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Cerebroprotective effect of Quercetin in Ischemia/reperfusion-induced oxidative stress in rats

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ABSTRACT

The most common causes of cerebrovascular disorders are ischemia/reperfusion (I/R) injury, stroke, and chronic hypo perfusion. Quercetin is a plant pigment that is reported to protect against stress. *Objective*: This research protocol was designed to evaluate the protective properties of quercetin in cerebral ischemia/reperfusion injuries. *Method:* Adult male rats were divided into four groups randomly (sham, quercetin, I/R, and I/R with quercetin). The induction of cerebral ischemia was done through the ligation of bilateral common carotid arteries for 30 minutes; then, reperfusion was allowed for four hours. *Result:* Quercetin ameliorates the harmful effect of I/R by reducing the oxidative stress. It reduced the high level of inflammatory cytokines, TNF-α and NF-κB, and improved the level of Bcl2. *Conclusion:* The present study reveals that quercetin protects the cerebral cortex against the injuries caused by I/R through its anti oxidative, anti-inflammatory and anti apoptotic effects. Furthermore, the results also suggest that the quercetin represents a hopeful therapy for neurological diseases due to oxidative stress and I/R.

Keywords: Quercetin, antioxidant, ischemia and reperfusion, oxidative stress, cerebral injury.

1. INTRODUCTION

Cerebrovascular disturbances are a significant reason of death and permanent handicap (Forman-Hoffman et al., 2015). The most common causes of cerebrovascular disorders are ischemia/reperfusion (I/R) injuries, stroke, and chronic hypo perfusion, which considerably impair the quality of life. Hypoxic cerebral damage in ischemia/reperfusion is increased following the restoration of blood supply (Teoh and Farrell, 2003). The return of the blood supply after cerebral ischemia may prevent the expansion of the infarction area; however, it is usually accompanied by more cerebral pathology (Xia et al., 2010). These pathological changes include oxidative stress that leads to the increment of reactive oxygen species, inflammation, neuronal apoptosis, leukocyte leakage, complement stimulation, BBB destruction (Koch et al., 2006; Pan et al., 2007; Doyle et al., 2008). Free radical formation has been shown to be prevalent during the cerebral ischemia and at the beginning of reperfusion (Ste-Marie et al., 2000). Oxidative stress causes cerebral damage due to the higher level of polyunsaturated fatty acids in the cerebrum and the lower levels of endogenous antioxidant enzymes (Juurlink and Sweeney, 1997). Oxidant stress-induced cell killing through the accumulation of calcium and the formation of superoxide by mitochondria, which ultimately lead to the membrane breakdown of the mitochondria (Chattopadhyay et al., 2010).

Recent researches have concentrated on natural plants that may improve the effects of brain damage caused by I/R. Quercetin is a plant pigment that is derived from the flavonoid group. It is present in many different plants and foods, such as onion, blueberry, citrus, capers, lovage, fennel, apple, green tea, Ginkgo biloba, grapes and olive oil (Tang et al., 2013; Atef et al., 2017). Quercetin is a prophylactic from diabetic cataracts, and it improves capillary viability. Additionally, it also inhibits platelet accumulation (Sexton and Jarow, 1997). Quercetin is used for treating atherosclerosis, high cholesterol, heart disease, and circulation problems. This flavonoid is also reported to protect against stress as it inhibits the release of cortisol (Cheng and Li., 2012).

Our aim was to assess the possible prophylactic effects of quercetin versus the harmful effects of I/R injury on the cerebral cortex of the adult male Wistar albino rats using oxidative stress biomarkers, inflammatory biomarkers, and light microscopes.

2. MATERIALS AND METHODS

Chemicals

Quercetin (≥95%) was procured from Germany Sigma Aldrich

Experimental animals and design

Forty (40) adult male Wistar albino rats (220 and 250 grams) were used in the present study. The rats were housed under a 12-hour light and dark cycle, with free access to feed and tap water. Room temperature and humidity were maintained at 23 ±1°C and 55 ± 5%, respectively. The rats were handled according to the International Animal Ethics Guidelines. The study was conducted from July 2017 to June 2019.

Ethical committee approval number and details

The research proposal was approved by the Institutional Animal Ethics Committee of the Shaqra University (approval number D170503/G01/N002).

Rats were randomly assigned into four (4) experimental groups of ten (10) rats each as follows:

Group I: Sham control.

Group II: Quercetin (50 mg/kg) intraperitoneal injection.

Group III: Ischemic, ligation of the bilateral common carotid artery (BCCA) for 30 minutes followed by 4 hours of reperfusion.

Group IV: Quercetin pretreatment (50 mg/kg) intraperitoneal injection 30 minutes before ligation of the BCCA followed by a four-hour reperfusion process.

Cerebral I/R

The induction of cerebral I/R were done by the method illustrated by Farbiszewski et al. (1995). Animals were anesthetized by intraperitoneal injection of Urethan (35 mg/kg).Groups III and IV were exposed to ischemia by ligation of the BCCA (Photo 1). After 30 minutes of ischemia, animals were subjected to reperfusion for four hours by withdrawing the ligation. At the end of the experiment, all the animals were killed by decapitation. The cerebral hemispheres of all the rats were isolated and rinsed with cold saline. Cerebral cortical tissues of the frontal and parietal lobes from the lateral surface of the brain were isolated. The cerebral

cortical tissues were then divided into two parts; one was merged in phosphate buffer saline with Teflon coated for biochemical studies and the other was used for the histopathological study.

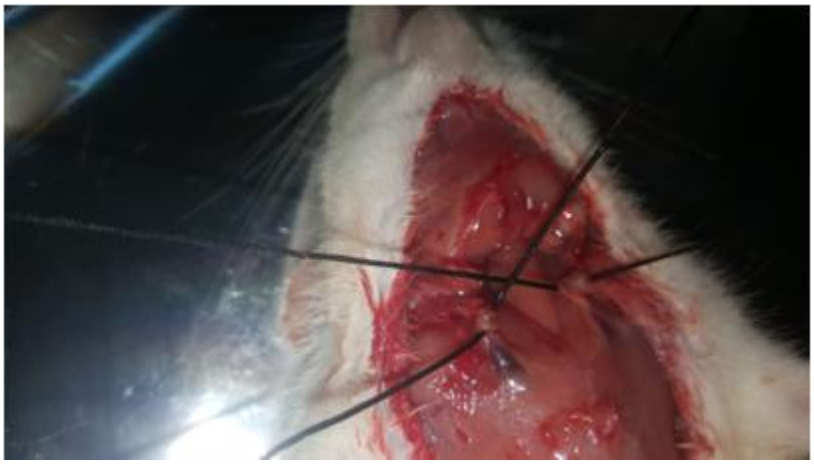

Photo 1 Photograph showing the *ligation* of bilateral common carotid arteries

Biochemical studies

Oxidative stress

Oxidative stresses in cerebral cortical tissues were determined by measuring the lipid peroxidation product by using commercially available kits and through a quantitative ELISA analysis. Malondialdehyde (MDA) and glutathione (GSH)from OxiSelect™ TBARS Assay Kit, Cell Biolabs Inc. (San Diego, USA), superoxide dismutase (SOD)from Superoxide Dismutase Assay Kit, Trevigen Inc. (Gaithersburg, USA) along with Catalase from Cat ELISA Kit, Eiaab®. (Wuhan, China).

TNF-α

The level of TNF-α in the cerebral cortex was determined by using the rat TNF-α ELISA Kit (Georgia, USA).

NF-κB

NF-κBin the cerebral cortex was determined by using the rat NF-κB ELISA Kit, Eiaab® (Wuhan, China).

Bcl2

Bcl-2 in the cerebral cortex was determined by using the rat Bcl2 ELISA kit, Eiaab® (Wuhan, China).

Histopathology study

The cerebral cortex specimens were fixed in 4% paraformaldehyde in 0.1 M phosphate-buffered saline for one day. The specimens were dehydrated in graded ethanol, cleared with xylene and embedded in paraffin. Sections were cut with microtome (Leica, Germany) into 5-µm thick sections. The obtained tissue sections were stained with hematoxylin and eosin (HE) (Bancroft and Stevens 1990). The slides were mounted using Entellan and covered with cover slips, then examined and photographed by a light microscope (Nikon Eclipse E-200 light microscope).

Data analysis

The data was presented as mean and standard errors of the mean. Data analysis was done using ANOVA, followed by Tukey's post hoc test. Values analyses were carried out using a software program (Prism 5, GraphPad, CA, USA). The degree of significance was considered at P-value <0.05.

3. RESULTS

Oxidative stress biomarkers

Table 1 show that the group treated with quercetin alone has no significant changes in the level of lipid peroxidation MDA, GSH, SOD and catalase in comparison to the control group. It also shows how ischemia followed by reperfusion results in a significant

increase in the level of MDA compared to the sham group in rat cerebral cortex. Pre-treatment with quercetin before I/R experiment significantly reduced the MDA level in rat cerebral cortex in contrastto the I/R group (Figure 1 A).

Table 1 Effect of I/R and quercetin on antioxidant enzymes (MDA, GSH, SOD, and catalase) in the cerebral cortex of rats.

	MDA (mmol/g)	GSH (µmol/g)	SOD activity Units/mg	Catalase Units/mg
Sham	0.36±0.024	18.2±1.6	11.3±1.06	3.4±0.37
Quercetin	0.37±0.022	18.4±1.9	11.2±1.02	3.5±0.35
I/R	0.59[a]±0.032	7.9[a]±1.02	5.4[a]±0.72	1.5[a]±0.20
Quercetin & I/R	0.45[b]±0.027	13[b]±1.33	8.1[b]±0.81	2.3[b]±0.24

Results are presented as mean ±SEM (n=10)

[a]P<0.05 (significantly different from the sham group)

[b]P<0.05 (significantly different from I/R group)

GSH level was reduced markedly in the I/R group in contrast to the sham control group. Quercetin treatment raised its level significantly in the quercetin I/R group in contrast to the I/R group (Figure 1 B). The Cerebroprotective effect of quercetin by increasing the level of GSH was observed. Furthermore, as observed in Table 1, the I/R reduced the SOD level significantly in contrast to the sham group. The present results illustrate that a high amount of ROS was developed through an I/R injury. Pre-treatment with quercetin elevated the lower SOD level in I/R. (Figure. 1C). Ischemia/reperfusion results in a significant deterioration of catalase in contrast to the control group. The catalase level was restored significantly through the quercetin pretreatment in the I/R group (Figure 1D).

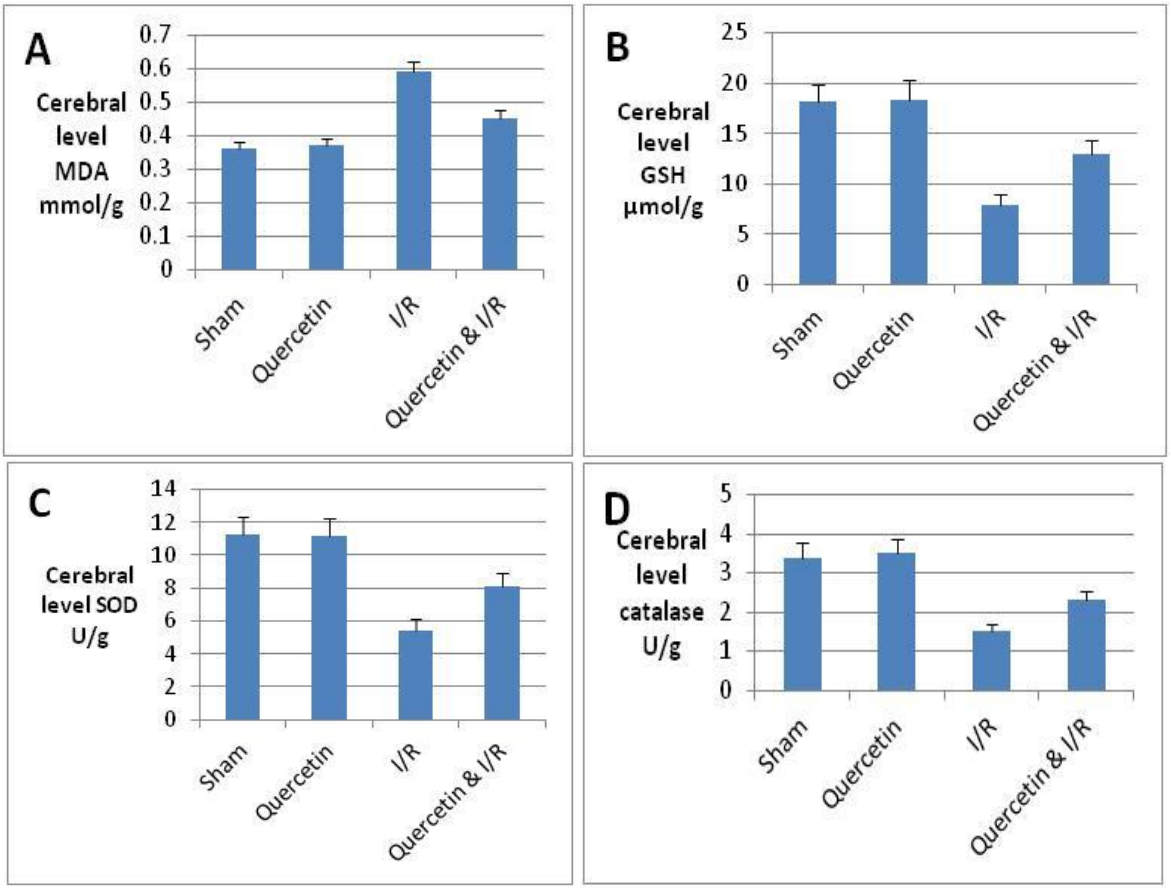

Figure 1 Effect of I/R and quercetin (50 mg/kg) on A – MDA, B – GSH, C – SOD, and D – Catalase levels in the cerebral cortex. Results are presented as mean ± SEM (n = 10)

Inflammatory cytokines and Bcl2

I/R in the cerebral cortex resulted in a significant increase in TNF-α and NF-κB levels and decrease in the levels of Bcl2 in comparison to the control group (P < 0.05) (Table 2). Pre-treatment with quercetin revealed a significant deterioration in the levels of TNF-α and NF-κB and rise in the Bcl2 level in comparison to the I/R group (P < 0.05) (Figure. 2, A, B, & C).

Table 2 Effect of I/R and quercetin (50 mg/kg) on TNF-α, NF-κB and BCL2.

	TNF-α (ng/g)	NF-κB (ng/g)	BCL-2(mg/ml)
Sham	8.36±0. 94	0.72±0.06	27.3±2.86
Quercetin	8.30±0.82	0.74±0.07	27.9±2.92
I/R	21.57[a]±2.42	3.19[a]±0.17	8.4[a] ±0.72
Quercetin & I/R	14.76[b]±1.88	1.73[b]±0.13	19.1[b]±2.01

Results are presented as mean ±SEM (*n*=10)

[a]*P*<0.05 (significantly different from the sham group)

[b]*P*<0.05 (significantly different from the I/R group)

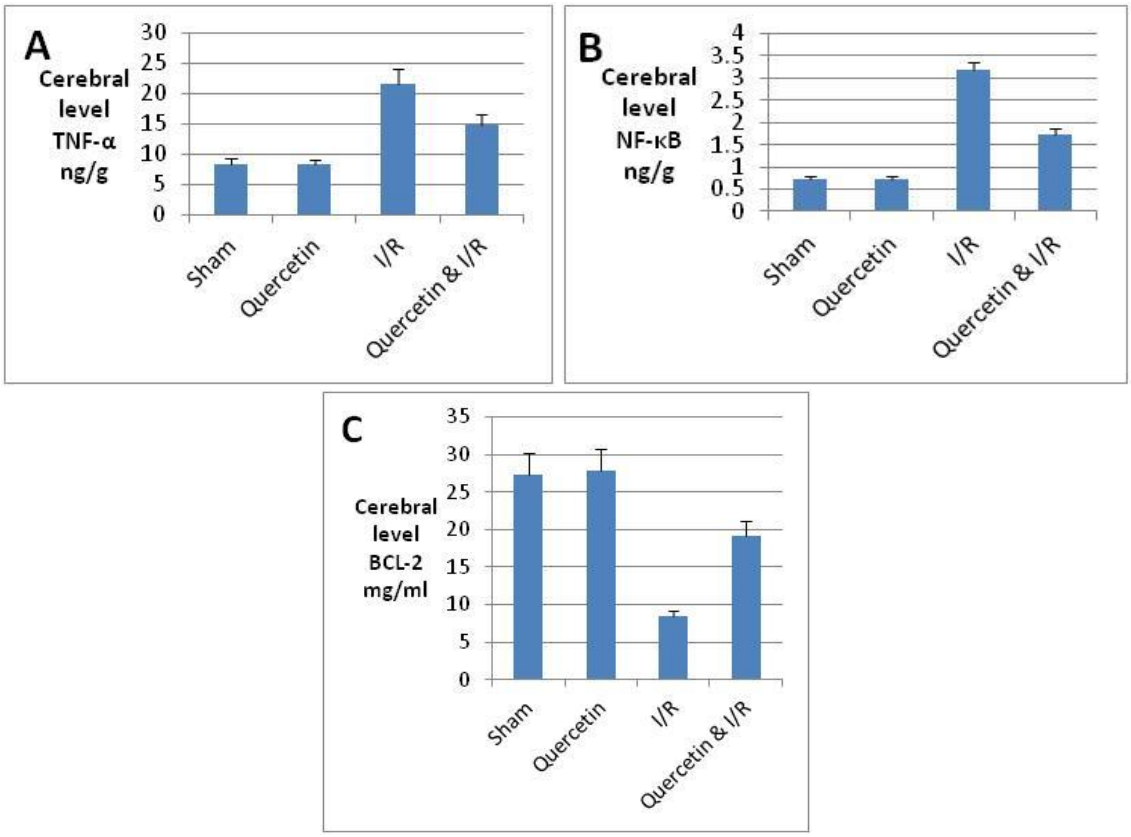

Figure 2 The effects of I/R and quercetin (50 mg/kg) on A –TNF-α, B –NF-κB, and C –BCL-2 in the cerebral cortex. Results are presented as mean ± SEM (n = 10)

The histological study of cerebral cortical tissue in the sham and quercetin groups showed a normal neuronal structure without any cellular pathology (Photo 1A and 2). The I/R group depicted the presence of neuronal degeneration with some shrunken neurons and pyknotic nuclei with the presence of peri-neuronal spaces (Photo 3A and 3B). Pretreatment with quercetin in the I/R group improve the pathological modification (Photo 4A and 4B).

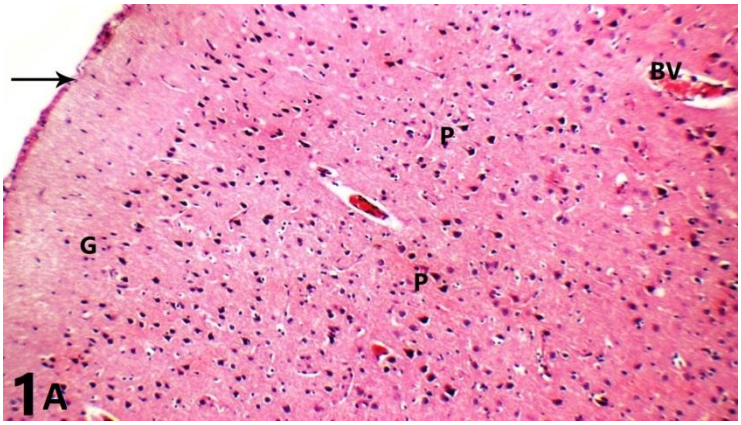

Photo 1A Histopathological section in the cerebral cortex of control adult rat showing the normal histological structure of the cerebral cortex. Notice, pia matter (arrow), granular cells (G), pyramidal cells (P) and blood vessel (BV) (H&E 100X).

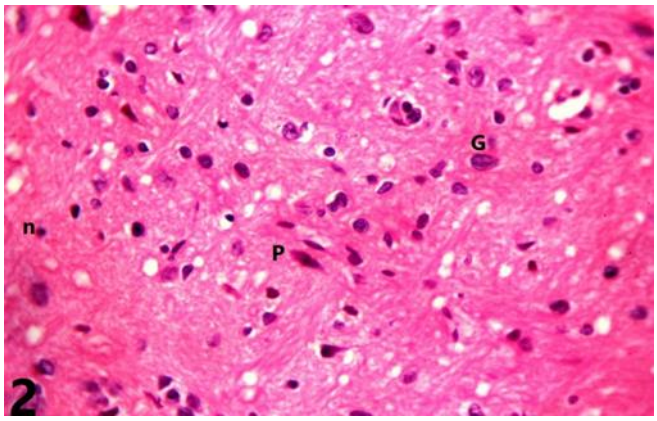

Photo 2 quercetin treated group of adult rat showing the normal histological structure of the cerebral cortex. Notice, granular cells (G), pyramidal cells (P) and neuroglia (n) with dense nuclei (H&E 400X).

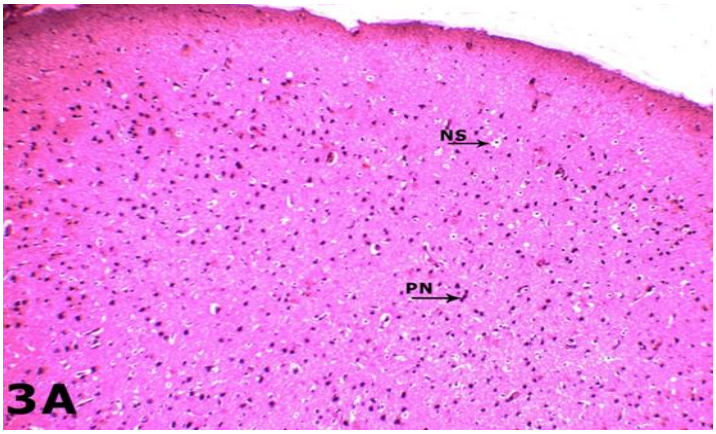

Photo 3A I/ R group of adult rat showing many pathological changes of the cerebral cortex. Notice, perinuclear space (PS) and pyknotic nuclei (PN) (H&E 100X).

4. DISCUSSION

Quercetin was administered in the present study through intra-peritoneal injections thirty minutes before the occlusion of the bilateral common carotid arteries. Another study revealed that the concentration of quercetin in the serum is significantly high after the intra-peritoneal injection, reaching a higher concentration ten minutes following the injection (Rivera et al., 2004). In another research for focal cerebral ischemia, quercetin administration was detected in the cerebral cortex thirty minutes after the intra-peritoneal injection (Chan et al., 2014). The concentration of quercetin cannot be noticed in the serum following oral doses as it is converted into its respective metabolites (Rivera et al., 2004).

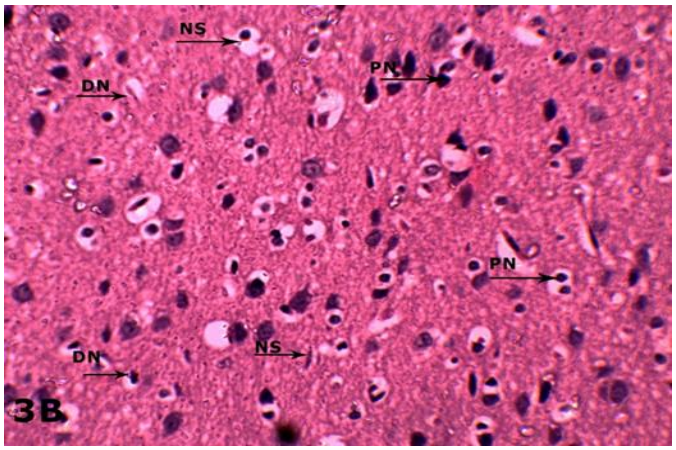

Photo 3B I/ R group of adult rat showing many pathological changes of the cerebral cortex. Notice, degenerative neuron (DN), perinuclear space (PS), neuron shrinkage (NS) and pyknotic nuclei (PN) (H&E 400X).

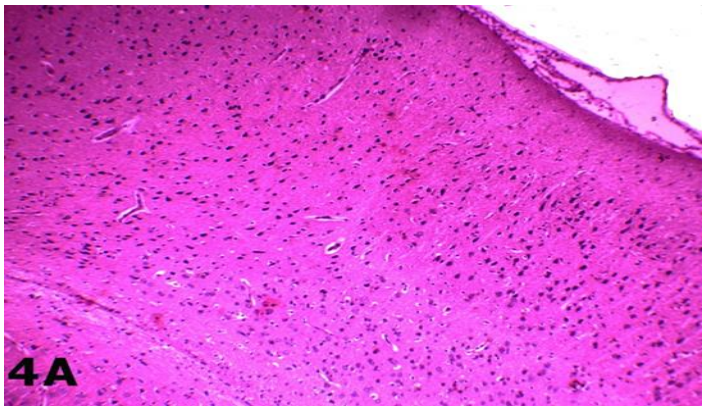

Photo 4A quercetin pretreated group followed by I/R of adult rat showing marked improvement of the pathological changes in comparison to I/R group (H&E 100X).

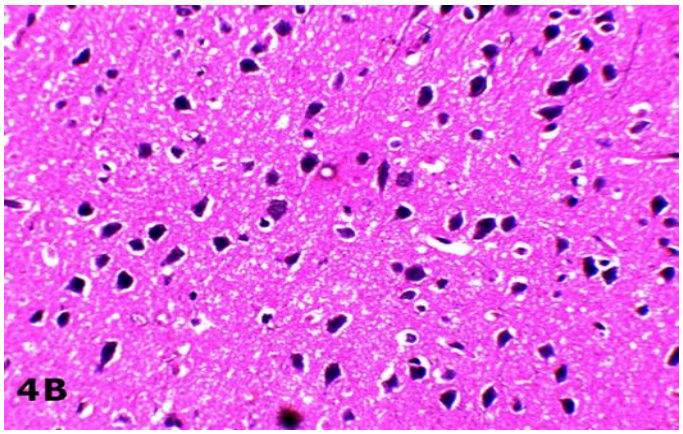

Photo 4B quercetin pretreated group followed by I/R of adult rat showing marked improvement of the pathological changes in comparison to I/R group (H&E 400X).

The present results have shown a significant increase of MDA and decrease of GSH, SOD, and catalase in rats belonging to the groups that suffered from I/R. GSH, SOD, and catalase are inhibited in cases of oxidative stress (Tao et al., 2014). The results demonstrate that I/R increased the production of ROS. Further production of ROS cannot be regulated by endogenous antioxidant defenses of the cerebral cortex (Sayre et al., 2008). Under I/R conditions, lipid per oxidation increases in the cerebral cortex due to the oxidative stress (Thiyagrajan and Sharma, 2004). MDA is a lipid per oxidation marker and represents the level of oxidative stress that is present (Shah et al., 2014). Our results revealed a higher degree of MDA in the cerebral cortex of the I/R group. Quercetin

pretreatment markedly improved the oxidative stress. In another study, the chronic treatment of rats by pesticides shows a higher level of MDA and low levels of GSH and catalase. The co-administration of quercetin and pesticides improved the levels of MDA, GSH and catalase to be nearly normal (Beghoul et al., 2017). GSH is an antioxidant that protects the brain tissues from oxidative stress. GSH share in the regulation of many anti-inflammatory genes. The plasma membranes of cerebral cells are more liable to destruction due to the lower level of GSH in I/R. The increased production of protein-glutathione mixed disulfide is the major reason for the inhibition of GSH in the course of oxidative stress processes in cerebral I/R (Reed, 1990). The overproduction of protein-glutathione mixed disulfide and low level of GSH in the cerebral cortex led to the destruction of the plasma membrane.

The GSH level in the I/R group is essentially different from the group with I/R and the administration of quercetin. Quercetin markedly improved the GSH levels. These data suggest that quercetin potentiates the defense mechanism against ROS and offers protection against oxidative damage. Quercetin is known for having strong antioxidant activity (Porritt et al., 2012). In another experimental study of middle cerebral artery ligation treated with selenium and melatonin, the researchers reported an increment in the antioxidant capacity (Ahmad et al., 2011). Quercetin has a positive consequence on antioxidant enzymes, glutathione and lipid peroxidation after I/R of the cerebral cortex (Rivera et al., 2008; Kumar et al., 2008). The quercetin treatment preserved the nervous tissue and raised the level of antioxidant enzymes and improved antioxidant capacity in the striatum of the cerebrum (Haleagrahara et al., 2013; Dong et al., 2014).

SOD is considered as an important antioxidant marker in the body and a major free radical scavenger to measure the extent of oxidative tension (Dong et al., 2013; Yan et al., 2013). I/R decrease the level of SOD, but prior treatment with quercetin significantly improve the SOD level. According to the present results, quercetin increased the level of SOD. Quercetin displays a protective action on the nervous tissue by suppression of oxidative process; causes decline inflammation, cerebral cell death (Yang et al., 2014; Du et al., 2016). The achieved results suggest that the quercetin can function as a probable medication for the management of neurological diseases and I/R due to oxidative stress.

Catalase is enzymes found in all living organisms (Vainshtein et al., 1981) and is expressed by neuronal and glial cells. It is responsible for catalyzing hydrogen peroxide into water and oxygen. The present study revealed that the catalase level in the I/R group was essentially different than the I/R with prior administration of quercetin. The level of catalase enzyme was notably low in patients with neurodegenerative diseases due to cerebral ischaemic strokes (Rosario de la Torre et al., 1996). Raising the level of catalase enzyme and its activity may be considered as a promising therapeutic plan for cases of cerebral stroke. Neuronal cells in the corpus striatum that have high levels of catalase are less liable to pathological changes after the transient ligation of the center cerebral artery (Gu et al., 2004). Quercetin elicits neuro protection in different oxidative tension models because of antioxidant function (Echeverry et al., 2010; Ghosh et al., 2013; Maciel et al., 2013).

TNF-α is a cell signaling factor and one of the major cytokines released during I/R. TNF-α can increase cellular inflammation, leading to more cerebral pathology (Yin et al., 2013; Marshall et al., 2014). Determination of the TNF-α stage can reflect the degree of inflammation and cerebral damage occurred during the I/R injury (Liu et al., 2016). In compliance with the present investigation, Yin and his collaborators (2013) noted that the level of TNF-α was highly moral in the I/R group of rats. Quercetin pre-treatment significantly drop the blood levels of TNF-α in I/R rats. The same results came in accordance with recent researches that reported that amount of TNF-α were suppressed by quercetin pre-treatment. The Cerebroprotective consequence of quercetin is partially related to its anti-inflammatory development (Ghosh et al., 2017). Isoquercetin, in another in vitro study, is shown to improve the cerebral damage of the rat hippocampus by decreasing the level of TNF-α (Wang et al., 2017). Quercetin suppressed TNF-α production as well as gene expression (Hirpara et al., 2009).

Nuclear factor-κB manages transcription factors, which controls many genes shared in the immunity and inflammatory processes (Oeckinghaus and Ghosh., 2009). In the present research, pretreatment with quercetin suppressed the NF-κB convinced by I/R. Quercetin has powerful antioxidant, anti-inflammation, and anti-fibrosis properties (Uzun and Kalender, 2013; Khaksary-Mahabady et al., 2018). These antioxidant and anti-inflammation properties of quercetin are due to its capacity to suppress the NF-κB production (Kang et al., 2013). BCL2 is a regulator protein that regulates cellular apoptosis (Tsujimoto and Shimizu, 2000). The inhibition of BCL2 after I/R was significant, as shown in the results. Pretreatment with quercetin increased the level of BCL2. The present results suggest that quercetin has anti-apoptosis effects, which is in accordance with the results of another study of a rat with cerebral bleeding (Zhang et al., 2015). The Cerebroprotective function of quercetin was led by the neuronal cell death abolition (Du et al., 2018). Quercetin improves cerebral ischemia by managing the aspect of various proteins in cases of focal cerebral injury (Shah et al., 2018). With reference to the pathological changes, the neuronal damage was revealed in the cerebral cortex of the I/R group. Cellular pathology illustrates the presence of pyknotic nuclei with marked perineuronal spaces. In the group pretreated with quercetin, the pathology of the cerebral cortex was ameliorated in contrast to the I/R group. Quercetin improves the neuronal cell death convinced by cerebral I/R (Pu et al., 2007). The outcomes of this research were in compliance with recent researches

demonstrating that quercetin may have cerebro protection in different models (Zhang et al., 2011; Kumar et al., 2014). The antioxidant properties of quercetin have been proven by their competency to scavenge free radicals (Pu et al., 2007). Quercetin supports beneficial therapeutic ability across neuronal pathology in different experiments on oxidative stress (Echeverry et al., 2010; Hwang et al., 2009).

5. CONCLUSION

This research protocol suggests that quercetin protects the cerebral cortex against I/R injury by way of its anti oxidative, anti-inflammation and anti-apoptosis properties. Quercetin can function as probable medication for the management of neurological diseases that result from oxidative stress and ischemic brain injury.

Conflicts of Interest:

The authors declare no conflict of interest.

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List of Abbreviations

ANOVA:	Analysis of variance
BBB:	Blood brain barrier
BCCA:	bilateral common carotid artery
Bcl2:	B-cell lymphoma 2
ELISA:	enzyme-linked immunosorbent assay
GSH:	Glutathione
I/R:	Ischemia/reperfusion
MDA:	Malondialdehyde
NF-κB:	Nuclear factor kappa-light-chain-enhancer of activated B cells
ROS:	reactive oxygen species
SEM:	Standard error of the mean
SOD:	Superoxide dismutase
TNF-α:	Tumor necrosis factor-α

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