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Influence of *Casearia esculenta* root extract on glycoprotein components in streptozotocin diabetic rats

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The present study was aimed to evaluate the role of the indigenous antidiabetic medicinal plant *Casearia esculenta* on glycoprotein components in streptozotocin-induced diabetic rats in plasma, liver, kidney and cardiac tissues. Streptozotocin injection (50 mg/kg body weight) caused massive elevation of glycoprotein components such as hexose, hexosamine, sialic acid and fucose in plasma and tissues of diabetic control and experimental animals. Oral administration of *C. esculenta* root extract (200 and 300 mg/kg body weight) for 45 days significantly reverted the hexose, hexosamine, sialic acid and fucose levels to near normal values. These results suggest a normalizing effect of *C. esculenta* on glycoprotein components in STZ diabetic rats.

1. Introduction

Glycoproteins (GP) are carbohydrate linked protein macromolecules found in the cell surface, which form the principal 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 secretion and absorption of macromolecules (Mittal et al. 1996).

Brownlee (1992) showed that the glycosylation process in rat epithelial cells is under hormonal regulation. The insulin receptor of the plasma membrane is a glycoprotein. Abnormal levels of glycoprotein components are important in the pathogenesis of liver and kidney diseases in diabetes mellitus (Mittal et al. 1996; Robinson et al. 1995). Administration of streptozotocin (STZ) induced altered glycoprotein synthesis and ultra structural abnormalities in tissues (Osterby and Gundersen 1980; Mohanan and Bose 1983). STZ induced hyperglycaemic effects may be related to altered glycoprotein components, which interfere with several subcellular functions (Maddux et al. 1995). Changes in the lysosomal enzymes involved in the degradation of glycoproteins may play an important role in the pathophysiology of diabetes (Rossetti et al. 1993). Several hypotheses have been proposed to explain glycoprotein component abnormalities and functions in diabetes and its secondary complications (Hunt et al. 1988; Brownlee et al. 1988). Thus the present study aimed to investigate the levels of glycoprotein components in normal, control, untreated diabetic and C. esculenta treated diabetic rats.

Casearia esculenta Roxb. (Flacourtiaceae), popularly known as "Kadala-Zhinjill", "Kottarkovai" in Tamil "Wild cowrie fruit" in English and "Saptarangi" in Sanskrit is a shrub richly distributed in Konkan plateau, South India. In Indian traditional medicine, the plant has been a popular remedy for the treatment of diabetes mellitus (Wealth of India 1992; Asolkar et al. 1992; Yoganarasimhan 2000). It is one of the major ingredients of D-400, a largest selling antidiabetic drug in India (Himalaya drug Co, Bangalore) (Mitra et al. 1995). In previous communications we reported the glycemic control achieved by by *C. esculenta* (Prakasam et al. 2002, 2003a), effects of plasma antioxidant status in STZ diabetic rats (Prakasam et al. 2003b) and hypolipidaemic action of *C. esculenta* on STZ diabetic rats (Prakasam et al. 2003c). The present study was carried out to evaluate the modulating role of *C. esculenta* root extract on glycoprotein components in control and experimental animals.

2. Investigations and results

The levels of plasma glycoprotein components such as hexoses, hexosamine, sialic acid and fucose of control and experimental animals are shown in Table 1. The levels of these parameters are significantly elevated in STZ diabetic rats ($p < 0.05$) when compared to control rats. Oral administration of *C. esculenta* for 45 days to the diabetic rats shows significant reversal of these elevated parameters to near normal values.

The levels of hexoses, hexosamine and sialic acid in the liver of control and experimental animals are shown in Table 2. The levels of these parameters are significantly elevated in STZ diabetic rats ($p < 0.05$) when compared to control rats. Oral administration of *C. esculenta* for 45 days of treatment to the diabetic rats shows significant reversal of these elevated parameters to near normal values.

The levels of hexoses, hexosamine and sialic acid in the kidney of control and experimental animals are shown in Table 3. The levels of these parameters are significantly elevated in STZ diabetic rats except sialic acid ($p < 0.05$) when compared to control rats. Oral administration of *C.*

Table 1: Levels of glycoprotein components in the plasma of control and experimental animals

Group	Treatment	Hexoses (mg/dL)	Hexosamine (mg/dL)	Sialic acid (mg/dL)	Fucose (mg/dL)
I	Control (3% gum acacia)	109.01 ± 3.47^{a}	47.00 ± 1.81^{a}	42.25 ± 4.17^{a}	9.99 ± 1.18^{a}
II	Diabetic control	157.50 ± 7.58^{b}	62.91 ± 2.57^{b}	64.16 ± 5.15^{b}	14.16 ± 1.22^{b}
III	Diabetic + *C. esculenta* (200 mg/kg body wt.)	137.0 ± 5.70^{c}	58.29 ± 5.37^{c}	58.33 ± 4.87^{c}	11.56 ± 0.65^{a}
IV	Diabetic + *C. esculenta* (300 mg/kg body wt.)	120.00 ± 4.47^{d}	50.66 ± 1.36^{a}	51.78 ± 3.74^{a}	10.93 ± 0.65^{a}
V	Diabetic + glibenclamide (600 µg/kg body wt.)	$115.83 \pm 7.35^{a,d}$	50.08 ± 2.01^{a}	47.31 ± 2.71^{a}	10.51 ± 0.73^{a}

Values are given as means ± SD for six animals in each group.
Values not sharing a common superscript differ significantly at $p < 0.05$, Duncan's Multiple Range Test (DMRT)

Table 2: Levels of glycoprotein components in the liver of control and experimental animals

Group	Treatment	Hexoses (mg/g wet tissue)	Hexosamine (mg/g wet tissue)	Sialic acid (mg/g wet tissue)
I	Control (3% gum acacia)	22.66 ± 3.50^{a}	7.37 ± 0.99^{a}	8.00 ± 0.89^{a}
II	Diabetic control	37.6 ± 3.32^{b}	15.50 ± 1.81^{b}	15.00 ± 1.67^{b}
III	Diabetic + *C. esculenta* (200 mg/kg body wt.)	29.83 ± 3.22^{a}	11.58 ± 1.59^{c}	12.16 ± 1.16^{a}
IV	Diabetic + *C. esculenta* (300 mg/kg body wt.)	25.0 ± 2.02^{a}	8.50 ± 1.81^{a}	10.83 ± 1.72^{c}
V	Diabetic + glibenclamide (600 µg/kg body wt.)	22.33 ± 2.90^{a}	7.75 ± 1.12^{a}	$8.33 \pm 0.81^{a,c}$

Values are given as means ± SD for six animals in each group.
Values not sharing a common superscript differ significantly at $p < 0.05$, Duncan's Multiple Range Test (DMRT)

Table 3: Levels of glycoprotein components in the kidney of control and experimental animals

Group	Treatment	Hexoses (mg/g wet tissue)	Hexosamine (mg/g wet tissue)	Sialic acid (mg/g wet tissue)
I	Control (3% gum acacia)	18.00 ± 0.89^{a}	4.00 ± 1.12^{a}	0.27 ± 0.02^{a}
II	Diabetic control	35.66 ± 2.58^{b}	11.25 ± 1.57^{b}	0.12 ± 0.01^{b}
III	Diabetic + *C. esculenta* (200 mg/kg body wt.)	20.83 ± 1.16^{a}	7.75 ± 1.47^{c}	0.17 ± 0.01^{c}
IV	Diabetic + *C. esculenta* (300 mg/kg body wt.)	18.66 ± 1.96^{a}	4.87 ± 0.91^{a}	0.23 ± 0.01^{a}
V	Diabetic + glibenclamide (600 µg/kg body wt.)	18.33 ± 1.36^{a}	4.87 ± 0.62^{a}	0.23 ± 0.01^{a}

Values are given as means ± SD for six animals in each group.
Values not sharing a common superscript differ significantly at $p < 0.05$, Duncan's Multiple Range Test (DMRT)

Table 4: Levels of glycoprotein components in the cardiac tissue of control and experimental animals

Group	Treatment	Hexoses (mg/g wet tissue)	Hexosamine (mg/g wet tissue)	Sialic acid (mg/g wet tissue)
I	Control (3% gum acacia)	21.16 ± 2.78^{a}	13.75 ± 1.75^{a}	6.66 ± 1.63^{a}
II	Diabetic control	35.33 ± 3.66^{b}	32.75 ± 1.12^{b}	13.16 ± 2.31^{b}
III	Diabetic + *C. esculenta* (200 mg/kg body wt.)	26.50 ± 3.08^{c}	20.50 ± 2.62^{c}	8.66 ± 1.21^{c}
IV	Diabetic + *C. esculenta* (300 mg/kg body wt.)	22.50 ± 1.87^{a}	14.87 ± 0.91^{a}	7.00 ± 0.89^{a}
V	Diabetic + glibenclamide (600 µg/kg body wt.)	22.33 ± 2.42^{a}	14.25 ± 0.82^{a}	6.66 ± 0.82^{a}

Values are given as means ± SD for six animals in each group.
Values not sharing a common superscript differ significantly at $p < 0.05$, Duncan's Multiple Range Test (DMRT)

esculenta for 45 days significantly brings the values to near normal.

The levels of hexoses, hexosamine and sialic acid in the heart of control and experimental animals are shown in Table 4. The levels of these parameters are significantly elevated in STZ diabetic rats ($p < 0.05$) when compared to control rats. Oral administration of *C. esculenta* for 45 days of treatment to the diabetic rats shows significant reversal of these elevated parameters to near normal values.

3. Discussion

Glycoproteins together with glycosaminoglycans form the major macromolecular components of connective tissue (Mathew et al. 1982). The luminal surface of epithelial cells in kidney tubules is also lined with a thick carbohydrate rich glycoprotein layer (Mittal et al. 1996). Diabetes mellitus is reflected in profound changes in the metabolism of glycoproteins (Crook and McClain 1996; Mahmood and Pinedo 1983) and by a reduction in membrane glycosylation in the kidney brush border membrane (McVerry et al. 1980).

Several workers (Guillot et al. 1994; Sekar et al. 1990) have reported elevated levels of plasma protein bound carbohydrate compounds in diabetes. An increase in plasma glycoprotein components has been reported to relate to the duration, severity and existence of degenerative vascular diseases (Methew et al. 1982). The liver is primarily responsible for producing a large amount of glycoproteins present in the blood. Guilot et al. (1994) have suggested that elevated levels of plasma glycoproteins in diabetic patients could be a consequence of abnormal carbohydrate metabolism.

Robinson et al. (1995) reported that glycogen synthesis in the rat liver and skeletal muscles were impaired in the diabetic state. Synthesis of glucosamine from glucose is an insulin dependent pathway despite going through glucose-6-phosphatase. It is therefore conceivable that, in insulin deficiency as in diabetes mellitus glucose is redirected to an insulin dependent pathway. This could lead to the accumulation of high levels of glucose in the blood, which may result in an increased synthesis of glycoproteins or glycosylated 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 basement membranes (Sekar et al. 1990). The epithelial cells of the glomerulus, which produce the capillary basement membrane may also be responsible for the synthesis of pathological glycoproteins deposited in the diabetic state. Thickening of capillary basement membrane is recognized an accompaniment of disturbed glycaemic control in diabetes mellitus (Williamson and Kilo 1983; Rasch et al. 1995.

The basement membrane proteins consist of glycoproteins and basement membrane thickening may be influenced by insulin deficiency. The resulting basement membrane thickening obviously impairs cellular transport mechanism and also alters receptor specificity (Marshall et al. 1991).

Long standing diabetes mellitus is attributed to the alteration of macromolecules, such as glycoproteins of blood vessel (Tschope 1995; He et al. 1995). The complexity of the problem further indicated that the changes in the glycosaminoglycans occurs in the diabetes mellitus pathway (Harlan et al. 1994). Hexoses, hexosamine and sialic acid are the basic components of the glycosaminoglycans and glycoproteins. Increased deposition of these components have been observed in the liver of STZ-induced diabetic rats (Mittal et al. 1996; Robinson et al. 1995). But the kidney of diabetic rats showed elevated levels of hexoses and hexosamine and a depletion of sialic acid.

4. Experimental

4.1. Plant material

Root of *Casearia esculenta* was collected from Western ghats of Tamil Nadu and the plant was botanical authenticated by Dr. C. Chelladurai, Research Officer, Survey Medicinal Plant Unit (S.M.P.U.), Central Council for Research in Siddha and Ayurvedic, Siddha Medical College, Palayamkottai, Tamilnadu. Voucher specimen was deposited in the (AU2145) Department of Botany, Annamalai University, Annamalainagar, Tamilnadu. The plant root was air dried at 25 °C in the room and the dried root was made into fine powder with auto-mix blender and the powdered part was kept in deep freezer until the time of use.

4.2. Preparation of aqueous extract

Dry fine powder (100 g) was suspended in 250 ml water for 2 h and then boiled at 60–65 °C for 30 min (since boiled decoction of root of this plant has been used as remedy for diabetes).

The extract was preserved and the processes were repeated for three times with the residual powder, each time collecting the extract. The collected extract was pooled and passed through a fine cotton cloth. The filtrate upon evaporation at 40 °C yielded 12% semi-solid extract.

4.3. Drugs and chemicals

Streptozotocin (STZ) was obtained form Sigma Chemical Co, St Louis, MA, USA. All other chemicals used were of analytical grade.

4.4. Animals

Male Wistar albino rats (weighing 140–160 g) were procured from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College, Annamalai University, Annamalai Nagar. Animals were maintained at Central Animal House and the animals were fed on standard diet (Hindustan Lever, Bangalore) and water *ad libitum.* All studies were conducted in accordance with the National Institute Health "Guide for the Care and Use of Laboratory Animals" (National Institute of Health 1985) and the study was approved by the Ethical Committee of Rajah Muthaih Medical College and Hospital, Annamalai University, Annamalainagar.

4.5. Experimental induction of diabetes

Adult (9 weeks old) male Wistar rats were made diabetic with an intraperitoneal injection of streptozotocin (STZ, 50 mg/kg body weight) dissolved in citrate buffer (0.1 M, pH 4.5). Streptozotocin injected animals exhibited massive glycosuria and hyperglycemia within a few days. Diabetes was confirmed in STZ rats by measuring the fasting blood glucose concentration, 96 h after injection with STZ. Albino rats with blood glucose level above 240 mg/dl were considered to be diabetic and were used in the experiment. Six rats received 2% gum acacia alone that served as control.

4.6. Animals

After the induction of diabetes the rats were divided into five groups of six animals each.

Group I: Control rats received vehicle solution (2% gum acacia); group II: Diabetic control; group III: Diabetic rats received *C. esculenta* root extract (200 mg/kg body weight) in 2% gum acacia using an intragastric tube daily for 45 days; group IV: Diabetic rats given *C. esculenta* root extract (300 mg/kg body weight in 2% gum acacia using an intragastric tube daily for 45 days; group V: Diabetic rats received glibenclamide orally (600 μg/kg body weight) as aqueous solution using an intragastric tube daily for 45 days.

After 45 days of treatment, the animals were sacrificed by cervical decapitation. The blood was collected in heparinised centrifuge tubes and the plasma were collected. The liver, kidney and cardiac tissues was dissected out immediately and washed with ice-cold saline. A portion of these tissues was weighed using an electronic balance and homogenized using a potter Elvejham homogenizer and centrifuged at $3000 \times g$ for 10 min. The supernatant were further used for the assay of glycoprotein components such as hexoses, hexosamine, sialic acid and fucose.

4.7. Biochemical analysis

4.7.1. Estimation of serum and tissue hexoses

Serum and tissue hexose content was estimated by the method of Niebes (1972). 0.2 mL of the serum or tissue homogenate are mixed with 8.5 mL of orcinol-H_2SO_4. The tubes were then heated at 80 °C for 15 min, cooled and read at 540 nm after 20 min. Standard and blank containing 0.2 mL of 0.2N H_2SO_4 were processed similarly. The hexose content of serum is expressed as mg/dL or mg/g wet tissue for tissues.

4.7.2. Estimation of hexosamine

Serum and tissue hexosamine content was determined by the method of Elson and Morgon (1933). The method involves 0.1 mL of serum or tissue homogenate in a test tube graduated at 10 mL. 5 mL of 95% ethanol was added and mixed well, centrifuged for 15 min, decanted, and the precipitate was suspended in 3 mL of 95% ethanol. The solution was centrifuged and decanted. To the precipitated protein 2 mL of 3N HCl were added and the solution was hydrolysed in a boiling water bath with an air condenser for 4 h.

The hydrolysate was neutralized with 3N NaOH; 1 mL of the acetyl acetone was added to 1 mL of the aliquot, 1 mL of water (blank) and 1 mL of standard. The tubes were capped with marbles to prevent evaporation and were placed in a boiling water bath for 15 min. The tubes were cooled in a tap water. 5 mL of 95% ethanol was added and the solution was mixed well. 1 mL of Ehrlich reagent was added, mixed well, and diluted to 10 mL with 95% ethanol. Absorbance was measured at 530 nm after 30 min. Hexosamine content of the serum is expressed as mg/dL or mg/g wet tissue for tissues.

4.7.3. Estimation of sialic acid

Sialic acid content in serum and tissues was estimated by the method of Welmer et al. (1952). 4.8 mL of 5% TCA was added slowly to 0.2 mL of serum or tissue homogenate, 0.2 mL of orosomucoid standard in separate tubes. The test tube was placed in a boiling water bath for exactly 15 min with a glass marble to prevent evaporation, then the tubes were cooled by immersion in water and filtered. 2 mL of clear filtrate was pipetted out of each tube; 4 mL of diphenylamine (DPA) reagent were added to one of each pair of tubes and 4 mL of acid-mixture without DPA into the other tube.

The reagent blank was prepared by adding 2 mL of 5% TCA and 4 mL of DPA reagent. The solutions were mixed well and capped with a glass marble and immersed in a boiling water bath for exactly 30 min. The tubes were cooled in water and the absorbance was determined at 530 nm with a reagent blank set at zero. Sialic acid content of the serum is expressed as mg/dL or mg/g wet tissue for tissues.

4.7.4. Estimation of serum fucose

Serum fucose content was estimated by the method of Dische and Shettles (1948). To 2.2 mL of serum, 4.8 mL of sulphuric acid reagent was added and heated in a boiling water bath for 3 min. The sample was cooled and 0.1 mL of cysteine hydrochloride reagent was added, 0.5 mL of 0.1 N NaOH was also treated in the same way for blank, after 25 min the optical density was measured at 393 and 430 nm. Fucose content is expressed as mg/dL for serum.

4.8. Statistical analysis

Values were represented as means ± S.D., Data were analysed using Analysis of Variance (ANOVA) and group mean were compared with Duncan's multiple range test (DMRT).

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