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Effect of *Chloroxylon swietenia* Dc bark extracts on STZ induced diabetic rats with special attention to its glycoprotein levels

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ABSTRACT

The aim of the present study is to find out the effect of *Chloroxylon swietenia* Dc bark methanol (CSBMEt) and aqueous extracts (CSBAEt) on glycoprotein levels of STZ induced diabetic rats liver and kidney. The level of glycoproteins such as hexose, hexosamine, fucose and sialic acid were significantly increased in STZ induced diabetic rats. Whereas, treatment with CSBMEt, CSBAEt and glibenclamide (standard drug) for 45 days, restored these levels to near normal. The results of CSBMEt and glibenclamide were comparable. On basis of these results it can be concluded that, *Chloroxylon swietenia* Dc bark extracts own noteworthy protective effect on glycoprotein metabolism in addition to its antidiabetic effect.

Key words: *Chloroxylon swietenia*, Glycoprotein, hexose, fucose

INTRODUCTION

Diabetes mellitus (DM) is a widespread health issue; occurrence of DM increasing promptly [1]. It affects carbohydrates, fat and protein metabolism. It has emerged as the major cause of adult morbidity and mortality worldwide [2]. In diabetic state hyperglycemia leads to decreased utilization of glucose by insulin dependent pathways, thus increases the formation of glycoproteins [3].

Glycoproteins are carbohydrate associated protein component appear in the cell surface, it form the principal component of animal cells Hexose, hexosamine, sialic acid and fucose are the fundamental components of the glycoproteins, they connect together covalently linked to polypeptide chain. It plays a central position in membrane transport, cell differentiation and recognition [4]. They include various and multifaceted role and are found as enzymes, hormones, blood group material and as component extracellular membranes [5].

Streptozotocin (STZ) is commonly used for experimental induction of type-I diabetes mellitus, which causes selective pancreatic islet β -cell cytotoxicity mediated through the release of nitric oxide (NO). This results in rapid reduction in pancreatic islet pyridine nucleotide concentration and subsequent β -cell necrosis. The action of STZ on mitochondria generates SOD anions, which leads to diabetic complications [6-8]. STZ partly destroys the beta cells bringing about inadequate insulin discharge creating type 2 diabetes [9,10].

Herbal drugs enjoy the advantages of comparatively less toxic than synthetic drugs [11]. Currently available antidiabetic synthetic drugs are associated with various toxicities, owing to which the developmental process in

antidiabetic drug discovery has shifted its focus on natural plant sources having minimal side effects and low cost [12]. The support of majority disease treatment is natural origin, and greater part of components as well as drugs of modern pharmaceuticals relies upon natural source [13]. *Chloroxylon swietenia* DC. (Rutaceae) is an important medicinal plant with several medicinal uses in both folklore and traditional systems of medicine. Various parts of the plant were utilized in treating wounds, cuts, burns and skin diseases. The plant is supposed to be ready to lend a hand in treating rheumatism, impotence, common cold as well as cough. Various extracts of the plant have been reported to possess hepatoprotective, antioxidant, anti-inflammatory, analgesic, antimicrobial and anthelmintic activities etc. by different authors. Less number of compounds from various parts has been reported previously [14]. Though it has been utilized in traditional system of medicine for antidiabetic activity it is short of scientific evidence. Based on these perspectives the present study was designed to find out the effect of *Chloroxylon swietenia* bark extracts (CSBMEt and CSBAEt) on glycoprotein levels in STZ (50 mg/kg b.w) induced diabetic rats liver and kidney.

MATERIALS AND METHODS

Chemicals

STZ and glibenclamide was obtained from Sigma- Aldrich Company (Bangalore). The other experimental chemicals used were analytical grade purchased from HiMedia (Mumbai, India).

Plant material collection and preparation of extracts

Chloroxylon swietenia bark (CSB) was gathered in the middle of December from Kalvarayan Hills Kallakurichi, Tamil Nadu, India. Dr. V. Chelladhurai (Research Officer—Botany Central Council for Research in Ayurvedha and Siddha, Govt. of India) have given the taxonomic distinguishing proof. It was shade dried and grinded in mechanical blender and packed in an airtight pack. 300 g of CSB was extracted with 1 L of methanol by soxhlet apparatus for 72 h, mixture was dried at 45°C in rotary evaporator (Heidolph, Germany). 300 g of powdered bark was soaked for 3 days with 1 L distilled water in room temperature, by periodic shaking and separated aside using cotton attachment took after by Whatman filter paper (no.1) and dehydrated at ambient temperature. The dried extracts were stored at 4 °C until further use.

Animals

Wistar albino rats of either sex (of age 6 months and weighing 350 g) was bred in the Institutional animal house. The animals was housed in standard polypropylene cages and maintained under controlled room temperature ($22 \pm 2^\circ\text{C}$) and humidity ($55 \pm 5\%$) with 12:12 hour light and dark cycle. All the animals were provided with commercially available rat normal pellet diet and water ad libitum. The guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) of the Government of India were followed and prior permission has obtained from the Institutional Animal Ethics Committee (IAEC) (Proposal No. 998, Reg.No.160/1999/CPCSEA).

Experimental induction of diabetes

Diabetes was induced by single intraperitoneal injection of freshly prepared streptozotocin (STZ) (50mg/ kg b.w) in 0.1 M citrate buffer (pH = 4.5) to overnight-fasted rats [15]. Diabetic rats were allowed to drink 20% glucose solution overnight to overcome the initial drug-induced hypoglycemic mortality. The fasting blood glucose level was determined after 7 days, those fasting glucose levels >250 mg/dL were considered as diabetic and taken for the study. Control rats were injected with 0.2ml of vehicle (0.1M citrate buffer, pH 4.5) alone.

Experimental Design for the treatment of STZ induced diabetic rats

In this experiment 30 rats (6 normal and 24 STZ diabetic existing rats) were employed. They were isolated into five groups of 6 rats each. The CSBMEt were dissolved in 2% CMC (Carboxyl methyl cellulose) in distilled water [16] CSBAEt and glibenclamide 0.5ml of 0.9% saline and administered orally (45 days).

Group I. Control rats (received 0.5ml of 0.9% saline orally for 45 days).

Group II. Diabetic group (STZ 50mg/kg b.w. i.p. single dose).

Group III. Diabetic rats received CSBAEt (250mg/kg b.w dissolved in 0.5ml of 0.9% saline) orally for 45 days [17].

Group IV. Diabetic rats received CSBMEt (250mg/kg b.w dissolved in 0.5ml of CMC) for 45 days [17].

Group V. Diabetic rats received Glibenclamide (600µg/kg b.w dissolved 0.5ml of 0.9% of saline) for 45 days [18].

Biochemical analysis

At the end of the study (45 days) of treatment the animals were sacrificed under chloroform anesthesia. Liver and kidney was dissected, a portion of tissues washed with saline. Delipidised residues of tissues were prepared according to the method of Folch *et al* [19]. A known amount of delipidised residues of the tissues were hydrolysed with 2.0 mL of 4 N HCl at 100 °C for 4 h. The hydrolyzed material was neutralized and used for the estimation of hexose Niebes (1972) [20], hexosamine content was estimated by the method of Wagner (1974) [21], sialic acid level was determined by the method of Warren (1959) [22], and fucose level was estimated by the method of Dische and Shettles (1948) [23].

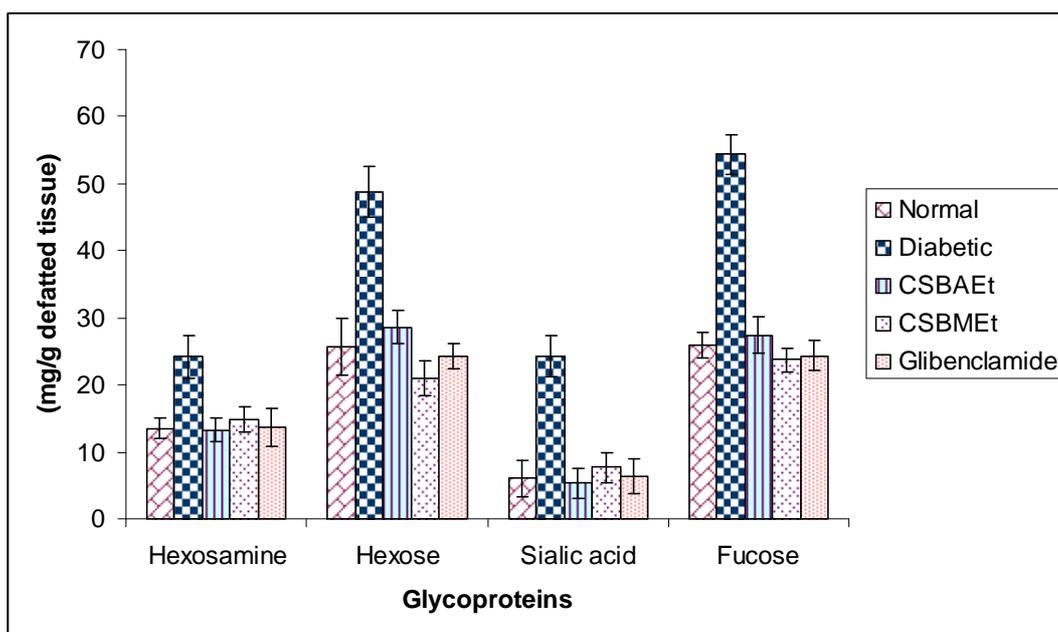
Statistical Analysis

All values were expressed as mean \pm SD. The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS and the Duncan's Multiple Range Test (DMRT). A value of $p < 0.05$ was considered to indicate a significant difference between groups.

RESULTS

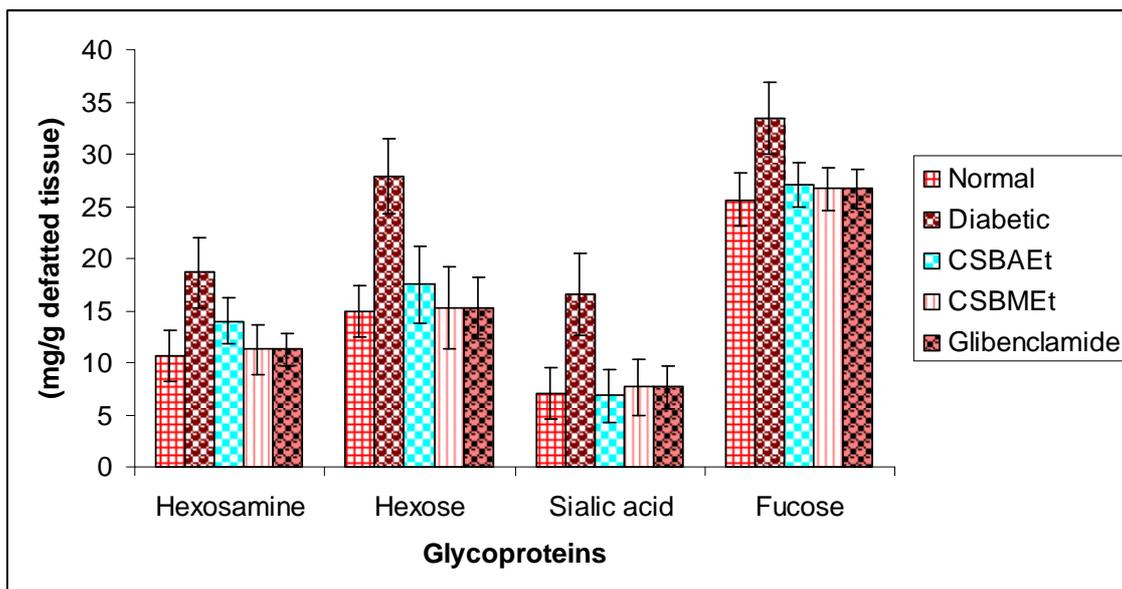
Fig 1 represents the effect of *Chloroxylon swietenia* Dc bark extracts on glycoprotein level of normal and experimental rats liver. The results reveal that hexosamine, hexose, sialic acid and fucose were found to be increased in STZ induced diabetic rats when compared to normal rats. Whereas, treatment with CSBAEt, CSBMEt and glibenclamide brings these values to normal.

Fig 1: Effect of *Chloroxylon swietenia* Dc bark extracts on glycoprotein level of normal and experimental rats liver



Values are expressed as Mean \pm SD for six rats ($n = 6$ rats in each group). Hexosamine, Hexose, Sialic acid and Fucose - mg/g defatted tissue. Statistical significance was evaluated by one-way analysis of variance (ANOVA) and the Duncan's Multiple Range Test (DMRT). $p < 0.05$ Experimental groups compared with diabetic control. $p < 0.05$ Diabetic rats compared with normal rats. CSBAEt - *Chloroxylon swietenia* bark aqueous extract (250mg/kg). CSBMEt - *Chloroxylon swietenia* bark methanol extract (250mg/kg).

Effect of *Chloroxylon swietenia* Dc bark extracts on glycoprotein level of normal and experimental rats kidney was recorded in Fig 2. The results of the current study make clear that STZ induced diabetic rats increased hexosamine, hexose, sialic acid and fucose. All these values tend to decline upon treatment with CSBAEt, CSBMEt and glibenclamide for 45 days. Result of the CSBMEt and glibenclamide were significant ($p < 0.05$).

Fig 2: Effect of *Chloroxylon swietenia* Dc bark extracts on glycoprotein level of normal and experimental rats kidney

Values are expressed as Mean \pm SD for six rats ($n = 6$ rats in each group). Hexosamine, Hexose, Sialic acid and Fucose - mg/g defatted tissue. Statistical significance was evaluated by one-way analysis of variance (ANOVA) and the Duncan's Multiple Range Test (DMRT). $p < 0.05$ Experimental groups compared with diabetic control. $p < 0.05$ Diabetic rats compared with normal rats. CSBAEt - *Chloroxylon swietenia* bark aqueous extract (250mg/kg). CSBMEt - *Chloroxylon swietenia* bark methanol extract (250mg/kg).

DISCUSSION

In general, increased production and decreased utilization of glucose of by the cell is the main cause of hyperglycaemia, it is the proven feature uncontrolled diabetes, which leads to protein glycation [24]. Augmented production of glucose tends to increased functioning of kidney. This is mainly due to lack or inadequate production of insulin from β cell. These conditions enhance the formation of glycoproteins in various tissues. Glycoproteins are extracellular components of cell membrane; it appears in various tissues [25], Hexose, hexosamine and sialic acid are the essential components of the glycoproteins [26]. Impaired metabolism of glycoprotein leads to the pathogenesis of diabetes mellitus [27].

In experimental diabetes, STZ put forth its toxic outcome on pancreatic β -cells and other organs such as liver, kidney [28]. In this study STZ is used as a diabetogenic agent. In STZ induced diabetic rats, glycoprotein components such as hexose, hexosamine, fucose and sialic acid observed to be high in liver and kidney tissues. Similar findings were reported previously [29,30]. Hyperglycemic state speeds up the synthesis glycoproteins [31]. The elevated level of hexose in diabetic rats may be associated with disturbances with carbohydrate metabolism. Declined insulin emission might be the reason behind increased production of hexosamine. Hoist in fucose levels could be due to increased glycosylation, activity of sialyltransferase converts sialic acid residues to the glycolipids and glycoproteins [32,33].

However, oral administration of CSBAEt, CSBMEt and glibenclamide for 45 days brought these values to near normal. The results of CSBMEt and glibenclamide are significantly comparable. This might be owing to the decreased hyperglycemic state with increased levels of plasma insulin in diabetic rats (unpublished data). Our results are in agreement with the previous researchers, administration of iridoid glucoside to diabetic rats decreased plasma the levels of glycoprotein components [11]. This could be due to the decreased hyperglycemic state with increased levels of plasma insulin in diabetic rats [34].

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