NEUROPROTECTIVE EFFECT OF QUERCETIN IN NEUROTOXICITY INDUCED RATS: ROLE OF NEUROINFLAMMATION IN NEURODEGENERATION

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ABSTRACT

Objective: The aim was to study the neuroprotective effect of quercetin in the animal model of neurodegeneration.

Materials and Methods: Quercetin (3,5,7,3',4'-pentahydroxy flavones) is a potential compound having both anti-inflammatory and anti-oxidant properties with low gastric and cardiac side effect. Different Cyclooxygenase (COX-2) inhibitors such as nimesulide, refecoxib and celecoxib have been proved to have their neuroprotective action in different animal models of neurodegenerative disorders, but they are burdened with high toxicity. Different neurodegenerative models like haloperidol-induced catalepsy, reserpine induced vacuous chewing movements and 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced neurodegeneration were evaluated with levadopa at a dose of (30 mg/kg i.p.) and quercetin at a dose of (25 mg/kg, p.o.) as standard and test drugs respectively.

Results: In the haloperidol-induced catalepsy model the increased cataleptic score was significantly reduced with both the standard drug levodopa and the test drug quercetin. The increased frequencies of vacuous chewing movements on administration of reserpine were reversed with the treatment of quercetin. The reduced actophotometer activity score due to reserpine was significantly reversed by quercetin. The decreased level of lipid per-oxidation and increased glutathione concentration by the administration of quercetin that reversed the toxicity of MPTP.

Conclusion: Quercetin is a potential compound having both anti-inflammatory and anti-oxidant properties. These effects of enlights the pharmacodynamic pathway of neuroprotective properties of quercetin in animal model study.

Keywords: Catalepsy, Cyclooxygenases, Neuroprotection, Quercetin.

INTRODUCTION

Based on the neuropathology, inflammation plays a remarkable role in the development of neuro-inflammatory and neurodegenerative disorders. Parkinson's disease (PD) is the second most neurodegenerative disease after Alzheimer's disease and first with movement disorders. Currently, about 2% of the worldwide population over the age of 60 are affected by this neurodegenerative disease [1,2].

Cyclooxygenases (COX) are expressed in different parts of the brain and therefore, it can be speculated that those are involved in the neuropathology of various central nervous system related disorders. COX type-2 is involved in the inflammatory components of the ischemic cascade, playing an important role in the progression of brain damage. Neuronal expression of COX-2 is increased in the course of catalepsy, parkinsonism, seizures and neuroprotection as demonstrated by COXs inhibitors in several models of neurodegeneration both in *in vitro* and *in vivo* [3,4].

Different COX-2 inhibitors such as nimesulide, refecoxib and celecoxib have been proved to have their neuroprotective action in different animal models of neurodegenerative disorders, but due to various adverse reactions like gastric ulceration, cardiotoxicity they are not regarded as a drug of choice in treatment strategies of neuronal diseases [5].

Quercetin (3,5,7,3',4'-pentahydroxy flavones) was chosen as the lead compound in the development of anti-inflammatory agent due to its low gastric ulcer side-effect and in addition to its inhibitory action on prostaglandins (PG) production, COX-2 expression and nuclear factor-κB (NF-κB) activation. Quercetin is a potential compound having both anti-inflammatory and anti-oxidant properties [6]. In this study, the neuroprotective effect of quercetin was studied in the animal model of neurodegeneration.

MATERIALS AND METHODS

Haloperidol induced catalepsy model

Animals

Wistar rats (180-250 g) of either sex, procured from Animal House of School of Pharmaceutical Sciences, Siksha O Anusandhan University (Regd No. 117/c//CPCSEA) were used. They were acclimatized to the laboratory conditions from 1 week before studies. The animals had free access to food and water and maintained at 12:12 hr light and dark cycles.

Drugs

Haloperidol (Sigma Alchid) at a dose of (10 mg/kg i.p.) was used as catalepsy inducing agent. Levadopa (Sun Pharma) at a dose of (30 mg/kg i.p.) and quercetin (Merk) at a dose of (25 mg/kg, p.o.) was used as standard and test drugs, respectively.

Cataleptic score study

The animals were divided into four groups (n=6). Group I served as control, Group II as toxic control (Haloperidol 1 mg/kg, i.p.), Group III treated with standard (Levodopa 30 mg/kg i.p.)+(Haloperidol 1 mg/kg, i.p). Group IV treated with quercetin (25 mg/kg, p.o.). Catalepsy was induced with haloperidol (1.0 mg/kg i.p.) and assessed at 15 minutes interval for 90 minutes on a standard bar test. Catalepsy was assessed in terms of the time for which the mouse maintained an imposed position with both front limbs extended and resting on a 4 cm high bar (1 cm diameter). The end point of catalepsy was considered to

occur when both front paws were removed from the bar or if the animal moved its head in an exploratory manner. A cut-off time of 5 minutes was applied. All observations were made between 10.00 and 16.00 hrs in a quiet room at about 30°C [7,8].

Reserpine induced dyskinesia

Animals

Wister rats (180-250 g) of either sex, procured from Animal House of School of Pharmaceutical Sciences, Siksha O Anusandhan University (Regd No. 117/c//CPCSEA) were used. They were acclimatized to the laboratory conditions from 1 week before studies. The animals had free access to food and water and maintained at 12:12 hr light and dark cycles.

Drugs

Reserpine (Sigma Alchid) at a dose of (10 mg/kg i.p.) was used as catalepsy inducing agent. Levadopa (Sun Pharma Ltd) at a dose of (30 mg/kg i.p.) and quercetin (Merk) at a dose of (25 mg/kg, p.o.) was used as standard and test drugs respectively.

Dyskinesia induced by acute reserpine administration in rats

Reserpine (1 mg/kg, s.c.) was repeatedly administered to rats on alternative days for a period of 5 days (days 1, 3, and 5) to induce oral dyskinesia. Total study period was 14 days. 24 rats were divided into 4 groups of 6 animals each.

Group 1: The animals served as control.
Group 2: The animals received reserpine (1 mg/kg, s.c.) and served as a negative control.
Group 3: The animals received reserpine (1 mg/kg, s.c.) injections and treated with standard (Levodopa 30 mg/kg i.p.).
Group 4: The animals received reserpine (1 mg/kg, s.c.) injections and treated with quercetin (25 mg/kg, p.o.).

Behavioral study

Assessment of orofacial dyskinesia in plexiglass cage

The rats were placed individually in a small plexiglass observation chamber for the assessment of oral dyskinesia. The animals were allowed 10 minutes to get used to the observation chamber before behavioral assessments. The number of vacuous chewing movements is referred to as single mouth openings in the vertical plane not directed toward physical material. Counting was stopped whenever rat began grooming and restarted when grooming stopped. Mirrors were placed under the floor and behind the back wall of the chamber to permit the observation of oral dyskinesia when the animal was facing away from the observer. The behavioral parameters of oral dyskinesia were measured continuously for a period of 5 minutes. The assessment was carried out in 14th day [9].

Actophotometer test

The animals were placed individually for 10 minutes in actophotometer. The activity score on the digital counter were noted [10].

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced neurodegeneration

Animals

Wister rats (180-250 g) of either sex, procured from Animal House of School of Pharmaceutical Sciences, Siksha O Anusandhan University (Regd No. 117/c//CPCSEA) were used. They were acclimatized to the laboratory conditions from 1 week before studies. The animals had free access to food and water and maintained at 12:12 hr light and dark cycles [11].

Drugs

MPTP (Sigma Alchid) at a dose of (10 mg/kg i.p.) was used as the inducing agent. Levadopa (Sun Pharma Ltd.) at a dose of (30 mg/kg i.p.)

and quercetin (Merk) at a dose of (25 mg/kg, p.o.) was used as standard and test drugs respectively.

Experimental protocol

Four groups were employed in the present study, each comprising of six animals.

Group 1: The animals served as control.
Group 2: As toxic control by administering MPTP (10 mg/kg) for the 7th, 9th, 11th, 13th, 15th days of treatment.
Group 3: Animals received levadopa (30 mg/kg, i.p.)+MPTP (10 mg/kg) for the 7th, 9th, 11th, 13th, 15th days, and all other days only levadopa (30 mg/kg, i.p.) upto 15th day.
Group 4: Animals received quercetin (25 mg/kg, p.o.)+MPTP (10 mg/kg) for the 7th, 9th, 11th, 13th, 15th days and all other days only quercetin (25 mg/kg, p.o.) up to 15th day.

Biochemical estimation

On 15th day, all the animals were sacrificed, and brain was isolated for biochemical estimation. The brain tissue homogenate was prepared with 0.1 M phosphate buffer (pH 7.4). Homogenized brain tissue was used to assess lipid peroxidation, catalase activity and glutathione (GSH) activity.

Measurement of lipid peroxidation

The formation of lipid peroxidase during lipid peroxidation process was analyzed by measuring the thiobarbituric-acid-reacting substances in cells. Briefly, the brain homogenized samples were mixed with 50 mm potassium phosphate monobasic buffer pH 7.4 and catalytic system of formation of free radicals ($FeSO_4$ 0.01 mm and ascorbic acid 0.1 mm), and then incubated at 30°C for 30 minutes. The reaction was stopped with 0.5 ml of trichoroacetic acid 10%, and then the samples were retried and mixed with 0.5 ml of thiobarboturic acid 0.8% then heated in a boiling water bath for 15 minutes. And after this period, immediately cooled in the ice bath. Lipid peroxidation was determined at 532 nm and expressed in μmol of malondialdehyde/g tissue [12,13].

Evaluation of GSH level

GSH levels were evaluated to estimate endogenous defenses against oxidative stress. The method was based on Elman's reagent (DTNB) reaction with free thiol groups. To brain homogenate sample, 0.02 M ethylenediaminetetraacetic acid was added with 50% trichloroacetic acid solution. After centrifugation (3000 rpm/15 minutes), the supernatant was collected, and production levels of GSH are determined as described by Sedlack and Lidsay. The samples were mixed with 0.4 M tri-HCl buffer, pH 8.0 and 0.01M DTNB. GSH levels was determined at 412 nm and reported as ng of GSH/g wet tissue [14].

RESULTS

Haloperidol induced catalepsy model

Cataleptic score

The cataleptic score was significantly reduced after 60 minutes, with both, the standard drug levodopa (30 mg/kg i.p.) and the test drug quercetin (25 mg/kg, p.o.). The reduction in cataleptic scores with quercetin (25 mg/kg, p.o.) was significant throughout the period of observations, till 120 minutes (Fig. 1). The reduction of cataleptic score with levodopa (30 mg/kg i.p.) and quercetin (25 mg/kg, p.o.) was seen only after 60 minutes of observation.

Reserpine induced dyskinesia

Assessment of orofacial dyskinesia

Reserpine (1 mg/kg, s.c.) treated animals shown increased frequencies of vacuous chewing movements and tongue protrusions compared with control. Treatment with levodopa (30 mg/kg i.p.) and quercetin (25 mg/kg, p.o.) significantly reversed reserpine induced vacuous chewing movements and tongue protrusion in animals (Fig. 2).

Actophotometer test

The locomotor activity score (mean±standard error of mean (SEM) in actophotometer are reported in Table 1. Reserpine (1 mg/kg, s.c.) significantly ($p<0.05$) reduced the activity score as compared to control. When levodopa (30 mg/kg i.p.) and the test drug quercetin (25 mg/kg, p.o.) were administered there was a significant increase in activity score as compared to control. Both levodopa (30 mg/kg i.p.) and the test drug quercetin (25 mg/kg, p.o.) significantly increase the decrease in activity score caused by reserpine (1 mg/kg, s.c.).

MPTP induced neurodegeneration

Biochemical estimation

Measurement of lipid peroxidation

The effect of levodopa (30 mg/kg i.p.) and quercetin (25 mg/kg, p.o.) on lipid peroxidation in MPTP (10 mg/kg) induced neurodegeneration model is given in Fig. 3.There is a significant ($P<0.05$) increase in lipid peroxidation in MPTP (10 mg/kg) administered rats when compared to control. On administration of levodopa (30 mg/kg i.p.) and the test drug quercetin (25 mg/kg, p.o.) there is a significant ($p<0.05$) decrease in lipid peroxidation.

GSH level

The effect of levodopa (30 mg/kg i.p.) and the test drug quercetin (25 mg/kg, p.o.) on GSH level in MPTP (10 mg/kg) induced neurodegeneration model is given in Fig. 4. The GSH level in rat brain is significantly ($p<0.05$) decreased by MPTP (10 mg/kg) in rats when

Table 1: Effect of levodopa (30 mg/kg i.p.) and the test drug quercetin (25 mg/kg, p.o.) on locomotor activity in reserpine (1 mg/kg, s.c.) induced rats using actophotometer test

Groups	Treatments	Activity score (mean±SEM)
I	Control	249.16±3.40
II	Reserpine (1 mg/kg, s.c.)	61.83±1.86*
III	Reserpine (1 mg/kg, s.c.)+ levodopa (30 mg/kg i.p.)	238.33±1.86*
IV	Reserpine (1 mg/kg, s.c.)+ quercetin (25 mg/kg, p.o.)	224.16±1.83*

SEM: Standard error of mean. The values are expressed as mean±SEM. Comparisons are made between: Group I with Group II and Group II with Group III and IV. Statistical significant test for comparison was done by one way ANOVA followed by Dunnet's t-test. *p<0.05
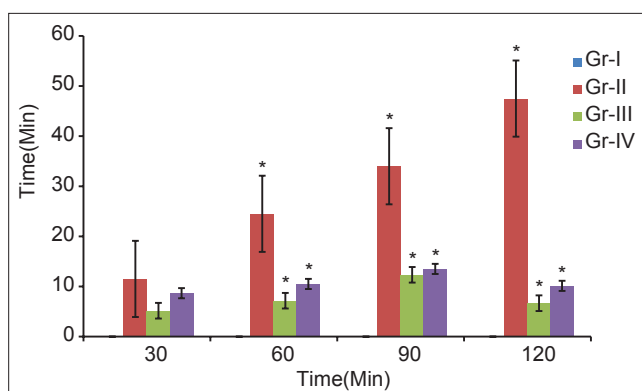

Fig. 1: Effect of levodopa (30 mg/kg i.p.) and the test drug quercetin (25 mg/kg, p.o.) on cataleptic score in haloperidol 1 mg/kg, i.p induced rats. The values are expressed as mean±SEM. Comparisons are made between: Group I with Group II and Group II with Group III and IV statistical significant test for comparison was done by one way ANOVA followed by Dunnet's t-test. *p<0.05
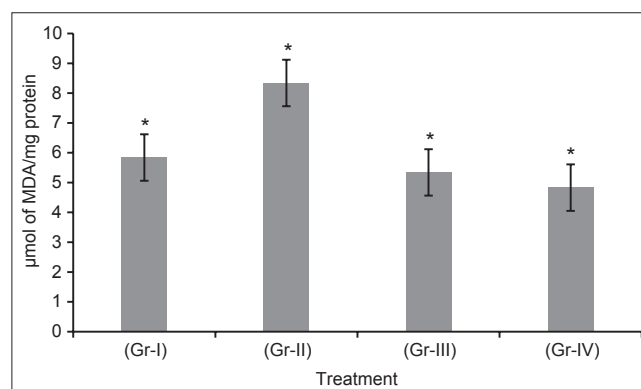

Fig. 3: Effect of levodopa (30 mg/kg i.p.) and the test drug quercetin (25 mg/kg, p.o.) on lipidperoxidation level in MPTP 10 mg/kg, i.p induced rats. The values are expressed as mean±SEM. Comparisons are made between: Group I with Group II and Group II with Group III and IV. Statistical significant test for comparison was done by one way ANOVA followed by Dunnet's t-test. *p<0.05
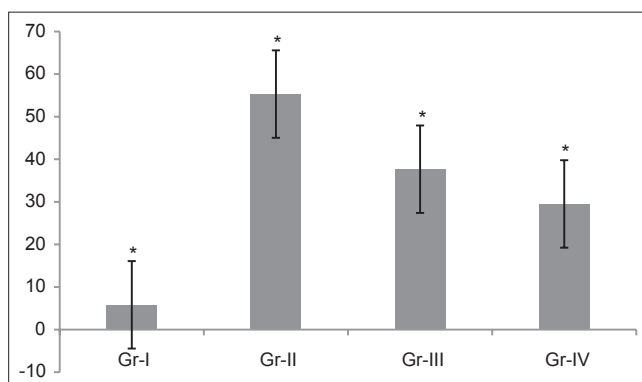

Fig. 2: Effect of levodopa (30 mg/kg i.p.) and the test drug quercetin (25 mg/kg, p.o.) on orofacial dyskinesiain reserpine (1 mg/kg, s.c.) induced rats. The values are expressed as mean±SEM. Comparisons are made between: Group I with Group II and Group II with Group III and IV. Statistical significant test for comparison was done by one way ANOVA followed by Dunnet's t-test. *p<0.05
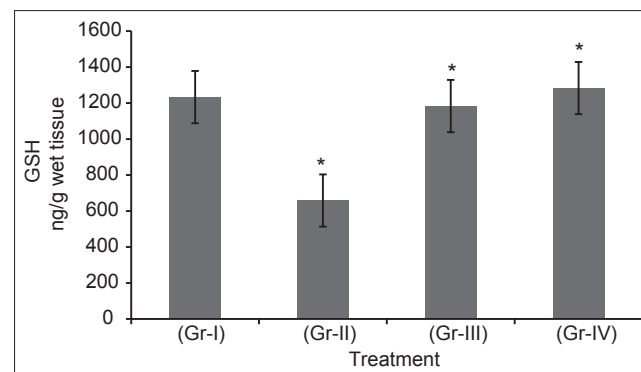

Fig. 4: Effect of levodopa (30 mg/kg i.p.) and the test drug quercetin (25 mg/kg, p.o.) on glutathion level in MPTP 10 mg/kg, i.p induced rats. The values are expressed as mean±SEM. Comparisons are made between: Group I with Group II and Group II with Group III and IV. Statistical significant test for comparison was done by one way ANOVA followed by Dunnet's t-test. *p<0.05

compared to control. On administration of levodopa (30 mg/kg i.p.) and the test drug quercetin (25 mg/kg, p.o.), there is significant (p<0.05) increase in GSH level.

DISCUSSION

In the mammalian brain, COX-II is constitutively expressed in specific neuronal population under physiological conditions. Neuronal expression of COX-II (m-RNAs and protein) is increased in brain of aging animals that suggest a role of their enzymes in neurodegeneration. COX-II expression is also increased during seizures and stroke. It has been shown that normally COX-II is expressed in low levels in nigral dopaminergic neurons, but it becomes upregulated in both patients and experimental models of. PD neuroprotection was demonstrated by COX inhibitors in several models of neurodegeneration both *in vitro* and *in vivo* [15-18].

Accumulating evidence suggests that inflammation plays an important role in the progression of neurodegenerative diseases. Among many inflammatory factors found in neurodegenerative brain, COX, specifically the inducible isoform, COX-II is believed to be a critical enzyme in the inflammatory response. Induction of COX-II is also found in an experimental model of PD produced by administration of MPTP. Genetic or pharmacological inactivation of COX-II, but not of COX-I is associated with neuroprotection in mice and rats exposed to MPTP [19,20].

Selective COX-II inhibitor valdecoxib and deficiency of COX-II (COX-II deficient mice or C57BL/6 mice) inhibits microglial activation and protects the nigrostriatal dopaminergic system against MPTP-induced neurotoxicity and behavioral deficits. Selective COX-II inhibitors like refecoxib offers protection against drug-induced catatonia and MPTP-induced striatal lesions possibly by modulating dopaminergic neurotransmission and oxidative stress. MPTP produces oxidative stress as demonstrated by increased lipid peroxides, nitrate and decreased antioxidant defense system in whole brain and striatal region in particular. In another study inhibition of either COX-I or COX-II by acetyl salicylic acid or preferentially COX-II by meloxicam provided a clear neuroprotection against MPTP-toxicity on striatal and nigral levels. Nimesulide also significantly reversed the behavioral, biochemical, mitochondrial and histological alterations by MPTP [21-24].

All aerobic organisms are susceptible to oxidative stress simply because there is reactive oxygen species like superoxide, hydrogen peroxide, etc. are produced by mitochondria during respiration. Brain is considered abnormally sensitive to oxidative damage because brain is enriched in the more easily peroxidizable fatty acids, consumes an excessive fraction (20%) of total oxygen consumption for its reactively small weight (1%) and not particularly enriched in antioxidant defenses. In addition, human brain has lighter levels of iron (Fe) in certain regions and in general has high levels of ascorbate. Thus, if tissue organizational disruption occurs, the Fe/ascorbate mixture is expected to be an abnormally potent pro-oxidant for brain membranes [25-28].

Activation of COX enzymes and oxidative stress are two separate pathogenic mechanisms, which have been implicated as major contributors to neurodegenerative diseases. The common link between these seemingly disparate processes is the oxidation of Arachidonic acid (AA) to yield bioactive oxidized lipids. Interestingly, both COX mediated and stress mediated oxidation of AA can lead to the generation of electrophitic lipid species containing unsaturated cyclopentenone ecosanoids rapidly form michael adducts with cellular thiols, including those found in GSH and proteins. These cyclopentenone ecosanoids in the brain may represent a novel pathogenic mechanism which contributes to many neurodegenerative conditions [29,30].

Reserpine produces spontaneous oral dyskinesia that develops as a result of persistent neuropathological changes in brain. Existing evidences strongly indicated that reserpine induced oral dyskinesia was closely associated with oxidative stress process. Free radicals

are effectively involved in the development of orofacial dyskinesia in rats. Reserpine treated animals showed an increase in levels of lipid peroxidation and also exhibited low levels of detoxifying enzymes such as Superoxide dismutase, catalase and GSH suggesting possible induction of free radicals generation [31-33].

The central PG synthesis may have a role in the development of cataleptic behavior. The cataleptic behavior induced by haloperidol was inhibited the dose-dependently by oral pre-treatment with COX inhibitors like aspirin and indomethacin.

CONCLUSION

Quercetin (3,5,7,3',4'-pentahydroxy flavones) is a bioflavonoid with strong antioxidant properties. It inhibits PGE2 production, COX-2 expression, inducible nitric oxide synthase expression and nuclear factor-κB activation. Our study found that the quercetin possesses neuroprotective effect and may be useful in preventing oxidative damage in the brain.

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