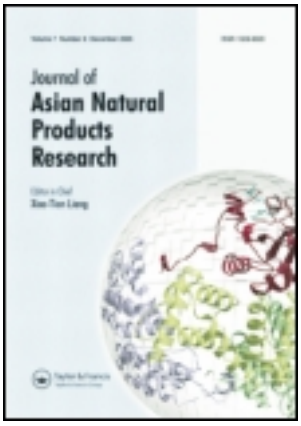

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Influence of 3-hydroxymethyl xylitol, a novel antidiabetic compound isolated from *Casearia esculenta* (Roxb.) root, on glycoprotein components in streptozotocin-diabetic rats

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Influence of 3-hydroxymethyl xylitol, a novel antidiabetic compound isolated from *Casearia esculenta* (Roxb.) root, on glycoprotein components in streptozotocin-diabetic rats

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Casearia esculenta root (Roxb.) is widely used in traditional system of medicine to treat diabetes in India. An active compound, 3-hydroxymethyl xylitol (3-HMX), has been isolated, and its optimum dose has been determined in a short duration study and patented. In addition, the long-term effect of 3-HMX in type 2 diabetic rats on antihyperglycemic, antioxidants, antihyperlipidemic, and protein metabolism and kidney marker enzymes was investigated, and its effect was shown previously. In this study, we investigated the effect of 3-HMX on plasma and tissue glycoproteins in streptozotocin-diabetic rats. Animals were divided into five groups *viz*., control group, 3-HMX (40 mg/kg of body weight) treated group, diabetic group, diabetic + 3-HMX (40 mg/kg of body weight), and diabetic + glibenclamide (600 μg/kg of body weight). 3-HMX was administered orally at a dose of 40 mg/kg of body weight for 45 days. The study shows significant increases in the level of sialic acid except kidney and elevated levels of hexose, hexosamine, and fucose in the liver and kidney of diabetic rats, and the treatment with 3-HMX and glibenclamide showed reversal of these parameters toward normalcy. Thus, the study indicates that 3-HMX possesses a significant beneficial effect on glycoprotein components.

Keywords: *Casearia esculenta*; 3-hydroxymethyl xylitol; streptozotocin; glycoproteins; glibenclamide

1. Introduction

Diabetes mellitus is a metabolic disorder, characterized by severe insulin deficiency, resulting in the impairment of glucose, lipids, proteins, and glycoproteins metabolism [1]. It is becoming increasingly accepted that the oligosaccharide moieties of glycoproteins: hexose, hexosamine, fucose, and sialic acid have an important role in protein stability, function, and turnover [2]. Glycoproteins are rich in extracellular matrix and they contribute a major source to the structure of the matrix. In the diabetic state, glucose is utilized by the insulin-independent pathways leading to the synthesis of glycoproteins and even mild deficiency of insulin influences thickening of basement membrane [3]. Raised levels of glycoproteins in diabetics may also be a predictor of angiopathic complications [3]. Medicinal plants continue to provide valuable therapeutic agents, both in modern medicine and in traditional system. The doubts about the

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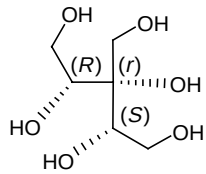

Figure 1. Structure of 3-HMX.

efficacy and safety of the oral hypoglycemic agents have prompted a search for safer and more effective drugs in the treatment of diabetes.

Casearia esculenta Roxb. (Flacourtiaceae) is one such plant in Indian traditional medicine and the plant has been a popular remedy for the treatment of diabetes. Previous studies from our laboratory demonstrated a significant reduction of blood glucose and glycoproteins in normal and streptozotocin-diabetic rats after oral administration of *C. esculenta* root extract [4]. In our early study, the active compound, 3-hydroxymethyl xylitol (3-HMX), was isolated from the root of *C. esculenta*, and at an optimum dose of 40 mg it decreased blood glucose level and improved body weight in a 15-day study [5]. 3-HMX exhibited antihyperglycemic [6] and antioxidant properties [7], hypolipidemic action [8], protein metabolism and nephritic marker enzymes in streptozotocin-diabetic rats after 45 days of treatment [9]. In this study, we have investigated the effect of 3-HMX on plasma and tissue glycoproteins in streptozotocin-diabetic rats. The structure of 3-HMX is depicted in Figure 1.

2. Results and discussion

2.1 Biochemical analysis

Table 1 shows the effect of administration of 3-HMX for 45 days on plasma glucose in normal and streptozotocin-diabetic rats. Plasma glucose significantly increased in diabetic rats. Both 3-HMX and glibenclamide significantly brought down the plasma glucose toward normal level in streptozotocin-diabetic rats. Normal rats treated with 3-HMX also decreased significantly the plasma glucose level, but not up to hypoglycemic level.

Tables 2 and 3 represent the levels of sialic acid and hexosamines in the plasma and tissues (liver and kidney) of normal and diabetic rats. The diabetic rats had increased levels of sialic acid and hexosamines in the plasma and tissues except kidney, in which sialic acid decreased, and the treatment with 3-HMX or glibenclamide showed reversal of these parameters toward normalcy.

Tables 4 and 5 represent the levels of fucose and total hexoses in the plasma and tissues (liver and kidney) of normal and diabetic rats. The diabetic rats had increased levels of fucose and total hexoses in the plasma and tissues, and the treatment with 3-HMX or glibenclamide showed reversal of these parameters toward normalcy.

2.2 Discussion

Generalized abnormalities in glycoprotein metabolism are observed in both naturally

Table 1. Influence of 3-HMX on plasma glucose level in normal and streptozotocin-diabetic rats.

	Plasma glucose (mg/dl)	
Group	0 day	45th day
Normal	77.40 ± 2.28^{a}	83.15 ± 6.33^{a}
Normal + 3-HMX (40 mg/kg b.wt.)	78.54 ± 1.89^{a}	69.83 ± 6.45^{b}
Diabetic control	246.54 ± 3.44^{b}	292.45 ± 4.85^{c}
Diabetic + 3-HMX (40 mg/kg b.wt.)	248.49 ± 2.78^{b}	122.21 ± 6.05^{d}
Diabetic + glibenclamide (600 μg/kg b.wt.)	253.67 ± 4.10^{b}	117.39 ± 5.94^{d}

Notes: Values are means ± SD for six rats. $^{a-d}$Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

Table 2. Influence of 3-HMX on sialic acid in the plasma and tissues of normal and streptozotocin-diabetic rats.

Group	Plasma sialic acid (mg/dl)	Sialic acid (mg/100 g wet tissue)	
		Liver	Kidney
Normal	55.44 ± 4.22[a]	8.43 ± 0.64[a]	7.12 ± 0.54[a]
Normal + 3-HMX (40 mg/kg b.wt.)	54.29 ± 4.13[a]	8.16 ± 0.61[a]	7.31 ± 0.55[a]
Diabetic control	78.20 ± 5.98[b]	15.29 ± 1.17[b]	4.14 ± 0.31[b]
Diabetic + 3-HMX (40 mg/kg b.wt.)	68.71 ± 5.23[c]	11.24 ± 0.85[c]	5.98 ± 0.45[c]
Diabetic + glibenclamide (600 μg/kg b.wt.)	60.57 ± 4.60[a]	9.89 ± 0.75[d]	6.49 ± 0.49[c]

Notes: Values are means ± SD for six rats. [a–d]Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

occurring and experimental diabetes. The increases in plasma glycoprotein components have been reported to be associated with severity and duration of diabetes. Elevation of serum glycoprotein components may be due to secretion or shedding from cell membrane glycoconjugates into the circulation. Glycoproteins found in a variety of tissues including the arterial wall are very similar in structure and composition to those in plasma [10]. Therefore, vascular complications that involve complex protein–carbohydrate molecules could contribute to an increase in the level of plasma glycoproteins. The liver is primarily responsible for producing a large amount of glycoproteins present in blood.

Guillot *et al.* [11] suggested that elevated levels of plasma glycoproteins in diabetic patients could be a consequence of abnormal carbohydrate metabolism. The biosynthesis of the carbohydrate moieties of glycoprotein forms the insulin-independent pathways for the utilization of glucose-6-phosphate. The deficiency of insulin during diabetes produces derangement of glycoprotein metabolism. The increased availability of glucose in the hyperglycemic state accelerates the synthesis of basement components, i.e. glycoproteins [12]. This is due to decreased utilization of glucose by insulin-dependent pathways, thereby enhancing the formation of hexose, hexosamine, and fucose for the accumulation of glycoproteins [13].

Alterations of enzymatic glycosylation processes detected as changes in sialic acid and fucose tissue contents have been previously reported in diabetic patients and animals [14]. The elevation of serum glycoprotein levels with a disproportionate increase in the level of serum protein-bound fucose has been reported. Studies have also indicated that serum and hepatic fucosyltransferase and fucosidase activities are increased in streptozotocin-induced diabetic rats [2]. In diabetes, three serum proteins (haptoglobin, α1-acid glycoprotein, and α1-antitrypsin) synthesized in the liver are mainly responsible for the increase in bound fucose levels [15].

Our findings also suggest that the increased fucosylated proteins in diabetic rats could be due to an increase in the synthesis and/or decrease in degradation of these proteins. In this study, the serum sialic acid level was found to be significantly increased, but tissues sialic acid was decreased in diabetic rats. The cleavage of sialic acid residue from circulatory and membrane glycoproteins might be the cause of high serum levels. Alterations in membrane protein glycosylation may also be important in membrane protein turnover. As carbohydrate groups such as sialic acid render protection against proteolysis of glycoproteins, a decrease

Table 3. Influence of 3-HMX on hexosamines in the plasma and tissues of normal and streptozotocin-diabetic rats.

Group	Plasma hexosamines (mg/dl)	Hexosamines (mg/100 g wet tissue)	
		Liver	Kidney
Normal	68.27 ± 5.19^{a}	10.34 ± 0.78^{a}	14.74 ± 1.12^{a}
Normal + 3-HMX (40 mg/kg b.wt.)	67.36 ± 5.12^{a}	10.02 ± 0.76^{a}	14.13 ± 1.07^{a}
Diabetic control	94.86 ± 7.26^{b}	17.56 ± 1.34^{b}	28.65 ± 2.19^{b}
Diabetic + 3-HMX (40 mg/kg b.wt.)	86.46 ± 6.58^{c}	13.11 ± 0.99^{c}	21.42 ± 1.63^{c}
Diabetic + glibenclamide (600 µg/kg b.wt.)	79.54 ± 6.05^{c}	11.36 ± 0.86^{a}	18.36 ± 1.39^{d}

Notes: Values are means ± SD for six rats. $^{a-d}$Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

in superficial carbohydrate moieties might increase proteolysis and hence membrane protein degradation [16]. Moreover, a decrease in sialic acid will induce a decrease in the negative electrostatic charge of endothelial cells that may participate in the hyperaggregability of circulating cells to the vascular endothelium [14]. The possibility of a general decrease in sialylation proteins in diabetes is further supported by the study of Pickup *et al.* [17], who found decreased activity of hepatic enzymes associated with sialic acid synthesis in diabetic rats, reduced sialylation of the erythrocyte membrane protein, glycophorin in human diabetes, and reduced sialylation of a glycoprotein associated with the insulin receptor or insulin action in diabetic rats. It has been postulated that an increased activity of sialidase (neuraminidase), an enzyme which catalyzes the removal of sialic acid residues from sialoconjugates, might be responsible for the depletion of tissue sialic acid content [18]. The decrease in the content of sialic acid in tissues may also be due to its utilization for the synthesis of fibronectin, which contains sialic acid residues in the core structure. The synthesis of fibronectin was reported to increase significantly in various tissues of diabetic animals and patients [19]. Administration of 3-HMX to diabetic rats significantly reversed all these changes to near normal levels. The antidiabetic action of 3-HMX, which is mediated via an enhancement of insulin action, as evidenced by the increased level of insulin in 3-HMX-treated diabetic rats (6), may be responsible for the reversal of glycoprotein changes associated with diabetes. It is likely that the changes in glycoprotein metabolism induced by hyperglycemia will have biological and possibly pathological importance in the development of diabetic complications.

Table 4. Influence of 3-HMX on fucose in the plasma and tissues of normal and streptozotocin-diabetic rats.

Group	Plasma fucose (mg/dl)	Fucose (mg/100 g wet tissue)	
		Liver	Kidney
Normal	24.69 ± 1.88[a]	14.56 ± 1.10[a]	13.11 ± 0.99[a]
Normal + 3-HMX(40 mg/kg b.wt.)	24.13 ± 1.83[a]	13.94 ± 1.06[a]	12.72 ± 0.96[a]
Diabetic control	43.34 ± 3.31[b]	28.25 ± 2.16[b]	26.65 ± 2.04[b]
Diabetic + 3-HMX (40 mg/kg b.wt.)	36.14 ± 2.75[c]	23.65 ± 1.80[c]	20.26 ± 1.54[c]
Diabetic + glibenclamide (600 μg/kg b.wt.)	32.46 ± 2.47[d]	19.33 ± 1.47[d]	17.34 ± 1.32[d]

Notes: Values are means ± SD for six rats. [a–d] Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

3. Materials and methods

3.1 Animals

Male albino rats of Wistar strain with body weight ranging from 180 to 200 g were procured from Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, and the animals were maintained in an air conditioned room (25 ± 1°C) with a 12 h light and 12 h dark cycle. Feed and water were provided *ad libitum.* Studies were carried out in accordance with Indian National Law on Animal Care and Use. Committee for the Purpose of Control and Supervision of Experiments on Animals of Rajah Muthiah Medical College and Hospital (Pro. No. 282, Reg No.160/1999/CPCSEA), Annamalai University, Annamalainagar, provided ethical clearance.

3.2 Chemicals

Streptozotocin was purchased from Sigma-Aldrich, St Louis, MO, USA. All other chemicals were of analytical grade and obtained from E. Merck or Himedia, Mumbai, India.

3.3 Experimental induction of diabetes

The animals were rendered diabetes by a single intraperitoneal injection of streptozotocin (40 mg/kg of body weight) in freshly prepared citrate buffer (0.1 M, pH 4.5) after an overnight fast. Streptozotocin-injected animals were given 20% glucose solution for 24 h to prevent initial drug induced hypoglycemic mortality. Streptozotocin-injected animals exhibited massive glycosuria (determined by Benedict's qualitative test), and diabetes in streptozotocin rats was confirmed by measuring the fasting blood glucose concentration, 96 h after injection of streptozotocin. The animals with blood glucose above 240 mg/dl were considered to be diabetic and used for the experiment.

3.4 Experimental design

The animals were randomly divided into five groups of six animals each. 3-HMX was dissolved in water and glibenclamide was suspended in distilled water for oral administration using intragastric tube. The duration of treatment was 45 days.

- Group I: Normal.
- Group II: Normal + 3-HMX (40 mg/kg of body weight)
- Group III: Diabetic control.
- Group IV: Diabetic rats + 3-HMX (40 mg/kg of body weight).
- Group V: Diabetic rats + glibenclamide (600 μg/kg of body weight).

Glibenclamide is a sulfonylurea antidiabetic agent, a class of drug used to treat type II diabetes mellitus. This disease is a

Table 5. Influence of 3-HMX on total hexoses in the plasma and tissues of normal and streptozotocin-diabetic rats.

Group	Plasma total hexoses (mg/dl)	Total hexoses (mg/100 g wet tissue)	
		Liver	Kidney
Normal	93.46 ± 7.11[a]	27.52 ± 2.09[a]	23.14 ± 1.76[a]
Normal + 3-HMX(40 mg/kg b.wt.)	92.37 ± 7.03[a]	26.17 ± 1.99[a]	22.68 ± 1.72[a]
Diabetic control	143.72 ± 11.00[b]	48.43 ± 3.70[b]	41.20 ± 3.15[b]
Diabetic + 3-HMX (40 mg/kg b.wt.)	118.64 ± 9.03[c]	37.12 ± 2.82[c]	31.15 ± 2.36[c]
Diabetic + glibenclamide (600 μg/kg b.wt.)	112.22 ± 8.54[c]	34.65 ± 2.63[c]	27.85 ± 2.12[d]

Notes: Values are means ± SD for six rats. [a–d] Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

chronic metabolic illness characterized by a deficiency of insulin, a hormone produced by the pancreas, which controls the sugar in the blood. For that, in this study, we are using glibenclamide as standard drug for the comparison of efficacy with 3-HMX-treated diabetic rats.

3.5 Sample collection

After 45 days of treatment, the animals were fasted for 12 h, anesthetized between 8:00 to 9:00 am using ketamine (24 mg/kg body weight, intramuscular injection), and sacrificed by decapitation. Blood was collected in tubes with a mixture of potassium oxalate and sodium fluoride (1:3) for the biochemical analysis. Liver and kidney were immediately dissected out and washed in ice-cold saline to remove the blood. Liver and kidney were sliced into pieces and homogenized in an appropriate buffer (pH 7.0) in cold condition to give 20% homogenate (w/v). The homogenates were centrifuged at 1000 rpm for 10 min at 0°C in cold centrifuge. The supernatants were separated and used for various biochemical estimations.

3.6 Biochemical analysis

Plasma glucose was estimated by the method of Trinder using a reagent kit [20]. For the estimation of glycoproteins, the tissues (liver and kidney) were defatted by the method of Folch *et al.* [21]. Hexose was estimated by the method of Niebes [22]. Hexosamine was estimated by the method of Elson and Morgan [23] with slight modifications of Niebes [22]. Sialic acid and fucose were determined by the method of Warren [24] and Dische and Shuttle [25].

3.7 Statistical analysis

Values were given as means ± SD for six rats in each group. Data were analyzed by a one-way analysis of variance followed by Duncan's multiple range test (DMRT) using SPSS version 10 (SPSS, Chicago,

IL, USA). The limit of statistical significance was set at $P < 0.05$.

4. Conclusion

The administration of 3-HMX to diabetic rats has a beneficial effect on the carbohydrate moieties of glycoproteins. Furthermore, molecular studies could be carried out to know the exact molecular mechanism.

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