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Research Article
Quercetin Inhibits Chronic Stress-Induced Depression Associated with the Inhibition of Nitrosative Stress and Apoptosis

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Abstract

Background and Objective: Exposure to chronic stress is harmful to vital organs such as the brain and the heart. The potential inhibitory effects of the polyphenolic compound quercetin on Chronic Unpredictable Stress (CUS)-induced depression and biomarkers of brain injury associated with the inhibition of Nitrosative Stress (iNOS) and the apoptotic axis p53-Bax-caspase-3 has not been investigated before. **Materials and Methods:** Rats were either exposed to a variety of unpredictable stressors daily before being sacrificed after 3 weeks or were treated for 3 weeks with quercetin (50 mg kg^{-1} b.wt./day). Animals were then culled and brain tissues were harvested. **Results:** CUS significantly ($p<0.05$) induced iNOS, MDA, p53, Bax and caspase-3, which were significantly inhibited by quercetin. Whereas, quercetin significantly increased brain tissue levels of SOD and Bcl-2. In addition, CUS caused a significant increase in animal immobility and a decrease in climbing ability and sucrose consumption, which were reverted by quercetin. Furthermore, a significant ($p<0.0001$) correlation between either cerebral cortex brain injury and biomarkers of apoptosis and survival, p53 and Bcl-2, or between p53 and Bcl-2 and biomarkers of nitrosative stress and depression were observed. **Conclusion:** Quercetin protects against CUS-induced cerebral cortex injury and depression, which is associated with the inhibition of the p53-Bax-caspase-3 axis and biomarkers of nitrosative and oxidative stress.

Key words: Brain injury, depression, chronic stress, apoptosis, quercetin, nitrosative, reactive oxygen species

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Repeated stress is a risk factor for depression, general anxiety disorder and neurological abnormalities in both humans and experimental animal models[1,2] and about 10% of the US population is inflicted with depression[3]. In addition, exposure to stressors for a prolonged period can affect other systems such as the endocrine and cardiovascular systems[4,5]. Indeed, chronic stress caused cardiac arrest and death in more than a quarter of the mice that had their anti-stress receptor, serotonin knocked out[6]. Also, mental stress, depression and anxiety are recognised amongst many risk factors that triggermyocardialinfarction[7].

To cope with stress, the body mobilizes physiological and psychological resources leading to alterations in the dynamic regulations of the autonomic, neuroendocrine and immune systems[8]. Accumulated evidence shows that chronic stress such as those arising from natural disasters, wars, economic deprivation or induced by experimental procedures, is a risk factor for the central and peripheral nervous systems[9,10]. For example, (i) exposure to various forms of psychological or occupational stressors resulted in the stimulation of the hypothalamic-pituitary-adrenal axis which leads to the increase in the secretion of the stress hormone cortisol[9] and (ii) oxidative damage to neurons has been implicated in the pathogenesis of depression[11]. Furthermore, tissue oxidative stress is believed to be the main cause behind damage that occurs in animals and humans exposed to traumatic events including chronic stress[12]. Stress-induced overproduction of Reactive Oxygen Species (ROS) caused apoptosis and enhanced levels of lipid peroxidation and peroxynitrite that damages DNA in the brain[13]. In addition, the role of lowered levels of antioxidants and high generation of ROS and nitrosative stress in the pathogenesis of depression have been reported, which have pointed to neuroinflammation, increased apoptosis rate and altered neurogenesis/ neuroplasticity[14].

Quercetin is a flavonol antioxidant compound that is found in many vegetables, fruits and grains[15,16]. Quercetin has many pleiotropic effects that demonstrated effective protection to the cardiovascular, kidney and liver[17-19]. Quercetin was also reported to protect against diabetes-induced depression in mice[20]. However, little is known about the protective effects of quercetin against chronic stress-induced brain injury.

Therefore, this study aimed to investigate the effects of quercetin on CUS-induced depression in rats and to monitor brain tissue levels of the p53-Bax-caspase-3 axis of apoptosis, the survival cell signalling and nitrosative stress in the presence and absence of quercetin.

MATERIALS AND METHODS

Study area: The study was carried out at the Research Center, College of Medicine, King Khalid University, Abha, Saudi Arabia from September-December, 2019.

Animals: All rat studies were overseen and approved by the Medical Research Ethical Committee at King Khalid University, Abha, Saudi Arabia. Ref no: (Rec. No. 2014-06-09). Male Wistar rats (total 24 rats) at 8 weeks and weighing 150-200 g were used for these studies. They had free access to food and water and were housed under a constant temperature of 23±1°C with a 12 hrs light/dark cycle.

Experimental design: After a one-week adaptation period, rats were randomly assigned into 4 groups (n = 6 each) as follows: 1. Control group: Received normal saline. 2. Quercetin treated group (QUR): Rats received QUR (50 mg kg^{-1})[20]. 3. Chronic unpredictable stress (CUS) group: A model group and were exposed to CUS protocol, as detailed below and received normal saline. 4. CUS+QUR treated group: were exposed to CUS with a concomitant daily dose of QUR (50 mg kg^{-1}). All treatments were administered as 1 mL, i.p. for three consecutive weeks daily.

Chronic unpredictable stress (CUS) protocol: CUS protocol was induced as previously described[21] with modification. Briefly, a set of chronic unpredictable stressors were used to induce brain injury in rats that lasted for 3 weeks.

Assessment of depressive-like behaviours; forced swimming and sucrose preference tests: The experiment of assessing depressive-like behaviour in the form of a forced swimming test was carried out as previously described[22] to induce brain injury in rats for three weeks. All scoring was done by a single trained person, blind to experimental conditions. The sucrose preference test procedure was performed as previously described[23]. The sucrose preference was calculated using the following formula:

$$\text{Sucrose preference (\%)} = \frac{\text{Sucrose consumption}}{\text{Water consumption+Sucrose consumption}} \times 100$$

Preparation of brain tissues for analysis: At the end of the experimental period, blood samples were collected by cardiac puncture under anaesthesia (sodium thiopental at 40 mg kg^{-1} b.wt.) and rats were then culled using cervical dislocation. Brain tissues were harvested and washed with iced Phosphate-Buffered Saline (PBS). The brains were cut into

longitudinal sections. Parts of the brains were homogenized in a cold phosphate buffer, pH 7.4, containing EDTA. The supernatant obtained was distributed in separate tubes and stored at -70°C for biochemical analysis. Other parts of the brains were stored at -70°C for RNA extraction.

Detection of p53 and Bax messenger RNAs by reverse transcriptase-polymerase chain reaction: As previously described[24], total RNAs were isolated from freshly dissected rats' brains using the RNeasy Mini Kit (Qiagen Pty, Victoria, Australia). The RNA was reverse-transcribed for a single strand cDNA synthesis (Invitrogen) and amplified by PCR for the tumour suppressor p53, apoptosis regulator Bax and β-actin. The PCR products were separated by 2% agarose gel electrophoresis and visualised by ethidium bromide. Gel images were scanned and quantified by densitometry using the NIH image software.

Determination of tissue levels of nitrosative and oxidative stress and apoptosis and survival biomarkers: ELISA kits were used according to the manufacturers' instructions to determine the tissue levels of malondialdehyde (MDA) for lipid peroxidation (Cat No. NWK-MDA01, NWLSS, USA), Superoxide Dismutase (SOD) (Cat. No. 706002, Cayman Chemical, Ann Arbor, MI, USA),Glutathione Peroxidase (GPx) (Cat. No. 703102, Cayman Chemical, Ann Arbor, MI, USA), caspase 3 (Cat. No. R5814), B-cell lymphoma 2 (Bcl-2) (Cat. No. R6813) and inducible Nitric Oxide Synthase (iNOS) (Cat. No R6663) were purchased from STZ ELISA (USA).

Statistical and morphometric analysis: Statistical analysis was performed by using the Graph pad Prism statistical software package (version 6). The data were expressed as mean±standard deviation (SD).One-way analysis of variance (ANOVA) was performed followed by Tukey's t-test. Using the "Leica Qwin 500 C" image analyzer (Cambridge, UK), the degree of cerebral cortex damage was obtained in 10 non-overlapping high-power fields/ rat of H and E-stained sections. Quantitative data were tabulated as a means and Standard Deviations (SD) and compared using analysis of variance (ANOVA) followed by *post hoc* analysis (Tukey test). A significant difference was considered when p-value 0.05. Calculations were made on SPSS software (version 19).

RESULTS

Quercetin inhibits CUS-induced brain injury biomarkers and depression: To test the hypothesis that quercetin in this modified model of CUS can protect against brain injury and depression caused by CUS, we measured iNOS, MDA as a by-product of lipid peroxidation and the antioxidants SOD and Gpx using the ELISA method in brain tissue homogenates of all groups of rats. Compared with the control untreated group, CUS significantly ($p<0.0001$) augmented iNOS (Fig. 1a) and MDA (Fig. 1b) and ameliorated SOD (Fig. 1c) and Gpx (Fig. 1d) tissue levels, which were significantly ($p<0.0001$; CUS *vs* CUS+QUR) protected by quercetin. However, the levels of MDA in the treated group (CUS+QUR) were still significant ($p<0.046$) to the two control groups (Fig. 1b) which mean, partial inhibition of MDA was achieved by quercetin. We further assessed depression-like behaviour levels in these animals in the presence and absence of quercetin. As shown in Fig. 2, CUS significantly ($p<0.0001$) inhibited mobility (Fig. 2a; increased immobility) and climbing ability (Fig. 2b) that were significantly ($p<0.0001$; CUS vs. CUS+QUR) protected by quercetin. However, exposing rats to CUS for 3 weeks did not affect their swimming ability (Fig. 2c). Sucrose consumption (Fig. 2d) was inhibited by CUS and protected with quercetin. The degree of protection exerted by quercetin was partial in (A).

Quercetin protects CUS-modulated apoptosis and survival biomarkers in brain tissues: We then tested the apoptotic p53-Bax-caspase-3 axis cell signalling in brain tissue CUS strongly (i) augmented p53 and Bax cDNA message (Fig. 3a-c); (ii) augmented caspase-3 protein expression (Fig. 3d) and (iii) inhibited the survival protein Bcl-2 (Fig. 3e), which were effectively ($p<0.0001$; CUS vs. CUS+QUR) protected by quercetin. However, complete protection was only seen in (E).

Correlation between p53 or Bcl-2 and biomarkers of depression and nitrosative and oxidative stress: The correlation between either p53 or Bcl-2 scorning and tissue levels of nitrosative and oxidative stress and depression biomarkers were determined to further support the link between p53 and Bcl-2 and brain injury and to further confirm and characterize the role of QUR as being stable and an appropriate agent in brain injury rats. A significant ($p<0.0001$) correlation was observed between p53 and iNOS ($r = 0.849$) (Fig. 4a), p53 and MDA ($r = 0.918$) (Fig. 4b), p53 and climbing ability ($r = -0.898$) (Fig. 4c), p53 and sucrose consumption ($r = -0.915$) (Fig. 4d), Bcl-2 and iNOS ($r = -0.936$) (Fig. 4e), Bcl-2 and MDA ($r = -0.946$) (Fig. 4f), Bcl-2 and climbing ability ($r = 0.921$) (Fig. 4g) and Bcl-2 and sucrose consumption ($r = 0.955$) (Fig. 4h).

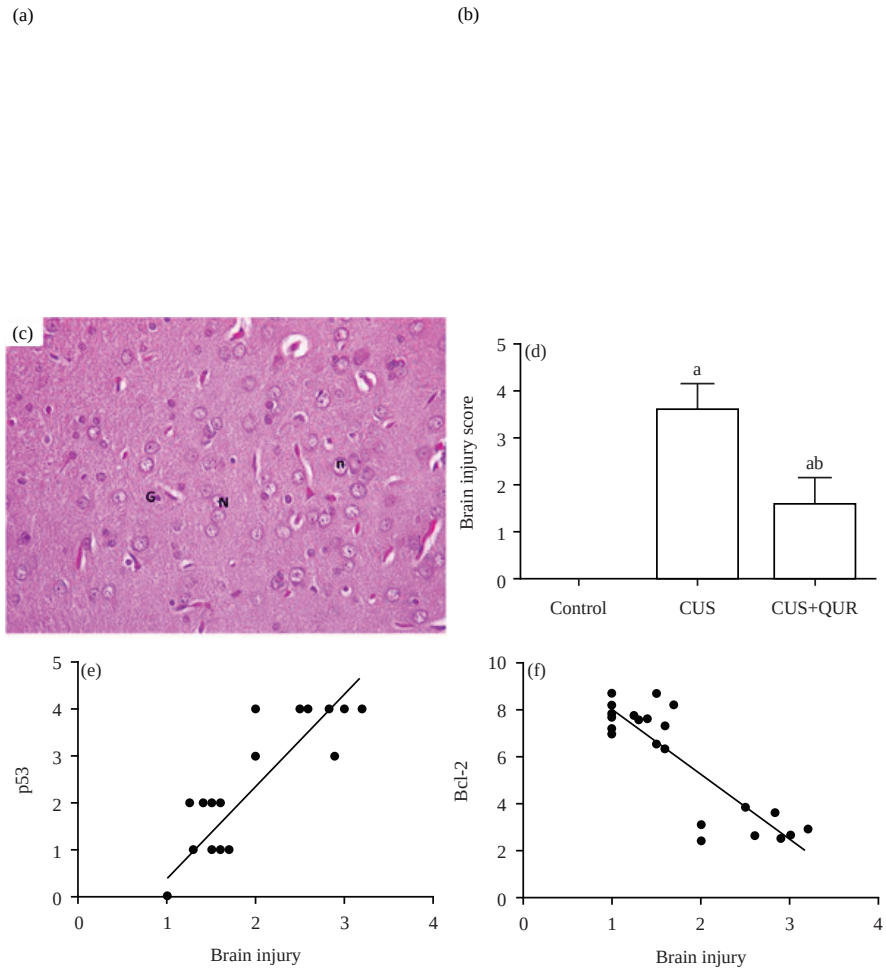

Fig. 5(a-f): Quercetin partially protects brain architecture against injury induced by CUS

(a) H and E stained images (400x) of harvested rat tissues obtained after 3 weeks from the brain cerebral cortex of the control group, (b) CUS group, (c) Protective group, CUS+QUR are visualized by light microscopy. Note that in (A), arrows point to the glial processes and in (B), point to the perineuronal vacuolation. N: Neurons, n: Nucleus, G: Glial cells. Histograms in (d) represent a quantitative analysis of the cerebral cortex brain injury in the three groups of rats. All shown p values are significant, [a]p<0.05 versus control, [b]p<0.05 versus QUR and (e-f) Correlation between the brain injury score versus (e) p53 and (f) Bcl-2. CUS: Chronic uncontrolled stress, QUR: Quercetin, p53: Tumour suppressor p53, Bcl-2: B-cell lymphoma 2

and nitrosative stress and p53 were reported to be involved in Alzheimer disease[26]. In addition, oxidative stress-activated a specific p53 transcriptional response, which regulates cellular senescence and ageing in mice and deletion of p53 and the redox that generates mitochondrial ROS by genetic means reduced apoptosis and increased longevity of transgenic mice[26]. Furthermore, overexpression of iNOS caused induction of vascular endothelial cell apoptosis and caspase-3 and 9activities,which were reduced by SMT, a selective iNOS inhibitor[33]. These reports are in agreement with our data that demonstrate the association between CUS-induced depression and apoptosis and oxidative and nitrosative stress (Fig. 1-4). Also, our data shown here are in agreement with our recently published report[24] that showed modulation of these parameters; p53, Bax, Bcl-2 and biomarkers of oxidative and nitrosative stress in acute liver injury. Finally, *in vivo* study of quercetin on humans is warranted to evaluate whether these animal findings can be translated into therapy. Also, a future study would use quercetin to augment the effects of an antidepressant drug in this model.

CONCLUSION

In summary, we have demonstrated that induction of CUS-induced depression in rats for three weeks causes the activation of brain tissue p53-Bax-caspase-3 axis of apoptosis associated with the induction of oxidative and nitrosative stress and inhibition of the survival protein Bcl-2 and quercetin can revert these parameters.

SIGNIFICANCE STATEMENT

This study represents a significant contribution in the study of brain injury and depression induced by chronic stress in rats since it investigates the brain tissue apoptotic and survival cell signalling molecules modulated by chronic stress and the potential protection of these molecules and inhibition of brain injury and depression by the antioxidant compound quercetin, which may offer therapeutic potential in humans.

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