Restoration of altered plasma, erythrocyte and liver antioxidant levels by 3-hydroxymethyl xylitol in streptozotocin-diabetic rats

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Abstract
The present study was aimed to investigate the effect of 3-hydroxymethyl xylitol (3-HMX) on lipid peroxidation, nonenzymic and enzymatic antioxidants in plasma, erythrocyte and liver of streptozotocin (STZ) diabetic rats. Adult male albino rats of Wistar strain, weighing 180–200 g, were included in the study. The rats were rendered diabetic by streptozotocin (STZ) (40 mg/kg BW.). The rats were divided into five groups of six rats each after the induction of diabetes: normal control; normal + 3-HMX (40 mg/kg BW.); diabetic control; diabetic rats given 3-HMX (40 mg/kg BW.); diabetic rats given glibenclamide (600 µg/kg BW.). After 45 days of treatment, a significant reduction in thiobarbituric acid reactive substances and hydroperoxides. 3-HMX (40 mg/kg BW.) also caused a significant increase in nonenzymic antioxidants (vitamin C, vitamin E and reduced glutathione) levels in plasma, erythrocytes and liver, and activity of enzymic antioxidants (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase in erythrocytes and liver of streptozotocin induced diabetic rats. The effect of 3-HMX (40 mg/kg BW.) was less effective than glibenclamide (600 µg/kg BW.) in restoring the values to near normal. Histological study of liver also confirmed the biochemical findings. The result of this study clearly shows the 3-hydroxymethyl xylitol possesses antioxidant properties.

Keywords: 3-hydroxymethyl xylitol; streptozotocin; lipid peroxidation; antioxidants.

INTRODUCTION
Reactive oxygen species (ROS) are an important part of the defense mechanism against infection, but excessive generation of free oxygen radicals may damage tissue (Steinberg et al., 1989). Formation of lipid peroxides by the action of free radicals on unsaturated fatty acids has been implicated in the pathogenesis of atherosclerosis and vascular disease (Stinger et al., 1989). Free radicals may also be a contributory factor in a progressive decline in the function of the immune system (Pike & Chandra 1995). Cooperative defense systems that protect the body from free radical damage include the antioxidant nutrients and enzymes (Kim et al., 2003). The antioxidant systems include Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GSH-Px) and indirectly glutathione reductase. The role as protective enzymes is well known and has been investigated extensively in diabetic patients and experimental diabetic animals.

Glibenclamide is a second-generation sulphonylurea known and has been investigated extensively in diabetic patients and experimental diabetic animals. Glibenclamide is a second-generation sulphonylurea which has been widely used in the management of non-insulin dependent diabetes mellitus (Feldman 1985).

Casearia esculenta Roxb. (Flacourtiaeae) is one such plant in Indian traditional medicine and the plant has been a popular remedy for the treatment of diabetes (Asolkar et al., 1992; Wealth of India, 1992; Yoganarasimhan, 2000). Preliminary research revealed a significant blood glucose lowering effect and antioxidant activity after oral administration of C. esculenta root extract in normal and streptozotocin-diabetic rats (Prakasam et al., 2002 and Prakasam et al., 2005). The active compound, 3-hydroxymethyl xylitol (3-HMX) was isolated from Casearia esculenta root and optimum dose was determined in a 15 day short duration study. 3-HMX at 40 mg dose decreased blood
Restoration of altered plasma, erythrocyte and liver antioxidant levels glucose level and gave a maximum improvement on body weight (Chandramohan et al., 2007). The antihyperglycemic activity of 3-hydroxymethyl xylitol also determined in streptozotocin-diabetic rats after a long term treatment (Chandramohan et al., 2008). In this study, we have investigated the effect of 3-HMX on lipid peroxidation and antioxidants status in streptozotocin-diabetic rats. The structure of 3-HMX is depicted below.

\[ \text{Figure 1: Structure of the 3-hydroxymethyl xylitol (3-HMX)} \]

## MATERIALS AND METHODS

### 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: 12 h dark cycle. Food 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.

### Chemicals

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

### Experimental induction of diabetes

The animals were rendered diabetes by a single intraperitoneal injection of streptozotocin (40 mg/kg 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 (Ramesh and Pugalendi, 2006). 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 with streptozotocin. Plasma glucose was estimated by the method of Trinder using a reagent kit (Trinder, 1969). The animals with blood glucose above 240 mg/dl were considered to be diabetic and included for this experiment.

### Experimental design

The animals were randomly divided into five groups of six animals each. Feeding was started by 9 a.m. and 3-HMX or glibenclamide were dissolved in water and administered post-orally using intragastric tube at 10.00 a.m. The duration of treatment was 45 days.

- **Group I**: Normal (water)
- **Group II**: Normal + 3-HMX (40 mg/kg BW.) in water
- **Group III**: Diabetic control
- **Group IV**: Diabetic rats + 3-HMX (40 mg/kg BW.) in water
- **Group V**: Diabetic rats + glibenclamide (600µg/kg BW.) in water

### Sample collection

After 45 days of treatment, the animals were fasted for 12 h, anaesthetized between 8:00 a.m. to 9:00 a.m. 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 was immediately dissected out, washed in ice-cold saline to remove the blood. Liver 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.

### Biochemical analysis

#### Estimation of TBARS

The concentration of TBARS in the plasma, erythrocytes and tissues was estimated by the method of Niehaus and Samuelson [1968]. In brief, 0.1 mL of tissue homogenate (supernatant; Tris-HCl buffer, pH 7.5) was treated with 2 mL of (1:1:1 ratio) TBA-TCA-HCl reagent (0.37% thiobarbituric acid, 0.25 N HCl, and 15% TCA), placed in water bath for 15 min, cooled, and centrifuged at room temperature for 10 min. The absorbance of clear supernatant was measured against reference blank at 535. The values were expressed as mmol/dL of plasma or mmol/100 g of tissues or nmol/mg of protein for erythrocytes.

#### Estimation of lipid hydroperoxides

The estimation of lipid hydroperoxides (HP) was done by the method of Jiang et al (1992). 0.1 mL of tissue homogenate (supernatant) was treated with 0.9 mL of Fox reagent (88 mg of butylated hydroxytoluene, 7.6
Ascorbic acid in the plasma, erythrocytes and tissues incubation, 2.5 ml of 85% sulfuric acid was added and incubated for 3h at room temperature. After 0.5 ml of supernatant, 0.5 ml of DNPH reagent (2% dinitrobenzene) was used as a substrate.

Method of Estimation of Ascorbic Acid (vitamin C)

The activity of catalase in the erythrocytes and tissues was determined by the method of Sinha (1972). CAT was assayed colorimetrically at 620 nm. The reaction mixture (1.5 mL, vol) contained 0.01 M (pH 7.0) phosphate buffer, 0.1 mL of tissue homogenate (supernatant), and 0.4 mL of 2 M H₂O₂. The reaction was stopped by the addition of 2.0 mL of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in a 1:3 ratio). The specific activity was expressed as μmol of H₂O₂ consumed/min/mg of protein for tissue or μmol of H₂O₂ consumed/min/mg of Hb for erythrocytes.

Estimation of α–Tocopherol (vitamin E)

α–Tocopherol in the plasma, erythrocytes and tissues was estimated by the method of Baker et al., (1980). Vitamin E was extracted from liver tissue by addition of 1.6 mL ethanol and 2.0 mL petroleum ether and centrifuged. The supernatant was separated and evaporated on air. To the residue, 0.2 mL of 0.2% 2,2′-dipyridyl, 0.2 mL of 0.5% ferric chloride was added and kept in dark for 5 min. An intense red coloured layer obtained on addition of 4 mL butanol was read at 520 nm. The values were expressed as mg/dL for plasma or μmol/mg protein for tissue or μg/mg of Hb for erythrocytes.

Estimation of superoxide dismutase

Superoxide dismutase in the erythrocytes and tissues was assayed by the method of Kakkar et al., (1978), based on inhibition of the formation of nicotinamide adenine dinucleotide, phenazine methosulfate, and amino blue tetrazolium formazan. A single unit of enzyme was expressed as 50% inhibition of nitroblue tetrazolium reduction per minute per milligram of protein.

Estimation of catalase

Protein in the tissues was determined after trichloroacetic acid precipitation by the method of Lowry et al., (1951), using bovine serum albumin as the standard at 660 nm. Values were expressed as mg/g of tissue.

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mg of xylene orange, and 9.8 mg of ammonium iron sulfate added to 90 mL of methanol and 10 mL of 250 mM sulfuric acid and incubated at 37°C for 30 min. The color developed was read at 560 nm colorimetrically. Lipid hydroperoxides were expressed as mmol/mL of plasma or mmol/100 g of tissues or μmol/mg of protein for erythrocytes.

Estimation of reduced glutathione (GSH)

Reduced glutathione (GSH) was determined by the method of Ellman (1959). One milliliter of supernatant was treated with 0.5 mL of Ellman’s reagent (19.8 mg of 5,5′-dithiobisnitrobenzoic acid in 100 mL of 0.1% sodium citrate) and 3 mL of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm in spectrophotometer. To prevent the autoxidation of GSH, the samples were reduced with potassium borohydride prior to analysis. GPx activity was expressed as grams of GSH consumed per minute per milligram of protein and reduced GSH as milligrams per 100 g of tissue.

Estimation of glutathione peroxidase (GPx)

GPx activity was measured by the method described by Rotruck et al., (1973). A known amount of enzyme preparation was allowed to react with hydrogen peroxide in the presence of GSH for a specified time period. Then the remaining GSH was measured by the method of Ellman. The glutathione S-transferase (GST) activity was determined spectrophotometrically by the method of Habig et al. in which 1-chloro-2,4-dinitrobenzene was used as a substrate.

Estimation of ascorbic acid (vitamin C)

Ascorbic acid in the plasma, erythrocytes and tissues was estimated by the method of Roe and Kuether (1943). To 0.5 mL of liver homogenate, 1.5 mL of 6% TCA was added and centrifuged (3500×g, 20 min). To 0.5 mL of supernatant, 0.5 mL of DNPH reagent (2% DNPH and 4% thiourea in 9N sulfuric acid) was added and incubated for 3 h at room temperature. After incubation, 2.5 mL of 85% sulfuric acid was added and colour developed was read at 530 nm after 30 min. The values were expressed as mg/dL of plasma or μg/mg of protein for tissue or μg/mg of Hb for erythrocytes.

Figure 2: (a) Hepatocytes of diabetic control rat showing fatty change with inflammation in portal triad. (b) Hepatocytes of diabetic rat treated with 3-HMX showing normal cells with mild inflammation in portal triad.
In Table 3, 45 days restored the antioxidant levels to near normal. Activities of SOD, CAT and GPx and treatment with 3-HMX for diabetic rats. Diabetic rats had decreased activities of CAT and GPx in the erythrocytes and liver of normal rats. However, the parameters studied in diabetic rats. However, the activities of SOD and CAT in diabetic rats. The effect exerted by 3-HMX (40 mg/kg bw) was less effective than glibenclamide. Oral administration of 3-HMX (40 mg/kg bw) to normal rats had no significant effect.

DISCUSSION

Lipid peroxidation is one of the characteristic features of chronic diabetes. Lipid peroxide mediated damage has been observed in the development of both type I and type II diabetes mellitus. It has been observed that insulin secretion is closely associated with lipoxygenase derived peroxides (Walsh & Pek 1984). Low levels of lipoxygenase peroxides stimulate the secretion of insulin, but when the concentration of endogenous peroxides increases it may initiate uncontrolled lipid peroxidation leading to cellular infiltration and islet cell damage in type I diabetes (Metz 1984).

In the present study, increased levels of lipid peroxidative products such as TBARS and hydroperoxides were observed in diabetic rats. Tremendous increase in lipid peroxidation observed in diabetic rats is attributed to chronic hyperglycemia which causes increased production of reactive oxygen species (ROS) due to the auto-oxidation of monosaccharides (Wolf & Dean, 1987), which cause tissue damage by reacting with polyunsaturated fatty acids (PUFA) in membranes (Das et al., 2000). The observed increase in these levels could be due to decrease in enzymatic and nonenzymatic defense system in streptozotocin diabetic rats. Treatment with 3-HMX prevented the increase of lipid peroxidation markers, which could be as a result of improved glycemic control.

GSH, being the most important biomolecule against chemically induced toxicity, can participate in the elimination of reactive intermediates by reduction of hydroperoxides in the presence of GPx. GSH also functions as free radical scavenger and in the repair of radical cause biological damage (Meister, 1984; Nicotera & Orrenius 1986). Under in vivo conditions, GSH act as an antioxidant and its decrease is reported in the liver of STZ-induced diabetic condition (Venkateswaran and Pari 2003). Administration of 3-HMX increased the content of GSH in erythrocyte and liver of STZ-diabetic rats. Activities of GPx, decrease significantly in diabetic rats. GPx an enzyme with selenium catalyses the reduction of hydrogen peroxide and hydroperoxide to non-toxic products (Bruce et al., 1982). Administration of 3-HMX increased the content of GPx in liver and kidney of STZ-diabetic rats.

Activities of Superoxide Dismutase (SOD) and Catalase (CAT) in plasma, erythrocyte and liver have been reduced in diabetic rats. SOD catalyses the dismutation of the highly reactive Superoxide anion to oxygen and hydrogen peroxide (McCord et al., 1976). Hemoprotein Catalase which catalyze the reduction of hydrogen peroxide and protects the tissues from highly reactive hydroxyle radicals (Chance et al., 1952), i.e., Reduction in the activities of SOD and CAT may result in a number of deleterious effects due to the accumulation of Superoxide anion radicals and hydrogen peroxide. Administration of 3-HMX, increase the activities of SOD and CAT in diabetic rats.
Decreased activities of the enzyme may result in the involvement of deleterious oxidative change due to the accumulation of toxic products (Venkateswaran and Pari 2003). Reports have shown that the activities of SOD, Catalase and GPx were lowered in tissues of diabetic rats (Anuradha and Selvam 1993). 3-HMX treated diabetic rats showed decreased lipid peroxidation associated with increased activities of the antioxidant enzymes.

Vitamin C is a potent antioxidant, which widely acts on oxygen free radicals (OFR) as well as interact with vitamin E (Garg and Bansal, 2000). Vitamin E is a lipophilic antioxidant and inhibits lipid peroxidation, scavenging lipid peroxyl radicals to yield lipid hydroperoxides and the α-tocopheroxyl radical (Stahl and Sies, 1997). Both the vitamins C and E significantly decreased in the plasma, erythrocytes and the liver of diabetic rats. The decreased level of α-tocopherol found in the diabetics as compared with control rats could be due to the increased oxidative stress, which accompanies the decrease in the level of antioxidants, and may be related to the causation of diabetes mellitus. In this context, Jennings et al., (1987) reduced plasma concentration of vitamin C in diabetics and Garg et al., (2000) reported the decreased level of plasma α-tocopherol in streptozotocin diabetic rats. Administration of 3-HMX increased the vitamin C and E levels.

Histological study of liver showed fatty changes surrounding portal triad in the liver of diabetic rats and treatment with 3-HMX prevented the damage by decreasing lipid peroxidation and improving antioxidants status.

In conclusion, the present study demonstrates that 3-HMX (at 40 mg/kg BW) exhibits antilipidperoxidative and antioxidant activities in streptozotocin-diabetic rats by decreasing the levels lipid peroxidation products and increasing the levels/activities of antioxidants. Further detailed investigation is necessary to find out its mechanism of action and to establish its therapeutic potential in the treatment of diabetes and diabetic complications.

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References


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