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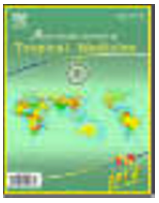

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Protective effect of *Cardiospermum halicacabum* leaf extract on glycoprotein components on STZ-induced hyperglycemic rats

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ABSTRACT

Objective: To investigate the protective role of *Cardiospermum halicacabum (C. halicacabum)* leaf extract on glycoprotein metabolism in streptozotocin (STZ)-induced diabetic rats. **Methods:** Diabetes was induced in male albino Wistar rats by intraperitonial administration of STZ. The *C. halicacabum* leaf extract (CHE) was administered orally to normal and STZ-diabetic rats for 45 days. The effects of *C. halicacabum* leaf extract (CHE) on plasma and tissue glycoproteins (hexose, hexosamine, fucose and sialic acid) were determined. **Results:** The levels of plasma and tissues glycoproteins containing hexose, hexosamine and fucose were significantly increased in STZ-induced diabetic rats. In addition, the level of sialic acid significantly increased in plasma and liver while decreased in kidney of STZ-induced diabetic rats. After administration of CHE to diabetic rats, the metabolic alteration of glycoprotein reverted towards normal levels. **Conclusions:** The present study indicates that the CHE possesses a protective effect on abnormal glycoprotein metabolism in addition to its antihyperglycemic activity.

1. Introduction

Diabetes mellitus (DM), a chronic disease affecting millions of individuals worldwide, characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism. Based on the World Health Organization (WHO) report, the number of diabetic patients is expected to increase from 171 million in year 2000 to 366 million or more by the year 2030 [1]. Hyperglycemia, due to uncontrolled glucose regulation is considered as the causal link between diabetes and diabetic complications. A number of studies emphasizes that alterations in glucose metabolism leads to hyperglycemia-induced cell damage by four key metabolic pathways, viz., increased polyol pathway flux, increased glycation of proteins (enzymatic or nonenzymatic), increased hexosamine pathway flux and activation of protein kinase C (PKC) isoforms [2]. Among the above stated possibilities, glycosylation of proteins has been the prime subject of much interest. Glycoproteins, a carbohydrate linked protein macromolecules found in the cell surface, serves as the principal component of animal cells. Alterations in glycoprotein level leads to the pathogenesis of diabetes mellitus [3]. Many studies confirm the involvement of glycoprotein in diabetic complications [4]. With increasing severity of diabetes, there is a parallel rise in glycoprotein levels [5]. During diabetes, utilization of glucose by insulin independent pathways leads to the synthesis of glycoprotein which may be a predictor of angiopathic complications [6].

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An increase in the biosynthesis and or a decrease in the metabolism of glycoproteins attributed 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 [7,8].

Plants used in traditional medicine to treat diabetes mellitus represent a valuable alternative for the control of this disease. *C. halicacabum* L. (CH) has been used in Chinese medicine for a long time in the treatment of rheumatism, lumbago, nervous diseases, as a demulcent in orchitis and in dropsy [9-11]. Various pharmacological actions of CH have been investigated in animal models [12]. The anti-inflammatory activity of ethanolic extract against inhibits LPS induced COX-2, TNF-alpha and iNOS expression in RAW264.7 cells [13]. Experimental pharmacological studies have shown the analgesic and vasodepressant activities [14], antipyretic activity against yeast-induced pyrexia in rats [15], antimalarial [16], antioxidant activity [17], suppressing the production of TNF- alpha and nitric oxide in human peripheral blood mononuclear cells [18, 19] and anti-ulcer activity against ethanol induced gastric ulcer in rats [20]. Our earlier study, reported that methanolic extract of *C. halicacabum* possesses antihyperglycemic and antioxidants potential against STZ-induced diabetic rats [21, 22]. Hence, the current work to investigate the protective role of *C. halicacabum* leaf extract (CHE) on glycoproteins metabolism in STZ-induced diabetic rats.

2. Materials and methods

2.1. Animals

Male albino Wistar rats (weighing 180-200 g, 9 weeks old) were procured from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, and maintained in an airconditioned room [(25±1) ℃] with a 12 h light/12 h dark cycle. Feed and water were provided *ad libitum*. The study was conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH, 1985) and the experimental study was approved by the Ethical Committee of Rajah Muthiah Medical College and Hospital (Reg No.160/1999/CPCSEA), Annamalai University, Annamalainagar.

2.2. Chemicals

Streptozotocin was obtained from Sigma-Aldrich Company (St. Louis, Missouri, USA). All other chemicals used were of analytical grade obtained from E. Merck, Mumbai and HIMEDIA, Mumbai, India.

2.3. Experimental induction of diabetes

The animals were made diabetic by an intraperitoneal injection of streptozotocin (STZ, 40 mg/kg body weight, between 8:00 AM to 9:00 AM) in a freshly prepared citrate buffer (0.1M, pH 4.5) after an overnight fast. STZ injected animals were given 20% glucose solution for 24 h to prevent initial drug-induced hypoglycaemic mortality. The animals exhibited massive glycosuria (determined by Benedict's qualitative test, Benedict 1911) and hyperglycaemia within a few days. Diabetes was confirmed by measuring the fasting blood glucose concentration 96 h after induction[23]. Albino rats with a blood glucose level above 220 mg/dL were considered diabetic and were used in the experiment.

2.4. Plant material

Leaves of *C. halicacabum* were collected from the local areas, Jeyankondam, Ariyalur district, Tamil Nadu, India. The plant was botanically identified and authenticated in the Department of Botany, Annamalai University, Annamalainagar, Chidambaram, Tamil Nadu, India and a voucher specimen was deposited at the herbarium of botany.

2.5. Preparation of plant extract

The plant leaf was shade dried at room temperature [(32±2) ℃] and the dried leaf was ground into fine powder using a pulverizer. The powered part was sieved and kept in deep freezer until use. 100 g of dry fine powder was suspended in 300 mL of ethanol for 72 h. The extract was filtered using a muslin cloth and concentrated at [(40±5) ℃].

2.6. Experimental design

The animals were randomly divided into five groups of six animals each. In our earlier study, the extract was suspended in 2% gum acacia vehicle solution and fed by intubation at three different doses such as 50, 100 and 200 mg/kg body w.t. The dose of 200 mg exhibited maximum reduction of blood glucose when compared to the other two doses in STZ-induced diabetic rats [22]. The active dose of 200 mg was used in this study.

Group I: Normal (2% gum acacia), Group II: Normal + CHE (200 mg/kg body wt.) in 2% gum acacia, Group III: Diabetic control rats, Group IV: Diabetic + CHE (200 mg/kg body wt.) in 2% gum acacia, Group V: Diabetic + glibenclamide (600 μg/kg body wt.) in 2% gum acacia.

After 45 days, the animals were anaesthetized using ketamine (24 mg/kg/body weight, intramuscular injection), and sacrificed by cervical dislocation. Between 8:00 am and 9:00 am blood was collected in tubes with a mixture of potassium oxalate and sodium fluoride (1:3) to get plasma for various assays. Tissues (liver and kidney) were collected and stored at 4 ℃ for the measurement of various parameters.

2.7. Biochemical estimations

Blood glucose was estimated by using the reagent kit method of Trinder [23]. Total hexoses, hexosamine, fucose and sialic acid were estimated by the methods of Niebes [24], Elson and Morgon [25], Dische and Shettles [26] and Welmer *et al* [27], respectively.

2.8. Statistical analysis

Values are given as means±S.D. for six rats in each group. Data were analyzed by one-way analysis of variance followed by Duncan's Multiple Range Test (DMRT) using SPSS version 10 (SPSS, Chicago, IL). The limit of statistical significance was set at $P \leqslant 0.05$.

3. Results

Table 1 shows the effect of the 45 day oral administration of CHE on blood glucose levels in normal and STZ-diabetic rats. Diabetic rats showed an elevated blood glucose level and administration of CHE and glibenclamide in diabetic rats showed a decreased blood glucose level.

Table 2 represent the levels of total hexoses and hexosamines in the plasma and tissues (liver and kidney) of normal and diabetic rats. The diabetic rats had increased levels of total hexoses and hexosamines in the plasma and tissues and treatment with CHE and glibenclamide showed reversal of these parameters towards normal levels.

Tables 3 represent the levels of fucose and sialic acid in the plasma and tissues (liver and kidney) of normal and diabetic rats. The diabetic rats had increased levels of fucose and sialic acid (except kidney) in the plasma and tissues while

Table 1
Effect of alcoholic leaf extract of *C. halicacabum* (CHE) on the glucose levels in the plasma of normal and STZ-diabetic rats.

Groups	Glucose (mg/dL)	
	0th day	After 45 days
Normal control (2% gum acacia)	66.54±5.89	74.55±4.34a
Diabetic control	246.63±9.45	290.52±16.24b
Normal + CHE (200 mg/kg body wt.)	72.48±5.52	68.93±3.94a
Diabetes + CHE (200 mg/kg body wt.)	246.37±12.39	138.45±7.10e
Diabetes + glibenclamide (600 μg/kg body wt.)	251.38±17.48	98.50±9.56f

Values are means ± SD of 6 rats from each group, values not sharing a common superscript differ significantly at $P< 0.05$ (DMRT).

Table 2
Effect of CHE on the hexoses and hexosamines in the plasma, liver and kidney of normal and STZ-diabetic rats.

Groups	Hexoses			Hexoseamines		
	Plasma (mg/dL)	Liver (mg/100 g tissue)	Kidney (mg/100 g tissue)	Plasma (mg/dL)	Liver (mg/100 g tissue)	Kidney (mg/100 g tissue)
Normal control (2% gum acacia)	102.27±6.89^{af}	22.22±0.78^{a}	20.05±1.11^{a}	45.12±1.17^{a}	8.11±1.94^{a}	5.48±0.49^{af}
Diabetic control	165.15±8.46^{b}	41.04±1.101^{b}	38.13±2.66^{b}	65.34±3.53^{b}	20.22±3.09^{b}	14.22±1.26^{b}
Normal + CHE (200 mg/kg body wt.)	99.24±7.83^{a}	20.98±1.15^{a}	18.18±1.07^{a}	43.34±2.27^{a}	7.45±1.76^{a}	4.51±0.21^{a}
Diabetes + CHE (200 mg/kg body wt.)	120.45±5.57^{e}	28.03±1.73^{e}	27.27±2.40^{e}	51.78±1.82^{e}	11.44±2.13^{d}	6.20±0.56^{e}
Diabetes + glibenclamide (600 μg/kg body wt.)	109.00±6.51^{f}	24.24±2.14^{f}	22.98±2.90^{f}	47.89±2.02^{f}	9.33±1.74^{ad}	5.19±0.47^{f}

Values are means ± SD of 6 rats from each group, values not sharing a common superscript differ significantly at $P< 0.05$ (DMRT).

Table 3
Effect of CHE on the fucose and sialic acid in the plasma, liver and kidney of normal and STZ-diabetic rats.

Groups	Fucose			Sialic acid		
	Plasma (mg/dL)	Liver (mg/100g tissue)	Kidney (mg/100g tissue)	Plasma (mg/dL)	Liver (mg/100g tissue)	Kidney (mg/100gtissue)
Normal control (2% gum acacia)	8.75±0.81^{a}	14.48±1.35^{a}	11.67±1.08^{a}	42.14±2.63^{a,f}	8.18±0.69^{a}	7.42±0.26^{a}
Diabetic control	15.11±1.16^{b}	27.16±2.46^{b}	25.42±1.94^{b}	67.14±2.02^{b}	17.86±0.88^{b}	2.90±0.16^{b}
Normal + CHE (200 mg/kg body wt.)	7.82±0.60^{a}	13.14±1.08^{a}	10.83±0.68^{a}	40.59±1.77^{a}	7.14±0.67^{c}	8.10±0.31^{c}
Diabetes + CHE (200 mg/kg body wt.)	10.32±0.94^{d}	17.08±1.54^{e}	15.83±1.36^{e}	50.35±2.38^{e}	10.24±0.60^{f}	6.07±0.27^{e}
Diabetes + glibenclamide (600 μg/kg body wt.)	8.91±0.81^{a}	15.83±1.24^{ae}	12.36±0.89^{a}	44.10±1.63^{f}	9.05±0.54^{a}	6.98±0.40^{f}

Values are means ± SD of 6 rats from each group, values not sharing a common superscript differ significantly at $P< 0.05$ (DMRT).

treatment with CHE and glibenclamide showed reversal of these parameters towards normal levels.

4. Discussion

Streptozotocin selectively destroys the pancreatic insulin secreting β-cells, leaving less active cells and resulting in a diabetic state [28,29]. The fundamental mechanism underlying hyperglycaemia in diabetes mellitus involves the overproduction (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues [30], and studies have shown that the level of blood glucose was elevated in STZ-induced diabetic rats. Hence, in the present study, we observed an increased level of blood glucose. Oral administration of CHE resulted in a significant reduction in blood glucose. Flavonoids are one of the most numerous and widespread groups of phenolic compounds in higher plants [31]. Some of them, due to their phenolic structure are known to be involved in the healing process of free radical mediated diseases including diabetes [32]. The plant leaf possesses several flavonoids such as apigenin, pinitol and luteolin [33], which are reported as the antidiabetic principles. Apigenin, a component of CHE, was also isolated from flowers of *Platycodon grandiflorum* and found to possess an inhibitory effect on the aldose reductase enzyme [34]. This enzyme is known to play a key role in the polyol pathway, by catalyzing the reduction of the glucose to sorbitol, which under normal conditions cannot diffuse out of cell membranes. Because of the intracellular accumulation of sorbitol, the chronic complications (such as neuropathy, retinopathy and cataracts) of diabetes can occur. Apigenin and luteolin were shown to possess antihyperglycaemic [35, 36], and antioxidant activity [36,37].

Glycoproteins are carbohydrate linked protein macromolecules found in the cell surface, which form the principle components of animal cells. They play an important role in membrane transport, cell differentiation and recognition, the adhesion of macromolecules to cell surface and the excretion and absorption of macromolecules [39]. Prolonged elevation of blood glucose in diabetes may result in structural and functional alterations of both circulating and membrane bound proteins [40]. Alterations in the diabetic state of the composition of the carbohydrate components of glycoproteins, especially serum glyloproteins and glycoproteins of the capillary basement membrane have been reported [41].

Protein bound hexoses in the cell membrane provides hydrophobic areas, whereas protein bound hexosamine provides cationic charges on the cell membrane surface and make the membrane more polar. The elevated level of hexoses in diabetic rats may be associated with disturbances with carbohydrate metabolism. Treatment with CHE and glibenclamide in diabetic rats showed significantly decreased hexoses due to improved glycemic control. Hexosamines function as physiologic glucose sensors that serve as an adaptor in diverting excess calories toward storage as fat [42]. One pathway through which glucose is sensed subacutely is hexosamine synthesis. The amination of fructose-6-phosphate to glucosamine-6-phosphate is rate limiting and is catalysed by glutamine fructose-6-phosphate aminotransferase (GFA) [43]. In accordance with previous report diabetic rats had elevated level of hexosamines, which could be due to, increased expression of GFA and increased plasma glucose. In our report, diabetic rats had elevated level of hexosamine in plasma and tissues when compared with normal rats. Diabetic rats treated with CHE and glibenclamide showed significantly decreased hexosamines in the plasma and tissues when compared to diabetic rats, which could be due to improved glycemic control.

Sialic acid is the terminal residue of the oligosaccharide side chain of glycoproteins and widely occurs in the exposed positions of molecules like hormones, enzymes and also on tissues. Elevated levels of serum sialic acid are considered to be a good predictor of cardiovascular disease [44]. Diabetic rats had increased level of sialic acid in the plasma and tissues [45]. In our study, the level of sialic acid in plasma and tissues of diabetic control rats significantly elevated when compared to normal rats. The elevated level of sialic acid in diabetic rats might be due to either enhanced sialic acid synthesis or decreased sialidase activity. Sialic acid contributes to the negative charges on this membrane, thus possibly playing a role in the selective glomerular permeability to negative charged proteins [46]. It has been postulated that an increased activity of sialidase, an enzyme which catalyses the removal of sialic acid residues from sialoconjugates which might be responsible for the depletion of glomerular sialic acid [47]. Treatment with CHE and glibenclamide had significantly decreased sialic acid in the plasma and tissues of diabetic rats, which could be due to the regulation of sialidase activity by insulin, since insulin is a more likely mediator of sialic acid changes than any other alterations in plasma glucose levels [45].

Fucose (6-deoxy-L-galactose) is a characteristic constituent of many glycoproteins], and is a mobile component of plasma glycoproteins of particular physiological and pathological significance. In our study, diabetic rats had elevated level of fucose, which could be due to elevated blood glucose level, which is in line with previous report [45]. Treatment with CHE and glibenclamide in diabetic rats had significantly decreased fucose levels,

which could be due to improved glycemic control.

The biosynthesis of the carbohydrate moieties of glycoprotein forms the insulin independent pathways for the use of glucose 6-phosphate. But the deficiency of insulin during diabetes produces derangement of glycoprotein metabolism, resulting in the thickening of the basal membrane of pancreatic beta cells. In hyperglycemic state, the excess availability of glucose accelerates the synthesis of glucose basement membrane components i.e., glycoproteins [47]. Agents with antioxidant or free radical scavenging property may inhibit oxidative reactions associated with glycation. In this context, previous studies have shown that decrease in hyperglycemia could lead to a decrease in glycoprotein levels [45]. Administration of CHE to diabetic rats resulted in a significant reversal of all these changes to near normal.

In conclusion, the decreased hyperglycemic state in CHE treated diabetic rats might have been responsible for the decrease of glycoproteins in plasma, liver and kidney. The observed effect of CHE on reversing the adverse effects of hyperglycemia provides an insight into the pathogenesis of diabetic complications, and may be used to advantage in therapeutic approaches.

Conflict of interest statement

There are no conflicts of interest.

Acknowledgments

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