EVALUATION OF CEREBROPROTECTIVE ACTIVITY OF QUERCETIN AND RUTIN IN ISCHEMIA REPERFUSION INDUCED CEREBRAL INFARCTION IN RATS

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Summary

Bioflavonoids quercetin and rutin were evaluated for their cerebroprotective role in experimental ischemia reperfusion induced cerebral infarction in rats. Percentage cerebral infarction, lipid peroxidation levels in brain tissue, superoxide dismutase, and catalase levels in brain tissue were estimated. Pretreatment with Quercetin and Rutin found to have cerebroprotective action in ischemia reperfusion induced cerebral infarction**.** There was a dose dependent and same degree of cerebroprotective activity in terms of reduction in infarct size, MDA, SOD, Catalse levels was observed with both the drugs. The observed cerebroprotection of Quercetin and Rutin might be due to enhanced antioxidant defense mechanisms like SOD and CAT and decrease in lipid peroxidation levels. This was clearly evidenced by the significant increase in SOD and CAT levels and significant decrease in tissue MDA levels when compared to untreated group animals.

Key words: Cerebral Infarction, Ischemia Reperfusion Injury, Bioflavonoids.

Introduction

Cerebral ischemic disease has become a worldwide health problem affecting all economic groups of society. Vigorous global research is underway in an effort to develop pharmacological means to control morbidity and mortality arising from cerebral ischemic disease. Drug therapy for ischemic stroke includes thrombolytics, defibrinogenating agents, anti-platelet agents, anti-coagulants etc. Thrombolytic therapy with various fibrinolytic agents and surgical interventions like carotid endarterectomy are used to re-establish the blood flow to the ischemic brain tissue can paradoxically produce a progressive destruction of cells, there by leading to tissue dysfunction and infarction. This injury is called reperfusion injury.

Research on antioxidants attempts were made to protect those cells from free radical attack in cerebral diseases. During ischemia and reperfusion, antioxidant compounds in form of Vitamins C, E and ß carotene or glutathione were tested to protect neuron from oxidative attack. Most *in vivo* and clinical studies of the effects of antioxidant supplementation in neurological diseases have focused on vitamin E. Increased the protection of reactive oxygen species during ischemia reperfusion injury leading to rapid consumption and depletion of endogenous scavenging antioxidants.

Numerous epidemiological studies suggest a protective role of dietary flavonoids against cerebral ischemic disease. Flavonoids possess a wide variety of potential cerebroprotective beneficial effects like antioxidant, anti-inflammatory, anti-platelet aggregatory activities and they can also restore endothelial function, prevent neutrophil accumulation and LDL oxidation. Many of them proved to be antithrombogenic. They also have been shown regulatory activity on certain hormones and enzymes (1).They has a gentle, beneficial action on numerous physiological processes in the body with minimal side effects. Also they inhibit the expression of heat shock proteins, which have a role in various diseases (2). Some of the diseases in which the therapeutic attempts have been made with flavonoids are diabetes, allergy, cancer, chemical oncogenesis, common cold, viral infection, ulcers, hypertension, cerebrovascular diseases, micro bleeding, atherosclerosis, hyperlipidaemia, pain and inflammation, night cramps, chronic prostatitis, asthma (2). Limited research work has been done on the cerebroprotective activities of various flavonoids in ischemia reperfusion injury in rats.

The present research work has been undertaken with an objective to investigate the cerebroprotective actions of flavonoids, Quercetin and Rutin in ischemia reperfusion induced cerebral infarction in rats. In our present study, the selected bioflavonoids namely Quercetin & Rutin were found to produce a dose dependent cerebral protective activity in terms of reduction in the infarct size in rats. Quercetin and Rutin are members of the class of flavonoids termed as flavonols. They are widely distributed in the plant kingdom. Quercetin is found abundantly in red wine, green tea, onions, berries, citrus fruits, parsely, apples, garlic. Rutin is abundantly found in black wheat and also in apple peels, garlic, tomatoes, and black tea. Commercially, quercetin is derived from blue-green algae (VITAMIN Retailer).Bioflavonoids quercetin and rutin were evaluated for their cerebroprotective role in experimental ischemia reperfusion induced cerebral infarction in rats. Percentage cerebral infarction, lipid peroxidation levels in brain tissue, superoxide dismutase, and catalase levels in brain tissue were estimated.

Materials and Methods

Chemicals:

- Thiopentone sodium (Neon-labs-Mumbai).
- T.T.C (2,3,4-tetrazolium chloride) (National Chemicals,Vadodara)
- PH 7.4: phosphate buffer solution (I.P).
- Rutin (Sigma Chemicals, U.S.A.)
- Quercetin (Sigma Chemicals, U.S.A.)

Unless otherwise specified all the chemicals and reagents used are of analytical grade.

Drug solutions:

As Rutin and Quercetin are very sparingly soluble in aqueous solutions, to solubilise these drugs, 99% dimethyl sulphoxide (DMSO) was used as vehicle and different concentrations (1mg/kg, 2mg/kg, 5mg/kg) were prepared. As DMSO has some cerebroprotective activity, animal group administered with only DMSO was kept as control group with which the activities of other groups were compared.

Experimental Work:

Design and surgical procedure:

In the present study, albino rats of either sex supplied by Chakraborthy Enterprises, Calcutta weighing 150 to 250gm were used. The rat was anaesthetized by giving Thiopentone sodium (40mg/kg) I.P. and was ventilated with room air using (Techno artificial respirator) positive pressure ventilator. Ventilator parameters were adjusted to maintain normal PH and satisfactory oxygenation.

Common Carotid Artery Ligation

Surgical technique for the induction of cerebral ischemia was adapted from earlier published method of Iwasaki et al, 1989. (3)Under anesthesia, a midline incision in neck was given. Common carotid arteries were identified and isolated carefully from vago-sympathetic nerve. Rats were made ischemia by occluding Bicommon Carotid Arteries (BCCA) with a silk thread for 30 minutes and reperfusion was allowed for 4hrs by removing the thread. Body temperature was maintained at about 37°C during the period with the help of a heating lamp. Drugs were administered intravenously though jugular vein, at the end of occlusion period i.e. at 28^{th} minute and before the beginning of the reperfusion.

Quantification of infarct size

Quantification of infarct size provides information regarding viable brain tissue, which is related to the functional capacity of the brain. Hence in the present study, Quantification of infarct size was measured by using 2,3,5- triphenyl tetrazolium chloride (TTC) stain as described by Johnson et al.

Determination of MDA levels

The assay is based upon the reaction of TBA with MDA, one of the aldehyde products of lipid peroxidation.MDA has been used as an index of tissue injury after ischemia-reperfusion by several investigators i.e Kokita et al., 1998; Ozden et al., 1998; Chen et al., 2000 etc. (4 &1) In the present study, MDA levels in brain tissue was determined after cerebral ischemia-reperfusion injury.

Superoxide Dismutase (SOD)

Superoxide dismutase (SOD) activity was determined by the method developed by Kakkar et al. The SOD level was expressed as Units mg protein^{-1}.(1)

Catalase (CAT)

Catalase activity was measured by the method of Aebi (1974). Activity of catalase was expressed as μmoles of H_2O_2 metabolized/mg protein/min.(1)

Experimental Groups:

Rats were randomly divided into nine groups, each containing of 5 animals. All the rats are treated with the drug just before reperfusion intravenously.

Group –I : sham control group.

Group –II : control group animals treated with saline.

Group –III : control group animals treated with DMSO.

Group –IV : animals treated with quercetin 1mg/kg.

Group –V : animals treated with quercetin 2mg/kg.

Group –VI : animals treated with quercetin 5mg/kg.

Group –VII : animals treated with rutin 1mg/kg.

Group –VIII : animals treated with rutin 2mg/kg.

Group – IX : animals treated with rutin 5mg/kg.

Data Analysis:

The results are expressed as mean ± SEM. Differences in percentage infarction of brain tissue, and tissue lipid peroxide levels were determined by Turkey test, one way - analysis of variance.

Results

In sham control group, percentage infarction of brain tissue, MDA, SOD, Catalase levels were found to be 6.28 ± 0.39, 11.15 ± 0.52 n.mol /gm, 42.84 ± 1.78 U/mg, 33.22 ± 2.01 u moles of H2O2 decomposed/mg protein/min respectively. Control group subjected to ischemia reperfusion and treated with the vehicle (saline), percentage infarction and MDA levels were significantly increased to 44.61 ± 1.43 and 115.2 ± 1.81 nmol MDA /gm tissue respectively.(Fig. 1to 4)

With control group animals treated with DMSO, percentage infarction and tissue MDA concentration were significantly decreased to 40.34 ± 1.39 and 109.56 ± 1.25 nmol MDA /gm respectively. In control group of animals subjected to ischemia reperfusion treated with the vehicle saline Antioxidant enzymes SOD and Catalse were decreased to 32.71 ± 0.82 U/mg protein) and 21.62 ± 0.77 u moles of H2O2 decomposed/mg protein/min respectively.(Fig.1 to 4)

With the control group animals treated with DMSO, tissue SOD level and catalase were significantly increased to 36.85 ± 1.60 (Units/mg protein) and 18.68 ± 1.40 (u moles of H2O2 decomposed/mg protein/min) respectively. The values obtained after the treatment with quercetin and rutin are compared with DMSO treated group. (Fig.1 to 4)

With the pretreatment of Quercetin at different dose levels i.e. 1mg/kg, 2mg/kg, 5mg/kg, there was a statistically significant reduction in the percent infarction of brain tissue and MDA levels in a dose dependent manner .The antioxidant Enzymes SOD and Catalse were significantly increased in a dose dependent manner. (Fig.1 to 4)

With the pretreatment of Rutin at different dose levels i.e. 1mg/kg, 2mg/kg, 5mg/kg, there was a statistically significant reduction in the percent infarction of brain tissue and MDA levels in a dose dependent manner. The antioxidant Enzymes SOD and Catalse were significantly increased in a dose dependent manner. (Fig. 1 to 4)

In the present study, it was found that bioflavonoids quercetin; rutin significantly reduced the cerebral infarct size, tissue MDA levels and significantly increased the tissue SOD, Catalase levels in experimental cerebral ischemia-reperfusion injury in rats. The observed cerebroprotective activity appears to be reducing lipid peroxidation and by increasing the antioxidant defense enzymes superoxide dismutase and catalase.

Fig – 1: Percentage Infarction of brain tissue of rats with sham control, saline control, DMSO control,and treated with quercetin and rutin (1mg/kg, 2mg/kg, 5mg/kg)

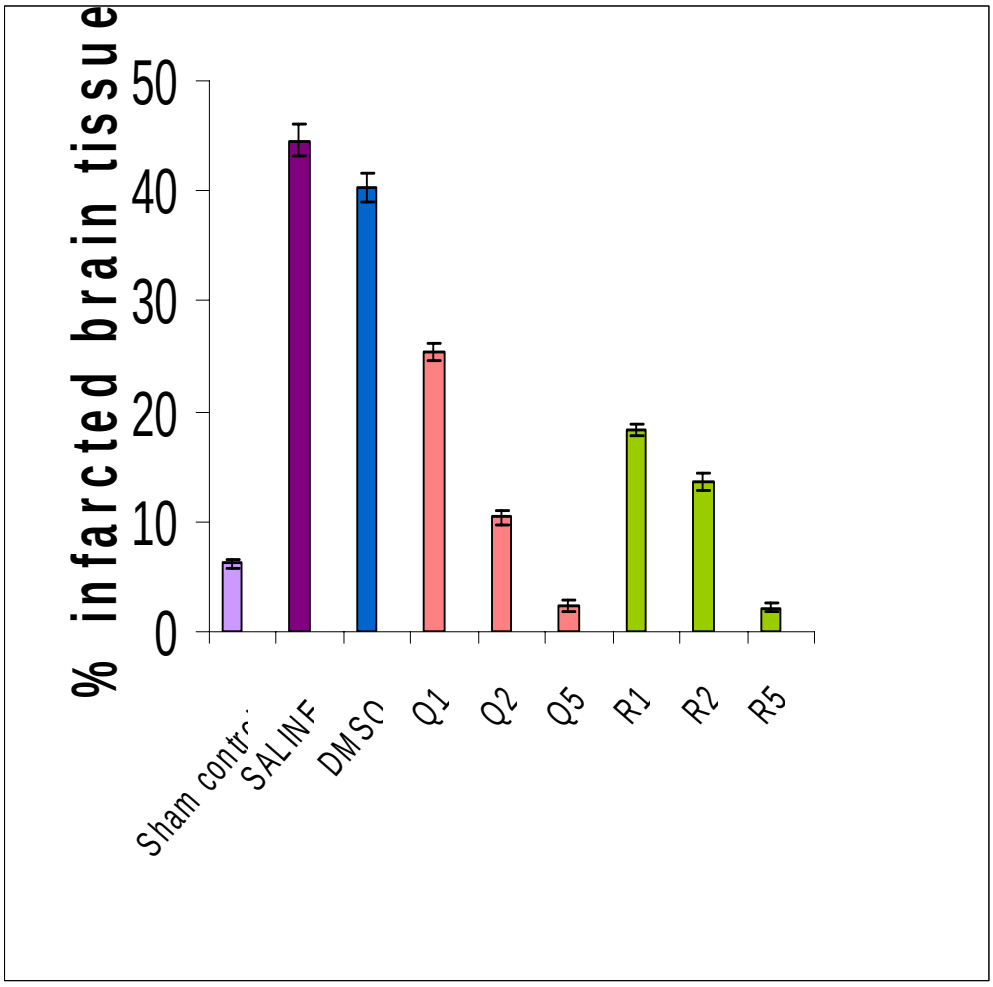


Fig – 2: MDA levels in brain tissue of animals with sham control, saline control,DMSO control, and treated with quercetin and rutin (1mg/kg, 2mg/kg, and 5mg/kg)

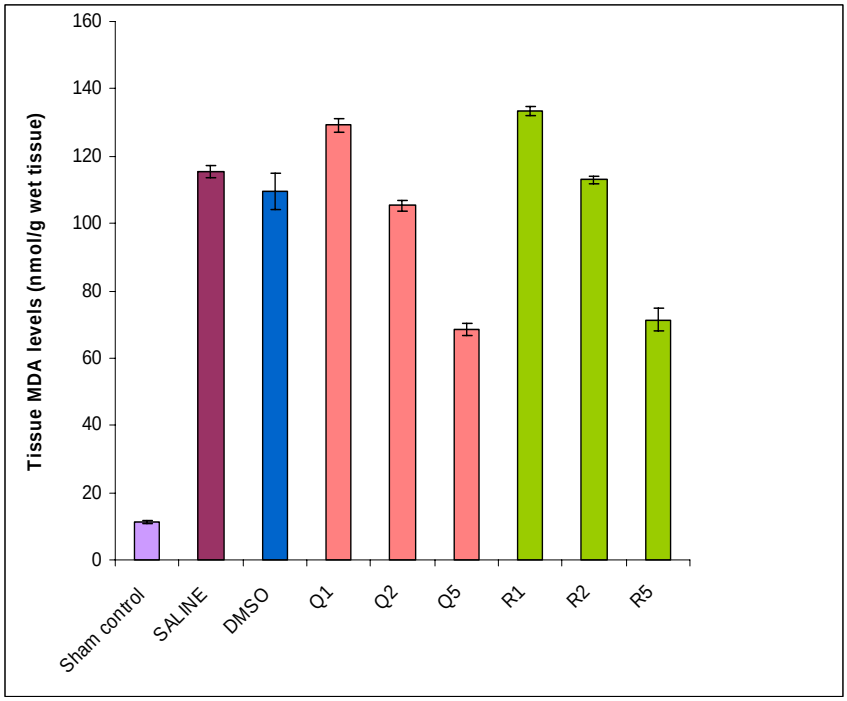


Fig – 3: Superoxide dismutase levels in brain tissue of animals with sham control, saline control, DMSO control, and treated with quercetin and rutin (1mg/kg, 2mg/kg, 5mg/kg)

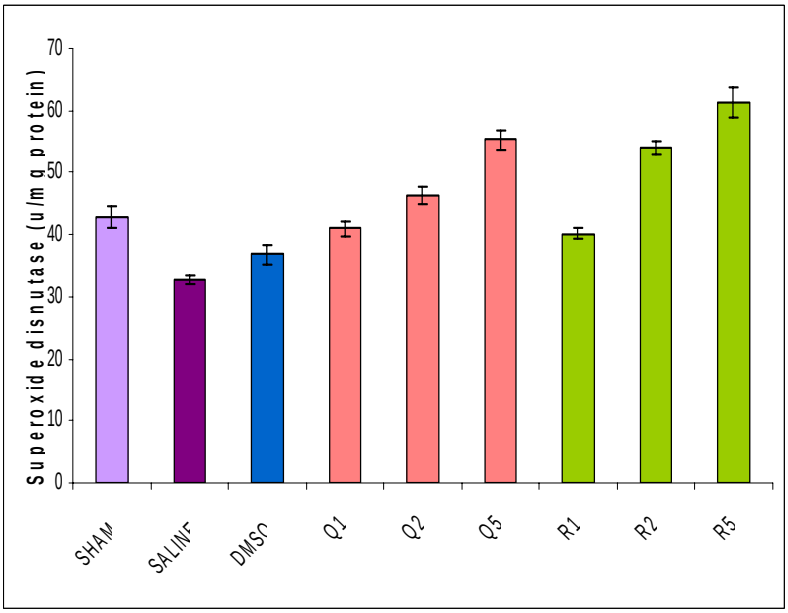
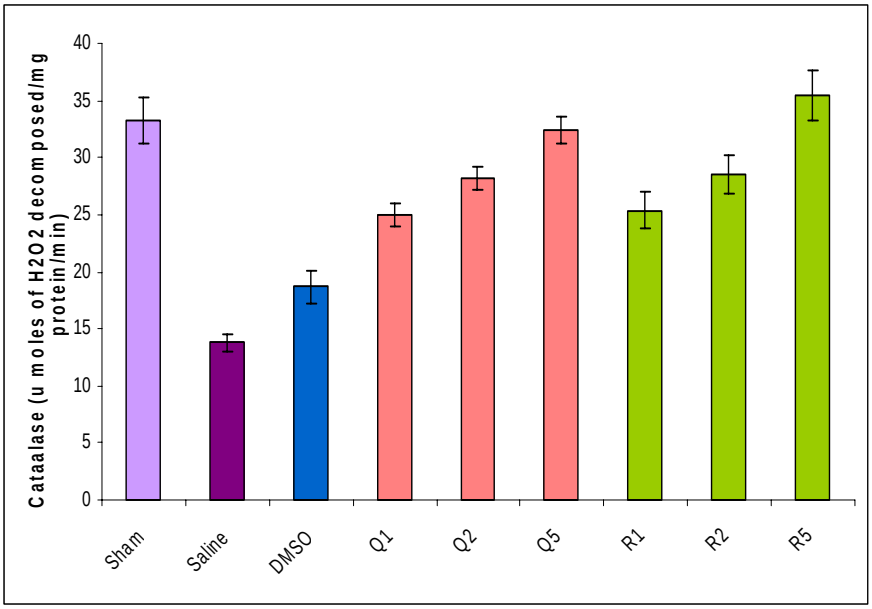


Fig – 4: Catalase levels in brain tissue of animals with sham control, saline control, DMSO control, and treated with quercetin and rutin (1mg/kg, 2mg/kg, 5mg/kg)



Discussion

Prolonged interruption of cerebral blood flow during ischemic disorders results in irreversible cell damage. Interventions such as thrombolyis, emergency percutaneous with surgical endarterectomy or angioplasty can restore blood flow to affected tissue. However, restoration of blood flow following acute disruption is also accompanied by a further injurious phenomenon known as cerebral reperfusion injury (5).

A balance is maintained between oxidative attack and antioxidant defense system prevailing in different tissues. But the brain has a low level of antioxidative defense (6).This increased level of oxidative stress seen in cerebral tissue is thus the major contributing factor for the development of neurodegenerative disease like cerebral ischemia reperfusion.

Oxygen free radicals that are generated during post ischemic reperfusion are causative agents of neuronal damage. Elevation of conjugated diene levels, the index of lipid peroxidation and cell damage was reported in the rat cerebral region after ischemia and reperfusion (7).

Numerous epidemiological studies have demonstrated an inverse correlation between flavonoids intake and the risk of death from cerebral disease. One large clinical study indicated that flavonoids may reduce mortality from cerebral disease. Due to the Anti-oxidant property of flavonoids, they act as cerebroprotective agents. Due to the beneficial effects on the stroke, the group of flavonoids has been the most studied. Many flavonoids were tried in the case of ischemia reperfusion injury and showed that flavone administration improved functional recovery in the rat brain during global ischemia and reperfusion.

Quercetin and Rutin are polyphenols present in relatively large concentration in red wine and, may play a role in this cerebro protective phenomenon. In the present study quercetin and rutin were evaluated for their cerebroprotective activity in ischemia reperfusion induced cerebral infarction in rats.

In vitro studies indicate that quercetin treatment significantly decreased the impairment of cerebral function following ischemia reperfusion. Also quercetin was demonstrated to have neuroprotective effect during experimental brain ischemia and it was examined in gerbils after transient forebrain ischemia. (1&2).Rutin is glycosylated form of quercetin. In 1994, Kanwaljit Chopra et al, through their studies assessed the ability of rutin to inhibit the infarct size. Also rutin was found to reduce ischemia reperfusion induced brain damage (8&9).

In our present study we have evaluated the cerebroprotective activities of different doses of quercetin and rutin in experimental ischemia reperfusion induced cerebral infarction in rats. They have offered very good cerebroprotective activity by significantly reducing the infarct size, when compare to control group. Also when three different doses (1mg, 2mg, and 5mg/kg) of rutin and quercetin were administered, both have offered same degree of cerebroprotective activity in terms of reduction in infarct size. At a dose level of 5mg/kg body wt, negligible infarction was observed i.e. complete cerebroprotective activity was observed, which was not reported earlier by any of synthetic drugs.

Though numerous mechanisms were proposed for occurrence of ischemic – reperfusion injury, oxidative stress by free radicals was found to have major contribution. Cells have developed a comprehensive assay of antioxidant defenses to prevent free radical formation.

They include sequestration of transition metal ions, removal of peroxides (by catalase, superoxide dismutase, α-tocopherols, etc.) and repair process (10).

Free radicals that are generated during post ischemic reperfusion are causative agents of neuronal damage. Elevation of conjugated diene levels, the index of lipid peroxidation and cell damage was reported in the rat cerebral region after ischemia and reperfusion (10).In our present study significant reduction in tissue lipid peroxidation in terms of MDA levels was found in the groups treated with different doses of quercetin and rutin. Even with the maximum dose 5mg/kg, which offered complete cerebroprotection, tissue MDA levels were not reduced to that of sham control levels. That means reduction in the tissue MDA levels might be partly responsible for the observed cerebroprotective activity of Quercetin and Rutin.

During cerebral ischemia and reperfusion, when free radicals production exceeds the buffering capabilities, both non- enzymatic scavengers (GSH) and antioxidant enzymes (SOD, GPx, CAT) decrease. Decrease in the free radical production was followed by a gradual normalization of both GSH and antioxidant enzymes (8).

Because of the brain's low concentrations of antioxidant substances like glutathione (9), antioxidative enzymes including superoxide dismutase (SOD), and catalase (CAT) (9&10), the brain is exceptionally vulnerable to ischemia and reperfusion induced oxygen free radicals, which cause oxidative damage to brain lipids, proteins, and nucleic acids, leading to brain dysfunction and cell death (11).

Flavonoids can interfere directly with different free radical producing systems or can also increase the function of the endogenous antioxidant (10 & 11).In line with the earlier reports, the present study also demonstrated the decreased levels of tissue SOD and catalase in normal control rats that were subjected to cerebral ischemia reperfusion injury. It was reported by the earlier experiment studies that Scutellaria flavonoid could increase the content of SOD in brain tissues after cerebral ischemia.(12).

Mice over expresses SOD so they are more resistant to ischemia associated with reperfusion, and conversely, larger infarcts develop in SOD knockout mice (6). The present study also supports the protective role of tissue SOD during cerebral ischemia reperfusion injury.

That was evidenced by the significant increase the tissue SOD levels in the treated groups than that of sham control group parallel to the reduction in the infarct size. The present study also demonstrated that Quercetin and Rutin treated animals were exhibited to increase in the tissue Catalase levels equal to that of sham control.

However bioflavone fraction was ineffective to increase CAT levels in the cerebellum region this may be explained by insufficient concentration and antioxidative capacity to defend adequately against oxygen free radicals generated in the brain region during ischemia.

The observed cerebroprotection of Quercetin and Rutin might be due to enhanced antioxidant defense mechanisms like SOD and CAT and decrease in lipid peroxidation levels. This was clearly evidenced by the significant increase in SOD and CAT levels and significant decrease in tissue MDA levels when compared to untreated group animals.

Conclusions

Cerebroprotective activity of Quercetin and rutin was evaluated in experimentally induced cerebral ischemia reperfusion injury in rat model and pretreatment with Quercetin and Rutin found to have cerebroprotective action in ischemia reperfusion induced cerebral infarction**.** There was a dose dependent and same degree cerebroprotective activity in terms of reduction in infarct size, MDA, SOD and Catalse levels in brain tissue. Complete cerebroprotective activity was observed with both quercetin and rutin at the dose of 5mg/kg body weight. The observed cerebroprotection of Quercetin and Rutin might be due to enhanced antioxidant defense mechanisms like SOD and CAT and decrease in lipid peroxidation levels. This was clearly evidenced by the significant increase in SOD and CAT levels and significant decrease in tissue MDA levels when compared to untreated group animals.

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