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AMELIORATIVE EFFECT OF Coccinia *grandis* LEAF EXTRACT ON NEUROCHEMICAL ALTERATIONS OF SCIATIC NERVE IN STZ INDUCED DIABETIC RATS.

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ABSTRACT

The role of *Coccinia* as a hypoglycemic agent in diabetic rats has been proved earlier. The present study investigates the effect of *Coccinia* on glucose metabolism of sciatic nerve in STZ induced diabetic rats, and reports its amelioration. *Coccinia grandis* leaf extract (200 mg/kg body weight) was administered orally to STZ induced diabetic rats for 3 weeks. Metformin (150mg/kg body weight) was used as standard reference drug. After 21 days of experimentation neurochemical alterations such as glucose, fructose, sorbitol and reduced glutathione were determined in sciatic nerve. Enzymes such as hexokinase, aldose reductase, sorbitol dehydrogenase and glucose-6-phosphate dehydrogenase were also determined after 3 weeks. A histopathological alteration of sciatic nerve was also studied. Plasma glucose levels were significantly reduced after oral administration of *Coccinia* to diabetic rats for 21 days. In normal glycolytic pathway glucose is channelized through it, but reduced hexokinase activity in STZ induced diabetic rats indicated a portion of the glucose was found to be directed to polyol pathway, which was evident by increased polyol pathway enzymes aldose reductase and sorbitol dehydrogenase activity, glucose-6-phosphate dehydrogenase causing accumulation of sorbitol and fructose. Decreased reduced glutathione content in sciatic nerve indicates its antioxidant effect. *Coccinia* treatment forbade activation of the polyol pathway and its metabolites accumulation. The sciatic nerves were also protected against the structural abnormalities found in diabetes with *Coccinia* treatment. The results suggest that it has the property to ameliorate the progression of diabetic complications in peripheral nerve with its efficient antidiabetic effect.

KEYS WORDS: Coccinia *grandis*, Polyol pathway, Metformin, Sciatic nerve.

INTRODUCTION

Hexokinase is the first enzyme in glycolysis which acts on cellular glucose phosphorylating to glucose 6-phosphate. Nonphosphorylated glucose which is minor part enters the so called polyol pathway, which is an alternate pathway of glucose metabolism. Aldose reductase (AR) is the primary enzyme in polyol pathway which reduces glucose into sorbitol, which is the rate limiting step of polyol pathway. This sorbitol is subsequently converted into fructose by sorbitol dehydrogenase, thus constituting the polyol (sorbitol) pathway. But under hyperglycemic condition hexokinase is saturated with ambient glucose, diverting most of this glucose through polyol pathway which accounts as much as one third of total glucose. [1] As a result it leads to overflow of the products of the polyol pathway along with depletion in NADPH (nictotinamide adenine dinucleotide phosphate) and the oxidized from of nictotinamide adenine dinucleotide (NAD), the cofactors used in the pathway. Thus elicits various metabolic imbalances in those tissues that undergo insulin independent uptake of glucose with activation of polyol pathway. Such metabolic disruption evokes the early tissue damage in target organs of diabetic complication, such as lens, renal glomerulus and peripheral nerve. [2, 3] In diabetes, advanced glycation end products accumulate in sciatic nerve as result of non-enzymatic glycation of structural proteins. Among both glucose and fructose, fructose is more potent as a glycation agent,[4] and the formation of fructose is augmented because of the accelerated flux of glucose through the polyol pathway. As a result the structure and function of various macromolecules in the sciatic nerve is altered resulting in increased non-enzymatic glycation causing basement membrane thickening, demyelination, and impaired axonal transport as a result of the glycation of myelin, tubulin and neurofilaments.[5]

Activation of polyol pathway results in enhanced glycation process by supplying a reactive glycation agent fructose. Under hyperglycemic condition accelerated polyol pathway is assigned for availability of reduced glutathione, depleting the cofactor NADPH for glutathione reductase, which will lead to oxidative stress. The toxic effects of hyperglycemia can be interrelated to each other and linked to enhanced polyol pathway activity which is the most acknowledged mechanisms. Plant products have been proven to be beneficial in the treatment of various diseases including diabetes.Coccinia *grandis*, the ivy guard, also known as baby watermelon, little gourd or gentleman's toes is a tropical vine. It belongs to family

cucurbitaceae. Since long before the leaves are consumed to control of hyperglycemia as indigenous system of medicine.[6, 7] Reduction of sugar absorption from the gut, increased insulin production from the pancreas, reduction of release of glucose from the liver, increasing glucose uptake by fat and muscle cells are probable mechanisms which may be involved. The aim of this study was to test efficacy of Coccinia *grandis* leaf extracton glucose metabolism and polyol pathway of sciatic nerve.

MATERIAL AND METHODS

Animals

All experiments were performed on Wistar adult male rat(National centre for Laboratory animal sciences, National Institute of Nutrition, Hyderabad), weighing 100-200 gms. The rats were housed six per cage and allowed free access to food {standard pellet diet (NIN)} and water. The animals were maintained in the climate-controlled animal facility (Dept. of Zoology, Osmania University, Hyderabad) with a 12-h light/12 h dark cycle at a stable temperature 18-22 ◦C. Corn cob was used as bedding material All experimental procedures were performed according to the ethical guidelines of the Institutional Animal Ethics Committee (CPCSEA No, 383/01/a/CPCSE).

Chemicals

STZ was obtained Sigma Chemical (USA).Metformin drug procured from Hetero drugs, INDIA. Other essential chemicals were obtained from SRL biochemical, INDIA.

Plant material and extraction

The fresh leaves of Coccinia *grandis* were collected locally. A voucher specimen (No.018) was deposited at Department of Botany, University College of Science, Osmania University, Hyderabad-500007. Leaves were then shade dried at room temperature. Dry material was coarsely pulverized to powder form. The powder was extracted with boiling water and methanol using rotary evaporator and the crude extracted was used for experiment.

Experimental design

About 30 rats were starved for 24 h and divided into control and experimental groups. Each experimental rat was injected Streptozocin 50 mg/Kg body weight in 100mM Citrate buffer pH 4.5. The control rats and the experimental rats randomly assorted into different groups were treated as follows. Rats were randomly allocated in five groups. The control group were treated physiological saline, this group served as control; Diabetic group were induced with

STZ, this group served as diabetic, Metformin group diabetic rats treated with metformin drug(150mg/kg body weight in RO water) served as Met; Coc+D group where the STZ induced diabetic animals treated with Coccinia *grandis* leaf extract (200mg/kg body weight in RO water);Coc+C group where control animals treated with Coccinia *grandis* leaf extract (200mg/kg body weight in RO water). The experiments were carried out to see the effect of diabetes for short-term on the sciatic nerve. The animals were sacrificed by cervical dislocation after 21 days and various biochemical and morphological studies were conducted on sciatic nerve.

Biochemical estimations

Preparation of tissue extracts

The animals were sacrificed by cervical dislocation after 21st day and sciatic nerves carefully dissected out avoiding extraneous tissue, washed with normal saline, blotted dry, the nerves were immediately transfer and kept at 80 $^{\circ}$C to be used later.10% tissue (sciatic nerve) homogenate was prepared in 50mM potassium phosphate buffer pH 7.2 and centrifuged at 25,000g for 30 min at 4°Cand the supernatant is used for all enzyme assays. For metabolite assays the frozen nerve tissue was homogenized in nine volumes of 1N perchloric acid. The homogenates were centrifuged at 6000×*g* for 10 min. The supernatants were neutralized with 2N KOH and centrifuged at 1200×*g* for 10 min to remove the $KClO_4$ resulting in clear extracts, these clear extracts were used for all metabolite determinations by coupling the reaction with purified enzymes using oxidation/reduction of NAD/NADP using a spectrophotometer as described by Gabbay, 1972.[8]

Enzymatic estimations

Hexokinase (EC 2.7.1.1)

Hexokinase enzyme activity was measured essentially by the method of Gumaa and McLean.[9] The total reaction solution mixture 1ml consisted of 0.1 M Tris-Hcl (pH 7.4), 8 mM $MgCl_2$ (pH 7.0), 0.4 mM NADP+, 8 mM/2mMofATP/Mg^{2+} (pH 7.2), 0.5mMglucose and one unit of purified glucose-6-phosphate dehydrogenase.100µl of supernatant was added to initiate reaction. O.D was followed for 5 min at 340nm contemplating the reduction of NADP as a measure of enzyme activity.

Estimation of Aldose Reductase (AR, EC.1.1.1.21)

The enzyme activity was measured essential by the method of Hayman and Kinoshita.[10]The assay mixture in a final volume of 1ml consisted of 50 µl mercaptoethanol, 50 µl of tissue

extract, 500 µl Potassium phosphate buffer pH 6.2, 100 µl of DL –glyceraldehyde, 100 µl of Liso4, 150 µl of double distilled water, and 50 µl of NADPH. The reaction was pre-incubated at 37°C for 5 min after which the reaction was started by the addition of NADPH and decrease in absorbance (of oxidation of NADPH is measured as an index of Aldose reductase activity) at 340nm was recorded for 5 min spectrophotometrically.

Sorbitol dehydrogenase (EC 1.1.1.14)

Sorbitol dehydrogenase activity was measured by the method of Gerlach and Hiby.[11] The final concentration of: 0.2mM of NADH, 300nM fructose and 0.107 M Triethanolamine buffer (pH 7.4) were added in total assay mixture of 1.5ml. The reaction was initiated by the addition of 0.05 ml of cytosol. The oxidation of NADH at 340 nm was recorded for 5 min spectrophotometrically.

Glucose-6-phosphate dehydrogenase (EC 1.1.1.49)

The reduction of NADP was measured as index of Glucose-6-phosphate dehydrogenase activity was assayed by the method of Baquer *et al.,* 1973.[12] Total reaction mixture consists of final concentrations: 10mMTris-HCL (pH 7.8), 0.25mM D-Glucose-6-phosphate, 6.5μMNADP+ and 0.25mM $MgCl_2$ in final volume of 1ml. The reaction was initiated by the addition of 0.05 ml of cytosol, absorbance were recorded at 340 nm was followed for 5 min.

Units of Enzyme

The oxidation/reduction of one µm of NADH or NADPH per g of tissue/min is defined as one enzyme unit.

Metabolite estimations

Glucose

It was estimated as described by Bergmeyer.[13]The total mixture of 3ml comprises of 100mMTris HclpH 7.8, 0.26 mM NADP+, 8.0 mM ATP, 5.0 mM MgCl2 and appropriate sample volume. Reaction was initiated by adding one unit of purified yeast Glucose-6-phosphate dehydrogenase.

Fructose

It was estimated as described by Bergmeyer.[13]The reaction mixture contained the following in final concentration of 0.25 M triethanolamine pH 7.5, 2.5mM MgSO4, 1.1mMATP, 1.5 unit of purified hexokinase, 0.8 Mm NADP and appropriate sample volume. The reaction was

initiated by adding one unit of purified yeast G-6PDH followed by addition of 3 units of Phosphoglucose isomerase.

Sorbitol

Sorbitol was measured by modified enzymatic method of Malone *et al.*[14] by Fluorescence spectrophotometer. The reaction mixture contained the following in a final concentration of: 0.05 M glycine buffer pH 9.4, 0.2 mM NAD+ and an appropriate amount of sample volume in a final volume of 3 ml. The reaction was initiated by adding 1 unit of sorbitol dehydrogenase. For all the above metabolites measurement the reduction of NAD+ and NADP+ or oxidation of NADH or NADPH was measured at 340 nm.

Reduced glutathione

GSH was estimated as described by Griffith.[15] The final concentration of reaction mixture contained: 0.20mMNADPH, 0.6mM5, 5-dithiobis-(2-nitrobenzoic acid, 0.5 units of glutathione reductase in 125 mM sodium phosphate buffer (pH 7.5), 6.3 mM EDTA and appropriate amount of sample volume. The rate of reduction of 5, 5- dithiobis-(2-nitrobenzoic acid) was measured at 412 nm.

Other estimations

Glucose oxidase-peroxidase (GOD_POD) (Glucose measuring kit from Beacon Diagnostics Pvt Ltd, New Delhi India) method was used for estimating blood glucose. Protein contents in nerve extracts were determined by the method of Lowry et al.[16]

Histological processing

Histology of nerve was studied by explained Federica Di Scipio et *al.*, 2008.[17]

Statistical analysis

Results are presented as mean ± S.E., six in each group. Statistical difference between control and various groups was determined by one-way ANOVA. *p*-values less than 0.05 were considered significant.

RESULTS

The body weight, serum glucose levels and neural proteins of all the Experimental groups are shown are shown in Fig 1, Fig.2 and Fig.3. STZ diabetes caused a significant (46%) decrease in the body weight with age-matched controls. There was no increase in the final body weight in the Met group as well as Coccinia treated animals. STZ-induced diabetes in rats caused

169% increase in the blood glucose levels in comparison to the control group which was restored to 43% in metformin treated animals and 43% in *Coccinia* treated animals. The total neural protein levels of sciatic nerve from diabetic group (38%) showed a significant decrease(p <0.05) as compared to that of control and the treated groups and that of met group showed 32% decreased, animals treated with *Coccinia* showed only 22% decrease.

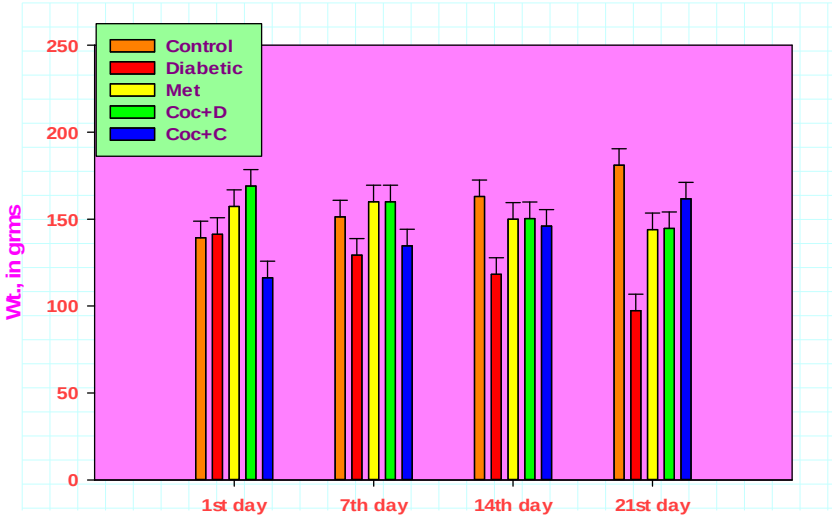


Fig.1.Effect of Coccinia *grandis* leaf extract on body weights of different experimental rats. (Body weight in grams) (*Values are given as mean ± S for groups of six animals each. Values are statistically significant at p<0.05.Significance Control vs Coc+C is < 0.006; Diabetic Vs Coc+C is < 0.009; Met+D Vs Coc+D is < 0.010 respectively*).

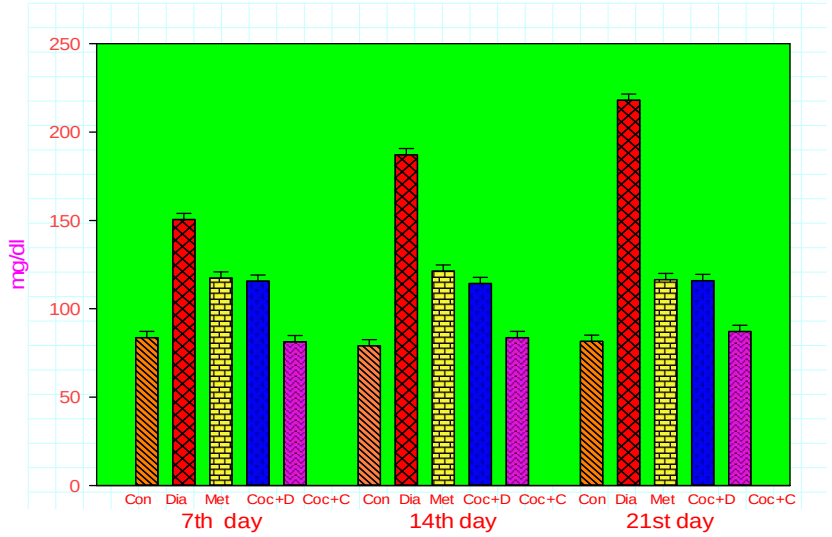


Fig.2 Serum glucose levels in Control, STZ induced diabetic rat and simultaneous treatment with Coccinia *grandis* leaf extract on 7th, 14th, and 21st day of experimental rats. (Serum glucose levels expressed in mg /dl) *(Values are given as mean ± S for groups of six animals each. Values are statistically significant at p<0.05.Significance Control vs Coc+c is < 0.003; Met+D Vs Coc+D is < 0.010 respectively).*

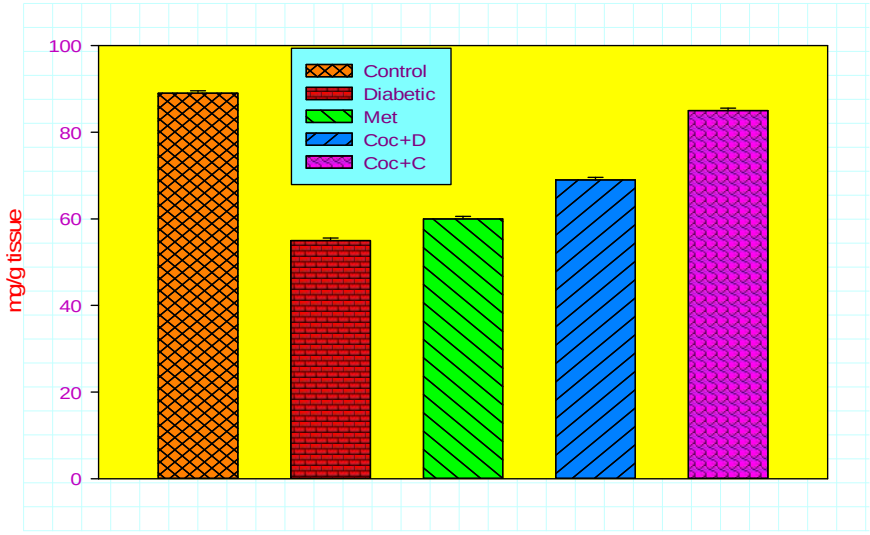


Fig.3. Total proteins of sciatic nerve in Control, STZ induced diabetic rat and simultaneous treatment with Coccinia on 21st day of experimental rats. (*Total proteins expressed in mg/g tissue) (Values are given as mean ± S for groups of six animals each. Values are statistically significant at p<0.05. Significance Control Vs Coc+C is < 0.003; Diabetes Vs Met+D is <0.01 respectively*)

Changes in glucose metabolising enzymes

Hexokinase

The hexokinase activity in sciatic nerve (-33%) was significantly (P<0.05) decreased in STZ induced diabetic rats on 21st day compared to control animals (Fig.4). The hexokinase activity in sciatic nerve was markedly recovered on 21st day after treatment with metformin (-11%) in STZ induced diabetic rats, whereas sciatic nerve has shown reversal in hexokinase activity by (-18%) after treatment of diabetic rats with *Coccinia*. Controls animals treated with Coccinia have shown the hexokinase activity in sciatic nerve is 0%.

Aldose reductase

There was significant increase in aldose reductase enzyme activity in sciatic nerve of diabetic animals (+83%) as compared to normal animals. Diabetic rats treated with *Coccinia* showed decrease in aldose reductase activity by 50% (Fig. 5). Percentage of variation of metformin treated diabetic was 61% and that of control animals treated with *Coccinia* was only 1%.

Sorbitol dehydrogenase

The sorbitol dehydrogenase activity in sciatic nerve (+17%) which was significantly (P<0.005) increased in STZ induced diabetic rats on 21st day (Fig.6). After simultaneous treatment of metformin in STZ induced diabetic rat (Met), the sorbitol dehydrogenase activity was markedly reversed in sciatic nerve on 21st day (+3). *Coccinia* treatment has shown a

marginal reversal as compared to metformin group by just +6%. Controls animals treated with *Coccinia* have shown the Sorbitol dehydrogenase activity in sciatic nerve is -5%.

Glucose 6-phosphate dehydrogenase

Glucose-6-phosphate dehydrogenase (G-6-PDH) activity was significantly ($p<0.05$) decreased in sciatic nerve on 21st day by -27.26% in STZ induced diabetic rat when compared to controls (Fig.7). The treatment of diabetic rats with metformin decreased G-6-PDH activity in sciatic nerve (-15.58%). However the G-6-PDH activity in sciatic nerve is partially regained by -16% when diabetic animals treated with *Coccinia*. Controls animals treated with *Coccinia* have shown the G-6-PDH activity in sciatic nerve is +2%.

Table.1 Effect of *Coccinia* on enzyme activities such as hexokinase, aldose reductase, sorbitol dehydrogenase, G-6-PDH of sciatic nerve on 21st day.(Expressed as µ moles of NADPH oxidized/ hour/100 mg of protein)

Enzymes	Control	Diabetic	Met	Coc+D	Coc+C
Hexokinase	0.2700± 0.00577	0.1800± 0.00577 (33%)	0.2400± 0.0057 (11%)* #	0.2200± 0.00577 (18%)$&	0.2570±0.00577 (7%) @
Aldose reductase	0.1200± 0.00577	0.2200± 0.00577 (-45%)	0.1933± 0.00333 (-58%)	0.1800± 0.00577 (-50%)µ	0.1213±0.05813 (0%)
Sorbitol dehydrogenase	0.5800± 0.00577	0.6867± 0.00333 (-17%)	0.6033± 0.00333 (-3%)^	0.5933± 0.00333 (22%)	0.5533± 0.00333 (5%)
G-6-PDH	0.2560± 0.00333	0.1860± 0.00333 (28%)	0.2160± 0.00333 (16%)	0.2030± 0.00333 (20%) ® β	0.2830± 0.00333 (-12%)

(The values given are mean ± std. error; the mean difference is significant at 0.05 level compared to control.(Percentage of variation).* Control Vs Met is < 0.1; $ Control Vs Coc+D is < 0.02; @ Control Vs Coc+C is < 0.005; # Diabetes Vs Met+D is < 0.009;& Diabetes Vs Coc+C is < 0.002; ^ Control Vs Met+D is < 0.001;µ Met Vs Coc+D is 0.01; ® Diabetes Vs Coc+D is < 0.01; β Met+D Vs Coc+D is < 0.04 respectively).

Changes in metabolites levels

Glucose

The percent variation of glucose content in STZ induced diabetic rats consistently increase in sciatic nerve (+180%) on 21st day when compared to controls (Fig.8). The diabetic rats treated with metformin have reversed the glucose levels by +26%. The glucose content after *Coccinia* administration has shown glucose levels better than metformin treated rats with +10% in sciatic nerve. The glucose levels in control animals treated with *Coccinia* is +2%.

Sorbitol

A spontaneous increase in sorbitol levels increase +76% in sciatic nerve on 21st day in the STZ induced diabetic rats group when compared to control (Fig.9). The sorbitol content in sciatic nerve after treatment with metformin of STZ induced diabetic rats it was gradually recovered on 21st day by +17%. After *Coccinia* treatment, Sorbitol content was prominently recovered in sciatic nerve with +13% when compared to control.

Fructose

The percent variation of Fructose content in STZ induced diabetic rats consistently increase in sciatic nerve (+89%) when compared to controls (Fig.10). The diabetic rats treated with metformin have reversed the fructose levels by +20% in sciatic nerve. The fructose content after Coccinia administration has shown fructose levels with +21%.

Reduced Glutathione

The STZ induced diabetic rats group has shown decrease in glutathione reduced content (% of variation) by -37% on 21st day when compared to control (Fig.11). The glutathione reduced content in sciatic nerve of STZ induced diabetic rats after treatment with metformin was gradually recovered on 21st day by -13%. After *Coccinia* treatment, glutathione reduced content was-21% compared to control in sciatic nerve.

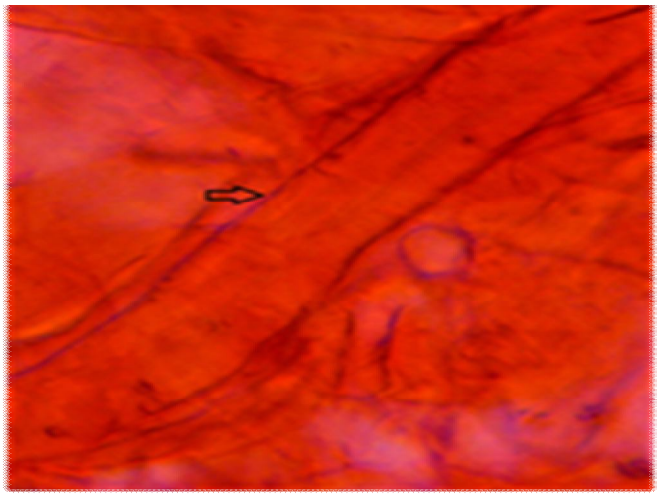
Table.2 Effect of *Coccinia* on neurochemical alterations such as glucose, sorbitol, fructose and reduced glutathione of sciatic nerve on 21st day.(Expressed as µ moles/gm tissue)

Neurochemical/exper. groups	Control	Diabetic	Met	Coc+D	Coc+C
Glucose	1.5200± 0.01856	4.2600±0.03333 (180%)	1.9300±0.03512 (26%)	1.6800±0.00333 (10%)	1.4800±0.00577 (-2%) @
Sorbitol	6.8000± 0.1000	12.0000± 0.0058 (76%)	8.0000± 0.0636 (17%)	7.7000±0.0577 (13%) %	6.6000± 0.0577 (-2%) #
Fructose	0.7460± 0.0033	1.4000±0.05774 (89%)	0.8930± 0.0033 (20%)	0.9030± 0.0033 (21%)*	0.7360± 0.0033 (-1%) &
Reduced glutathione	1.0367± 0.0033	0.6400± 0.00577 (37%)	0.8960± 0.0033 (13%)	0.8100± 0.00577 (21%)	1.0300± 0.00577 (0%) ®

(The values given are mean ± std. error; the mean difference is significant at 0.05 level compared to control.(Percentage of variation) Significance @ Control Vs Coc+C is < 0.1; # Control Vs Coc+C is < 0.05; % Met Vs Coc+D is < 0.005;& Control Vs Coc+C is < 0.06; * Met Vs Coc+D is < 0.07; ® Control Vs Coc+C is < 0.003respectively)

Histological changes in sciatic nerve

Longitudinal Sections of sciatic nerve of all experimental groups are shown in Fig.4. Fig. A is the L.S of sciatic nerve of control rat showing normal histological features with thick myelin membrane. Fig B is the L.S of sciatic nerve of STZ-induced diabetic rat. Myelin membrane was thin at certain regions (←) indicating the segmental demyelination of neuropathy.Fig.C is the L.S of sciatic nerve of Metformin treated diabetic rat which is showing rough myelin membrane indicating the attachment of RER. These RER (Rough endoplasmic reticulum) are attached for protein synthesis suggesting the regeneration of sciatic nerve. Fig D represents the L.S of sciatic nerve of Coccinia treated diabetic rat. It is showing thick membrane (←) indicating the protective capacity of *Coccinia*. Fig.E is L.S of sciatic nerve of *Coccinia* treated control rats (Coc+C) showing normal histological features without disruptions.



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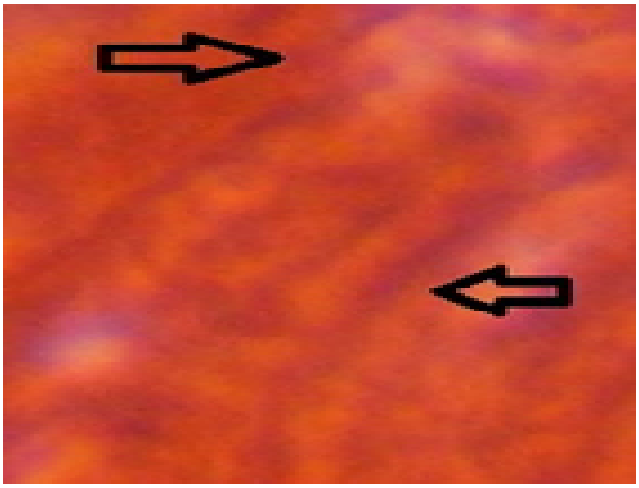

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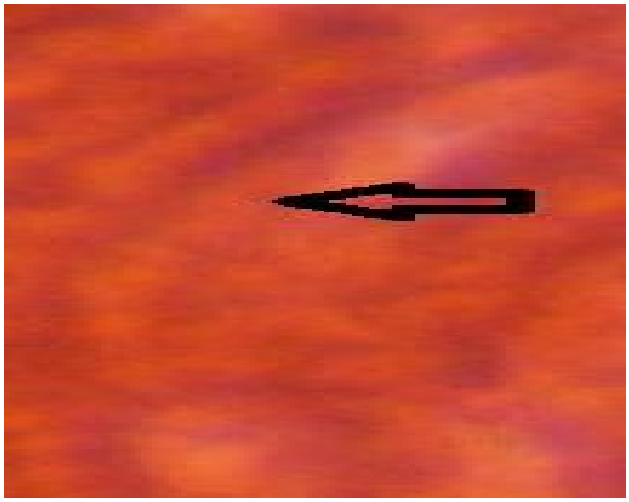

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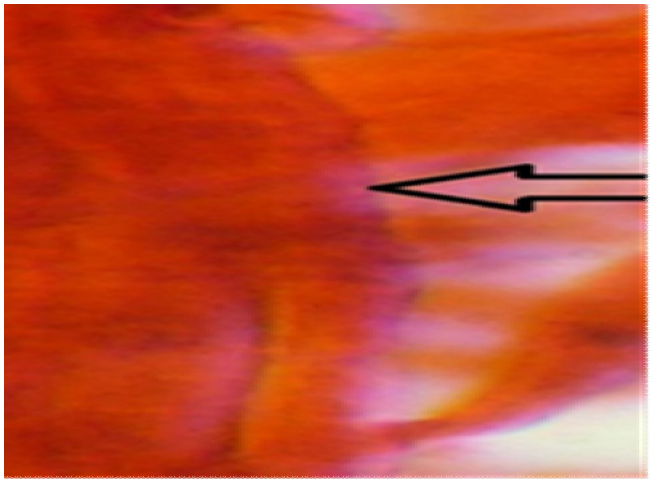

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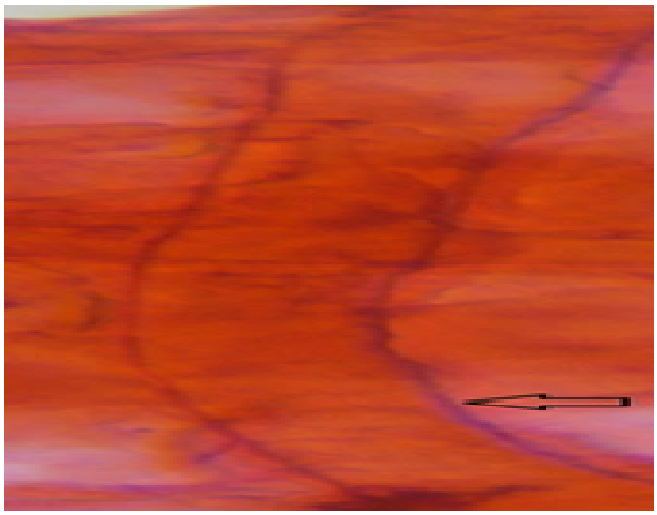

E

Fig.4

Fig. 11 Photomicrographs showing the longitudinal sections of the sciatic nerves. Fig. A is of Control rat (5µm thick, 100X+Immersion oil) showing normal histological features with thick myelin membrane indicated by arrow directing right side (→). B is the photomicrograph of longitudinal section of Sciatic nerve of STZ-induced diabetic rat. Myelin membrane thin at certain regions (→) (←) indicating the segmental demyelination of PN. C is longitudinal section of metformin treatment diabetic rat, which is showing rough myelin membrane (←) suggesting the regeneration of myelin. D represents the L.S of Sciatic nerve of *Coccinia* treated diabetic rat. It shows thick membrane (←). E is of *Coccinia* treated control rat, showing normal histological features without disruptions.

DISCUSSION

One of the microvascular complications of diabetes is diabetic neuropathy effecting peripheral nerves, with widely varying pathology.[18] Elevated blood glucose and several interactive pathogenetic mechanisms have been identified in progression of neuropathy in diabetic state. Polyol pathway activation has been reported to play vital role in diabetic neuropathy among various pathways. Although there is cross talk between these mechanisms, results of several studies suggest that oxidative stress is a major determinant in diabetic complications.[19]

In diabetic rats free radical generation in sciatic nerve have been substantiated from various studies.[20] Hence, the amelioration of oxidative stress using potent antioxidants can be beneficial in diabetic neuropathy. As Coccinia was already proved to be potent antioxidant,[21] this study evaluates the ameliorative effect of Coccinia on polyol pathway of sciatic nerve. Long standing hyperglycemia is known for inducting events in development diabetic neuropathy which initiate progression of structural changes within the nerves.[22] Significant decrease of plasma glucose levels were noticed with administration of Coccinia in STZ induced diabetic rats as compared to that of untreated diabetic rats. Decreased protein synthesis in nerve may be attributed to decreased synthesis of myelin protein in diabetic state, which is responsible for delamination in myelin sheath.

In diabetic neuropathy nerve dysfunction may be due to abnormal glucose metabolism, thereby deranging the normal glycolytic pathway and activating the polyol pathway.[23]In diabetic sciatic nerve glucose utilization via glycolysis may be decreased. There is accumulation of glucose in sciatic due to its free permeability to nerve tissue, [24] which is metabolized, via the activated sorbitol pathway enzymes. Glucose is phosphorylated by hexokinase which makes the first step in glycolysis. Present study has shown decreased activity of hexokinase in sciatic nerve of STZ-induced diabetic rats on 21st. Activation of polyol pathway was indicated by decrease in the hexokinase activity diverting the high glucose through this pathway. The subsequent treatment of diabetic rats treated with leaf extract of *Coccinia* has predominantly reversed the hexokinase activity. Aldose reductase enzymes is the primary enzyme in polyol pathway converting glucose to sorbitol in the presence of cofactor NADPH, this NADPH is competitively utilised by glutathione reductase for generation of reduced glutathione (GSH) an important antioxidant which protects against oxidative stress. The pathophysiological consequences of GSH depletion have been

extensively studied, which promotes generation of reactive oxygen species and oxidative stress with the subsequent cascade of effects affecting the functional and structural integrity of nerve membrane.[25] In the present study level of antioxidant reduced glutathione was decreased in diabetic nerve, is in agreement with earlier studies.[26] Treatment with *Coccinia* increased GSH content, this increased GSH protects cellular proteins against oxidation through the GSH redox cycle and also directly detoxify the reactive oxygen species generation induced by exposure to streptozotocin.[25]Sorbitol dehydrogenase catalyzes the conversion sorbitol to fructose in the presence of NAD. Sorbitol dehydrogenase activity has been reported to be elevated in diabetic rats, leading to increased fructose conversion. The elevated activity of Sorbitol dehydrogenase in diabetic rats may have been due to the increased availability of sorbitol. Sorbitol dehydrogenase activity was found to be significantly reduced after *Coccinia grandis* leaf extract treatment.

Present results show increase in the accumulation of sorbitol and fructose in the nerves from diabetic rats, which were found to be in accordance with the earlier studies.[27] The sorbitol and fructose does not diffuse out of the cells and their accumulation may be responsible for the structural abnormalities of axons due to increase in osmolarity. The accumulation of these sugars also causes an increase in oxidative status of the cell, where the polyol pathway is known to be very active.[28] Diabetes induces the generation of free radicals and their products that manifest structural changes in the nerve fibres over its prolonged duration.[29]

The histological abnormalities observed in diabetic sciatic nerve were not observed when treated with *Coccinia*. *Coccinia grandis* by maintaining good glycemic control aided the restoration of the nerve morphology, as good glycemic control is known to prevent the onset and delays the progression of neuropathy in diabetes. [22] The accumulated polyols may cause oxidative stress in diabetes and may inhibit glycolytic pathway enzymes [30] as evident in the reduced G6PDH activity which derives substrate from glycolysis.

CONCLUSION

In conclusion the polyol pathway is the major contributor to the generation of hyperglycemic oxidative stress in sciatic nerve. Complete reversal was seen in the activity of hexokinase, *Coccinia* treatment appear to attenuate hyperglycemia reduce the influx of glucose through polyol pathway and thus ameliorative effects on progression of diabetic complications in sciatic nerve.

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