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Neuroprotective Effect of Flavonoids in Global Cerebral Ischemic Conditions

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ABSTRACT

To investigate the neuroprotective effect of flavonoids on cerebral ischemia by four vessel occlusion method. 30 spraguedawley rats were studied. In the present study, the animals were pretreated with quercetin(Capsicum parvifolium) for a period of 1 week(200 and 400mgkg^{-1})p.o. Cerebral ischemia was established by permanent occlusion of vertebral artery followed by 10mins occlusion of bilateral carotid arteries. The treatment was continued for another week after surgery with quercetin and the animals were evaluated for sensorimotor functions. The animals were sacrificed and brain was removed and homogenized. The homogenized content was used for the estimation of enzymatic and non enzymatic antioxidant levels. The group treated with 200 and 400mgkg^{-1}quercetin showed significant improvement in sensorimotor functions when compared to ischemic groups. Significant increase in brain antioxidant enzymes was observed in quercetin treated groups compared to ischemic groups. The quercetin treated groups exhibited a significant decrease in the levels of lipid peroxide and nitrites. Protein estimation and histopathological studies were further performed on quercetin treated and ischemic groups. The results obtained were compared with memantine (20mgkg^{-1})i.p, the standard drug. Quercetin can improve the neurological status and may reduce the cerebral ischemia-reperfusion injured rats.

Keywords: Quercetin, Capsicum parvifolium, cerebral ischemia, 4 vessel occlusion method.

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INTRODUCTION

Neurodegenerative disorders are characterized by progressive and irreversible loss of neuron from specific region of the brain. Prototypical neurodegenerative disorder includes common and debilitating disorder such as Parkinson's disease, Alzheimer's disease, Huntington's disease and Amyotrophic lateral sclerosis (ALS). Though stroke is a cardiovascular disease affecting the blood vessels that supply blood to the brain. It is ultimately leads to neurodegenaration.[1]

Focal ischemia decreases the cerebral blood flow (CBF) to below 8-10ml/100mg/min produces rapid neuronal cell death. However this densely ischemic core and normally perfused brain (CBF of 50-55ml/100mg/min), there exist a zone of moderately reduced blood flow, the extent of which depends on the collateral supply from surrounding arteries.[2] In this zone, called penumbra, oxygen delivery become insufficient to allow normal level of antioxidant metabolism. (Pulsinelli . 1992 and Heiss. et al 1994). This produces lactic acidosis and curtails the production of ATP, the energy source of cellular ionic pumps.[3] Failure of the sodium-potassium ion pumps results in a rapid loss of potassium ion from the neurons and massive neuronal depolarization occurs.[4] This opens voltage ssensitive calcium channels and leads to an extra cellular build up of excitatory amino acids, which over stimulate excitatory amino acid receptors . Acute elevation of glutathione activate the glutamate receptors of which, notably, the NMDA and AMPA receptors are clearly implicated in the neurotoxic process. AMPA-receptor activation contributes to the depolarization that is held responsible for the lift of the magnesium block of the NMDA-receptor channels, which subsequently open and allow a sustained influx of calcium ions.[5] When ATP dependent efflux of calcium ion and active uptake of calcium ion level will remain elevated, producing a range of consequences. One pathway of cytotoxic calcium ion leads to generation of reactive oxygen species via activation of NOS and formation of excessive amount of NO.[6] Elevated calcium ions in mitochondria uncouple phosphorylation. This leads to further decrease of energy supply and increase of free radical.

There is however, evidence that reperfusion of ischemic neurons is also associated with formation of oxygen radicals and that these radicals causes additional neuronal damage. A wide variety of cells organelles and enzymes may be involved in the free radicals formed by ischemia and reperfusion;[7] these include neutrophills, xanthine oxidase, cyclooxygenase and lipooxygenase, auto oxidation of catecholamines, mitochondria and the sarchoplasmicreticulum. There are many ways in which free radicals (including NO) and peroxinitrite may be generated during ischemia.[8]

The active constituents of capsicum or pepper are the flavonoid (quercetin of about 33.75). Flavonoids are phenolic compounds that give vegetables, fruits, grains, seeds, leaves and bark their colour.[9] Quercetin, an important constituent of Capsicum parvifolium possesses antioxidant, anticancer. Quercetin belongs to a group of plant pigments called flavonoids that give many fruits, flowers, and vegetables their color.[10]

Flavonoids such as quercetin are antioxidants -- they scavenge damaging particles in the body known as free radicals, which damage cell membranes, tamper with DNA, and even cause cell death. Antioxidants can neutralize free radicals and may reduce or even help prevent some of the damage they cause.[11] They also help keep LDL ("bad") cholesterol from being damaged, which scientists think may contribute to heart disease. In test tubes, quercetin has strong antioxidant properties, but researchers aren't sure whether taking quercetin (and many other antioxidants) has the same effects inside the body.[12]

Quercetin acts like an antihistamine and an anti-inflammatory, and may help protect against heart disease and cancer. Fruits and vegetables -- particularly citrus fruits, apples, onions, parsley, tea, and red wine -- are the primary dietary sources of quercetin. Olive oil, grapes, dark cherries, and dark berries -- such as blueberries, blackberries, and bilberries -- are also high in flavonoids, including quercetin.[13]

Memantine is a non competitive, low to moderate affinity NMDA receptor antagonist, which binds with the receptor and thereby decreases the efflux of increase Ca+ inside the cell.[14]

Oxidative stress plays a major role in the pathogenesis of ischemic damage in the present study an attempt has been made to observe the neuroprotective effect of flavonoids as free radical scavenger.[15]

MATERIALS AND METHODS

PLANT EXTRACTION AND HYDROLYSIS:[16]

Flavonoids were determined in dried plant samples after extraction and hydrolysis of the flavonoid glycosides. The plants were cleaned, oven-dried at 40 °C, and ground, and 62.5% aqueous methanol containing 2 g/L TBHQ was added. To every 20 mL of aqueous methanol used was added 5 mL of 6M HCl. Extraction solution thus obtained consisted of 1.2M HCl in 50% aqueous methanol, v/v. These were carefully mixed and refluxed at 90 °C for 2 h. The extract was cooled, filtered using a Bu¨ chner filter, made to 50 mL with methanol, and filtered again with 0.45 ímWhatman membrane filter before injection into the HPLC.[17] The extracts were kept in airtight amber bottles and stored in the freezer until analyzed.

ANIMALS

Adult inbred female Sprague dawley rats weighing 200 – 250gm were used for the study. Animals were procured from the Central Animal House of the institute.[18] They were housed in groups of 5 – 6 in colony cages at an ambient temperature of 25±20C and 45 – 55% relative humidity with 12 hrs light or dark cycle. They are free access to pellet chock (Brook Bond, Lipton India) and water ad libitum.[19] Experiments were performed between 9:00 to

17:00 hours. The project was approved by them Institutional Animal Ethical committee under the regulation of CPCSEA, New Delhi.

DRUG ADMINISTRATION:[20]

A total of 30 rats were randomized into six different treatment groups (n = 5 each group): the groups were sham-operated controls, vehicle group, Quercetin (200 & 400mg/kg), and memantine group.[21] Quercetin was dissolved in 4% dimethyl sulfoxide solution. Quercetin was administered at 200 & 400mg/kg/day orally respectively, for 7 days before the 4-VO and 7days after the 4-VO. The same times and routes of administration of 4%DMSO solution were used for the sham-operated and 4-VOischemia vehicle control rats. Memantine was administered at a dose of 20mg/kg i.p similar to that of test groups. All drug solutions were freshly prepared before use. [22]

INDUCTION OF GLOBAL ISCHEMIA[23]

Rat was weighed and the dose of anaesthetic was calculated as 40mg/kg of Thiopentone i.p. The rat was placed on a surgical table with a heating pad for maintaining body temperature at 370C and to prevent hypothermia. The vertebral artery arising from the external carotid was occluded permanently with a wire of .5mm thickness. [24] After 24hrs, both the carotid arteries were exposed and occluded simultaneously for 10 min. During both the occlusions, body temperature was maintained at 370C. After both the occlusions proper suturing and topical antibiotic application was done. On recovering, the rat was isolated to cage. Rat was give Ibuprofen (10mg/kg) and Ampicillin (100mg/kg/oral) daily as post surgical treatment for 5 days or till sacrifice whichever is earlier.[25]

HANGING ON WIRE-GRIP STRENGTH

The rat was suspended on its forelimbs from a wire which is held in position at about 3 feet from the table surface.[26] The time taken by the rat to hold itself without falling down is noted down for a maximum of 2 minutes.[27]

ROTAROD – GRIP AND BALANCE:

Rats were placed onto the rotating rod(speed 25mm/sec) and the time the rat can balance itself on the rod is noted down for a maximum of 3 min.[28]

BRAIN ISOLATION:[29]

Rats were sacrificed by decapitation after anaesthesia. Brains were quickly removed and weighed. One half of the brain was processed as follows:

Hippocampus, Pons medulla, Striatum and cortex were identified, dissected and weighed immediately. The weighed samples were homogenised immediately with Elvenjan hand homogeniser in chilled 10% Kcl solution (10ml/gm tissue). Homogenised sample were centrifuged at 2000 rpm for 10 min. Clear supernatant was taken for biochemical estimation. The other half of brain was stored in FAM mixture (40% formaldehyde, acetic acid and methanol in the ratio 1:1) and used for histopathological analysis.[30]

BIOCHEMICAL ESTIMATIONS:

ANTIOXIDANT PROFILE:

TOTAL GLUTATHIONE:

Stock buffer for the estimation of glutathione was prepared as follows:

125mM Sodiumphosphate, 6.3 mM Sodium EDTA was adjusted to pH 7.5. Three working solutions were made up in stock buffer. 0.3 mM NADPH, 6 mM DTNB and approximately 50 units of glutathione reductase/ml. Solutions were kept at 40C. During assay 700µl of NADPH, 100 µl of DTNB, 25 µl of Glutathione sample, 10 µl og Glutathione reductase were incubated at 300C and volume was made upto 1ml with deionized water. Absorbance was readout at 420nm.[31]

SUPEROXIDE DISMUTASE[32]

The Activity of SOD was estimated following the method of Kakkar et al; 1984. It involves the measurement of the formation of blue colored Formozan from nitro blue tetrazolium (NBT) in the presence of phenazinemetho sulphate (PMS) and reduced nicotinamide adenine dinucleotide (NADH). The whole brain was dissected out following the procedure mentioned earlier. One unit of activity of SOD was defined as the amount of the enzyme that inhibits the rate reaction by 50% under specific conditions. The incubation mixture consisted of sodium pyrophosphate buffer (pH8.3, 0.052M,1.2ml), phenazine methosulphate(186µM), nitroblue tetrazolium (300µM) and NADH(780µM,0.2ml). The reaction was initiated by the addition of NADH; following incubation was done for 90 seconds at 370C. The reaction was terminated by the addition of glacial acetic acid (1ml). n-Butanol(4ml) was added, shaken vigorously, centrifuged at 4000rpm for 1 min and the upper butanol layer was read at 560nm against butanol blank.

CATALASE:[33]

Catalase (CAT) measurement was done on the ability of CAT to inhibit oxidation of hydrogen peroxide (H2O2). 2.25ml of potassium phosphate buffer(65nM, pH7.8) and 100µl of the brain homogenate or sucrose (0.32M) were incubated at 250C for 30 minutes. H2O2(7.5mM;650µl) was added to initiate the reaction. The change in absorption at 240nm

was measured for 2-3 minutes, dy/dx for every min for each assay was calculated and the result are expressed at CAT units of protein.

CAT (U) in 100µl of sample=(dy/dx)X0.0003/38.3956X10-6

THIOBARBITURIC ACID REACTIVE SUBSTANCE:[34]

Ohkawa et al method was used to estimate total amount of lipid peroxidation product (Thiobarbituric acid reacting substance) in the homogenate. The incubation mixture consists of

INGREDIENTS	VOLUME
Brain homogenate (supernatant)	0.5ml
8% sodium dodecyl sulphate (SDS)	0.2ml
20% Acetic acid solution (Adjusted to pH 3.5 with 1N NaOH/0.1N Hcl)	1.5ml
0.9% aqueous solution of thio barbituric acid (Adjusted to pH 7.4 with 1N NaOH/0.1N Hcl)	1.5ml

The incubation mixture was made up to 5.0ml with double distilled water and then heated in boiling water for 30mins. After cooling, the red chromogen was extracted into 5ml of the mixture of n-butanol and pyridine (15.1v/v) centrifuged at 4000rpm for 10min. The organic layer was taken and its absorbance at 532nm was measured. 1, 1, 3, 3 tetra ethoxypropane (TEP) was used as an external standard and the levels of lipid peroxides was expressed as nmole of MDA/100g protein. The calibration curve of TEP was prepared by the above procedure taking 80-240 nmoles of TEP as standard over which, linearity was obtained.[35]

TOTAL PROTEINS:[36]

Protein was estimated following the methods . 50µl of brain homogenate was incubated at room temperature for 20min along with 450µl of distilled water and 5ml of copper reagent. After 20min, 5ml of 1N Folins phenol reagent was added and samples were vortexed. After 15min the colour intensity was read at 640nm. Protein calibration curve was prepared by taking bovine serum albumin (100mg/ml) as standard. It is expressed as mg/protein.[37]

ESTIMATION OF NITRITES:[38]

Nitrite concentration in brain homogenate was determined as nitrates by using Griess reagent (Green et al., 1982). 50µl of homogenate was mixed with 150µl of Griess (A+B) and absorbance was measured at 540nm by using Biorad ELISA reader. The nitrate levels were expressed as µg/ml of plasma.

HISTOPATHOLOGICAL EVALUATIONS:[39]

Histopathological analysis was performed for the ischemic and sham groups (Jaspers et al., 1990). Brains were extracted and embedded in paraffin and saggital sections of 5µm thickness were used for eosin and hemotoxillin (Kluver and Barrera, 1954) and used for assessing general features.

STATISTICAL ANALYSIS:[40]

Data are expressed as mean±S.E.M. The data on behavioural activity was subjected to one-way analysis of varience (ANOVA) followed by Dunnett multiple comparison test biochemical data were subjected to one way ANOVA followed by Newmann Keuls multiple comparison post hoc test, using Graph Pad Prism version 3.00 for windows (Graphpad Software, San Diego, California, USA). A value of less than 0.05 has been taken as significant.

RESULTS

ISCHEMIA- GENERAL OBSERVATION

After 7 days of 4 vessel occlusion method, Sprague Dawley rats produced severe ischemic condition. Animals have shown motor incordination and loss of grip strength. Animals have shown anxiety in general observation. Weight loss and decrease in daily food intake was also observed in ischemic animal in comparison with control group.

ROTAROD

In comparison to control non ischemia animals, quercetin treated ischemic rats significantly spent less time in the rotarod experiment. However it was found to be significantly higher than the vehicle treated ischemic rats, indicates attenuation of ischemic effect with quercetin treatment. Memantine significantly protected the ischemic rats in the motor coordination studies.(Table 1)

TABLE-1: Effect of memantine, quercetin on rotarod performance in global ischemic rats

GROUPS	TIME SPENT (SEC)
Control	57.16±3.74
Ischemia	6.33±0.61**
Quercetin (200mg/kg)	15.00±0.89**+
Quercetin (400mg/kg)	18.83±1.66**++
Memantine(20mg/kg)	24.66±3.65**++

Data are expressed as Mean±SEM (n=6)
**p<0.01*p<0.05- Compared with control group
++p<0.01 +p<0.05- Compared with ischemic group

GRIP STRENGTH

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Induction of ischemia in rats lowered grip strength. No significant increase in grip strength was observed with quercetin treated animals. Memantine significantly prevented the ischemic damage as observed by increase in time holding the wire. (Table 2)

TABLE-2: Effect of memantine, quercetin on Grip strength (Hanging on wire) in global ischemic rats

GROUPS	TIME SPENT (SEC)
Control	31.16±4.40
Ischemia	2.50±0.56**
Quercetin(200mg/kg)	5.50±0.56**
Quercetin(400mg/kg)	12.16±0.79**+
Memantine(20mg/kg)	20.33±3.24**++

Data are expressed as Mean±SEM (n=6)
**p<0.01 *p<0.05- Compared with control group
++p<0.01 +p<0.05- Compared with ischemic group

BIOCHEMICAL CHANGES DURING ISCHEMIA

Ischemia has produced oxidative stress in animal as seen with decreased endogenous antioxidative enzymes and more circulating lipid peroxide level.

CATALASE

Inducement of ischemia in rats decreased the catalase level in comparison to non ischemic rats. Treatment of quercetin dose dependently increased the catalase level. (Table-3)

TABLE-3: Effect of memantine, quercetin on Catalase in global ischemic rats

GROUPS	HIPPOCAMPUS	STRIATUM
Control	27.66±1.20	28.33±2.02
Ischemia	12.66±0.88**	5.00±0.57**
Quercetin(200mg/kg)	13.14±4.8++	12.39±1.32**+
Quercetin(400mg/kg)	24.8±0.99*	12.30±1.2**+
Memantine(20mg/kg)	24.29±3.63*	21.7±1.9*++

Data are expressed as Mean±SEM
**p<0.01 *p<0.05- Compared with control group
++p<0.01 +p<0.05- Compared with ischemic group

GLUTATHIONE

Depleted glutathione level was observed in hippocampus and striatum region of ischemic rats. Treatment of quercetin at higher dose (400mg/kg) and memantine significantly

elevated the glutathione as observed by increased glutathione level in hippocampus and striatum regions. (Table-4)

TABLE-4: Effect of memantine, quercetin on Total Glutathione in global ischemic rats

GROUPS	HIPPOCAMPUS	STRIATUM
Control	6.80±0.12	6.89±0.12
Ischemia	1.78±0.01**	1.80±0.05**
Quercetin(200mg/kg)	1.25±0.52**	1.08±0.40**
Quercetin(400mg/kg)	5.31±1.17++	5.52±1.47+
Memantine(20mg/kg)	6.36±0.80++	7.32±1.40++

Data are expressed as Mean±SEM
**p<0.01 *p<0.05- Compared with control group
++p<0.01 +p<0.05- Compared with ischemic group

SUPEROXIDE DISMUTASE (SOD)

Inducement of ischemia decreased the SOD level in hippocampus and striatum. Treatment of quercetin dose dependently elevated the SOD level in hippocampus and striatum region indicating antioxidant property of quercetin. Standard drug memantine protected the SOD levels during ischemia. (Table-5)

TABLE-5: Effect of memantine, quercetin on Superoxide Dismutase in global ischemic rats

GROUPS	HIPPOCAMPUS	STRIATUM
Control	0.6926±0.14	0.6556±0.053
Ischemia	0.176±0.020**	0.176±0.020**
Quercetin(200mg/kg)	0.247±0.043**	0.130±0.054**
Quercetin(400mg/kg)	0.409±0.033**	0.39±0.641**++
Memantine(20mg/kg)	0.410±0.011**	0.405±0.032**++

Data are expressed as Mean±SEM
**p<0.01 *p<0.05- Compared with control group
++p<0.01 +p<0.05- Compared with ischemic group

THIOBARBITURIC ACID REACTIVE SUBSTANCE (T-BAR)

Increased T-BAR level was observed with ischemia indicating more circulating lipid peroxide level. Treatment of quercetin in striatum protected the lipid peroxides level; at lower doses no significant effect on lipid peroxides level was observed. Memantine significantly protected the cells against ischemic damage. (Table-6)

TABLE-6: Effect of memantine, quercetin on T-BAR in global ischemic rats

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GROUPS	HIPPOCAMPUS	STRIATUM
Control	0.25±0.04	0.28±0.01
Ischemia	0.85±0.03**	0.86±0.018**
Quercetin(200mg/kg)	0.66±0.07**	0.72±0.05**++
Quercetin(400mg/kg)	0.48±0.03++	0.35±0.13++
Memantine(20mg/kg)	0.36±0.01++	0.42±0.03+

Data are expressed as Mean±SEM
**p<0.01 *p<0.05- Compared with control group
++p<0.01 +p<0.05- Compared with ischemic group

NITRITES

Ischemia generation in rats leads to increased nitrite level in comparison to control rats. Decreased nitrite level was observed with quercetin treatment in comparison to vehicle treated control rat. Similarly memantine reduced the nitrite level. (Table-7)

TABLE-7: Effect of memantine, quercetin on Nitrites in global ischemic rats

GROUPS	HIPPOCAMPUS	STRIATUM
Control	0.61±0.18	0.58±0.14
Ischemia	1.78±0.01**	1.78±0.01**
Quercetin(200mg/kg)	0.65±0.23++	0.42±0.07++
Quercetin(400mg/kg)	1.04±0.09+	0.90±0.31+
Memantine(20mg/kg)	0.77±0.24++	0.89±0.18+

Data are expressed as Mean±SEM
**p<0.01 *p<0.05- Compared with control group
++p<0.01 +p<0.05- Compared with ischemic group

PROTEINS

Protein level of various experiment are represented in table 8 in the hippocampus and striatum region. There is no significant difference in protein level of various drug treatment groups was observed. (Table-8)

TABLE-8: Effect of memantine, quercetin on Total Proteins in global ischemic rats

GROUPS	HIPPOCAMPUS	STRIATUM
Control	0.43±0.003	0.44±0.012
Ischemia	0.69±0.008**	0.78±0.015**
Quercetin(200mg/kg)	0.39±0.007++	0.37±0.010**++
Quercetin(400mg/kg)	0.37±0.011**++	0.37±0.012**++
Memantine(20mg/kg)	0.39±0.010++	0.40±0.004++

Data are expressed as Mean±SEM
**p<0.01 *p<0.05- Compared with control group
++p<0.01 +p<0.05- Compared with ischemic group

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HISTOPATHOLOGICAL RESULTS

Degrading of CA1 neurons was observed with vehicle ischemic rats. The histopathology results with eosin and haemotoxylin staining have shown that quercetin has protective effect in dose dependent manner. Memantine has also shown protection in comparison with ischemic group.

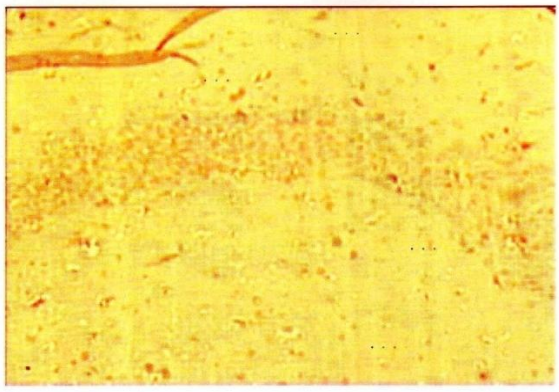

FIG 1 Represent the CA 1 region of ischemic rat

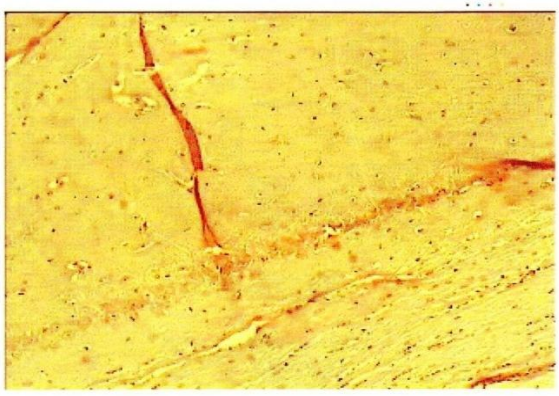

FIG 2 Represent the CA 1 region of Quercetin 200mg/kg treated rat

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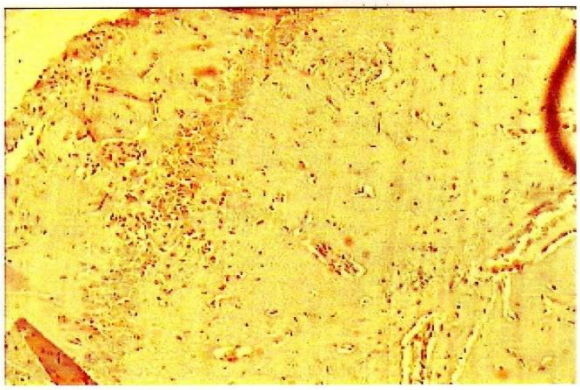

FIG 3 Represents the CA 1 region of Quercetin 400mg/kg treated rat

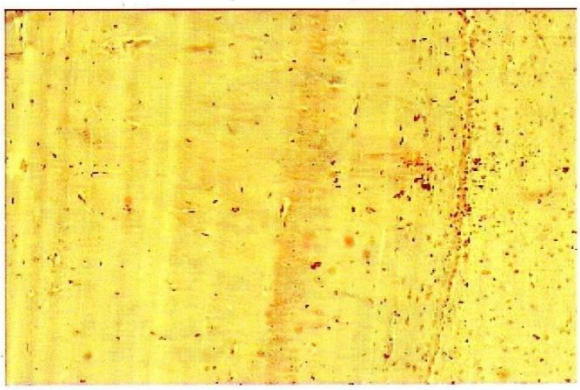

FIG 4 Represents the CA 1 region of memantine 20mg/kg treated rat

DISCUSSION

The present work was carried out to evaluate the effect of quercetinon ischemia. The querctin effect was compared with standard drug memantine. In comparison to control group, ischemic group animals lost the muscle coordination and exhibited decreased anti oxidant enzymes. The 4-vessel occlusion model of Pulsinelli et al 1979 is reliable model to develop global ischemia in rats. The neurological damage induced by 4-vessel occlusion method was found to be effective in measuring the sensory motor properties.[41] The ischemic damage is mainly focussed on the hippocampal region, striatal and cortical region in the penumbra. For biochemical estimations, anti oxidant properties of the striatal and hippocampal region of the ischemic rats was evaluated. [42]

Ischemic condition in brain produce changes in chemical, ionic, protein and enzyme environment. The alteration in cell function and structure is the proximal cause of narcotic and apoptotic cell changes.[43] Alteration in membrane transport (permeability) for ion and metabolites leads to cell death. There is also some evidence that free radical generation following ischemic insult can inhibit the Na+K+pumps. The ischemic condition also involves mitochondrial changes. Alteration in mitochondria therefore has enormous potential for causing damage. Inhibition of mitochondrial oxidation phosphorylation or significant uncoupling will lower ATP level, increased mitochondrial free radical production, and remove the buffering ability of the organelle. Low ATP level, overproduction of free radical and initiation of apoptosis are the main cause of cell death resulting in mitochondrial dysfunction.[44]

Ischemic insult and reperfusion leads to generation of excess of free radicals, the decrease in level of endogenous antioxidant enzymes such as glutathione, SOD, catalase. Free radicals affect the structure of both lipids and proteins.[45] They interact strongly with unsaturated bonds in lipid, leading to chain reaction formation of peroxides, hydroperoxides and aldehydes. Reactive oxygen species (ROS) has shown to influence gene expression and play a role in the events leading to neuronal death.[46] Glutamate is the major excitatory amino acids in the brain, acting mainly through activation of its through ionotropic receptors such as AMPA, kainic acid and NMDA receptors. Activation of these receptors leads to entry of positively charged ions in the cells which leads to depolarization and neuronal excitation. It is evidence that there is several fold increase in extracellular glutamate during global ischemia. [47]

Depletion of glutathione level after ischemia implies that the free radical generation is immediate onset during and after ischemic induction. During the reperfusion process, production of radical ions has been reported. The production of radical ions mainly superoxide gets reduced to hydrogen peroxide metabolism in mitochondria. During this process glutathione was found to be utilized and it has been replaced from cytosol. However in ischemic condition lack of ATP molecules hinder the synthesis of glutathione resulting in the mitochondrial overload of peroxides. This condition leads to excessive accumulation of free radicals and leading to depletion of glutathione from the cytosol. Other than this mechanism, glutathione can also be present in both reduced and oxidized form. Oxidized glutathione convert to reduced glutathione with the help of glutathione reductase. Hence depletion of glutathione level in the store can alter the glutathione reductase, peroxidase and various biochemical parameters like superoxide dismutase, nitrites and T-BAR level.[48] In ischemic rats along with depleted glutathione level, the level of SOD and nitrites was also found to be decreased. These indications also lead to elevated circulating lipid peroxide level.

Quercetin offers significant neuroprotection in middle cerebral artery occlusion induced cerebral ischemia. In the present study we have induced global ischemia by 4-vessel occlusion method and the extent of neuroprotection with quercetin in dose dependent manner has been studied. The neuroprotection activity of quercetin has been attributed to inhibition of lipid

peroxidation, increase in endogenous anti oxidant defense enzymes and reduction in peroxy nitrite formation. Memantine is a non competitive, low to moderate affinity NMDA receptor antagonist.[49] Memantine produces improved neurotransmission and activation of neurons. However, when glutamate release is excessive, memantine inhibits the excitatory action of glutamate by antagonizing NMDA receptors. So the memantine drug either modulates the glutaminergic neuro transmission or antagonizes the NMDA receptors by which blocks excessive glutaminergic stimulation. This prevents the increase in calcium influx and subsequently results in decreased cell death.[50]

Pretreatment with quercetin (200 mg/kg and 400 mg/kg p.o.) for 7 days significantly reduced the elevated biochemical markers in a dose dependent manner. Treatment with 400 mg/kg of quercetin produced the neuroprotective activity comparable to that of memantine 15mg/kg i.p.

SUMMARY AND CONCLUSION

The present study was designed to evaluate the protective effect of quercetin in global cerebral ischemia and to measure free radical generation in post-cerebral ischemic condition and to reproduce real time clinical stroke conditions.

Adult inbred female Sprague dawley rats weighing 200-250gm were used for the study. Animals were procured from the Central Animal House of the Institute. The project was approved by the Institutional Animal Ethical Committee under the regulation of CPCSEA.

Induction of global cerebral ischemia in rats was carried out by 4-vessel occlusion method of Pulsinelli et al. Animals were assigned into different groups such as control, quercetin, and memantine treated groups. Complete neuroprotective evaluation was planned by assessing the effect of drug treatment on post ischemic groups on various tests like sensorimotor performances, levels of enzymatic and non enzymatic anti oxidants which were finally correlated to neuropathological outcome.

After 7 days of 4-vessel occlusion method in SD rats produced severe ischemia. Animals have shown motor incoordination and loss of grip strength. Animals also exhibited anxiety in general observation. Weight loss and decrease in daily food intake was also observed in ischemic animal in comparison with control groups.

In comparison to control non ischemia animals, quercetin treated ischemic rats significantly spent less time in the rotarod experiment. However it was found to be significantly higher than the vehicle treated ischemic rats, indicates attenuation of ischemic effect with quercetin treated animals. No significant increase in the grip strength was observed with quercetin treated animals. Memantine significantly protected the ischemic rats in the motor coordination studies.

Induction of ischemia in rats decreased the catalase level, depleted the glutathione level in comparison to non ischemic rats. Treatment of quercetin dose dependently increased the antioxidants level. In striatum all the drug treatment increased the antioxidant enzyme level indicating the antioxidant effect of the drugs under study. Induction of ischemia decreased the SOD level in hippocampus and striatum. Treatment of quercetin dose dependently elevated the SOD level in hippocampus and striatum region. Increased T-BAR and nitrite levels were observed in ischemic group indicating more circulating lipid peroxide level and nitrite level. Treatment of quercetin in striatum protected the lipid peroxide levels; at lower doses no significant effect on lipid peroxides level was observed. No significant alteration in the protein level was observed.

It can be concluded that ischemia in rats produced damage in the hippocampal and striatal regions, which affected the behaviour of the animals. Treatment of quercetin at higher dose significantly protected the ischemic damage.

REFERENCES

[1] Arora M, Nair G, Strasburg GM. Free Radical Biol Med 1998; 24:1355-1363.
[2] Beers RF, Sizer IW. J Biol Chem 1952; 195:133-140.
[3] Benavente-Garcia, Castillo J, Martin FR, Ortune A, Del Rio JA. J Agric Food Chem 1997; 45:4505-4515.
[4] Benveniste H, Dreger J, Schousboe A, Diemer NH. J Neurochem 1984; 43:1369-1374.
[5] Bondy SC. Proc Soc Exp Biol Med 1995; 208:337-345.
[6] Bruce-Keller AJ, Li Y-J, Lovell A, Kramer PJ, Gray DS, Brown RR, Marksberry WR, Mattson MP. J Neuropathol Exp Neurol 1998; 57:257-267.
[7] Bullock R, Zauner A, Woodward J, Young HF. Massive persistent release of excitatory Aminoacids following human occlusive stroke 1995; 26:2187-2189.
[8] Cazevielle C, Muller A, Meyner F, Bonne C. Free Radic Biol Med 14: 389-395.
[9] Chan PH, Chu L, Fishman RA. Brain Res 1988; 439:388-390.
[10] Chan PK. J Cerebral Blood Flow Metab 2001; 21: 2-14.
[11] Chance B, Sies H, Boveris A. Physiol Rev 1979; 59: 527-605.
[12] Choi DW. Trends Neurosci 1988; 11:465-469.
[13] Cooper AJL, Pulsinelli WA, Duffy TE. Glutathione and ascorbate during ischemia and Post ischemic reperfusion in rat brain, 1980, 35: 1224-1245.
[14] Coyle JT, Puttfarcken P. Oxidative stress, glutamate and neurodegenerative disorders. Science (Wash DC) 1993; 262:689-695.
[15] Dangles O, Fargeix G, Dufour C. J Chem Soc Perkin Trans 2000; 2:1653-1663.
[16] Das D, De PK, Banerjee RK. Biochem J 1995; 305:59-64.
[17] Davalos A, Castillo J, Serena J, Noya M. Duration of glutamate release after acute Ischemic stroke, 1997; 28: 708-710.
[18] Decker EA, Faustman C, Lopez-Bote CJ. Antioxidants in Muscle Foods, Nutritional Strategies to Improve Quality, New York, John Wiley & Sons, Inc 2000.
[19] Diesseroth A, Dounce AL. Physiol Rev, 1970; 50:319-375.

[20] Demirkaya S, Topcuoglu MA, Aydin A, Ulas UH, Isimer AI, Vural O. Eur J Neurol 2000; 8: 43-51.
[21] Doble A. Pharmacol Ther 1999; 81:163-221.
[22] Faden AI, Demediuk P, Panter SS and Vink R. Science (Wash DC) 1989; 244: 798-800.
[23] Fischer M, Schaebitz W. An overview of acute stroke therapy. Arch Intern Med 2000; 160:3196-3206.
[24] Fridovich I. Annual Review. Pharmacol Toxicol 1983; 23:29-257.
[25] Fridovich I. Arch Biophys 1986; 247: 1-11.
[26] Fujimura M, Morria-Fujimura Y, Kawase M, Copin J C, Calgui B, Epstein CJ, Chan P.H. J Neuroscience 1999; 19:3414-3422.
[27] Fukuyama N, Takizawa S, Ushida H, Hoshiai K, Shinohara Y, Nakazawa H. J cerebral blood flow Metab 1998; 18:125-129.
[28] Grisham MB and Mccord M. J Phy oxygen radical (Taylor A.E, Matolon S and Ward P) 1986; 1-18.
[29] Halliwell B, Gutteridge JM. Free radicals in biology and medicine 1999, 3rd edition, Oxford University press, New York, NY.
[30] Harman D. Drug aging 1993; 3:60-80.
[31] Jenner P and Olnaw CW. Neurology 1996; 47:161-176.
[32] Johnson MA, Fisher JG. Food Technol 1994; 48:112-120.
[33] Jones EA, Shoskes DA. J Urology 2000; 163:999-1004.
[34] Kimihoko Hattari Hanna Lee, Patricia D, Hurn, Barbara J, Crain Richard J, Traystrm A, Courtney De vries 2000; 20:1690-1701.
[35] Kornhuber J, Quack G. Cerebrospinal fluid and serum concentrations of N-methyl-D-Aspartate (NMDA) receptor antagonist memantine in man 1995; 195: 137-139.
[36] Lee JM, Zipfel GJ, Choi DW. Nature 1999; 399:A7-A14.
[37] Love S. Oxidative stress in brain ischemia. Brain pathol 1999; 9:119-131.
[38] Lowery OH, Rosebrough NJ, Farr AL and Randall RJ. J Biol Chem 1951; 193:265-275.
[39] Luo Y, Hattori A, Munoz J, Qin ZH, Roth GS. Molecular Pharmacology 1999; 56(2): 254-264.
[40] Ohkawa H, Ohishi N, Yogi K. Anal Biochem 1979; 95-351.
[41] Schugens M, Egerter R, Daum I et al. Neurosci Lett 1997; 224:57-60.
[42] Siesjo BK. Ann Emerg Med 1993; 22:959-969.
[43] Szabo C, Dawson VL. Trends Pharmacol Sci 1998; 19:287-298.
[44] Tietze F. Anal Biochem 1969; 27:502-522.
[45] Watson BD, Busto R, Goldberg WJ, Santisto M, Yoshida S, Ginsberg MD. J Neurochem 1984; 42:268-274.
[46] Ferrer I, Planas AM. J Neuropathol Exp Neurol 2003; 62:329–339.
[47] Bickler PE, Fahlman CS. Neuroscience 2004; 127:673–683.
[48] Oyama Y, Fuchs PA, Katayama N & Noda K. Brain Res 1994; 635:125-129.
[49] Arredondo F, Blasina F, Echeverry C, Morquio A, Ferreira F, Abin JA, Lafon L & Dajas F. J Ethnopharmacol 2003.
[50] Welton AF, Tobias LD & Fiedler-Nagy C. Progress in Clinical and Biological Res 1986; 213:231- 242.