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GLUCOSE METABOLISM AND CYCLOOXYGENASE ACTIVITY IN BRAIN OF STZ INDUCED DIABETIC RATS TREATED WITH CURCUMIN

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ABSTRACT

The objective of this research was to study the glucose metabolism and cyclooxygenase metabolism in brain of rats and amelioration of curcumin treatment. This study reports the effect of curcumin (200mg/kg per body wt., / day) on polyol pathway, pentose pathway, Cyclooxygenase metabolism and histological alterations in hippocampus and cerebral cortex of rat with STZ induced diabetes for 3 weeks. Metformin (150mg/kg body weight) was used as standard reference drug. The activities of aldose reductase (AR); sorbitol dehydrogenase (SD) Cyclooxygenase (COX), PG peroxidase were increased whereas the activities of the Glucose 6-phosphate and Na+K+ATPase activity were decreased, and glucose and sorbitol content were increased in diabetic rat brain. The significant decrease AR and SD with curcumin treatments shows its protection against diabetic complications. Decreased COX and PG peroxidase suggests its protections against inflammation. STZ-induced brain damage in the cortex and regions within the hippocampus was seen but histological alterations induced by diabetes in Brain were restored with Curcumin treatment. These results suggest that Curcumin exerts, efficiently, an attenuating effect on the progression of hyperglycemia and also some hyperglycemia-induced complications in rat brain, when compared to Metformin.

KEY WORDS

Diabetic rat brain, Glucose, Hippocampus, Cerebral cortex, Cyclooxygenase, PG peroxidises Aldose reductase.

INTRODUCTION

Hyperglycemia, which occurs under the diabetic condition, is the major culprit in all diabetic complications including brain damage (1, 2). Hyperglycemia will lead to increased activity of polyol pathway in brain. Hyperglycemia is also among the major contributing factors in complications through excessive production of reactive oxygen spices (2). Apart from that, intermediary metabolites of the polyol pathways, also disturbances in NADPH and NADH balances, beside reduction in glutathione level are seen in diabetes. These all may contribute to the aetiology of diabetic complications including neuropathy (3). Changes in glucose metabolites in polyol pathway also targets Hippocampus of Brain (4). As hippocampus is critical integration centre for cognitive functions such as learning and memory. Morphological alterations to hippocampus in the form of impairment in synaptic plasticity and CA-1 field of the hippocampus lead to impairment in memory, learning and cognition in diabetic patients. And in Cerebral cortex neuropathological changes such as damage in neuron, Schwann cells, axons and inflammation have also been reported in several diabetic cases (5)

During inflammation Cyclooxygenase (COX) is an enzyme that plays a vital role in conversion of arachidonic acid to prostaglandins (PGs) (6). Release of these PGs will exacerbate the inflammation further. Hence natural products which inhibit COX are

considered to be analgesics because of management of inflammation by inhibiting COX.

Curcumin is a polyphenolic compound derived by one of the famous spices of India i.e Turmeric. Although various studies have proved that curcumin possess adverse pharmacological properties such as antibiotic, anti-inflammatory, antioxidant etc., Even though the protective effects of curcumin has been various investigated but not firmly stated. Curcumin is also reported to have antiamoebic and antiHIV activities.The curcumin has also been proved to efficient in reducing various diabetic secondary complications such as diabetic nephropathy/renal lesions, retinopathy and reduction of advanced glycation end products. Its potentials as a hypoglycaemic agent have also been proved by various studies both in animals and also in humans.

The present study investigated the enzyme of polyol pathway (AR, SOD) and pentose phosphate pathway (glucose-6-phosphate dehydrogenase), Hexokinase, COX, PG peroxidase, Na+K+ATPase activity and certain substrates of these enzymes such as glucose and sorbital in whole brain and in histological analysis the amount damage to the different regions of hippocampus as well as cortex neurons.

MATERIAL AND METHODS

Experimental animals:

Male Wistar rats weighing 160± 20 g were used for the study. The animals were maintained in the climate-controlled animal facility (Zoology department, Osmania University, Hyderabad), with a 12-h light/12 h dark cycle at a stable temperature 18-22 ◦C. The animals were fed with standard pellet diet (NIN) and tap water *adlibitum*; Corn cob was used as bedding material. The study had approval of Animal Ethical Committee (CPCSEA No, 383/01/a/CPCSE).

Experimental designed:

The rats were divided into five groups.

Group-I served as Control: These animals were treated with physiological saline.

Group-II served as Diabetic: The STZ-induced diabetic animals.

Group-III served as Met: The STZ induced diabetic animals treated with Metformin (150mg/kg body weight in RO water).

Group-IV served as Cur+T: The STZ induced diabetic animals treated with Curcumin (200mg/kg body weight in RO water).

Group-V served as Cur+C: Control animals treated with Curcumin (200mg/kg body weight in RO water).

The animals were sacrificed by cervical dislocation after 21 days; biochemical estimation was conducted in total brain and histological studies were done on hippocampus and cerebral cortex.

Induction of diabetes

Streptozotocin was prepared in freshly prepared citrate buffer (100mM pH 4.5) and was injected intraperitoneally at a concentration of 50 mg per kilogram of body weight of rat. Control rats were injected with citrate buffer only.

Chemicals

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

Biochemical estimation

Preparation of tissue extracts

All the animals were sacrificed by cervical dislocation after 21st day and Brains were carefully isolated and washed in normal saline, stored at -80 ◦C to be used later. 10% of Brain tissue homogenate was prepared in 50mM potassium phosphate buffer pH 7.2 and centrifuged at 25,000g for 30 min at 4°C and the supernatant is used for all enzyme assays. Frozen Brain tissue was homogenized in 9 volumes of 1N perchloric acid for metabolite assays. This homogenate were centrifuged at 6000xg for 10 min, from which the supernatants were neutralized with 2N KOH and centrifuged at 1200xg fir 10 min., to remove the $KCLO_4$ resulting in clear extracts, which was used for metabolite determination by coupling the reaction with purified enzymes using oxidation/reduction of NAD/NADP using a spectrophotometer as described by Gabbay, 1973 (7).

Enzymatic estimations

Hexokinase (EC 2.7.1.1)

Gumaa and McLean, 1972 (8) method was used for measuring Hexokinase enzyme activity.

Estimation of Aldose Reductase (AR, EC.1.1.1.21)

Hayman and Kinoshita, 1965 (9) method was used for measuring AR activity by the oxidation of NADPH was used as index.

Sorbitol dehydrogenase (EC 1.1.1.14)

Gerlach and Hiby, 1974 (10), method was used for measuring Sorbitol dehydrogenase.

Glucose-6-phosphate dehydrogenase (EC 1.1.1.49)

Baquer et al., 1973 (11), method was for measuring Glucose-6-phosphate dehydrogenase activity by studying the reduction of NADP was measured as index. The oxidation/reduction of one µm of NADH or NADPH per g of tissue/min is defined as one enzyme unit.

Na^+K^+ATPase Enzyme (EC 3.6.1.3)

Na^+K^+ATPase were estimated according Kaplay 1978 (12).

Metabolite Estimations

Glucose

Bergmeyer, 1974 (13) method was used for measuring Tissue Glucose.

Sorbitol

Malone *et al*., 1980 (14) modified form of method was used for measuring Sorbitol on fluorescence spectrophotometer.

Other estimations. Protein contents in brain extracts were determined by the method of Lowry *et al*., 1951. (15). The extent of protein oxidation was determined by measuring the protein carbonyl content of soluble protein of tissues (Brain) spectrophotometrically using 2, 4,-dinitro phenyl-hydrazine (16).

Biochemicals estimations

Estimation of Cyclooxygenase (COX assay) - Oxygen consumption test using the biological oxygenmeter was used for Cyclooxygenase assay was performed with the method of oxygen consumption test using the biological oxygenmeter. In translation of Arachidonic acid to PGH2 one molecule of oxygen was utilized, which is measured with the biological oxygen Clark electrode. The oxygen consumption rate is proportional to the enzyme activity.

Preparation of microsomes as a source for Cyclooxygenase - The homogenization buffer in cold condition consist 0.05 Tris-Hcl (pH 8.0), 0.1mM EDTA disodium salt, 0.1mM diethyldithiocarbamate and 0.01% sodium azide was prepared in which 25% homogenization was done. Supernatant was take for COX assay after centrifuging at 21,000rpm for 30 min. Measurement of Cyclooxygenase activity: Add 900 µl of oxygenated phosphate buffer pH 8.0 into mitocel chamber, add 100 µg of protein (source for COX if that expressed) and add 50 µl (10 µM) of Hemin stir well with magnetic stirrer when electrode is stable initiate the reaction with 50 µlAA (100 µM) observe the oxygen deflection curve on computer screen save and calculate the oxygen consumption rate with provided software of biological oxygen meter. Run the reaction 1 to 3 minutes [17].

Estimation of Prostaglandin peroxidase (Prostaglandins Assay) –

Procedure: The PGG to PGH peroxidase activity was determined by the measure of the enzyme-catalysed oxidation of tetramethylenediamine by hydrogen peroxide. The blue reaction product is measured at 610nm in a double-beam spectrophotometer. The experiment conducted at room temperature. 3ml of incubation buffer Tris-Hcl pH 8.0 in to test tube add the enzyme 2-30 µg of protein, 10 µl of Hemin solution at time of reading; add 100 µl of TMPD solution and 100µl H_2O_2 9mM mix well and keep the solution for reading, Set the spectrophotometer on time scan absorbance for every 30 sec up to 2 minutes. For calculation 12,000 liters mol-1cm-1 is found for the molar absorption coefficient of the oxidation product of TMPD. Definition of Unit: one unit of activity is defined as the amount of enzyme required to convert 1 µmol of hydrogen peroxide at 25°c in min [18].

Histological processing

Brains were stored in 10% formaldehyde and later carefully hippocampus and cerebral cortex were care dissected. These tissues were dehydrated, cleaned and embedded in paraffin. They were cut into section with microtome of 5 µm thicknesses, mounted on glass slides and stained with routine hematoxylin and eosin technique (19).

Statistical analysis

Results are presented as mean ± Std. Error., 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 significance.

RESULTS

The total protein levels and protein carbonyls of total brain tissue are shown in Fig.1 and Fig.2. Diabetic rats showed protein and protein carbonyls decrease as compared to the control and other treated groups. Protein carbonyls in diabetic rat caused 27% increase in comparison to control group which was restored to 12% in Metformin treated animal and almost similar trend was shown when treated with curcumin.

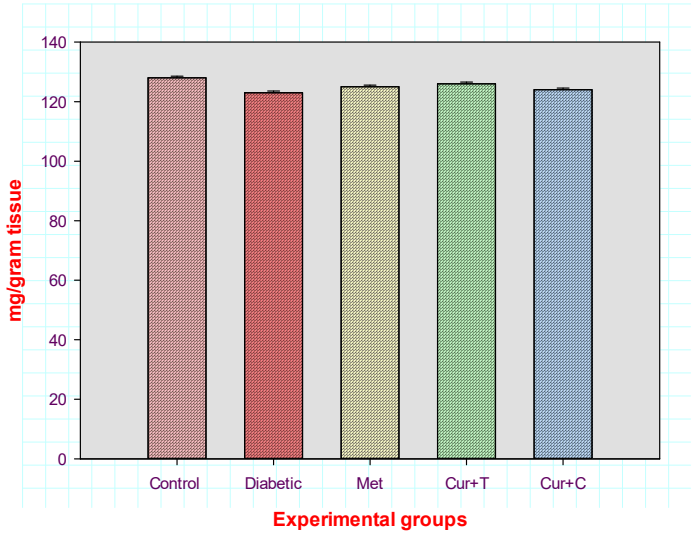

Fig.1: Changed levels of proteins in Brain on treatment with Curcumin on 21st day. (Proteins expressed in mg/gram tissue) (Values are given as mean ± Std. Error for groups of six animals each. Values are statistically significant at p<0.05. Significance)

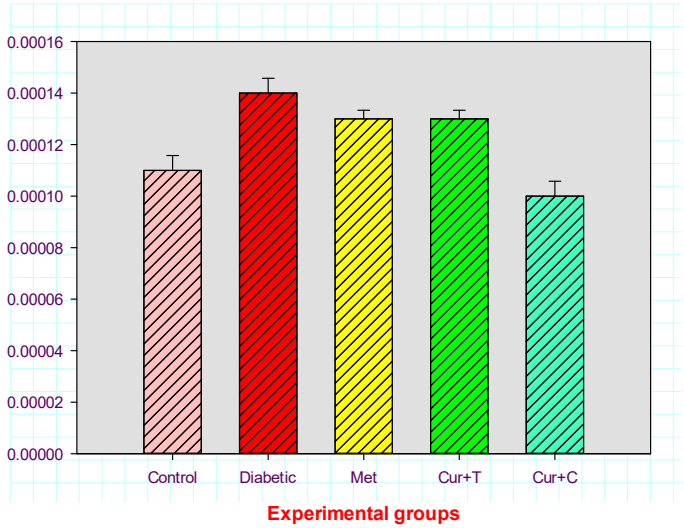

Fig.2: Changed levels of protein carbonyls in Brain on treatment with Curcumin on 21st day. (Proteins expressed in ng/gram tissue) (Values are given as mean ± Std. Error for groups of six animals each. Values are statistically significant at p<0.05. Significance)

Hexokinase

The Hexokinase activity in Brain (-12%) was significantly (P<0.05) decreased in STZ induced diabetic rats on 21st day compared to control animals (Fig.3). The Hexokinase activity in brain (55%) was markedly recovered on 21st day after treatment with Metformin in STZ induced diabetic rats, whereas brain has shown reversal in Hexokinase activity by (-55%) after treatment of diabetic rats with Curcumin. Controls animals treated with Curcumin have not shown any disturbance in Hexokinase activity in Brain.

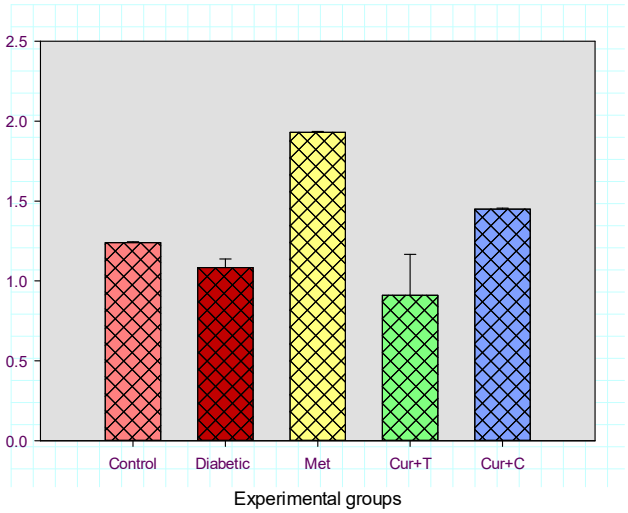

Fig 3: Effect of Curcumin on Hexokinase activity of Brain in Control and other Experimental Rats on 21st day. (Expressed as µ moles of NADPH oxidized/ hour/100 mg of protein) (Values are given as mean ± Std. Error for groups of six animals each. Values are statistically significant at p<0.05. Significance Control Vs Met+D is < 0.1, Control Vs Cur+D is < 0.5, Diabetes Vs Met+D is< 0.009, Diabetes Vs Cur+D is < 0.002, Met+D Vs Cur+C is < 0.1, Met+D Vs Cur+D is < 0.4, Cur+D Vs Cur+C is < 0.5 respectively).

Aldose reductase

There was significant increase in Aldose reductase enzyme activity in Brain of diabetic animals (+11.67%) as compared to normal animals. Diabetic rats treated with Curcumin showed decrease in Aldose reductase activity by 18% (Fig. 4). Percentage of variation of Metformin treated diabetic was 18% and that of control animals treated with Curcumin was -34%.

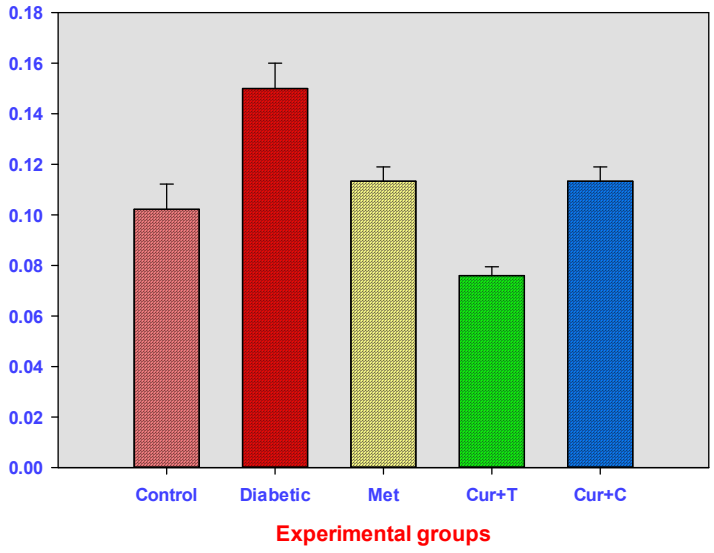

Fig 4. Effect of Curcumin on AR activity of Brain in Control and Experimental group of Rats on 21st day. (Expressed as µ moles of NADPH oxidized/ hour/100 mg of protein) (Values are given as mean ± Std. Error for groups of six animals each. Values are statistically significant at p<0.05Significance Diabetes vs Cur+C is<0.2 respectively) .

Sorbitol dehydrogenase

The Sorbitol dehydrogenase activity in Brain (+18.39%) was significantly (P<0.005) increased in STZ induced diabetic rats on 21st day (Fig.5). After simultaneous treatment of Metformin in STZ induced diabetic rat (Met), the Sorbitol dehydrogenase activity was

markedly reversed in sciatic nerve on 21st day (+4.01%). Curcumin treatment has shown a marginal reversal as compared to Metformin group by +7%. Controls animals treated with Curcumin have shown the Sorbitol dehydrogenase activity in Brain is -4.01%.

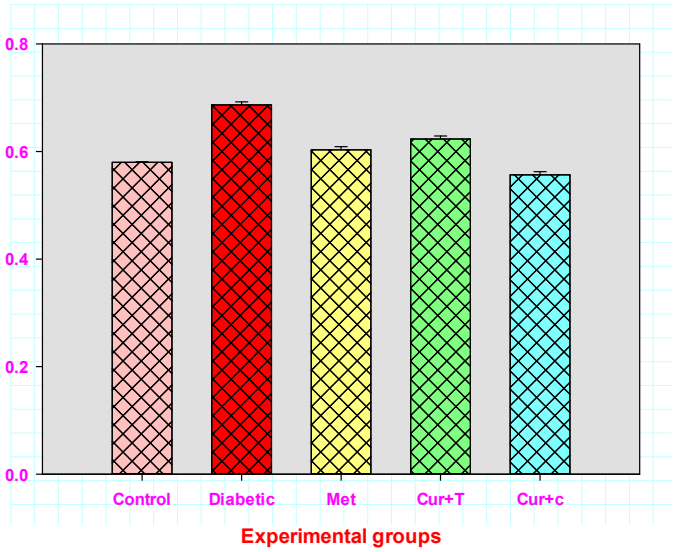

Fig.5: Effect of Curcumin on Sorbitol dehydrogenase activity of sciatic nerve in Control and Experimental group of Rats on 21st day. (Expressed as µ moles of NADPH oxidized/ hour/100 mg of protein)

(Values are given as mean ± Std. Error for groups of six animals each. Values are statistically significant at p<0.05SignificanceControl Vs Met+D is < 0.001, Control Vs Cur+C is < 0.001, Met+D Vs Cur+D is < 0.002 respectively

Glucose 6-phosphate dehydrogenase

Glucose-6-phosphate dehydrogenase (G-6-PDH) activity was significantly (p<0.05) decreased in Brain on 21st day by -19.65% in STZ induced diabetic rats when compared to control (Fig.6). After treatment of diabetic rats with Metformin decrease of G-6-PDH activity in Brain was -7.89%. However, the G-6-PDH activity in Brain is partially regained by -6.50 % when diabetic animals treated with Curcumin. Control animals treated with Curcumin have shown decreased 8.72% the G-6-PDH activity in Brain.

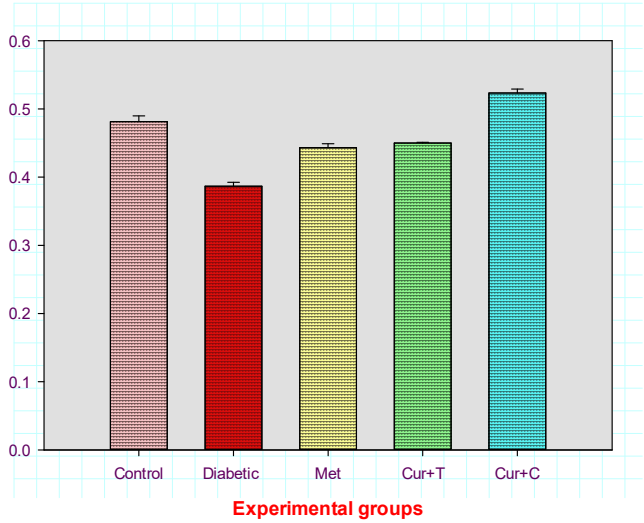

Fig 6. Effect of Curcumin on G-6-PDH activity of Brain in Control and Experimental groups of Rats on 21st day. (Expressed as µ moles of NADPH oxidized/ hour/100 mg of protein)

(Values are given as mean ± Std. Error for groups of six animals each. Values are statistically significant at p<0.05Significance Control Vs Cur+C is < 0.5, Diabetes Vs Cur+D is < 0.04, Met+D Vs Cur+D is < 0.01 respectively)

Substrates

Tissue Glucose

The tissue glucose levels of all the experimental groups are shown in Fig.7. STZ-induced diabetes in rats caused 32.86% increase in the glucose levels in comparison to the control group which was restored to 2% in Metformin treated animals and 0.96% in Curcumin treated animals.

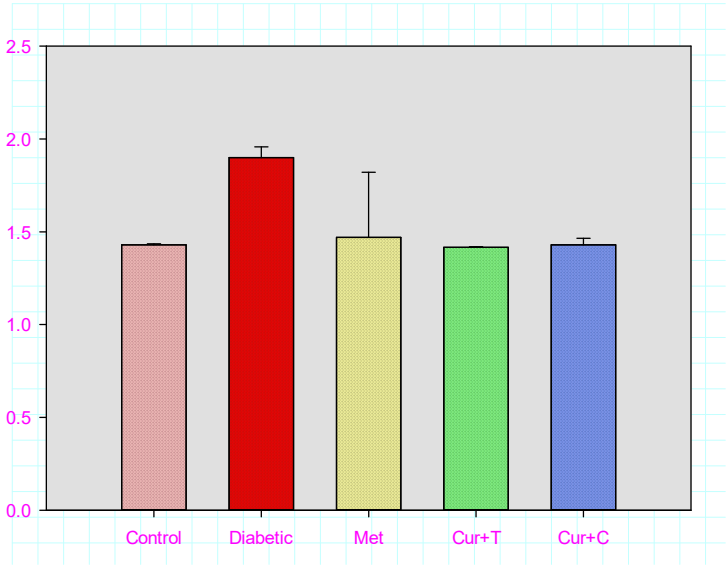

Fig 7: Effect of Curcumin on glucose of Brain in Control and Experimental Rats on 21st day. (Expressed as µ moles/gm tissue)

Sorbitol

A spontaneous increase in Sorbitol level was observed (+44.05%) in Brain on 21st day in the STZ induced diabetic rats group when compared to control (Fig.8). The Sorbitol content in Brain after treatment with Metformin of STZ induced diabetic rats was gradually recovered on 21st day by +18%. After Curcumin treatment, Sorbitol content was recovered in Brain with +25% when compared to control.

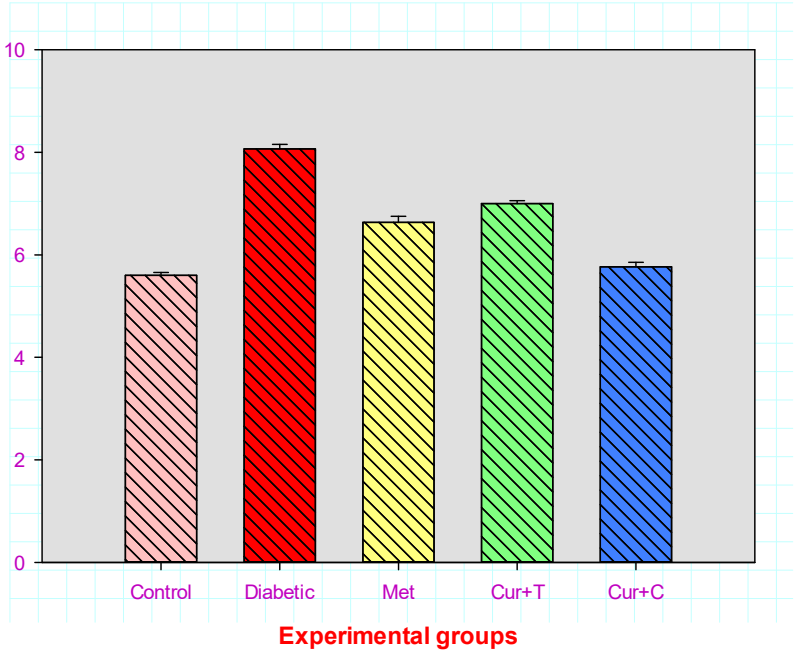

Fig 8: Effect of Curcumin on Sorbital of Brain in Control and Experimental Rats on 21st day. (Expressed as µ moles/gm tissue)

Sodium potassium ATPase enzyme

The tissue levels of this enzyme of all the experimental groups are shown in Fig.9. STZ-induced diabetes in rats caused -2.78% decreased activity in comparison to the control group almost trend was shown (-2%) in Metformin treated animals and -1.85% in Curcumin treated animals.

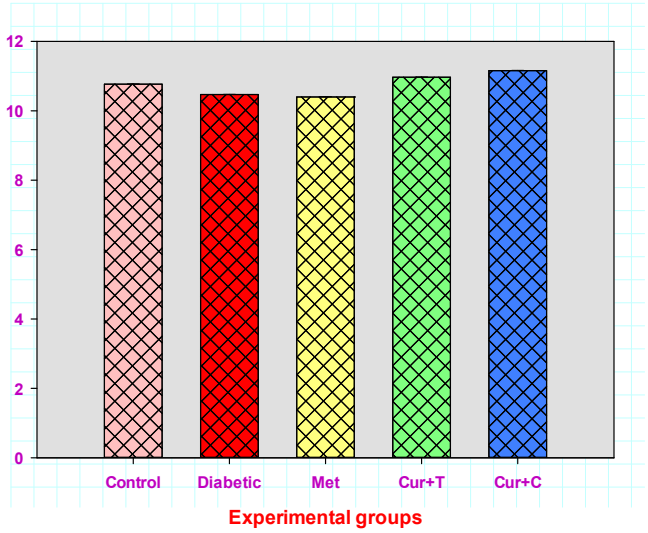

Fig 9: Effect of Curcumin on Sodium potassium ATPase enzyme of Brain in Control and Experimental Rats on 21st day. (Expressed as µ moles of pi/ hour/mg wt. of tissue)

Cyclooxygenase

The Cyclooxygenase (COX) activity in Brain of control and experimental animals is presented in Fig.10. In STZ induced diabetic rats a significant ($p<0.05$) increase in Cyclooxygenase (COX) activity was observed in Brain +184.89% on 21st day when compared to the control group. The Cyclooxygenase (COX) activity was predominantly recovered in Brain (+104.48%) when diabetic animals which were treated with Metformin. Curcumin treatment of diabetic rats has shown gradual recovery of Cyclooxygenase (COX) activity in Brain (+63.67%).

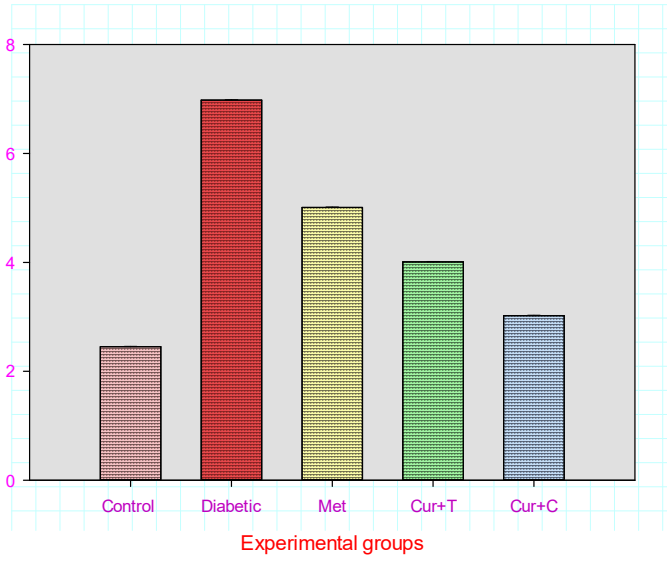

Fig.10: Effect of Curcumin on Cyclooxygenase (COX) activity in Brain on 21st day. (Expressed as µM Oxygen Consumption/min/100mg protein/ 1ml). (Values are given as mean ± Std.E for groups of six animals each. Values are statistically significant at $p<0.05$).

Prostaglandin Peroxidase (PG peroxidase) activity

Prostaglandin Peroxidase (PG peroxidase) activity was significantly ($p<0.05$) increased in Brain on 21st day by +143.63% in STZ induced diabetic rat when compared to controls (Fig.11). After treatment of diabetic with Metformin decrease of PG peroxidase activity in Brain was +49.21%. However, the PG peroxidase activity in Brain recovered by +1.77%, when diabetic animals treated with Curcumin.

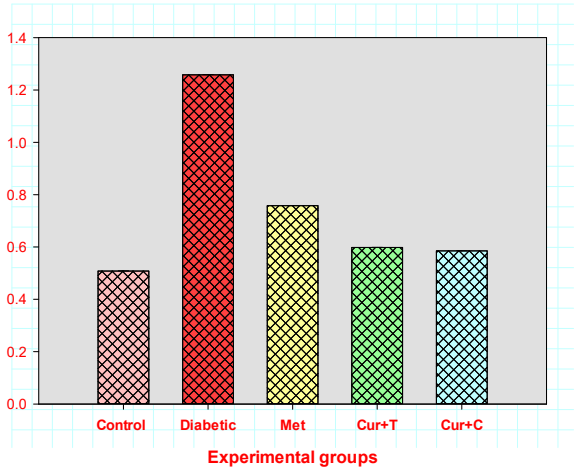

Figure 11: Effect of Curcumin on Prostaglandin Peroxidase (PG peroxidase) activity in Brain on 21st day. (Values are given as mean ± Std.E for groups of six animals each. Values are statistically significant at $p<0.05$)

Effect of Curcumin on the number of surviving neurons of hippocampus (Fig.12&13).

Light microscopic analysis:

CA1 region of the hippocampus: Microscopic examination of H&E stained sections of all groups except showed significant and reliable changes in rat hippocamps. Surviving neurons showed significant decrease in diabetic rats as compared with metformin treated as well as curcumin treated rats after 21days of STZ treatment**.**

CA2 regions of the hippocampus: The CA2 regions of all groups except control observed significant decreased after 21 days in diabetic groups as compared to metformin treated and curcumin treated rats.

Cerebral cortex of control groups showed normal histological strucutre with spherical or pyramidal cells whose nuclei were large and regular pattern of neurons. (**Figure: 14**). Whereas diabetic groups has shown many pathological changes, whereas the sections that were treated with Curcumin showed significant protection of neurons.

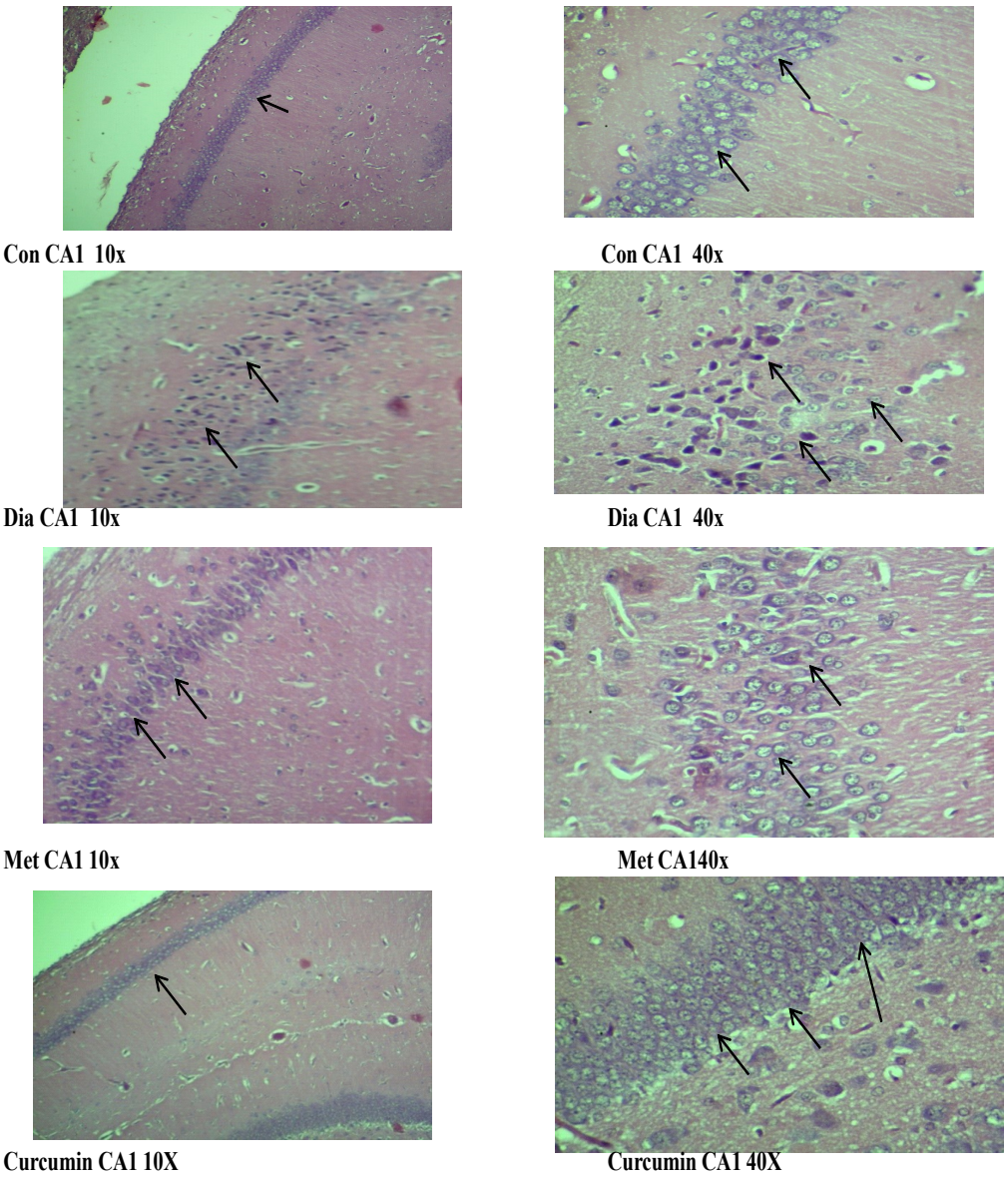

Fig.12: H&E stained photomicrographs (Control,diabetic,metformin and curcumin treated) of 21 days, STZ induced diabetic rat showing less number of surviving neurons in CA1 region of hippocampus.(10x and 40x).

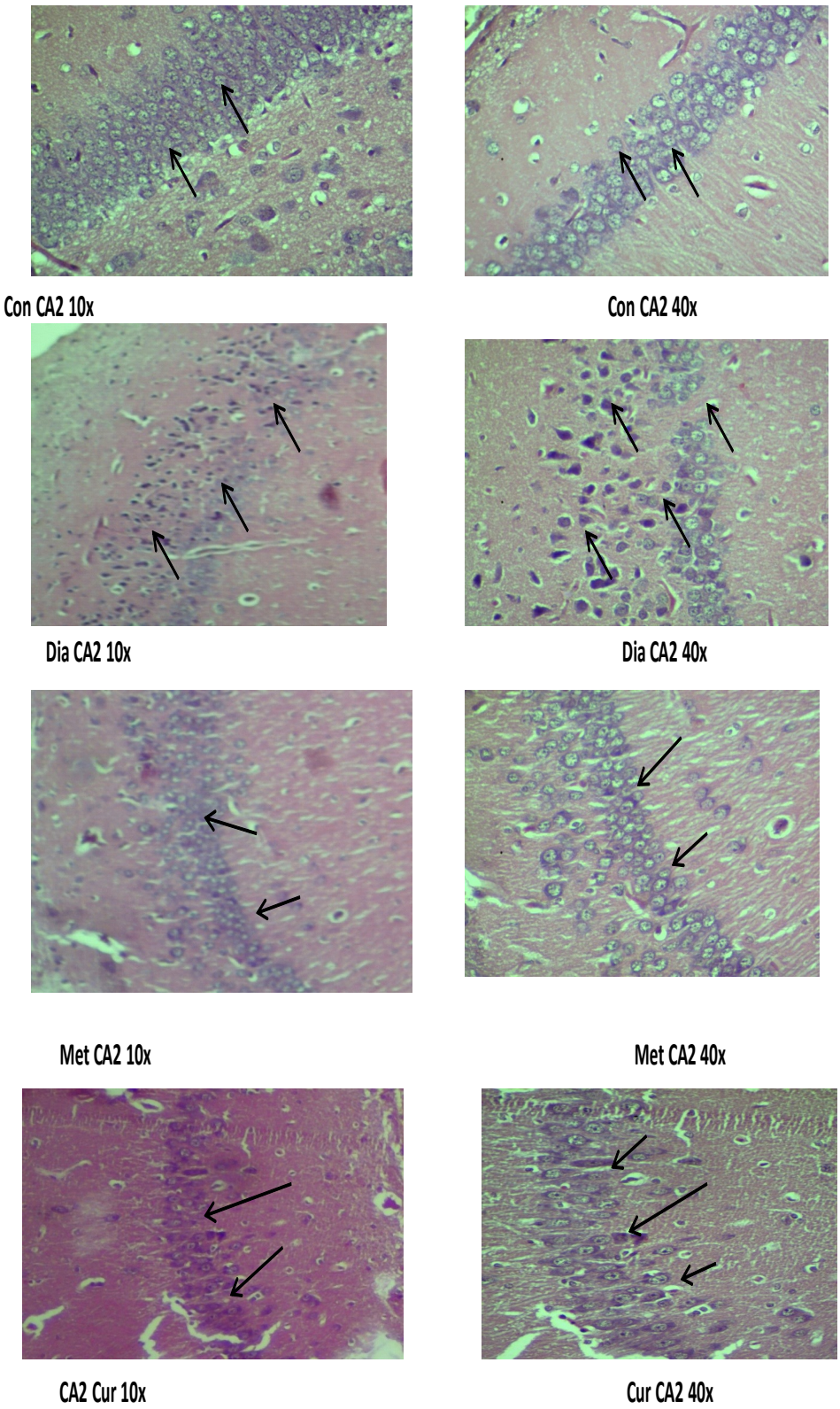

Fig.13: H&E stained photomicrographs (Control,diabetic,metformin and curcumin treated) of 21 days, STZ induced diabetic rat showing less number of surviving neurons in CA2 region of hippocampus.(10x and 40x).

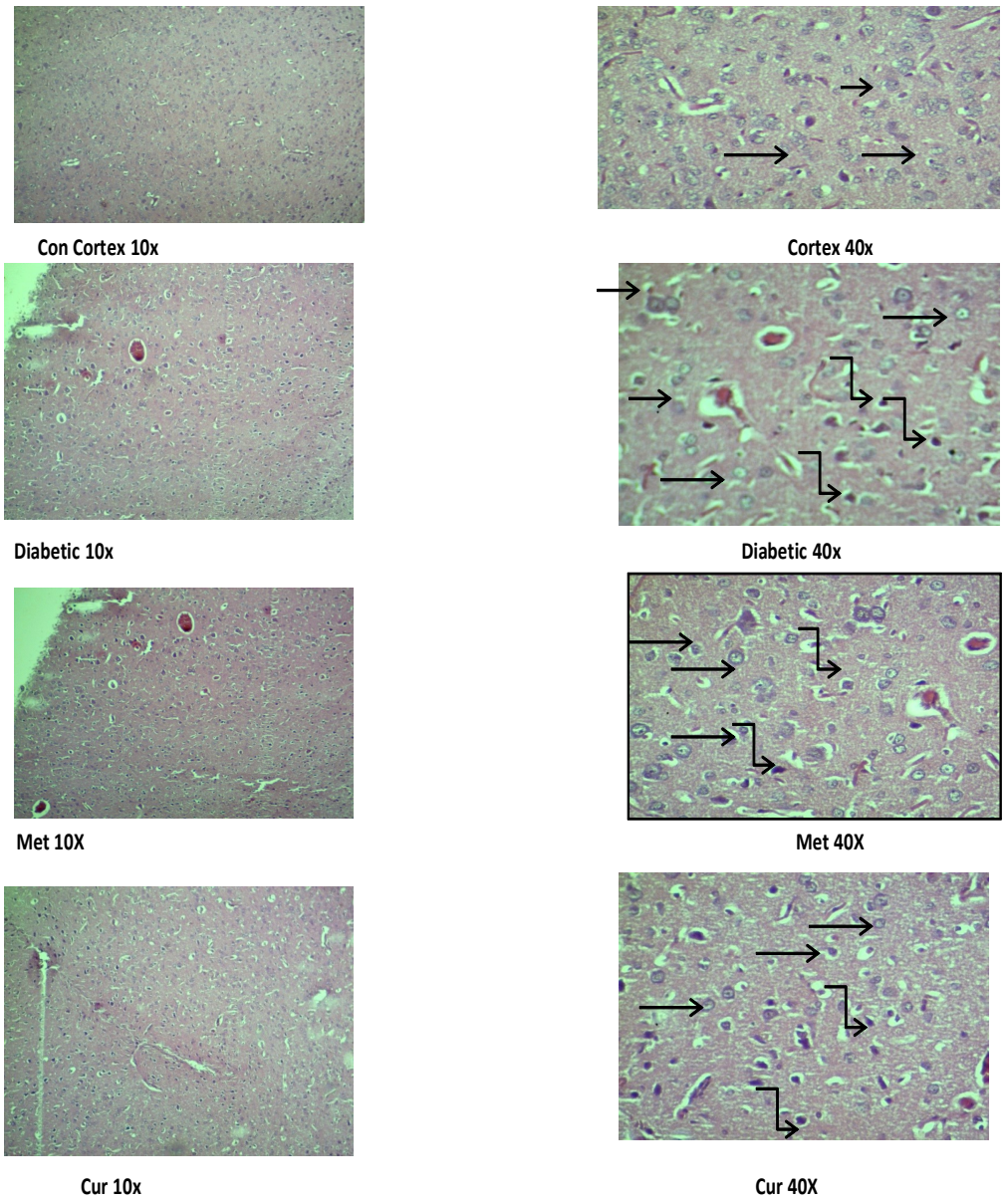

Fig.14: H&E stain of cerebral cortex show the pyramidal cells distribution neurocyte chromatolysis (arrow) and bended arrows degenerated neurons of Control, diabetic, Metformin and Curcumin treat rats (10X and 40X).

DISCUSSION:

Oxidative stress which plays a vital role in pathogenesis of many neurological diseases including diabetes, this led many workers to search for natural antioxidants that might play a role in reducing the damaged caused by diabetes. In such study curcumin has emerged as one such product which has natural antioxidant property along with properties such as anti-inflammatory (20), antibacterial (21), antifungal (22) antiamoebic (23) antiHIV (24) activities, antioxidant activity (25), antitumor (26) and anticarcinogeni (27) etc. So, present study was designed to investigate the potency of Curcumin in STZ induced diabetic on brain using Metformin as reference drug. Our results showed that administration of curcumin (250Mg/kg for 21 days) to the diabetic rats decreased Protein carbonyls. Several studies have shown that elevated protein carbonyls is considered as a broad marker of oxidative stress (28) and which are in high rise in both type-I and type-II diabetes (29,30). In this study also, there is increased quantity of protein carbonyls indicating oxidative stress. Increased PCO's is also reflected by decreased quantity of proteins. Treating the diabetic animals with curcumin has decreased the PCO's indicating its strong antioxidant property (31). In relation to the reference

drug Metformin, Curcumin have shown similar levels of both proteins and PCO's.

Diabetes is associated with several adverse effects on the brain, some of which may result primarily from direct consequences of chronic hyperglycemia. In normal human beings' glucose is the most predominant metabolic fuel source of the brain. In this normal condition brain cannot neither synthesize nor store glucose for longer periods of time, it has to be transported via facilitative glucose transporter (32), to facilitate this it is essential that proper glucose regulated in the periphery. Once glucose metabolism is disrupted due to poorly controlled diabetes in PNS it immediately affects the Brain (33). Once the glucose is accumulated in Brain it will be elevated another metabolic pathway for its metabolism. This is indicated by decrease in Hexokinase enzyme in diabetic rats. Normally the glucose will be metabolized by glycolysis under normal conditions but in hyperglycemic condition it will be diverted away from glycolysis. Current study has shown the subsequent treatment of diabetic rats with curcumin has predominantly reversed the Hexokinase activity. It can be attributed that in our previous study the glucose concentration in peripheral nerve was decreased after treating diabetic rats with curcumin. Hence if glucose is maintained low in PNS there won't be abnormal transport of glucose to the brain (34). In current study, diabetic rats showed higher activity of Aldose reductase and Sorbitol dehydrogenase as compared to normal animals. These results are consistent with earlier report (35). Increased Aldose reductase enzyme activity indicates the changed pathway of glucose metabolism i.e. activation of polyol pathway which might attribute mainly to hyperglycemia. The ability of Curcumin to reduce the activity of AR might be because of diverting the high glucose of brain via increasing its utilization especially through glycolysis which is also reflecting by showing higher activity of Hexokinase enzyme. Hence, the ability of curcumin to reduce AR activity can be attributed to its decreasing effect on the high brain glucose content via increasing its utilization especially through glycolysis. The stimulatory effect of curcumin on the glycolytic pathway seems to be related to its combination of AR inhibition and powerful antioxidant potential could have been more potential. And also, the ability of curcumin in restoring increased Sorbitol dehydrogenase of brain compared to diabetic rats can be related to the increase level of brain GSH by treatment. The results showing decrease amount of glucose, Sorbitol also confirm it. Glucose metabolism in the form of polyol pathway has shown discrete results when related with reference drug but the results of curcumin treatment are mostly consistent with control animals.

In the present study Curcumin treatment significantly reversed the Na^+ K^+ATPase. The ability of curcumin ability to increase Na^+ K^+ATPase activity in brain shows in potential in elevating the excitability of neuronal tissue. The mechanism by which it has reversed the Na^+ K^+ATPase activity compared to diabetic rats, may be because of activation of Phospholemman (PLM) an accessory protein associated with Na^+ K^+ATPase (36). This protein (PLM) has been shown to modulate the enzyme activity in cardiac and skeletal muscle sacrolemma of rat (37). Recently it has been shown the presence of PLM in certain parts of brain (38). Increased activity of Na^+ K^+ATPase by curcumin can be attributed to consequence of an activation of PLM. And also, it has been shown that Na^+ K^+ATPase activity is sensitive to lipid peroxidation, and antilipidperoxidative activity of Curcumin may be responsible for the activation of Na^+ K^+ATPase activity.

Inflammation has been another characteristic feature of diabetes. It has been shown that, in inflammatory prostaglandins, synthesized by both COX-1 and COX-2, may play a role in the sensitization of nociceptors (39, 40) and in the sensitization of neurons in the Central nervous system (41, 42). In current study curcumin has decreased both Cyclooxygenase and prostaglandins activity. Mechanism of action may be due to its anti-inflammatory property appears to be mediate through inhibition of induction of COX-2 and suppression of prostaglandins synthesis (43, 44). Importantly, an increase in PGs might influence both PNS and CNS in human beings and experimental animals, and numerous studies have illustrated the role of PGs on neuropathy, they not only thought to play a key role in Nociception and hyperalgesia in PNS but also appears to be involved in a wide variety of other functions, including vasodilation, altered microvascular permeability and febrile responses (45,46). Cyclooxygenase (COX) is an important enzyme in conversion of arachidonic acid to PGs. Cox is of two different isoforms, named as COX-1 and COX-2. Mostly COX-1 is not present in brain rather than COX-2 is constitutively expressed only in brain and spinal cord tissue. Hence inhibiting COX-2 will further

lower the synthesis of PGs, which will lead to lower inflammation. When inflammation was compared with reference drug and curcumin, curcumin was proved to be more potent than drug.

Morphology of brain especially hippocampus and cortex will show several adverse effects due to diabetes, which may result primarily from direct consequences of chronic glucose level. Diabetic animal hippocampus sections revealed distinguished effects of diabetes in the form of disruption of normal layer organization, cell death at certain regions. Certain areas were associated by clumping of neuronal processes (excess eosinophilia) which is indicative of damage to neurons (47). Uncontrolled glucose increases the NMDA receptor-mediated calcium entry into the neurons and will induce neuronal excitotoxicity through an activation cascade ending by the release of ROS (48). According to Jayanarayanan *et al* (49) Curcumin has the capacity to block NMDA receptor; significantly maintain optimum glucose level and insulin levels; this might have prevented damage caused by diabetes. Previous studies have shown that damage inflicted to neuron in hippocampus in experimental diabetes after 30 days (50). But according to our present study accountable histological alteration are seen in the regions of hippocampus of diabetic rats when compared to normal control group within 21st day. In addition to these changes, brain cerebral cortex of diabetic rat showed hispathological changes which may due to dysfunctioning of glucose metabolism i.e., activation of the polyol pathway (54) which may further lead to glucose autooxidation (53), formation of AGE' products (55), therefore leading to inactivation of antioxidant defense system. Activation of polyol pathway can promote oxidative imbalance, generating free radicals which can damage the regions such as hippocampus and cortex. These morphological changes cause abnormalities in neurobehavior of diabetic animals. Our previous work has shown behavioral abonormalities such as physical balance, coordination, pain, NCV and other nociceptive condition (51). These studies are consistent with different studies reported (52). Various other studies have shown that Diabetes leads in disturbance in oxidative stress and neuronal pathology. This oxidative stress in directly related with morphological alteration in different areas of brain especially chronic exposure to hyperglycemia. Hyperglycemia has shown to promote oxidative imbalance, favouring production of oxygen free radicals and reduction of antioxidative defence along with activation of other pathways such glucose autooxidation (53), activation of the polyol pathway (54), formation of advanced glycation end products (55), nitrogen species production by the mitochondrial respiratory system (56), antioxidant enzyme inactivation and an imbalance of glutathione redox status (57).Such modification will effect several functions, metabolism and gene expression, by damaging major components of the cellular structure, including nucleic acids, proteins, lipids amino acids (58) , which in turn can cause other pathological conditions(59). Curcumin treatment for 21 days decreased AR activity and also decreased quantity of tissue glucose. This proves inhibiting of polyol pathway which in turn indicates protection glutathione reductase. Along with it its property to control PCO's, proves its cellular antioxidant defense. This defense mechanism might have improved pathological alteration in diabetic rats.

CONCLUSION

In conclusion our study shows STZ diabetes induces brain damage both morphologically and biochemically, which was attenuated by Curcumin administration in potent manner than reference drug. And by inhibiting AR, conversion of glucose to Sorbitol is also inhibited, which is a crucial pathway for activating polyol pathway. Curcumin has shown neuroprotection by directly improving effect of hyperglycemia as well as its antioxidant and radical scavenging properties. Moreover, by attenuating active polyol pathway and COX pathway and histological damage, shown in diabetic rat brain, will finally state the neuroprotective efficiency of curcumin.

CONFLICTS OF INTERESTS

All authors have none to declare.

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