* fruits on streptozotocin (STZ)-induced experimental diabetes (Senthilkumar et al. 2006). Hence, the present study was aimed to evaluate the

pharmacological effect of ethanolic extract of *T. chebula* on plasma and tissue glycoproteins in both normal and STZ-induced diabetic rats. The effects of *T. chebula* were compared to glibenclamide, which is often used as a standard drug.

## MATERIALS AND METHODS

### *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. V. Kaviyaran, CAS in Botany, University of Madras, and a voucher specimen (107/05) was deposited at the herbarium of Botany, University of Madras.

### *Preparation of T. chebula fruit extract*

The fruits were shade dried and powdered in a pulverizer and stored at 4–5 °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 about 8.5 g/100 g.

### *Animals*

Adult male albino rats of the Wistar strain weighing approximately 150 to 180 g were procured from Tamil Nadu Veterinary and Animal Sciences University, Chennai, India. They were acclimatized to animal house conditions, and 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 the Ministry of Social Justice and Empowerment, Government of India and the guidelines of the Institutional Animal Ethics Committee (Approval No. 01/030/04).

### *Induction of experimental diabetes*

The animals were fasted overnight and diabetes was induced by a single intraperitoneal injection of freshly prepared solution of Streptozotocin (55 mg/kg body weight) in 0.1M cold citrate buffer pH 4.5 (Rakieten et al. 1963). The animals were allowed to drink 5% glucose solution overnight to overcome drug-induced hypoglycaemia. The control rats were

injected with citrate buffer alone. After a week's delay for the development of diabetes, the rats with moderate diabetes, i.e. with glycosuria and hyperglycaemia (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 four groups as

follows, with six animals in each group:

Group I: Control rats.

Group II: Diabetic control rats.

Group III: Diabetic rats administered orally with *T. chebula* fruit extract (200 mg/kg body weight/day/rat) in aqueous solution for 30 days (Senthilkumar et al. 2006).

Group IV: Diabetic rats administered with glibenclamide (0.6 mg/kg body weight/day/rat) in aqueous solution orally for 30 days (Ananthan et al. 2003).

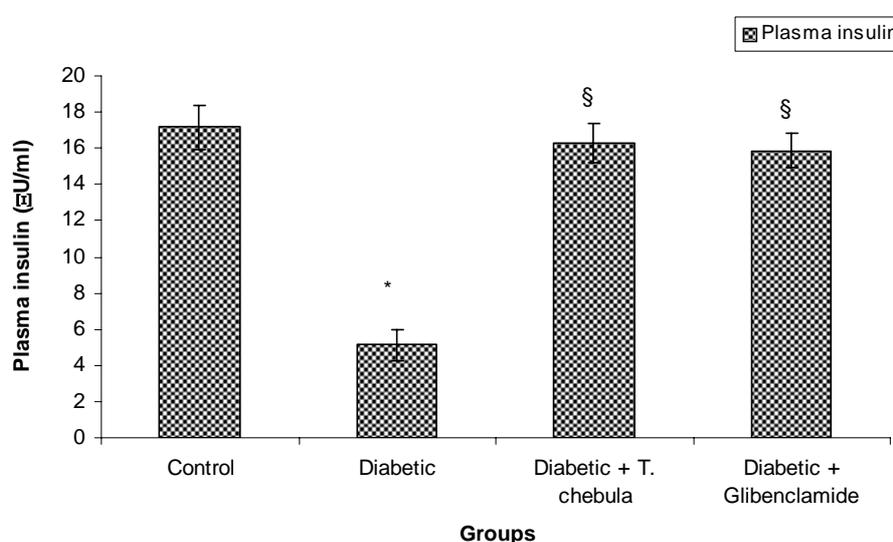


Fig. 1. Plasma insulin level of control and experimental groups of rats.

Values are given as mean  $\pm$  SD for groups of six animals each

\*Values are statistically significant as compared with controls

§Values are statistically significant as compared with diabetic animals

#### Biochemical assays

After 30 days of treatment, the rats were fasted overnight and sacrificed by cervical dislocation and the blood was collected using EDTA as an anticoagulant. The whole blood was used for the estimation of glucose (Sasaki et al. 1972), glycosylated haemoglobin (Nayak and Pattabiraman 1981) and urea (Natelson et al. 1961). Plasma insulin and C-peptide assays were performed using a radioimmunoassay (RIA) kit for rats supplied by Linco Research Laboratories, USA. The plasma protein levels were estimated according to the method of Lowry et al. (1951) and serum creatinine was estimated by the method of Brod and Sirota (1948).

#### Extraction of glycoproteins

The tissue samples were defatted before estimation. A weighed amount of defatted tissue was suspended in 3.0 ml 2M HCl and heated at 90 °C for 4h. The sample was cooled and neutralized with 3.0 ml 2M NaOH. Samples from this were used for the estimation of hexose, hexosamine, fucose and sialic acid. The plasma and tissue hexose content was estimated by the method of Niebes (1972) Sialic acid in plasma and tissues were estimated by the method of Warren (1959) and hexosamine by the method of Wagner (1979). Fucose was estimated by the method of Dische and Shettles (1948).

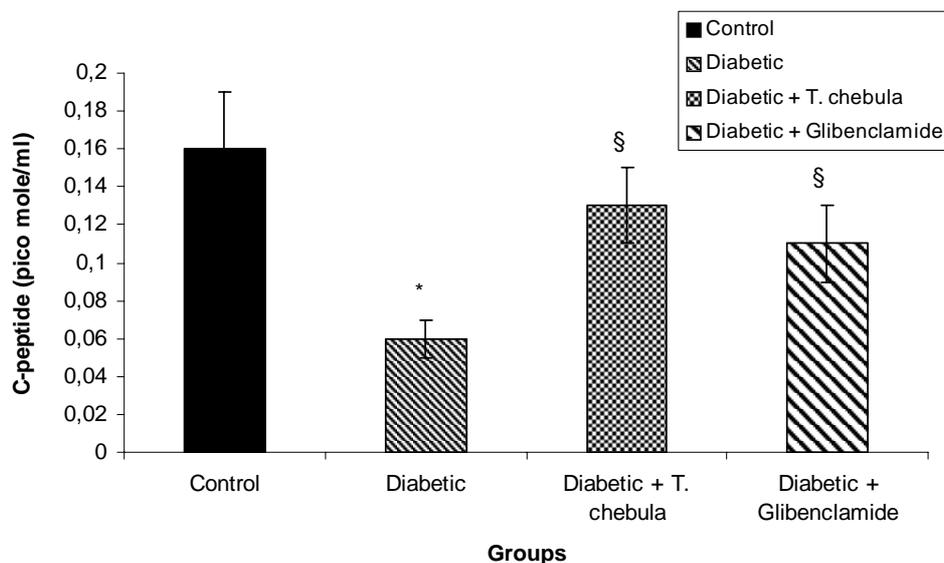


Fig.2. Changes in the plasma C-peptide levels in control and diabetic rats.

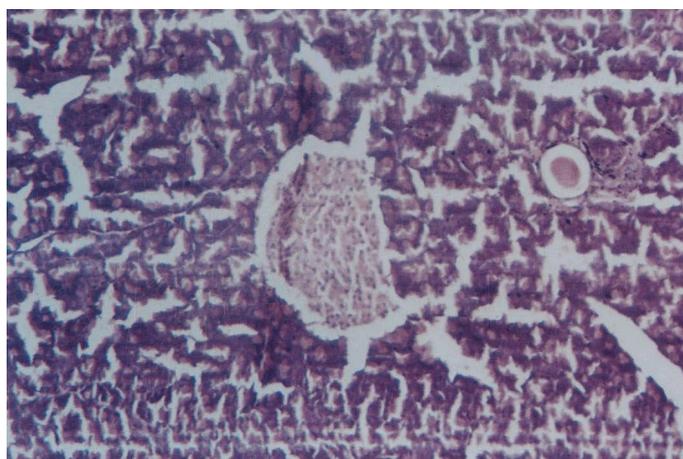


Fig. 3: Section of pancreatic tissue from a control rat showing normal architecture.

#### *Histopathological studies*

A portion of the pancreatic tissue was fixed in 10% buffered neutral formal saline for histological studies. After fixation, pancreas were embedded in paraffin, solid sections were cut at 5  $\mu$ m and stained with aldehyde. The sections were examined under a

light microscope and photomicrographs were obtained (Gordon and Bradbury 1990).

#### *Statistical analysis*

All the grouped data were statistically evaluated with SPSS/10 software. Hypothesis testing methods

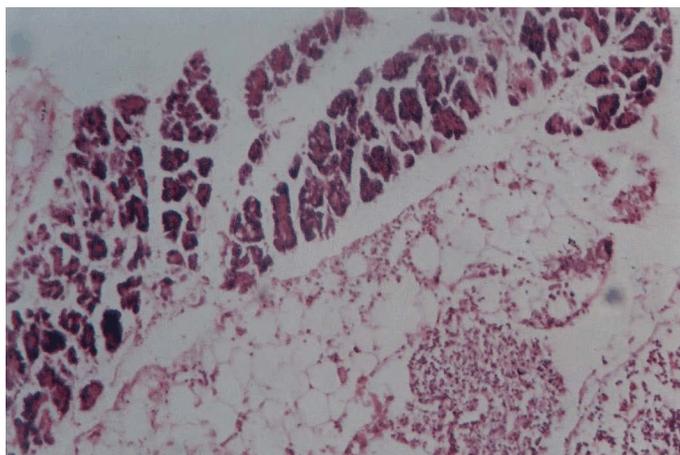


Fig. 4. Section of pancreatic tissue from a diabetic rat showing vascular degenerative changes in the islets and decrease in the number of  $\beta$ -cells.

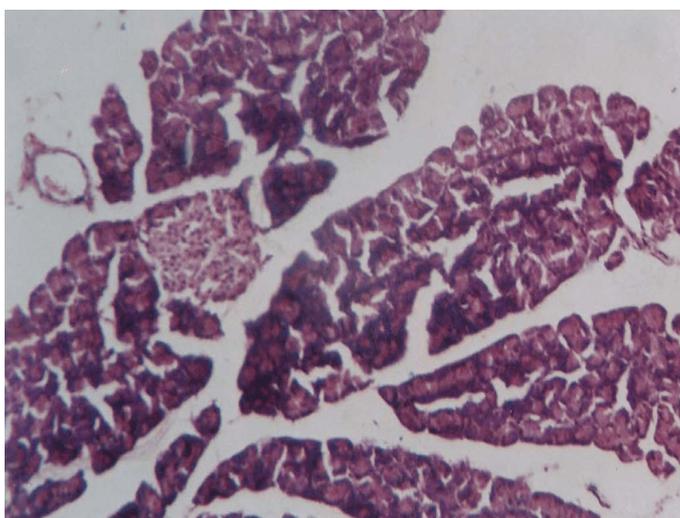


Fig. 5. Section of pancreatic tissue from a diabetic rat treated with *T. chebula* showing initial stages of regenerating islets.

included one-way analysis of variance (ANOVA) followed by the 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  $\pm$  Standard Deviation (SD) for six animals in each group.

## RESULTS

The diabetic rats exhibited a significant increase in blood glucose and glycosylated haemoglobin levels

when compared with control rats (Table 1). Upon oral administration of *T. chebula* extract as well as glibenclamide, the levels were found to be similar to those of control rats with the effect being more pronounced in the group of rats treated with *T. chebula* extract.

A marked decrease in the level of plasma insulin and plasma C-peptide was observed in the diabetic rats when compared with the control rats (Fig.1 and Fig.2). Treatment with *T. chebula* fruit extract as well as glibenclamide improved the levels to near normal.

Table 1 shows the levels of total proteins, blood

urea and plasma creatinine of the control and experimental groups of rats. These biochemical variables were significantly altered in diabetic rats when compared to control rats. Upon oral administration of *T. chebula* fruit extract as well as glibenclamide, these levels were found to be regulated to control rats and effect was more pronounced in the group of rats treated with *T. chebula* extract.

Tables 2 to 4 represent the levels of glycoproteins (hexose, hexosamine, fucose and sialic acid) in plasma and tissues (liver and kidney) of normal and diabetic rats. Significantly higher levels of glycoproteins were observed in the plasma and tissues of the diabetic control rats when compared with the normal control rats. Treatment with *T. chebula* of the diabetic rats resulted in a

significant reduction of glycoproteins in the plasma and tissues when compared with the diabetic control rats.

#### *Histological changes*

The histopathological examination revealed extensive alterations in the pancreas of STZ-induced diabetic rats (Fig.3 to Fig.6). The pancreas of the control rat showed normal islets (Fig.3). The pancreatic tissue of diabetic rats showed (Fig.4) atrophy of  $\beta$ -cells and vascular degenerative changes in the islets. Both the *T. chebula* (Fig.5) and glibenclamide (Fig.6) treated diabetic rats showed the initial stages of regenerating islets and an increase in the islets as compared with the diabetic control group.

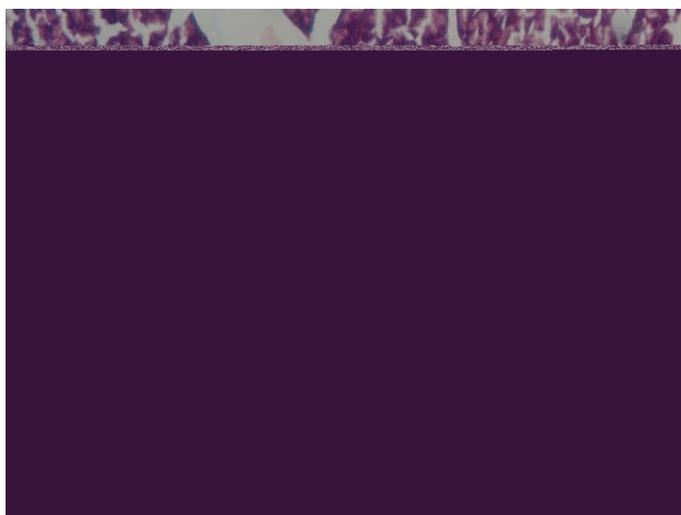


Fig. 6. Section of pancreatic tissue from a diabetic rat treated with glibenclamide showing apparently normal architecture.

## DISCUSSION

In the present study STZ-induced diabetes was chosen as the animal model because it resembles many of the features of human diabetes mellitus (Tomlinson et al. 1992). The results of the present study showed that the oral administration of *T. chebula* extract significantly decreased the levels of blood glucose and glycosylated haemoglobin in STZ-induced diabetic rats. Recently, we have reported that the oral administration of *T. chebula* fruit extract to STZ-induced diabetic rats optimized the activities of carbohydrate metabolizing enzymes and thus maintained blood glucose levels

(Senthilkumar et al. 2006). *T. chebula* may bring about its hypoglycaemic action through stimulation of the surviving or remnant  $\beta$ -cells of islets of Langerhans to release more insulin.

This was further evidenced by the observed increase in the levels of plasma insulin and C-peptide in the diabetic rats treated with *T. chebula* fruit extract. The presence in *T. chebula* of biologically active ingredients such as gallic acid, chebulic acid, 1,6-di-O-galloyl- $\beta$ -D-glucose, punicalagin, 3,4,6-tri-O-galloyl- $\beta$ -D-glucose, casuarinin, chebulanin, corilagin, neochebulinic acid, terchebulin, ellagic acid, chebulagic acid, chebulinic acid, and

1,2,3,4,6-penta-O-galloyl- $\beta$ -D-glucosein might be responsible for its medicinal properties (Juang et al. 2004).

In diabetes, the glycation and subsequent browning reaction are enhanced by increased glucose levels, and there is some evidence that glycation itself may induce the formation of oxygen-derived free radicals (Gupta et al. 1997). The levels of glycosylated haemoglobin are monitored as a reliable index of glycaemic control in diabetes (Cerami et al. 1978).

C-Peptide has insulin-mimetic effects on its own by activating insulin receptors, and increases glycogen synthesis and amino acid uptake. The C-peptide promotes insulin action at low concentrations and inhibits it at high levels, suggesting a modulatory effect by C-peptide on insulin signalling (Gurnberger and Sima 2004). In the present study oral administration of *T. chebula* fruit extract to diabetic rats increased the level of C-peptide and decreased the blood glucose level. This decrease in blood glucose level may be due to the increased formation of glycogen and amino acid uptake by C-peptide. The increased level of insulin and C-peptide in *T. chebula* extract treated diabetic rats may be due to the activation of remnant  $\beta$ -cells in the pancreas, which was in accordance with the observed histological observations.

There was increased protein catabolism with the flow of amino acids into the liver, which feeds gluconeogenesis during diabetes (Rannels et al. 1997). Dighe et al. (1984) have reported that accelerated proteolysis of uncontrolled diabetes

occurs as a result of the deranged glucagon mediated regulation of cAMP formation in insulin deficiency. This accounts for the observed decrease in the total protein content in the STZ-induced diabetic rats. Administration of *T. chebula* extract to diabetic rats significantly inhibits proteolysis caused by insulin deficiency and thus maintains the levels of total proteins.

Diabetic animals manifest negative nitrogen balance related to enhanced proteolysis in skeletal muscle and other tissues. Impaired nitrogen balance coupled with lowered protein synthesis leads to increased concentrations of urea and creatinine in the blood. This ultimately results in an impaired renal function in diabetic animals (Asayama et al. 1994). Administration of *T. chebula* extract to diabetic rats significantly decreased the level of blood urea and creatinine.

Increased glycosylation of various proteins in diabetic patients has been reported (Rahman et al. 1990). In this study, we have observed increased levels of hexose, hexosamine, fucose and sialic acid in the plasma and tissues of streptozotocin induced diabetic rats. The increase in plasma glycoprotein components has been associated with the severity and duration of diabetes. In hyperglycaemia, free amino groups of proteins react slowly with the carbonyl groups of reducing sugars such as glucose, to yield a Schiff's-base intermediate (Maillard reaction). These Schiff-base intermediates undergo Amadori rearrangement to a stable ketoamine derivative (fructosamine) (Bucala 1999).

Table 1. Level of blood glucose, glycosylated hemoglobin, total proteins, blood urea and plasma creatinine in control and experimental groups of rats

Parameters	Control	Diabetic control	Diabetic + <i>T.chebula</i>	Diabetic + Glibenclamide
Blood Glucose (mg/dl)	83.27 $\pm$ 6.73	268.18 $\pm$ 19.25*	90.53 $\pm$ 7.14*§	103.65 $\pm$ 8.37§
Glycosylated hemoglobin (%HbA <sup>1C</sup> )	6.47 $\pm$ 0.51	12.57 $\pm$ 1.07*	6.93 $\pm$ 0.63*§	7.10 $\pm$ 0.57§
Total proteins (g/dl)	6.85 $\pm$ 0.76	4.26 $\pm$ 0.69*	6.42 $\pm$ 0.59*§	6.12 $\pm$ 0.54*§
Blood urea (mg/dl)	23.12 $\pm$ 2.4	45.32 $\pm$ 3.12*	22.17 $\pm$ 1.17*§	21.67 $\pm$ 1.22*§
Plasma creatinine (mg/dl)	0.89 $\pm$ 0.05	2.36 $\pm$ 0.12*	0.94 $\pm$ 0.19*§	0.97 $\pm$ 0.13*§

values are presented as mean  $\pm$  SD six animals each

\* statistically significant as compared with controls

§ statistically significant versus diabetic rats

Table 2. Levels of glycoproteins (mg/dl) in plasma of control and experimental groups of rats

Groups	Hexoses	Hexosamine	Fucose	Sialic acid
Control	83.41 ± 5.77	64.27 ± 4.85	34.27 ± 2.53	57.43 ± 4.25
Diabetic control	124.13 ± 8.59*	85.34 ± 6.15*	45.32 ± 3.21*	77.35 ± 5.84*
Diabetic + <i>T. chebula</i>	86.65 ± 6.45*§	68.19 ± 5.21*§	36.47 ± 2.75*§	61.12 ± 4.81*§
Diabetic + Glibenclamide	88.53 ± 6.51*§	71.35 ± 5.93*§	37.51 ± 2.67*§	63.24 ± 4.67*§

Symbols as in Table 1

Table 3. Levels of glycoproteins (mg/g) in liver of control and experimental groups of rats

Groups	Hexoses	Hexosamine	Fucose	Sialic acid
Control	33.14 ± 2.85	17.48 ± 1.35	13.24 ± 1.35	7.59 ± 0.95
Diabetic control	56.28 ± 4.57*	29.11 ± 2.17*	21.53 ± 1.91*	14.67 ± 1.43*
Diabetic + <i>T. chebula</i>	35.17 ± 2.98*§	18.23 ± 1.83*§	14.36 ± 1.43*§	8.75 ± 0.87*§
Diabetic + Glibenclamide	36.34 ± 3.15*§	19.15 ± 1.85*§	15.17 ± 1.42*§	9.17 ± 1.1*§

Symbols as in Table 1

Fucose is a member of a group of eight essential sugars that the body requires for the optimal functioning of cell-to-cell communication and its metabolism appear to be altered in various diseases such as diabetes mellitus (Mondoa and Kitei 2001). A rise in fucose levels could be due to increased glycosylation in the diabetic state. Sialic acid is a terminal component of the non-reducing end of the carbohydrate chains of glycoproteins and glycolipids, which are essential constituents of many hormones and enzymes present in serum and tissues. Serum sialic acid is almost completely bound to glycoproteins and lipids. Total sialic acid in the serum has received considerable attention as a possible marker for cardiovascular disease and mortality (Moussa et al. 2004). In diabetes mellitus, the serum concentration of serum sialic acid was found to increase significantly (Gavella et al. 2003).

In our studies, a significant increase in total sialic acid levels in the serum was observed when

compared with the control group. Various factors might cause an elevation in the concentration of serum sialic acid. Among these factors, the first is an increase in the synthesis of sialic acid in insulin-independent tissues, such as the liver and the brain, and the second is an increase in the activity of sialyltransferase, which transfers the sialic acid residues to the glycolipids and glycoproteins. In our study, administration of *T. chebula* extract decreased the content of sialic acid in the serum of STZ-diabetic rats.

In the diabetic state, a deficiency in insulin secretion causes derangement of glycoprotein metabolism, which results in basal membrane thickening. Excess availability of glucose in the hyperglycaemic state accelerates the synthesis of glucose basement membrane components i.e. glycoproteins (Spiro and Spiro 1971). *T. chebula* administration to diabetic rats decreased the levels of glycoproteins in plasma and tissues. The

decreased hyperglycaemic state with increased levels of plasma insulin observed in *T. chebula* treated diabetic rats might have been responsible for

the decrease of glycoproteins in plasma, liver and kidney. Our results are also in line with the previous report (Ramkumar et al. 2007).

Table 4. Levels of glycoproteins (mg(g) in kidney of control and experimental groups of rats

Groups	Hexoses	Hexosamine	Fucose	Sialic acid
Control	25.17 ± 1.87	18.24 ± 1.48	11.87 ± 0.95	6.34 ± 0.83
Diabetic control	43.08 ± 3.15*	26.31 ± 2.18*	23.15 ± 2.27*	11.53 ± 1.10*
Diabetic + <i>T. chebula</i>	26.85 ± 2.10*§	20.53 ± 1.95*§	12.68 ± 1.32*§	7.27 ± 0.92*§
Diabetic + Glibenclamide	27.43 ± 2.27*§	21.17 ± 1.87*§	13.17 ± 1.18*§	7.38 ± 0.89*§

Symbols as in Table 1

In conclusion, treatment with *T. chebula* exhibits a beneficial effect on glycoproteins, as well as a protective effect against STZ-induced diabetes in rats and thus provides a rationale for the use of *T. chebula* in Ayurvedic medicinal treatment.

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