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CENTRAL NERVOUS SYSTEM PROTECTION BY *Catharanthus roseus* LEAF EXTRACT IN STREPTOZOTOCIN-INDUCED DIABETES IN RAT BRAIN.

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Abstract- In diabetes mellitus 60% to 70% of morbidity causes due to Diabetic neuropathy. It is a complication of long term diabetes mainly caused by hyperglycemia and produces oxidative stress in the central nervous system which causes an imbalance in the oxidative status of nervous tissue and leads to micro vascular cerebral diseases. The reason for high risk of micro vascular cerebral diseases, despite the fact that brain consumes 20% of the oxygen in the body, is that it has a low content of antioxidants and high content of unsaturated fatty acids and catecholamines that are easily oxidized, making the brain more vulnerable to oxidative damage than any other organs in the body. Oxidative stress plays important role in the pathogenesis of neurodegenerative changes.
The objective of the present study was to investigate the possible neuroprotective effect of *Catharanthus roseus* leaf extract against streptozotocin-induced hyperglycaemia in the rat brain. Thirty two adult male Wistar rats were divided into four groups as follows: control group, control *C. roseus* treated group, streptozotocin-induced diabetic-untreated group, and streptozotocin-induced diabetic *C. roseus* -treated group. Diabetes was induced by a single injection of streptozotocin (STZ) (55 mg/kg body weight). Three days after streptozotocin injection, *C. roseus* (100 mg/kg body weight) was given orally daily over 8 weeks to the rats in the treatment group. Eight weeks later, all rats from each group were killed and the brain was removed and used for biochemical studies (lipid peroxidation measuring malondialdehyde [MDA], xanthine oxidase [XO], Sorbitol DH [SD] and glutathione). MDA, XO and Sorbitol DH levels in brain of streptozotocin-induced diabetic-untreated group increased significantly. Treatment with *C. roseus* significantly reduced MDA, XO and Sorbitol DH production and increased glutathione levels when compared to the streptozotocin-induced diabetic-untreated group. This study demonstrates that *C. roseus* leaf extract is a potent neuroprotective agent against diabetic oxidative damage.
Keywords- *C. roseus*, diabetic rat brain, SD, XO, MDA, GSH

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Introduction

Diabetic neuropathy is a complication of long-term diabetes and it is estimated that 60-70% of diabetics have mild to severe forms of nervous systems damage. Neuropathy is one of the major complications contributing to morbidity in patients with diabetes mellitus. Hyperglycemia-induced oxidative stress has been implicated in the development of diabetic neuropathy in the peripheral (PNS) and central nervous system (CNS) [7-10,17]. Diabetes may have a deleterious effect on the CNS [15]. Diabetes is characterized by hyperglycemia and metabolic abnormalities due to decreased insulin levels, causing metabolic and physiological changes in various organs including brain [11]. Glucose uptake in peripheral nerves is not dependent on insulin. Under hyperglycemic condition high glucose flux through the sorbitol pathway accounts for one-third of glucose metabolism [12]. Therefore, high blood glucose levels in diabetes lead to high nerve glucose concentrations. In diabetic tissues excess glucose is metabolized to sorbitol, resulting in an accumulation of the intracellular organic osmolyte sorbitol, and causes the depletion of myo-inositol and taurine, an organic intracellular physiologic osmolytes. This depletion causes slowing of nerve conduction and leads to neuropathy, cataract formation and renal abnormalities. In addition to the changes in the intermediary metabolites of the polyol pathway, disturbances in NADPH and NADH balances, beside a reduction in glutathione

level were also encountered in diabetes. All may contribute to the etiology of diabetic neuropathy.

Brain consumes oxygen more rapidly due to high ATP demand and thus susceptible to interference with mitochondrial function, which in turn leads to increased superoxide radical formation, leads to tissue oxidative damage. Oxidative damage in rat brain is increased by experimentally induced hyperglycemia [2]. Cellular biomolecules like lipids, DNA, and proteins are the most susceptible to oxidatative damage. Reaction of ROS with lipids leads to the highly damaging reaction, lipid peroxidation and forms lipid hydroperoxides that breakdown to malondialdehyde (MDA) several products of lipid peroxidation and causes haemolysis of erythrocytes, damage to cardiomyocytes and degeneration of cellular membranes of brain cells and nervous tissues. During lipid peroxidation the products formed such as lipid hydroperoxides can alter the physical characteristics of the membrane, loss of its bound enzymes and can attack other cellular targets including DNA and proteins. Several lines of evidence indicate that the modified oxidative state induced by chronic hyperglycemia [2] may contribute to nervous tissue damage. Cell death leads to metabolism of purines by the induction of an enzyme xanthine oxidase [4,5], which plays an important role in contributing free radical mediated damage. Thus enhanced xanthine oxidase activity may also contribute to the generation of oxygen free radicals and oxidative stress.

Materials and Methods

Chemicals and drug

Streptozotocin (STZ) was purchased from Sigma-Aldrich Co. (St. Louis, MD, USA). Other chemicals and reagents used were of Analytical grade.

Plant material

Fresh mature leaves of *C. roseus* (white variety) were collected during Sep 2006, from University campus and taxonomically authenticated by Department of Botany, Sri Krishnadevaraya University, Anantapur, A.P. Leaves were shade dried and then grinded into fine powder.

Preparation of *C. roseus* leaf extract

The aqueous extract of fresh *C. roseus* leaf powder was orally administered at a dosage of 100 mg kg^{-1} body weight to treated rats once a day for 60 days. Normal and diabetic untreated rats were treated with distilled water.

Animals

Male albino wistar rats between 1 to 2 months of age and weighing 130-150 gm were used for the present study. The animals were procured from Sri Venkateswara enterprises, Bangalore. Animals were maintained as per the guidelines of NIN animal user's manual. Animals were acclimatized for 7 days to our animal house, maintained at temperature of 22 to ± 2^{o}C. The light source in the animal room was regulated with 12 hr light period followed by12 h dark schedule. The rats were fed on a standard pellet diet purchased from Sai Durga Feeds and Foods, Bangalore, and water *ad libitum*.

Induction of experimental diabetes mellitus in rats

Diabetes was induced in rats by a single intraperitoneal injection of freshly prepared STZ with a dosage of 55 mg kg^{-1} body weight, in 0.05 M citrate buffer (pH 4.5) after overnight fasting for 16 h. Seventy-two hours after STZ administration, plasma glucose level of each rat was determined for confirmation of diabetes. Rats with fasting plasma glucose greater than 300 mg/100 ml were considered diabetic and used for further studies in the present investigation.

Experimental design

In the experiment a total number of 40 rats, (20 normal and 20 STZ diabetic surviving rats) were used. The rats were divided into four groups of ten rats each.

Group 1: Normal rats (N).
Group 2: Normal rats treated with *C. roseus* (NT).
Group 3: Diabetic untreated rats (DUT).
Group 4: Diabetic rats treated with *C. roseus* (DT).

After the experimental period, all animals were sacrificed by cervical dislocation, immediately whole brain was dissected out, washed in ice cold saline to remove the blood. The brains were weighed and 10% tissue homogenate was prepared in 0.15M potassium chloride by using potter-elvehjem homogenizer at 4^{0}C. The whole homogenate was divided into two parts, one part i.e. the whole homogenate was used for estimation of glutathione and lipid peroxidation. Another part is centrifuged at 12,000 rpm for 45 min at 0-4^{0}C. After centrifugation, the clear supernatant was used for the assay of xanthine oxidase and sorbitol DH enzyme activities.

Biochemical analysis

Lipid peroxidation

The extent of lipid peroxidation was determined by assaying malondialdehyde (MDA) formation by the method of Utley *et al.,* (1967). Malondialdehyde, formed from the breakdown of polyunsaturated fatty acids serves as a product of lipid peroxidation that reacts with thiobarbituric acid to give a red coloured complex measured at 535 nm.

Reduced Glutathione (GSH)

Reduced glutathione was measured by following the method of Ellman's (1959). This method was based on the development of a yellow colour, when 5,5^{1}-dithio-2-nitro benzoic acid (DTNB) reacts with the compounds containing sulfhydryl groups with a maximum absorbance at 412 nm.

Xanthine Oxidase

Xanthine oxidase catalyses the oxidation of xanthine to uric acid. The activity of an enzyme was assayed by following the increase in uric acid concentration which was measured spectrophotometrically at 290 nm (Roussos GG, 1967).

Sorbitol dehydrogenase (EC 1.1.1.14)

Sorbitol dehydrogenase activity was measured by the method of Gerlach and Hiby as described by Berg Meyer and Bernt (1974). SDH catalyses the reduction of fructose to sorbitol in the presence of NADH as a reducing agent. The activity was measured by monitoring the decrease in the absorbance at 340 nm.

Statistical analysis

The percent change in the studied biochemical parameters of DT rats were calculated using the corresponding values of N and DUT rats and these values were subsequently used for calculation of percent recovery. The results are expressed as means ± S.E.M. Data was analysed using Duncan's Multiple Range (DMR) test p values of <0.05 and Student's t-test p values of <0.001 p values were considered to be statistically significant.

Results

The diabetic state is associated with hyperglycemia and an increased in tissue oxidative stress, which might be reflected in the changes in the tissue enzyme activities.

Effect on LPO and Reduced GSH

As shown in table (1) and Fig. (1), STZ-induced diabetes caused significant elevation in brain MDA (108%) and significant decrease in content of reduced glutathione (56%) compared to normal group. Administration of *C. roseus* to diabetic rats significantly decreased the levels of MDA and increased the content of reduced glutathione. Treatment of normal rats with *C. roseus* did not show significant change in lipidperoxidation and the content of reduced glutathione.

Table 1- Effect of C. roseus treatment on lipid peroxidation and glutathione of Brain tissue in STZ induced diabetic rats

Groups	Lipid peroxidation (n moles of MDA formed/mg protein)	Glutathione (µg of GSH/ mg protein)
Normal	1536.5±34[b]	0.222±0.007[a,b]
Normal C. roseus treated	1389.9±48[a]	0.242±0.01[a]
Diabetic untreated	3193.7±50[d]	0.145±0.006[c]
Diabetic C. roseus treated	2473.7±24[c]	0.203±0.003[b]

Values are mean ± S.E.M Values with different superscripts across the row are significantly different at P<0.05 (Duncan's multiple range test).

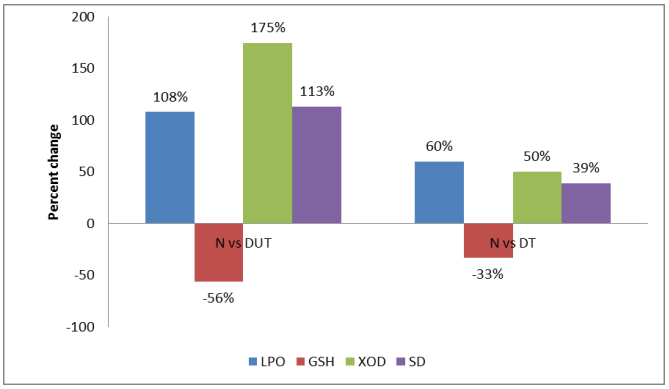

Fig.1- Effect of *C. roseus* treatment on recovery of enzyme activities of brain tissue in stz induced diabetic rats

Effect on XOD and SD

Table (2) and Fig. (1) shows significantly increased in XOD (175%) and SD (113%) activities in STZ-induced diabetic control rats, compared to normal group. Treatment of the diabetic animals with *C. roseus* reversed the changes of these enzymes to control levels and showed the maximum restoratation activities.

Table 2- Effect of C. roseus treatment on Xanthine oxidase & Sorbitol DH of Brain tissue in STZ induced diabetic rats

Groups	Xanthine oxidase (µmoles of uric acid formed/min/mg protein)	Sorbitol DH (n moles of NADH consumed/min/ mg protein)
Normal	3.54±0.174 [a]	0.61±0.24[a]
Normal *C. roseus* treated	2.74±c0.19 [b]	0.53±0.21[b]
Diabetic untreated	10.6±0.52[c]	1.30±0.52[c]
Diabetic *C. roseus* treated	6.16±0.32 [d]	0.85±0.34[d]

Values are mean ± S.E.M Values with different superscripts across the row are significantly different at P<0.05 (Duncan's multiple range test).

Discussion

STZ-induced diabetes provides a relevant example of endogenous chronic oxidative stress and hyperglycemia. Thus, we evaluated the brain oxidative stress and neuropathy induced by STZ in wistar rats and examined the potential protective effects of *C. roseus* against the STZ induced changes. In the present study, rats subjected to STZ-diabetes showed significant increase in brain LPO, XOD, SD activities and decreased GSH content, compared to normal values.

The brain is deficient in oxidative defense mechanisms and hence is at great risk of damage mediated by reactive oxygen species (ROS) resulting in molecular and cellular dysfunction and interacts with cellular components such as lipids, proteins and DNA [13]. The central nervous system (CNS) is vulnerable to free radical damage because of brain's high oxygen consumption, its abundant lipid content, and the relative paucity of antioxidant enzymes as compared with other tissues [24]. Reactive oxygen species (ROS) or oxygen-free radicals, especially superoxide anion radical, hydroxyl radical and alkyl peroxyl radical, are potent initiators of lipid peroxidation. MDA is one of the end products of the peroxidation of membrane lipids, currently considered to be basic markers of oxidative stress, which accumulates when lipid peroxidation increases. Our reports of increase in extent of LPO in diabetic rat brain was supported by earlier studies [18,23] suggesting that the increase in extent of LPO plays a role in the development of diabetic complications in brain. Treatment of *C. roseus* decreases in LPO or reduces free radical mediated damage and development of diabetic complications in brain.

Increase in MDA levels are much less than that of xanthine oxidase. Moreover, the measurement of MDA levels is complicated and time consuming as compared to the assay of xanthine oxidase activity. Xanthine oxidase is an important source of free radical generation. During oxidative stress, the adenosine nucleotide pool is degraded to hypoxanthine and xanthine, along with conversion of xanthine dehydrogenase to xanthine oxidase [20]. Xanthine oxidase acts on xanthine and hypoxanthine with the resultant production of oxygen free radicals [22]. Highly significant increase in the activity of xanthine oxidase in the brain of STZ-induced diabetic group indicates that the measurement of xanthine oxidase activity may be used as a biochemical marker of neuropathy [4]. Treatment of *C. roseus* decreases in XOD and reduces production of oxygen free radicals.

Previous researches revealed an accumulation of sorbitol and fructose in neural tissues of diabetic animals [1]. Increased activity of brain SD might attribute mainly to the marked elevation in glu-

cose content demonstrated in diabetic rat brain by many investigators [14,16]. On the other hand, the increase in brain SD activity of diabetic animals might be considered as an enzymatic adaptation which facilitates the degradation of the accumulated sorbitol [21]. Sorbitol doesn't readily diffuse across the cell membrane and tends to accumulate in the cell. On the other hand, accumulation of sorbitol in nervous tissue of diabetic animals increased the cellular osmolarity resulting in water retention, cell oedema and an increase in cytosolic Na+ concentration. Enhanced metabolism of glucose via the polyol pathway play an important role in the pathogenesis of diabetic complications [19] due to depletion of NADPH, necessary for synthesis of GSH. The ability of *C. roseus* to reduce significantly the increased brain AR activity might be attributed to its decreasing effect on the high brain glucose content via increasing its utilization especially through glycolysis. The stimulatory effect of *C. roseus* on the glycolytic pathway seems to be related to its powerful antioxidant property which may preserve the easily oxidizable sulfhydryl groups of rate limiting enzymes of the glycolytic pathway. However, the ability of *C. roseus* in restoring the increased activity of brain SD, observed in diabetic rats, might be related to the obtained increase in the level of brain GSH by this treatment. Increased cellular GSH level was considered as one of the inhibitory factors for SD activity [6].

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