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Influence of *Terminalia belerica* fruits extracts on glycoprotein components in streptozotocin induced diabetic rats

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ABSTRACT

The present study investigated the effect of aqueous bark extracts of *Terminalia belerica* (*T. belerica*) on dearrangement in glycoprotein levels in the streptozotocin-induced diabetic rats. The extract of *T. belerica* (150 mg/kg b.w/day) was administered orally for 30 days to normal and diabetic rats. The effects of extract on glucose and plasma glycoproteins were studied. The effect of fruit extract was compared with gliclazide, a reference drug. The levels of glucose, glycosylated haemoglobin and plasma glycoproteins containing hexose, hexosamine, fucose and sialic acid were increased significantly in diabetic rats. Administration of T.belerica (150 mg/kg) to diabetic rats significantly restored the level of glucose, glyosylated haemoglobin and plasma glycoproteins near normal. The present study indicates that the fruit extract of *T. belerica* possesses a significantly beneficial effect on the glycoprotein moiety in addition to its anti-diabetic effect.

Key words: *Terminalia belerica*, Streptozotocin, glycoprotein components and Diabetes.

INTRODUCTION

Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism characterized by increased fasting and post prandial blood sugar levels. The global prevalence of diabetes is estimated to increase, from 4% in 1995 to 5.4% by the year 2025[1]. It is defined as a state in which homeostasis of carbohydrate and lipid metabolism is improperly regulated, resulting in elevated blood glucose levels. Accumulation of lipids in diabetes is mediated through a variety of derangements in metabolic and regulatory processes, especially insulin deficiency, thereby rendering the diabetic patient more prone to hypercholesterolemia and hypertriglyceridaemia[2,3].

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[4]. Impaired metabolism of glycoproteins plays a major role in the pathogenesis of diabetes mellitus[5].

Hyperglycaemia in experimental diabetic rats leads to a decreased utilization of glucose by insulin dependent pathways, thereby enhancing the formation of glycoproteins[6]. 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[7].

T. belerica Roxb. (Combretaceae) commonly known as Bahera is one of the ingredients of Ayurvedic purgative medicament "Triphala". Traditionally the fruits of this plant were reported greater therapeutic value in the treatments of liver disorders and indigestion[8]. The fruits of *T. belerica* were also reported to have pungative[9], and antimicrobial activity[10]. In the current literature there is not much data concerning the effect of *T. belerica* on the STZ induced diabetes and their activities, which are abnormally altered due to diabetes mellitus. Hence, the present study was aimed to evaluate the pharmacological effect of ethanolic extract of *T. belerica* on plasma glycoproteins in both Control and STZ-induced diabetic rats. The effects of *T. belerica* were compared to Glyclazide, which is often used as a standard drug.

MATERIAL AND METHODS

Plant Material

Fresh mature *T .belerica* fruits were collected from a tree in Kolli Hills, Namakkal District, Tamil Nadu, India. The plant was identified by Dr. P. Ponmurugan Department of Biotechnology, K.S.R College of Technology, Tiruchengode.

Preparation of *T. belerica* fruit extract

The fruits were shade dried and powdered in a pulverizer and stored at 4-5°C until further use. 100g of the powder was extracted with petroleum ether solvent (60-80°C) to remove lipids particles. It was then filtered and the filtrate was discarded. The residue was extracted with 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 6.2 g/100 g.

Animals

Male Wister rats of body weight 150-180g were obtained from Nandha College of Pharmacy, Erode, India. The animals were maintained in The Department of Pharmacology, Nandha College of Pharmacy, Erode, India and fed on standard pellet diet (AMRUT, PUNE, INDIA) and water adlibitum. The protocol of this study was approved by Institutional ethical committee of Nandha College of Pharmacy.

Chemicals

Streptozotozin was purchased from Himedia, Bangalore, India. All other chemicals used were of analytical grade.

Induction of diabetes

Experimental diabetes was induced by single intraperitoneal injection of streptozotocin (55 mg /kg b.w) dissolved in 0.1 M of cold citrate buffer (pH 4.5)(11); 15 min after the intraperitoneal administration of nicotinamide (110 mg /kg b.w)(12) in 12 hrs fasted rats. Because STZ is capable of inducing fatal hypoglycemia as a result of massive pancreatic insulin release, the rats were provided with 10% glucose solution after 6 hrs of STZ administration for the next 24 h to prevent hypoglycemia. Neither death nor any other adverse effect was observed. After a week in time for the development and aggravation of diabetes, rats with moderate diabetes (i.e. blood glucose range, >260 mg /dl) that exhibited glycosuria and hyperglycemia were selected for the experimental work.

Experimental design

After the successful induction of experimental diabetes, the rats were divided into four groups each comprising a minimum of six rats.

Group 1: Control rats.
Group 2: Diabetic control
Group 3: Diabetic rats administered with *T.belerica* (150 mg/kg/b.w)in aqueous solution orally for 30 days.
Group 4: Diabetic rats administered with glyclazide (5 mg/ kg b.w) in aqueous solution orally for 30 days(13) .

Body weight and blood glucose level measurements were conducted periodically.

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[14], glycosylated hemoglobin[15]. The plasma hexose content was estimated by the method of Niebes[16], Sialic acid in plasma were estimated by the method of Warren[17] and hexosamine by the method of Wagner[18]. Fucose was estimated by the method of Dische and Shettles[19].

Statistical analysis

All the grouped data were statistically evaluated with SPSS\10.0 software. Hypothesis testing methods included one way analysis of variance(ANOVA) followed by least significant difference(LSD) test; p value of less than 0.05 were considered to indicate statistical significance. All the results were expressed as the Mean±S.D.for six animals in each group

RESULTS

The diabetic rats exhibited a significant increase in blood glucose and glycosylated hemoglobin levels when compared with control rats (Table 1). Upon oral treatment of *T. belerica* extract as well as standard drug, the levels were found to be similar to those of control rats

Table 1: Level of blood glucose and Glycosylated Hemoglobin in control and experimental groups of rats

Groups	Blood glucose (mg/dl)	Glycosylated Hemoglobin(% Hb)
Control	87.41 ± 2.12	5.56 ± 0.49
Diabetic Control	273.33 ± 6.45[a*]	13.21 ± 0.95[a*]
Diabetic + T.belerica	96.10 ± 2.10[b*]	7.56 ± 0.66[b*]
Diabetic + Glyclazide	94.32 ± 1.82[c*]	6.48 ± 0.57[c*]

Values are given as mean ± S.D for groups of six animals each.
Values are statistically significant at *p< 0.05.
Statistical significance was compared with in the groups as follows:
[a]Diabetic rats were compared with control rats; [b] *T. belerica* treated diabetic rats were compared diabetic rats; [c] Glyclazide treated diabetic rats were compared with diabetic rats.

Table 2: Levels of glycoproteins in plasma of control and experimental groups of rats

Groups	Hexoses (mg/dl)	Hexosamine (mg/dl)	Fucose (mg/dl)	Sialic acid (mg/dl)
Control	85.21 ± 2.25	62.67 ± 2.63	35.22 ± 1.14	55.45 ± 2.28
Diabetic control	121.32 ± 3.69[a,*]	84.21 ± 2.58[a,*]	46.56 ± 1.56[a,*]	76.41 ± 2.15[a*]
Diabetic+ T.belerica	88.71 ± 2.99[b*]	64.35 ± 2.69 [b*]	37.52 ± 1.55 [b*]	60.19 ± 1.42 [b*]
Diabetic+ Glyclazide	86.10 ± 3.11[c*]	63..12 ±1.95 [c*]	36.85 ± 2.36 [c*]	59.69 ± 1.67 [c*]

Values are given as mean ± S.D for groups of six animals each.
Values are statistically significant at *p< 0.05.
Statistical significance was compared with in the groups as follows:
[a]Diabetic rats were compared with control rats; [b] *T. belerica* treated diabetic rats were compared diabetic rats; [c]Glyclazide treated diabetic rats were compared with diabetic rats.

Table 2 represent the levels of glycoproteins (hexose, hexosamine, fucose and sialic acid) in plasma of control and diabetic rats. Significantly higher levels of glycoproteins were observed in the plasma s of the diabetic control rats when compared with the control rats. Treatment with *T. belerica* of the diabetic rats resulted in a significant reduction of glycoproteins in the plasma when compared with the diabetic control rats.

DISCUSSION

Streptozotocin is a commonly used compound for the induction of diabetes[21] that causes rapid depletion of β -cells, which leads to a reduction in insulin release. The hypoglycaemic effect of plant extracts is generally dependent upon the degree of β -cell destruction[21]. Treatment of moderate STZ-diabetic rats with medicinal plant extracts resulted in activation of β-cells and granulation returned to normal, showing an insulinogenic effect[22]. The increased levels of insulin in the present study indicate that T.belerica stimulate insulin secretion from remnant β -cells or from regenerated β -cells. In this context, a number of other plants have also been reported to have an antihyperglycaemic effect and a stimulatory effect on insulin release[23,24].

During diabetes, the excess of glucose present in the blood reacts with hemoglobin to form glycosylated hemoglobin. Thus, the hemoglobin level is decreased in diabetic rats[25]. The rate of glycosylation is proportional to the concentration of blood sugar at the peak of the glucose tolerance curve, correlates with glycosylation[26] and, with an improvement in glyceamic control, glycosylated hemoglobin also decreases. Hence, estimation of the glycosylation of hemoglobin is a well accepted parameter used in the management and prognosis of the disease[27]. In the present study, the administration of *T.belerica* tended to bring the altered levels of glycosylated hemoglobin significantly towards normal values.

Increased glycosylation of various proteins in diabetic patients had been reported earlier (28) .In this study, we have observed increased levels of hexose, hexosamine, fucose and sialic acid in plasma of STZ-treated diabetic rats. The increases in plasma glycoprotein components have been associated with the severity and duration of diabetes. In hyperglycemia, free amino groups of proteins react slowly with the carbonyl groups of reducing sugars such as glucose, to yield a Schiff-base intermediate (Maillard reaction). These Schiff-base intermediates undergo Amadori rearrangement to stable ketoamine derivative (fructosamine)[29]. Rahman *et al.* (1990) have shown increased serum fructosamine concentrations in diabetic patients[28].

Fucose is a member of the group of eight essential sugars the body requires for optimal function of cell-to-cell communication and its metabolism appears to be altered in various diseases such as diabetes mellitus[29]. A raise in fucose levels could be due to increased glycosylation in the diabetic state[30]. Elevated levels of fucose in experimental diabetes were reported by other researchers[31]. Sialic acid is found in a wide variety of substances and tissues in animals and humans, occurring most abundantly in glycoproteins and glycolipids. Sialic acid bound to membrane glycoproteins and glycolipids apparently enters the circulation by either shedding or cell lysis[32]. Increased levels of sialic acid were reported in STZ-induced diabetic rats[33] and in diabetic patients[34].

In the diabetic state, deficiency of insulin secretion causes derangement of glycoprotein metabolism those results in the basal membrane thickening. Excess availability of glucose in the hyperglycemic state accelerates the synthesis of basement membrane glycoprotein components[35].

T. belerica administration to diabetic rats decreased the levels of glycoproteins in plasma. Decreased hyperglycemic state observed in *T. belerica* -treated diabetic rats might be responsible for the decrease of glycoproteins in plasma. In this context, other researchers have shown that decrease in hyperglycemia could lead to a decrease in glycoprotein levels[36].

In conclusion, administration of *Terminalia belerica* demonstrated a beneficial effect on carbohydrate moieties of glycoproteins, as well as a protective effect against Streptozotozin challenge, and thus provides a scientific rationale for the use of *Terminalia belerica* as an antidiabetic drug. The

isolation of bioactive compounds in the extract would certainly help to ascertain the medicinal value of the extract, which could be further exploited for use by the food and pharmaceutical industries.

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