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Cerebral Ischemia/Reperfusion Injury in Rat Brain: Effects of Hesperidin and Hesperetin

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ABSTRACT

In the present study, the neurological activity was determined by evaluating the infarct size and antioxidant parameters before and after surgical procedures. Rats were anesthetized by administering thiopental sodium (45 mg/kg) by i.p. route. Focal cerebral ischemia-reperfusion (I/R) was produced by occluding the common carotid cerebral artery (BCCAO method) for 40 min and reperfusion for 5 h was allowed. Hesperidin and Hesperetin were administered in three variable doses (viz., 30, 60, and 90 mg/kg., i.p.) for focal. The long-term cerebral hypoperfusion was induced by doubly ligating the common carotid arteries for 28 days by the BCCAO method. The drug Hesperidin and Hesperetin were administered in three variable doses (viz., 30, 60, and 90 mg/kg., I.p.) for 28 days. Ischemia-induced neuronal damage was assessed by cerebral infarct area and biochemical estimations. The brain-infracts area and oxidative changes due to induction of ischemia injury were significantly attenuated by intraperitoneal treatment of Hesperidin and Hesperetin(viz., 30, 60, and 90 mg/kg., i.p.). These observations suggest that the drug Hesperidin and hesperetinposed neuroprotective actions in cerebral ischemic injury by antioxidant mechanisms, and be useful as an adjunct in the treatment of stroke.

INTRODUCTION

Cerebral/ Brain ischemia or stroke is a state where the blood source to the brain cell is inadequate for a certain period. In general medicine, ischemia is well-defined as a constraint in blood source to tissues which causes a scarcity of both oxygen and glucose required for cellular metabolism (Taylor FC and Kumar SK, 2012).

Stroke is a clinical disease, which can be alienated into two comprehensive categories which explain its pathophysiology: Ischemic strokes are produced by sudden occlusion of arteries delivering blood to the brain, either due to a thrombus at the spot of occlusion or formed in an alternative part of the circulation (Chen L *et al.,* 2013). It accounts for 50% - 85% of all strokes worldwide. Hemorrhagic stroke is produced by subarachnoid hemorrhage (bleeding from one of the brain's arteries into the brain tissue) or intracerebral hemorrhage (arterial bleeding in meninges between the spaces) (Humphrey PR and Marshall J, 1981). This category of stroke accounts for 1% - 7% and 7% - 27% respectively of all strokes worldwide. However, reperfusion after cerebral ischemia leads to more brain damage as well as mitochondrial dysfunction, cerebral edema, cerebral hemorrhage, neuronal death, and dopamine toxicity (Wu J and Hecker JG, 2009).

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Ischemic strokes can be treated with inoculations of a medication known as alteplase, which dissolves blood clots and reinstates the bloodstream to the brain (Ueshima H *et al.,* 2004). A minor amount of severe ischemic strokes may be attended to by an emergency process known as thrombectomy. This helps in removing blood clots and also helps in restoring the bloodstream to the brain (Schrader J *et al.,* 2005).

Certain effects in the brain are due to the neuroprotective activity of dietary flavonoids, with a potential to guard neurons counter to injury made by neurotoxins, a capability to suppress neuroinflammation, and the possibility to enhance learning recollecting and cognitive function (Keyser JD *et al.,* 1999).

Since flavonoids have poor solubility and bioavailability, in the present study, we have selected Hesperetin to explore its neuroprotective activity against two cerebral ischemic models i.e. focal cerebral ischemia and global cerebral ischemia.

MATERIAL AND METHODS

Experimental animals

Healthy Wister albino rats of bodyweight 200-250 g of either sex were procured from, CPCSEA approved vendors, were housed in polypropylene cages in a fully ventilated room, and maintained under standard conditions like 12:12 h light and dark cycle and were housed at the temperature of 25 ± 2°C. Experimental animals were fed with a standard diet and water *ad libitum*. The animals were maintained under CPCSEA guidelines for the use and care of laboratory animals.

Drugs and chemicals

Hesperidin, and Hesperetin, were purchased from Sigma-Aldrich Co. USA. All other chemicals were used of analytical grade.

BCCAO induced Focal cerebral Ischemia

Rats were randomly divided into 11 groups, each group consisting of 6 animals. Group 1: sham-operated control without I/R, Group 2: rats received 0.2 ml of saline and served as a control subjected to ischemia-reperfusion (with I/R) as vehicle control, Group 3: Hesperidin (30 mg/kg), Group 4: Hesperidin (60 mg/kg), Group 5: Hesperidin (90 mg/kg), Group 6: Hesperetin (30 mg/kg), Group 7: Hesperetin (60 mg/kg), Group 8: Hesperetin (90 mg/kg).

Group 9: Allopurinol (10 mg/kg), Group 10: Allopurinol (10 mg/kg) + Hesperidin (90 mg/kg).

Group 11: Allopurinol (10 mg/kg) + Hesperetin (90 mg/kg)-10 min before reperfusion. Focal cerebral I/R injury in rats was induced by BCCAO for 50 min followed by 5 h of reperfusion. Rats were anesthetized using thiopental sodium (45 mg/kg) intraperitoneally and fixed on a surgery board in the flat position. Body temperature was kept at 37±0.5ºC with the help of a heating lamp source throughout the surgery. Ischemia is induced by occluding the bi common carotid artery (BCCA) with a thread for 50 mins by the longitudinal incision of 1.5 cm in length of ventral cervical skin on the neck was made to expose the carotid arteries and isolated with careful conservation of vagosympathetic nerve or vagus nerve. Both left and right carotid arteries were ligated with the help of releasers. After 5 h of reperfusion, rats were decapitated in the focal

group. In operation of the sham group was performed with a similar surgical procedure, except the carotid arteries were not occluded.

Induction of Global chronic Cerebral Ischemia

Rats were randomly divided into 11 groups, each group consisting of 9 animals. Group 1: sham-operated control without I/R, Group 2: rats received 0.2 ml of saline and served as a control subjected to ischemia-reperfusion (with I/R) as vehicle control, Group 3: Hesperidin (30 mg/kg), Group 4: Hesperidin (60 mg/kg), Group 5: Hesperidin (90 mg/kg), Group 6: Hesperetin (30 mg/kg), Group 7: Hesperetin (60 mg/kg), Group 8: Hesperetin (90 mg/kg). Group 9: Allopurinol (10 mg/kg), Group 10: Allopurinol (10 mg/kg) + Hesperidin (90 mg/kg). Group 11: Allopurinol (10 mg/kg) + Hesperetin (90 mg/kg)-for 7 days followed by surgery. After separating both left and right carotid arteries from the vagus nerve, the carotid arteries were doubly ligated with silk thread and cut in between. The sham group received a similar operation without ligation of the carotid arteries. The skin at the surgery site was sutured and the animals were returned to the home cage. Then the dose of hesperetin 30 mg/kg & hesperidin methyl chalcone 30 mg/kg was continued up to the 7th post-surgical day. On the 7thday, 60 min after the last dose of the drug, the animals were examined for locomotor activity testing and decapitation as the endpoint (Nakamura H *et al.,* 2010).

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Infarct Size Measurement

For the infarct size measurement from each group, two animals were decapitated under deep anesthesia and the brains were removed and coronally sliced into 2 mm thick sections, later the slices were incubated with phosphate-buffered saline (pH 7.4) which contains 2% of TTC at 37°C for 30 min. And sections were fixed in 10% neutral buffered formalin overnight, which is expressed as a percentage of the total measured brain area. During this particular period, TTC was converted to red formazan pigment, NAD+, and dehydrogenase which were present in living cells. Therefore, the viable cells got stained with red. The unstained dull yellow cells were infarcted. Whole-brain slices were weighed. The dull yellow infarcted part was separated, weighed, and articulated as % of the total weight of the brain (Farkas E *et al.,* 2007).

% Brain Infarction= (Weight of white infarct / Weight of whole Brain slice) X 100

Biochemical estimations

Superoxide Dismutase (SOD): Cerebral ischemia-reperfusion injury-induced oxidative stress by causing mitochondria dysfunction which resulted in increased levels of ROS and decreased levels of antioxidants like SOD (Guo *et al.* 2012). Cytoplasmic superoxide radicals play a key role in the pathogenesis of ischemic tissue. Activation of SOD may be a therapeutic strategy for inhibition of reperfusion injury caused by oxidative stress. Oxidative phosphorylation of Mitochondria is an important source of ROS. Mitochondrial manganese superoxide dismutase (Mn-SOD) detoxifies the ROS to produce H2O2 which is converted to water by glutathione peroxides (GPx) or catalase (Wang et al., 2012). Among these methods, using NBT an indirect method is used commonly due to its convenience and ease of use (Farkas E et al., 2004).

Malondialdehyde (MDA): Lipid peroxidation is a well-recognized mechanism of cellular injury in the biological system of plants and animals. The mechanism includes the oxidation of unsaturated lipids to form radical species as well as toxic byproducts. The polyunsaturated lipids are more susceptible to oxidative cellular damage and react to form lipid peroxides. Lipid peroxides are unstable and undergo decomposition to form a series of carbonyl compounds, which further react to form malonaldehyde (MDA). Lipid peroxidation is the most frequently reported analytical procedure for estimating oxidative stress effects on lipids (Spencer J, 2009).

Myeloperoxidase (MPO): MPO is a hemoprotein, that has bactericidal and centrally linked innate host defense activities. The hemoprotein consists of two dimmers which are linked by a disulfide bridge. MPO is present in human polymorphonuclear neutrophils (PMN). It is also confined to monocytes and tissue macrophages (Spencer J, 2009). MPO is observed in the azurophilic granules of polymorphonuclear leukocytes. MPO plays a major role in the propagation and initiation of chronic and acute vascular inflammatory disease and is strongly involved in the regulation of cellular homeostasis (Lau and Baldus 2006).

Estimation of TNF-α: Tumour necrosis factor-alpha (TNF- α) is a strong pro-inflammatory cytokine with both effective and destructive properties of CNS. In ischemia and reperfusion injury, inflammation was robust to cause tissue apoptosis. In the present study quantification of TNF- α levels in brain tissue were estimated by using the TNF- α Rat ELISA kit as per the procedure mentioned (Block F and Schwartz M, 1997).

Estimation of IL-6: The cytokine IL-6 is an important proinflammatory cytokine, it plays a key role in the immune system to transition from 1innate to acquired immunity. In the present study quantification of IL-6 levels in brain tissue was done by Rat IL-6 ELISA kit as per the procedure mentioned (Gupta YK and Seema B, 2004).

Statistical analysis: All the values are expressed as Mean ± SEM, (n=5). Biochemical parameters SOD, MDA, MPO, IL6, and TNF α were determined by factorial one-way ANOVA. The individual group was compared against ischemic control using Dunnett's test. Values ranging between $P<0.001$ to $P<0.05$ were considered statistically significant. Statistical analysis was performed using GraphPad Prism 5 (version 5.01).

RESULTS

Effect of hesperidin and hesperetin on % of Brain Infarct

In the present study, during ischemia-reperfusion injury, there was a significant ($p<0.01$) increase in % infarct size in vehicle control groups when compared to sham-operated groups. Hesperidin 30 mg showed moderate ($P<0.05$) while 60 and 90 mg/kg showed a highly significant ($P<0.01$; $p<0.001$) decrease in cerebral infarct size compared to the I/R vehicle group in both the models. Hesperetin 30 mg showed moderate ($P<0.05$) while60 and 90 mg/kg showed a highly significant ($P<0.01$; $p<0.001$) decrease in cerebral infarct size compared to the I/R vehicle group in both the models.

Table 1: Effect of hesperidin and hesperetin on % of Brain Infarct

Groups	% infarct (Focal)	% infarct (Global)
Sham-operated control without I/R.	21.00±3.21***	11.33±2.84***
vehicle control Rats received 0.2 ml of 10% DMSO and served as a control subjected to ischemia-reperfusion (with I/R)	92.33±5.17	87.00±2.64
Rats received Hesperidin 30 mg/kgbefore reperfusion	69.54±0.33*	62.33±2.33**
Rats received Hesperidin 60 mg/kgbefore reperfusion	59.67±0.33**	52.33±1.01**
Rats received Hesperidin 90 mg/kgbefore reperfusion	51.43±0.33***	49.21±0.23***
Rats received Hesperetin 30 mg/kgbeforereperfusion	68.34±0.33*	65.21±1.3**
Rats received Hesperetin 60 mg/kgbeforereperfusion	56.32±0.23**	51.23±1.0**
Rats received Hesperetin 90 mg/kgbeforereperfusion	50.53±0.33***	51.11±1.3***
Rats received Allopurinol 10 mg/kg before reperfusion	45.23±1.3***	40.32±1.2***
Rats received Allopurinol 10 mg/kgand Hesperidin 90 mg/kg before reperfusion	37.1.2±0.3***	36.21±0.6***
Rats received Allopurinol 10 mg/kg and Hesperetin 90 mg/kg before reperfusion	39.3±0.9***	37.21±0.7***

Each value is expressed as mean ± SEM, n=4, and statistical analysis was performed by using one-way ANOVA followed by Dunnett's post hoc test where *=p<0.05, **=p<0.01, and ***=p<0.001 compared to saline group.

Effects of hesperidin and hesperetin on biochemical parameters in the Focal model

After 40 min of focal cerebral ischemia and 5 h of reperfusion, a significant reduction in SOD level in the brain was observed in the I/R control i.e. Saline group as compared to the sham group. This reduction was reversed by hesperidin and hesperetin 30 mg/kg (p<0.05) and hesperidin and hesperetin60 and 90 mg/kg showed increased significance (p<0.01; p<0.001) level when compared to vehicle I/R control group.

Treatments with hesperidin and hesperetin60 and 90 mg/kg reduced the MPO level compared to I/R treated group i.e. saline significantly (P<0.01) and moderately significant (P<0.05) with hesperidin and hesperetin30mg/kg respectively.

The amount of MDA formed in the vehicle control group was high as compared to the sham group. Treatments with hesperidin and hesperetin 30, 60, and 90 mg/kg reduced the MDA level compared to I/R treated group i.e. saline moderately significantly (P<0.05; p<0.01 and p<0.001).

Table 2: Effects of hesperidin and hesperetin on biochemical parameters in the Focal model

Groups	**SOD (units/mg)**	**MPO (pg/ml)**	**MDA (pg/ml)**
Sham-operated control without I/R.	14.89±0.85***	5.39±0.73***	5.29±0.77**
vehicle control Rats received 0.2 ml of 10% DMSO and served as a control subjected to ischemia-reperfusion (with I/R)	1.93±0.44	16.96±0.7	45.25±8.0
Rats received Hesperidin 30 mg/kgbefore reperfusion	5.69±0.35*	11.90±0.97**	33.32±3.22*
Rats received Hesperidin 60 mg/kgbefore reperfusion	7.34±2.12**	9.84±1.1**	21.23±2.3**
Rats received Hesperidin 90 mg/kgbefore reperfusion	11.32±1.3***	7.24±1.3**	18.32±4.31***
Rats received Hesperetin 30 mg/kgbeforereperfusion	5.67±2.1**	12.43±0.1***	23.54±4.43*
Rats received Hesperetin 60 mg/kgbeforereperfusion	7.3±01.3**	8.24±1.2**	18.58±4.32**
Rats received Hesperetin 90 mg/kgbeforereperfusion	10,31±1.1***	5.21±0.5***	17.65±3.22***
Rats received Allopurinol 10 mg/kg before reperfusion	12.4 ±0.13***	7.54±1.4**	24.33±6.32**
Rats received Allopurinol 10 mg/kgand Hesperidin 90 mg/kg before reperfusion	11.2±0.1***	5.56±0.1***	17.58±5.88***
Rats received Allopurinol 10 mg/kg and Hesperetin 90 mg/kg before reperfusion	10.2±1.0***	5.98±1.3***	16.65±3.34***

Each value is expressed as mean ± SEM, n=4, and statistical analysis was performed by using one-way ANOVA followed by DUNNET'S post hoc test where *=$p<0.05$, **=$p<0.01$, and ***=$p<0.001$ compared to saline group.

Effects of hesperidin and hesperetin on biochemical parameters in the Global model

After 40 min of global cerebral ischemia and 5 h of reperfusion, a significant reduction in SOD level in the brain was observed in the I/R control i.e. Saline group as compared to the sham group. This reduction was reversed by hesperetin 30mg/kg and HMC 30mg/kg showed increased significant ($p<0.01$) and moderately significant ($p<0.05$) levels when compared to the I/R control group (saline).

Treatments with hesperidin and hesperetin60 and 90 mg/kg reduced the MPO level compared to I/R treated group i.e. saline significantly ($P<0.01$) and moderately significant ($P<0.05$) with hesperidin and hesperetin30mg/kg respectively.

The amount of MDA formed in the vehicle control group was high as compared to the sham group. Treatments with hesperidin and hesperetin 30, 60, and 90 mg/kg reduced the MDA level compared to I/R treated group i.e. saline moderately significantly ($P<0.05$; $p<0.01$ and $p<0.001$).

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Table 3: Effects of HMC on biochemical parameters in Global model

Groups	SOD (units/mg)	MPO (pg/ml)	MDA (pg/ml)
Sham-operated control without I/R.	16.26±0.96***	4.94±0.95***	5.60±0.70***
vehicle control Rats received 0.2 ml of 10% DMSO and served as a control subjected to ischemia-reperfusion (with I/R)	1.623±0.2473	20.07±2.49	48.04±4.87
Rats received Hesperidin 30 mg/kgbefore reperfusion	4.12±0.09***	11.93±1.04*	12.85±1.730***
Rats received Hesperidin 60 mg/kgbefore reperfusion	9.93±0.466***	9.66±0.9**	10.58±2.207***
Rats received Hesperidin 90 mg/kgbefore reperfusion	13.56±0.3***	6.22±1.2***	8.23±1.23***
Rats received Hesperetin 30 mg/kgbeforereperfusion	5.03±0.9***	12.56±0.04*	12.32±2.12***
Rats received Hesperetin 60 mg/kgbeforereperfusion	9.23±0.3***	9.33±1.1**	9.56±2.34***
Rats received Hesperetin 90 mg/kgbeforereperfusion	14.42±0.3***	6.64±1.0***	7.89±2.34***
Rats received Allopurinol 10 mg/kg before reperfusion	7.12±1.09***	9.76±1.0**	11.56±2.4***
Rats received Allopurinol 10 mg/kgand Hesperidin 90 mg/kg before reperfusion	13.43±0.4***	7.66±0.9**	7.45±2.33***
Rats received Allopurinol 10 mg/kg and Hesperetin 90 mg/kg before reperfusion	13.56±0.3***	6.22±1.2***	7.78±3.21***

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Each value is expressed as mean ± SEM, n=4, and statistical analysis was performed by using one-way ANOVA followed by DUNNET'S post hoc test where *=p<0.05, **=p<0.01, and ***=p<0.001 compared to saline group.

Effects of hesperidin and hesperetin on inflammatory parameters in Focal and Global model

The level of inflammatory parameters was found to be more in-group compared to the sham group. The level of inflammatory parameters has been found to be less compared to the saline group after being treated with hesperidin and hesperetin 30, 60, and 90 mg/kg in both the models.

Table 4: Effects of hesperidin and hesperetin on inflammatory parameters in Focal and Global model

Groups	Focal		Global	
	TNF-α	IL-6	TNF-α	IL-6
Sham-operated control without I/R.	102.4±3.60 ***	284.1±11.11***	101.2±2.12***	232.1±10.0***
vehicle control Rats received 0.2 ml of 10% DMSO and served as a control subjected to ischemia-reperfusion (with I/R)	206.4±1.53	448.1±2.41	203.7±1.2	423.2±2.56
Rats received Hesperidin 30 mg/kgbefore reperfusion	120.0±0.75 **	318.9±1.83*	118.3±1.75**	313.2±1.80*
Rats received Hesperidin 60 mg/kgbefore reperfusion	112.1±0.20 **	307.0±1.55**	101.3±2.20**	336.0±1.22**
Rats received Hesperidin 90 mg/kgbefore reperfusion	103±1.3***	298±0.99***	97±1.02***	302±1.0***
Rats received Hesperetin 30 mg/kgbeforereperfusion	125.0±0.34 **	313.2±1.21*	112.3±1.32**	310.0±1.54*
Rats received Hesperetin 60 mg/kgbeforereperfusion	110.0±1.2**	302.0±1.5**	99.3±1.20**	333.0±2.2**
Rats received Hesperetin 90 mg/kgbeforereperfusion	99±0.9***	296±1.9***	95±1.0***	297±1.2***
Rats received Allopurinol 10 mg/kg before reperfusion	137.0±0.7**	372±1.8**	115.8±0.75**	310.2±1.8**
Rats received Allopurinol 10 mg/kgand Hesperidin 90 mg/kg before reperfusion	115.1±0.2***	302.0±1.5**	101.3±2.2**	331.0±1.2**
Rats received Allopurinol 10 mg/kg and Hesperetin 90 mg/kg before reperfusion	97±1.1***	278±1.3***	99±1.0***	312±1.0***

Each value is expressed as mean ± SEM, n=4, and statistical analysis was performed by using one-way ANOVA followed by DUNNET'S post hoc test where *=$p<0.05$, **=$p<0.01$, and ***=$p<0.001$ compared to saline group.

DISCUSSION

The protective effect of hesperidin and hesperetin in cerebral ischemia-reperfusion and long-term cerebral hypoperfusion in rats by using bilateral common carotid artery occlusion (BCCAO), was explored by evaluating its effects on some specific parameters such as percentage infarct size measurement; biochemical estimations like brain SOD level, MDA level, MPO level and Inflammatory parameters like TNF-α and IL-6 test.

The only usual therapy for ischemic stroke is reperfusion. Even if cerebral blood flow is re-established quickly enough to prevent immediate cell death, however, a large population of initially surviving neurons will die within the first few hours after reperfusion. According to current knowledge, major mechanisms involved in such delayed post-ischemic infarct development include energy failure, excitotoxicity, accumulation of reactive oxygen species (ROS), and apoptotic signaling as well as inflammatory processes (Ghoneim AI *et al.,* 2002).

Ischemic hypoperfusion produces cell injury by inhibition of active ion transmembrane transport, free radicals generation, elevated production of vasoconstrictive agents, and increased leukocyte-endothelial cell and neutrophil-neutrophil interactions (Markus JW *et al.,* 2003).

Oxidative stress is increased in the ischemic condition in the brain, the levels of oxidatively modified DNA and nitrosylated proteins are elevated and antioxidant defense enzymes are impaired (Won SJ *et al.,* 2002).

The endogenous antioxidant enzyme activity of the brain impaired by I/R is particularly important and measurement of those antioxidant enzymes after reperfusion can assess the vulnerability of the brain injury (Slikker W *et al.,* 1999).

During reperfusion, perturbation of the antioxidative defense mechanism is a result of the overproduction of oxygen radicals, inactivation of detoxification systems, consumption of antioxidants, and failure to adequately replenish antioxidants in the ischemic brain tissue (Devasagayam TPA *et al.,* 2003).

Triphenyl tetrazolium chloride (TTC) staining has been employed in the present study to determine the area and weight of infractions in brain tissue. TTC is a water-soluble dye that is reduced to form a zone by the enzyme succinate dehydrogenase and cofactor NAD, present in mitochondria and stains viable tissue deep red. Ischemic tissue with damaged mitochondria remains unstained. Treatment with hesperidin and hesperetin after ischemia-reperfusion and after long-term cerebral hypoperfusion have markedly attenuated cerebral infarct size. Significant reduction in infarct area indicates the neuroprotective potential of hesperidin and hesperetin in focal and global cerebral ischemia is moderately reduced compared to the control group in both the focal and global model of cerebral ischemia.

In this study, SOD levels were decreased in the ischemic reperfused group compared to the sham group, which is a consequence of the previous study. This may be due to the excessive formation of superoxide anions. The decline in the enzyme level may be explained by the fact that excessive superoxide anions may inactivate SOD, thus resulting in activation of the H_2O_2scavenging enzyme. The reduced SOD level was increased by administration of hesperidin and hesperetin (30, 60, and 90 mg/kg., i.p.) in both the ischemic model.

The finding suggested that hesperidin and hesperetin could considerably improve cellular anti-oxidative defense against oxidative stress.

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Free radicals promote lipid peroxidation, which results in the alteration in permeability and fluidity of the membrane. Reactive oxygen species (ROS) produce malondialdehyde (MDA), an end product of lipid peroxidation. MDA reacts with thiobarbituric acid (TBA) and is thus estimated as TBARS (Devasagayam TPA *et al.,* 2003). Therefore, in the present study MDA level was measured to estimate the extent of ROS. Our result showed that treatment with hesperidin and hesperetin in both focal cerebral ischemia-reperfusion and long-term cerebral hypoperfusion significantly decreased the brain MDA level in a given dose.

Inflammation is an important component of the pathogenesis of cerebral ischemia. Numerous pro-inflammatory molecules such as nitric oxide synthase-2 (NOS-2), chemokines, and adhesion molecules have been implicated in the development of cerebral ischemic injury (Markus JW *et al.,* 2003). Myeloperoxidase (MPO), is a heme enzyme secreted by activated phagocytes at the site of inflammation. MPO is an abundant enzyme that is involved in the production of free

radicals. Indeed, MPO uses hydrogen peroxide (H_2O_2) and NO_2^- to generate reactive nitrogen species.

The levels of enzyme MPO were found to be increased in the ischemic reperfused group compared to the sham group. Administration of hesperidin and hesperetin (30, 60, and 90mg/kg., i.p.) reduced MPO levels effectively in the treatment group in both models in a dose-dependent manner.

Inflammation is closely associated with ischemia and reperfusion of injured brain tissues. Tumour necrosis factor-alpha (TNF-α) is a potent pro-inflammatory cytokine with both beneficial and destructive properties for the central nervous system (Cui *et al.,* 2012). In ischemia and reperfusion injury, inflammation was robust to cause tissue apoptosis. Cytokines are important regulators of physiological inflammation. The aberrant cytokine expressions contribute to pathogenesis in inflammatory associated diseases (Samavedam*et al.*, 2012). The cytokine IL-6 is an important proinflammatory cytokine, it plays a key role in the immune system to transition from innate to acquired immunity. The level of TNF-α and IL-6 was found to be increased in the ischemic reperfused group compared to the sham group. Administration of hesperidin and hesperetin (30, 60, and 90mg/kg., i.p.) was reduced effectively in the treatment group in both models.

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CONCLUSION

The present study revealed that hesperidin and hesperetin have a Cerebro-protective effect against cerebral ischemia reperfusion-induced cerebral infarction. The neuroprotective effect of hesperidin and hesperetin was confirmed by evaluating various biochemical markers like SOD, MPO, and MDA along with inflammatory parameters like TNF-α and IL-6 and Infarct size measurement. The exact mechanisms of action for the neuroprotective effect of hesperidin and hesperetin can be further explored in future studies.

Conflict of interest: NIL

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