new drug development and therapeutic strategies remain urgent and important requirements.

The progressive neural cell loss has been indicated as a common hallmark of neurodegenerative diseases. Although activation of intrinsic regenerative adult neural stem cells occurs to replace the lost neural cells, but it is not adequate (Zeng and Wang, 2015; Khairallah et al., 2016). Therefore, pharmacological stimulation of endogenous neural stem cell proliferation may be a new perspective in the treatment of neurodegenerative diseases. Accordingly, quercetin, a natural polyphenolic flavonoid, has been shown to enhance neural stem/progenitor cells (NS/PCs) proliferation in *in vitro* and *in vivo* studies. For example, quercetin and CTN-986, a quercetin derivative, have significantly increased hippocampal progenitor cells proliferation (Tchantchou et al., 2009; Zhang et al., 2009). Furthermore, quercetin has enhanced proliferation of neural stem cells in adult rat's (subventricular zone) SVZ after focal cerebral ischemia (Zhang et al., 2011). Moreover, CTN-986 has been shown to increase proliferation of dentate gyrus progenitor cells in chronically stressed-mice (Zhang et al., 2009). Despite the evidences indicating the stimulatory effect of quercetin on neural stem cells, the mechanism by which quercetin promotes NS/PCs proliferation is still unclear. Unraveling the mechanism by which quercetin affects NS/PCs proliferation can guide us to develop new drugs in treating neurodegenerative diseases. In this context, two known cellular antioxidant mechanisms involved in cell proliferation i.e. nuclear transcription factor erythroid 2 (Nrf2) and proteasome systems can be suggested.

Nrf2 is a transcription factor that regulates the endogenous antioxidant responses through activation of antioxidant-responsive element/electrophile-responsive element (ARE/EpRE)-dependent gene expression. Under basal condition, Nrf2 is associated with kelch-like ECH-associated protein 1 (Keap1) which represses Nrf2 activation and enhances its degradation by 26S proteasomes (Silva-Islas and Maldonado, 2018). Under oxidative stress conditions, Nrf2 translocates into the nucleus (Silva-Islas and Maldonado, 2018) and exerts its antioxidant effect. It has been indicated that the intracellular redox state is an important factor in cell self-renewal (Wang et al., 2013) and Nrf2 has been shown to improve survival and proliferation of NS/PCs with a reduction in oxidative condition (Kärkkäinen et al., 2014; Robledinos-Antón et al., 2017). Notably, quercetin and dihydroquercetin with antioxidant properties have been shown to offset oxidative stress-induced cellular damage by stimulating the Nrf2-ARE pathway (Arredondo et al., 2010; Granado-Serrano et al., 2012; Liang et al., 2013; Saw et al., 2014; Costa et al., 2016). Taken together, there are evidences indicating that Nrf2 is involved in cell proliferation and quercetin is an Nrf2 activator (Tanigawa et al., 2007), therefore, it can be proposed that quercetin may enhance NS/PCs proliferation through Nrf2-ARE pathway.

The proteasome system is a major regulator of protein homeostasis that balances protein synthesis and degradation for maintaining normal cellular function (Morimoto and Cuervo, 2014). The proteasome has important role in cell survival and adaptation to oxidative stress (Kwak et al., 2007) through the removal of damaged and ubiquitinated proteins (Chondrogianni and Gonos, 2005). The 20S protoseame, a core of proteasome machinery, regulates degradation of oxidized protein, while 26S proteasome is mainly involved in degradation of ubiqutinated native and misfolded proteins (Chapple et al., 2012). Lately, a significant function of proteasome in promoting self-renewal of neural progenitor cells (NPCs) during aging has been reported (Zhao et al., 2016). Remarkably, quercetin has been shown to activate proteasome in Huntington expressing Neuro2a cell line (Chakraborty et al., 2015) and HLF-1 human primary cells (Chondrogianni et al., 2010). In view of proteasome role in NPCs proliferation and enhancing effect of quercetine on proteasome, it can be suggested that the quercetin possibly improve NS/PCs proliferation via proteasome activation.

In summary, quercetin has been shown to improve proliferation of neural stem/progenitor cells; but its underlying mechanism of action is still elusive. Considering the roles of Nrf2 and proteasome activity in cell proliferation, the existence of a cross-talk between Nrf2 and proteasome pathways (Chapple et al., 2012; Jang et al., 2014) and evidences indicating quercetin as activators of Nrf2 and proteasome, it is possible that quercetin may exert its effect on NS/PCs proliferation through Nrf2 and/or proteasome pathways. However, no studies have yet evaluated

the effects of quercetin on Nrf2 and proteasome in relation to NS/PCs proliferation. Therefore, the aim of this study was to determine the effect of quercetin on proliferation capacity, protosomal activity and Nrf2 protein levels in rat fetal ganglionic eminence NS/PCs.

Materials and methods

Chemical and reagents

Dulbecco's modified Eagle's medium/ nutrient mixture F-12 (DMEM F12), B27 serum free supplement, N2 supplement, penicillin/streptomycin (pen-strep), 0.25% trypsin-EDTA and GlutaMAX TM were purchased from Gibco. Heparin sodium salt, trypsin inhibitor, laminin and bromodeoxyuridin (BrdU) were from Sigma. The following were purchased from the companies as indicated: recombinant murine epidermal growth factor (EGF) and FGF-basic (Peprotech); ketamine10% and xylazine2% (Alfasan), protease and phosphatase inhibitors (Thermo Fisher Scientific), proteasome activity kit (Merk-Millipore, APT280), mouse anti-BrdU monoclonal antibody (Santa Cruz), mouse anti-nestin primary antibody (Santa Cruz), Alexa flour 647 secondary antibody (Invitrogen), anti-Nrf2 antibody (abcam), rabbit monoclonal β-actin antibody and horseradish peroxidase (HPR)-conjugated anti-rabbit IgG secondary antibody (Cell Signaling).

Experimental animals

Fourteen-day pregnant Sprague-Dawley rats were purchased from the center of comparative and experimental medicine of Shiraz University of Medical Sciences. All animal procedures were performed according to National Institute of Health guideline for the care and maintenance of laboratory animals (NIH publication ≠85- 23, revised in 1985) and approved by the local Research Ethics Committee of Shiraz University of Medical Sciences (approval ID; IR.SUMS.REC.1397.S1196)

Isolation and culture of neural stem/progenitor cells

Rats were anesthetized with ketamine10% (90mg/kg) and xylazine2% (10mg/kg) (Stickrod, 1979). After removal of embryonic ganglionic eminence, cells were cultured according to the method described by Azari et al. (2011). Briefly, cells were seeded at a density of 2×10^5 cells/ml in DMEM-F12 supplemented with B27, N2, Glutamax, EGF (20ng/ml), bFGF (20ng/ml), heparin and pen-strep and incubated at 37°C in an atmosphere of 95% air/ 5% CO_2 humidified incubator in order to form neurospheres. After 5-7 days, the neurospheres were collected and dissociated by exposing them to 0.25% trypsin-EDTA at 37°C for 3-5min. Before the following experiments, the dissociated cells were passaged once at a cell density of 5×10^4 cells/ml.

Identification of neural stem/progenitor cells

Cells were fixed on laminin-coated 96-well plate with 4% paraformaldehyde. Non-specific binding sites were blocked with PBS solution containing 1% bovine serum albumin (BSA) and 5% normal goat serum. Then, cells were exposed to mouse anti-nestin primary antibody (1:200) overnight at 4°C. On next day, cells were incubated with goat anti-mouse fluoro-conjugated secondary antibody (1:600) for 60 min at room temperature. Cells were counterstained with 4,6-diamidino-2-phenylindole (DAPI) to stain nuclei. The nestin expressing cells and total numbers of cells were counted under fluorescence microscopy (Olympus).

Cell treatment

Cells were treated with quercetin at concentarions of 1, 5 or 15μM, DMSO (0.05% v/v, vehicle) or only culture media (control) for 7 days. On day 7, fresh cells were used for the determination of cell viability and proliferation and cells for western immunoblotting and proteasome activity assay were kept at -80°C until use.

Measurement of cell viability

Cell viability was measured by MTT assay based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide. Cells were seeded on laminin (2μg/cm^2)-coated 96-well plate at cell density of 5000 cells/well. On day 7, cells were incubated with a fresh media containing 0.5mg/ml MTT solution at 37°C for 4 h, then 100μl DMSO was added to each well to solubilize the MTT formazan crystals. Ten minutes later, absorbance was measured at 570nm using a microplate ELISA reader (BioTek). Wells containing only DMSO, without cell and media, were used as blanks. All experiments were carried out in triplicates. The cell viability was

expressed as the percentage of optical density (OD) as follows: (OD treatment– OD blank/OD control) ×100.

Assessment of cell proliferation

BrdU assay

The dissociated cells were seeded on laminin-coated 96-well plate ($5x10^3$/well). On day 6, cells were incubated with BrdU (10μM final concentration) for 24 hours. On next day, cells were fixed with 1% paraformaldehyde, washed and then blocked with 1% BSA for 1h at room temperature. After blocking, cells were incubated with mouse anti-BrdU monoclonal primary antibody (1:30) overnight at 4°C. The next day, cells were incubated with anti-mouse fluorescein isothioocyanate (FITC) -labled secondary antibody (1:200) for 2 h at room temperature. DAPI (1g/ml) was used for staining the nuclei of cells. Images were taken by fluorescence microscopy (Olympus) and the quantitative analysis was performed (Jensen, 2013) using Fiji processing package/ImageJ software (2.0.0-rc-69/1.52i).

Neurosphere forming assay

The dissociated cells ($5x10^3$/well) were seeded on 96-well plate. On day 7 of the treatment period, images of neurospheres were taken under inverted light microscopy, then the sizes and numbers of neurospheres were counted using infinity software, version 4.6.0 (Lumenera Corporation).

Quantification of Nrf2 protein levels

The Nrf2 protein level was quantified by western immunoblotting. Cells were washed with ice cold PBS and then sonicated with radioimmunopercipitation assay (RIPA) lysis buffer containing protease and phosphatase inhibitors. The cell lysate was centrifuged at 14000rpm at 4^oC for 20 min. Protein concentration was determined using Lowry method (Lowry et al., 1951). The 60μg protein of each sample was subjected to 10% SDS-polyacrylamide gel electrophoresis, followed by blotting onto polyvinylidenedifluoride (PVDF) membrane using semidry transferring (Biorad). Non-specific bindings were blocked with 5% BSA (1h, room temperature). Subsequently, blots were incubated with primary anti-Nrf2 antibody (1:450), rabbit monoclonal β-actin antibody (1:4000) and anti-Histone H3 antibody (1:1000) overnight at 4^oC. On next day, blots were washed 3 times for 10 minutes and then incubated with HRP-conjugated anti-rabbit IgG secondary antibody (1:6000) for 2h at room temperature. Bands were visualized using enhanced chemiluminescence detection system (GE Healthcare) and chemidoc imaging system (Biorad, XRS^+). Relative optical densities of bands were analyzed using Fiji processing package/imageJ software (2.0.0-rc-69/1.52i). The relative optical density of Nrf2 band was normalized against its relevant β-actin (internal control) density.

Proteasomal activity test

Proteasome chemotrypsin-like activity was measured using proteasome activity kit according to the manufacture's instruction. Briefly, cells were centrifuged and washed with ice cold PBS. The pellets were sonicated in lysis buffer (50Mm HEPES, 5Mm EDTA, 150Mm NaCl and 1%TritonX-100). The lysates were then centrifuged at 12000g for 20min. at 4°C. Protein concentration was determined by the Lowry method (Lowry et al., 1951). To measure 20s proteasome activity, 50μg protein of each sample was incubated with the fluorogenic proteasome substrate i.e. LLVY labeled with the fluorophore 7-Amino-4-methylcoumarin (AMC) for 2h at 45°C. After the cleavage from LLVY-AMC by cellular proteasome, the free AMC fluorescence was quantified at 380nm excitation and 460nm emission wavelengths using a fluorescence microplate reader (BioTek). Different concentrations of free AMC were used as standards and the free AMC concentration of each sample was extrapolated from the standard curve. The fluorescence intensity reflects the enzymatic activity of cellular proteasome.

Statistical analysis

All data are presented as mean±SD. Kruskal-Wallis was used to compare the mean differences of cell viability and proliferation and proteasome activity among groups followed by Dunn's test to compare the mean differences between each two groups. Western immunoblotting data were analyzed by one-way ANOVA followed by Tukey's post hoc test. A concentration-response relationship for each variable was determined using linear regression analysis. Graphpad prism 6 (GraphPad Software) was used for statistical analysis and $P<0.05$ was considered statistically significant.

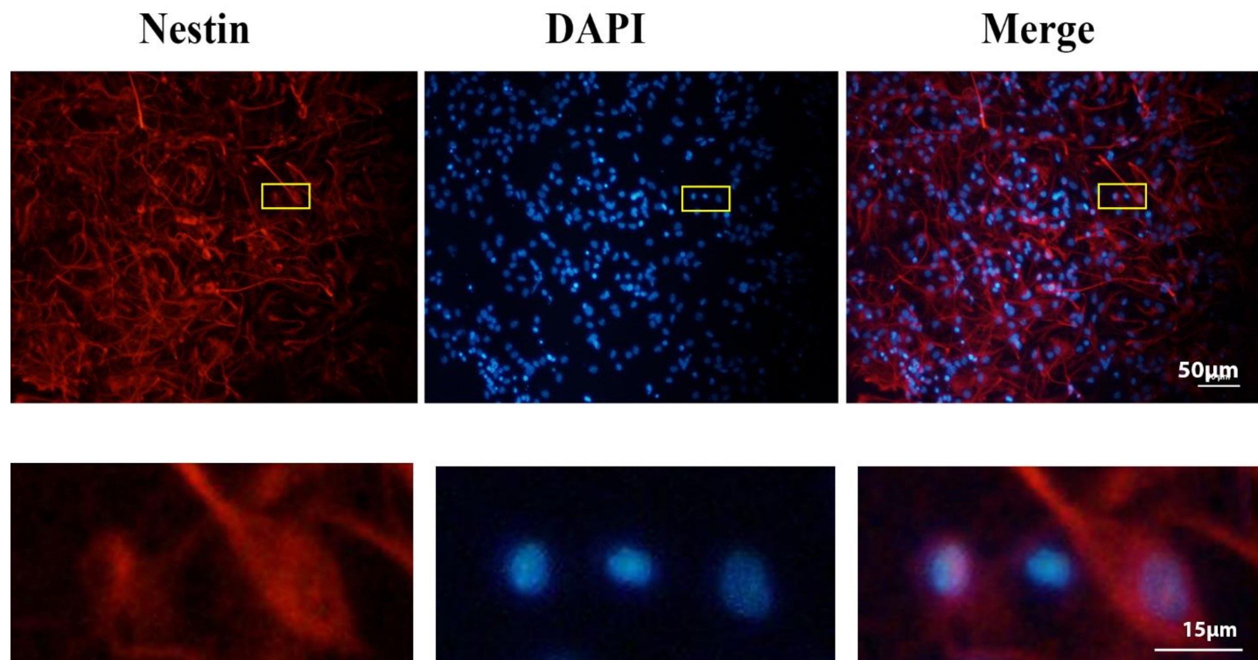

Fig.1. Immunofluorescence staining of neural stem/progenitor cells. Cultures of rat fetal ganglionic eminence were incubated with anti-nestin primary antibody to identify neural stem/progenitor cells (Red fluorescences). Cell nuclei were stained with DAPI (blue fluorescence) for counting the total number of cells. Merged image shows the percentage of cells expressing nestin. More than 80% of cultured cells were neural stem/progenitor cells. Scale bar: 50µm and 15µm (magnified images).

Results

Identification of neural stem cells

Immunocytochemistry analysis revealed that more than 80% of cultured cells expressed nestin, a marker of the NS/PCs (Fig. 1).

Effect of quercetin treatment on NS/PCs viability

MTT assay showed that quercetin at concentration of 15µM significantly increased NS/PCs viability (71.09%) as compared to DMSO treated cells (P<0.05, Fig. 2). In addition, 1 and 5µM quercetin increased NS/PCs viability by 18.55% and 32.26%, respectively, in comparison to DMSO treated cells, but these changes did not reach significant levels. There was no significant difference in cell viability between non-treated (control) and DMSO-treated cells. Linear regression analysis showed that a linear relationship existed between quercetin concentration and cell viability (r^2=0.67, P<0.001).

Effect of quercetin treatment on NS/PCs proliferation rate

Quercetin at concentration of 15µM significantly increased the numbers (55.9%) and diameters (91.2%) of neurospheres in comparison to DMSO (P<0.05, Figs 3A-C). Increases of numbers and diameters of neurospheres by 1 and 5µM quercetin did not reach significant levels in comparison to DMSO. In addition, there were no significant differences in numbers and diameters of neurospheres between control and DMSO groups. Increases in quercetin concentration resulted in a linear enhancement of numbers and diameters of neurospheres (r^2=0.73 and r^2=0.64, respectively, P<0.001).

The BrdU-incorporation assay demonstrated that the percentage of BrdU positive cells was markedly increased following treatment of cells with 15µM quercetin (89.8%) in comparison to DMSO (P<0.001, Fig. 4). Although 1 and 5µM quercetin increased the percentage of BrdU positive cells by 51.8% and 56.5%, respectively, in comparison to DMSO, however, these changes were not statistically significant. No significant difference in percentage of BrdU positive cells was found between control and DMSO-treated cells. A concentration-dependent increase in the percentage of BrdU positive cells was observed following quercetin treatment (r^2=0.63,

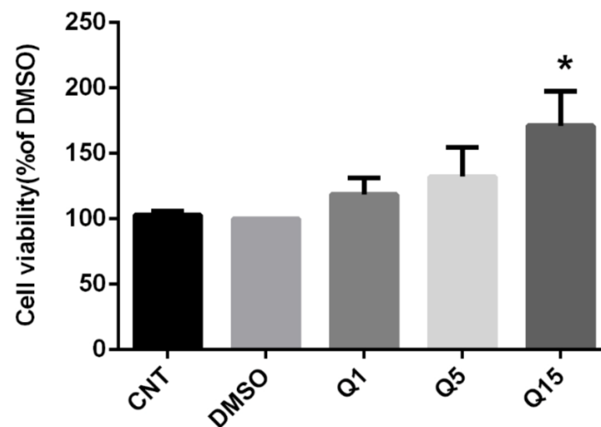

Fig.2. Effect of quercetin on cell viability. Cell viability was determined by MTT assay following 7-days treatment of cultured neural stem/progenitor cells with quercetin or DMSO. Bars represent mean+SD of cell viabilities (percentages of DMSO) for various concentrations of quercetin (1,5 and 15μM; Q1-Q15), DMSO and control (CNT) (n=3/group). *$P<0.05$ significantly different from DMSO. MTT: 3-(4,5-Cimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide.

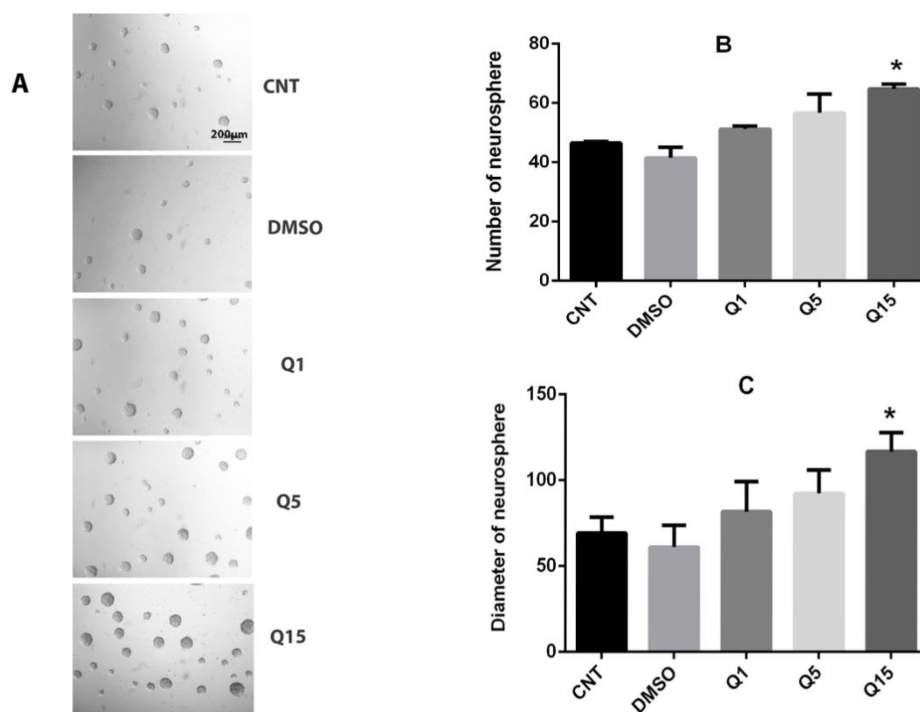

Fig.3. Effect of quercetion on proliferation of neural stem/progenitor cells. Total numbers and diameters of neurospheres were assessed under an inverted light microscope following 7-days treatment of cultured cells with quercetin or DMSO (A), scale bar: 200μm. Bars represent mean+SD of numbers (B) and diameters (C) of neurospheres for different concentrations of quercetin (1, 5 and 15μM; Q1-Q15), DMSO or control (CNT) (n=3/group). *$P<0.05$ significantly different from DMSO.

$P<0.001$).

Effect of quercetin treatment on Nrf2 protein levels in NS/PCs

One-way ANOVA revealed that there were significant differences in Nrf2 protein levels between studied groups ($P<0.001$). Tukey's post hoc comparisons showed that 15μM quercetin significantly increased Nrf2 protein levels in comparison to DMSO-treated cells (292%, $P<0.01$, Fig. 5). Quercetin at 1 and 5μM concentrations non-significantly increased Nrf2 protein levels by 31% and 100%, respectively. Increased Nrf2 protein levels by 15μM quercetin was significantly different from that of 1μM quercetin ($P<0.01$). No significant change in Nrf2 protein level was observed between control and DMSO-treated cells. There was a linear relationship between quercetin concentrations and increases in Nrf2 protein levels ($r^2=0.48$, $P<0.001$).

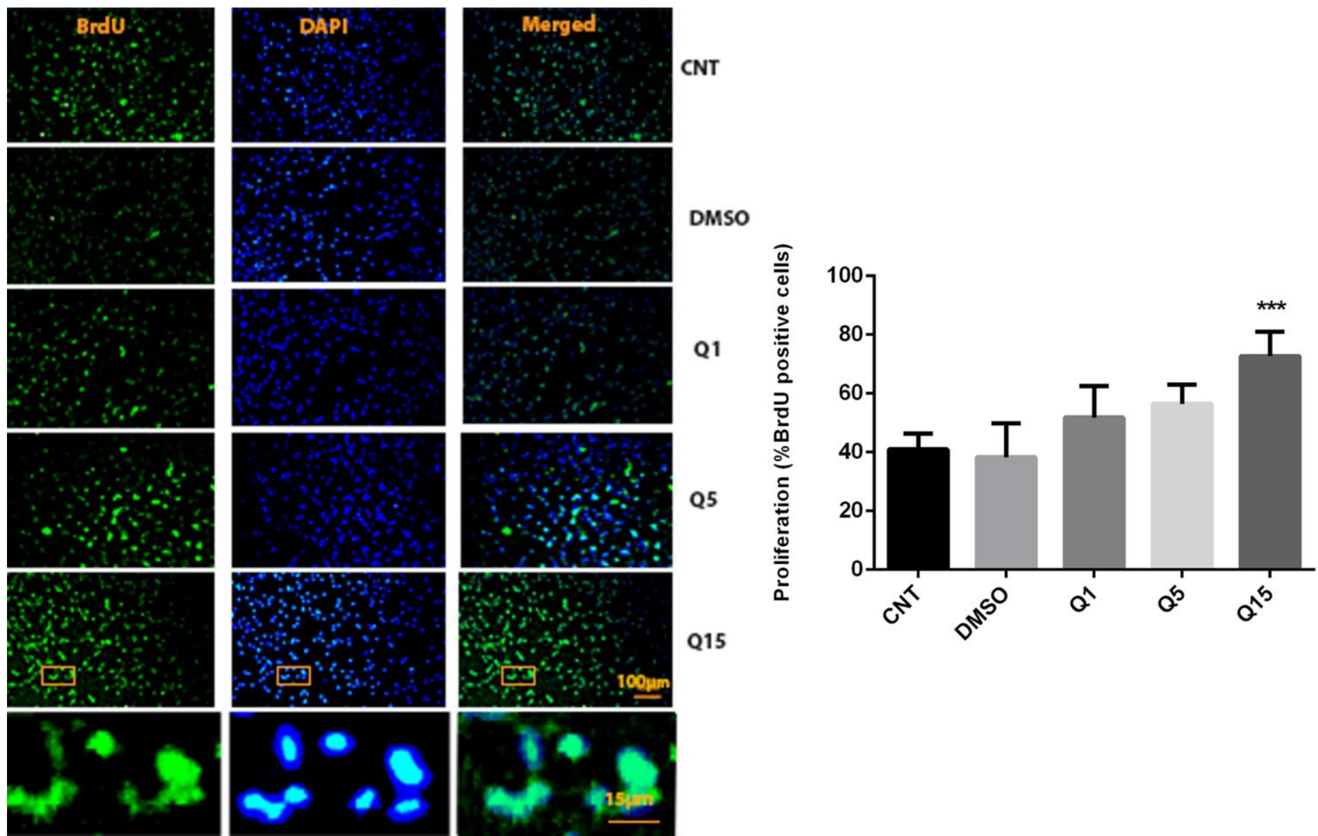

Fig.4. Representative immunofluorescence images of stained BrdU positive cells. On day 7 of treatment period, proliferation of neural stem/progenitor cells was assessed using mouse anti-BrdU-monoclonal primary antibody. (A) BrdU incorporated cells (green fluorescences) were quantified by fluorescent microscopy analysis. DAPI was used to stain cell nuclei (blue fluorescence) (scale bar: 100µm). (B) Bars represent mean+SD of the percentages of BrdU positive cells for different concentrations of quercetin (1,5 and 15µM; Q1-Q15), DMSO and control (CNT) (n=3/group). ***P<0.001 significantly different from DMSO.

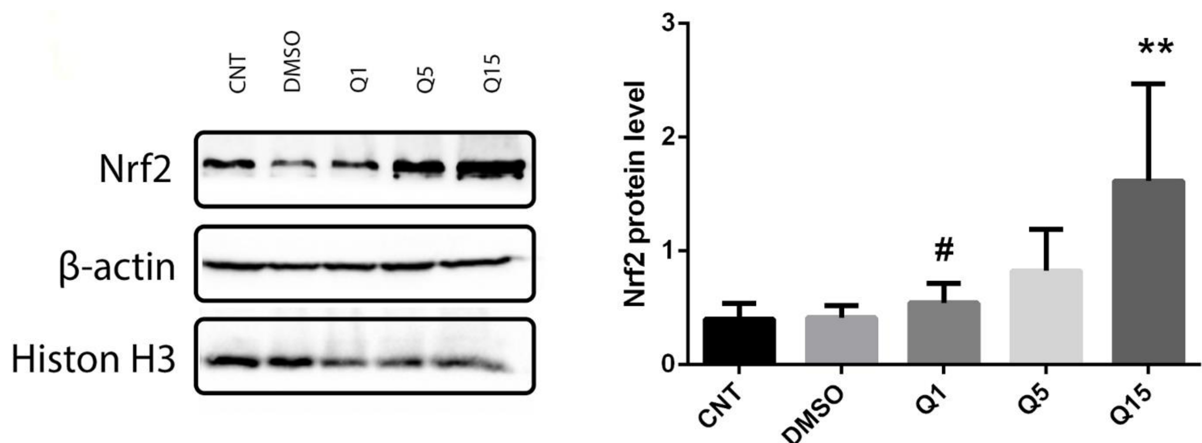

Fig.5. Effects of quercetin on Nrf2 protein levels in NS/PCs. Western blot analysis was used to measure Nrf2protein levels in neural stem/progenitor cells following 7-day treatment with quercetin or DMSO. Bars represent the mean+SD (n=5/group) of Nrf2 signal intensities normalized against β-actin as an internal control for different concentrations of quercetin (1,5 and 15 µM; Q1-Q15), DMSO and control (CNT) (arbitrary units). ***P<0.001 significantly different from DMSO, #P<0.01significantly different from Q15.

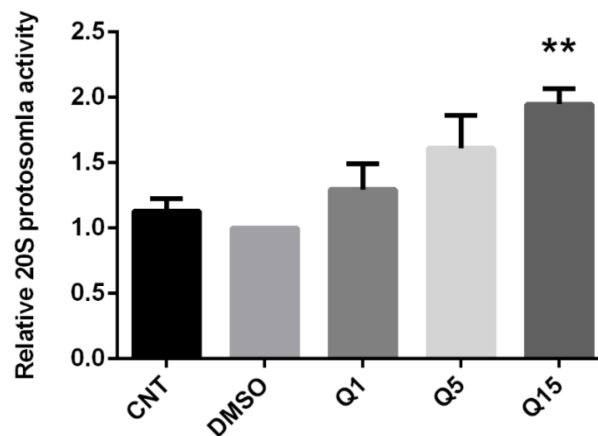

Fig.6. Effects of quercetin on 20S proteasomal activity in NS/PCs. Following 7-day treatment with quercetin or DMSO, 20s proteasomal activity was determined in whole cell lysate by quantifying free fluorophore 7-Amino-4-methylcoumarin (AMC) using fluorescent plate reader with 380/460nm filter set. Bars represent mean+SD of 20S proteasomal activity relative to DMSO for different concentrations of quercetin (1,5 and 15 μM; Q1-Q15), DMSO and control (CNT). $^{**}P<0.01$ significantly different from DMSO.

Effects of quercetin treatment on proteasomal activity in NS/PCs

The 20S protosomal activity was significantly increased in NS/PCs treated with 15μM quercetin in comparison to DMSO-treated cells (94%, $P<0.01$, Fig. 6). However, increases in protosomal activity by 1μM (28.6%) and 5μM (60.7%) quercetin in NS/PCs were not significantly different from that of DMSO. In addition, there was no significant difference in 20S protosomal activity between control and DMSO-treated cells. The effect of quercetin on protosomal activity was concentration-dependent i.e. increasing the concentration of quercetin caused an increased effect on protosomal activity ($r^2=0.85$, $P<0.001$).

Discussion

The findings of this study indicated that quercetin enhanced proliferation capacity of mice embryonic NS/PCs of ganglionic eminence in a concentration-dependent manner. It was also revealed that quercetin increased Nrf2 protein levels and 20S proteasomal activity in parallel to NS/PCs proliferation. Therefore, the stimulatory effect of quercetin on NS/PCs proliferation may be mediated through the enhancement of Nrf2-proteasome pathway. In the present study, quercetin concentration-dependently improved proliferation of NS/PCs of rat fetal ganglionic eminence. This was implied by the observation that 7-day quercetin treatment linearly increased the number of BrdU positive cells, sizes and numbers of neurospheres and viability of NS/PCs. These findings are in agreement with a previous report that quercetin (3-15μM) increased proliferation of rat fetal hippocampal progenitor cells in a concentration-dependent manner (Tchantchou et al., 2009).

In addition, *in vivo* administration of quercetin increased proliferation of neural stem cells (NSCs) of SVZ after focal cerebral ischemia (Zhang et al., 2011). Furthermore, it was shown that CTN986 (a quercetin derivative) and Q3GA (a quercetin metabolite) have increased the proliferation of mice hippocampal NPCs and human embryonic NSC, respectively (Zhang et al., 2009; Baral et al., 2017). In contrast, quercetin (20-60μM) has reduced the viability of human embryonic NSCs in a concentration-dependent way. This contradictory result might be due to the hormetic role of quercetin in viability and proliferation of different cell types. It means that quercetin at low concentration stimulates, while at high concentration inhibits cell proliferation. Thus, high concentrations of quecetin (usually higher than 40μM) have been reported to inhibit cell proliferation in various cancer cell lines such as malignant mesothelioma, H2452 (Lee et al., 2015) and NT2/D1 cells (Mojsin et al., 2013). However, low concentrations of quercetin used in this study as well as other previous studies had a stimulatory effect on cell proliferation. In order to provide some insights into the mechanism underlying the quercetin action on NS/PCs proliferation, we evaluated the effects of

quercetin on Nrf2 and proteasome systems which are two known cellular antioxidant mechanisms involved in cell proliferation.

In this study, quercetin increased Nrf2 protein levels in NS/PCs in a concentration-dependent manner. We detected Nrf2 band at 110 KDa which was at its biologically relevant (Lau et al., 2013) and predicted molecular weight based on antibody manufacturer's data sheet. Our finding of the increased Nrf2 protein level in NS/PCs by quercetin is in line with the previous reports of Nrf2 up-regulation by quercetin in human HepG2 cells (Khadrawy et al., 2019) and in a mice model of traumatic brain injury (Li et al., 2016). In addition, quercetin increased Nrf2 protein level at concentration of 25μM, while decreased Nrf2 mRNA levels at higher concentrations (50 and 75μM) in breast cancer cells (Mostafavi‑Pour et al., 2017). These opposite effects ofquercetin on Nrf2 protein levels at low and high concentrations are similar to those reported for cell proliferation as mentioned above.

The mechanism by which quercetin up-regulated Nrf2 in NS/PCs is unclear at present. Nevertheless, it can be suggested that quercetin might have up-regulated Nrf2 protein levels in NS/PCs through enhancing Nrf2 gene expression and/or preventing Nrf2 from degradation. In support of these possibilities, it has been shown that quercetin has increased Nrf2 protein levels in HepG2 cells at both transcriptional and posttranscriptional levels (Khadrawy et al., 2019). At posttranscriptional level, quercetin has increased Nrf2 stability in HepG2 cells by down-regulating keap1 (Khadrawy et al., 2019). Whether quercetin increased Nrf2 levels in NS/PCs in the present study through increased Nrf2 mRNA levels or preventing Nrf2 from degradation needs to be clarified in further studies.

The increased Nrf2 protein levels may be accounted for the enhancing effects of quercetin on NS/PCs proliferation and viability observed in this study. Accordingly, Nrf2, as a regulator of cellular oxidative stress, has been shown to improve survival and proliferation of NS/PCs with a reduction in oxidative condition (Kärkkäinen et al., 2014; Robledinos-Antón et al., 2017). In addition, a reduced NS/PCs proliferation under oxidative stress conditions and aging has been related to a decline in Nrf2 expression (Corenblum et al., 2016). Furthermore, it has been demonstrated that Nrf2 activation was depended on its protein stabilization (Nguyen et al., 2003; Dreger et al., 2009; Nguyen et al., 2009; Itoh et al., 2010). Taken together, the increased Nrf2 levels in NS/PCs by quercetin in the current study might be suggestive of enhanced Nrf2 activation and subsequently the increased NS/PCs proliferation and viability. To further support this implication, quercetin has been reported to increase Nrf2 stability in association with the increased ARE-mediated gene expression of NQO1 in HepG2 cells (Khadrawy et al., 2019). In addition, SFN which is an Nrf2 inducer (Tarozzi et al., 2013) has promoted NSC proliferation (Han et al., 2017). Therefore, the concurrent improving effects of quercetin on NS/PCs proliferation and increased Nrf2 levels observed in this study can be associated. Nevertheless, this proposition has to be further confirmed by the use of Nrf2 inhibitors and/or measurement of Nrf2-mediated expression of antioxidant genes in NS/PCs exposed to quercetin in forthcoming studies.

The findings of this study revealed that quercetin (1-15μM) concentration-dependently increased 20S proteasomal activity in NS/PCs. This is in agreement with the previous reports that quercetin has increased proteasomal activity in a cell line model of Huntington disease (Chakraborty et al., 2015) and in HFL-1 human primary cells (Chondrogianni et al., 2010). On the contrary, inhibitory effects of quercetin on proteasome activity have been reported in cell free systems or in tumor cells at concentrations over 25μM (Chen et al., 2005). These evidences suggested that the effect of quercetin on proteasomal activity was concentration-dependent and cell-type specific resembling the reported effects of quercetin on cell proliferation and Nrf2 pathway as mentioned above.

In the present study, increased 20S proteasomal activity may be another mechanism by which quercetin affected NS/PCs proliferation and viability. This can be supported by the evidence that impaired proteasomal activity was related to a decrease in self-renewal of NPCs during aging (Zhao et al., 2016) and increased proteasome expression was associated with self-renewing human embryonic stem cells (Jang et al., 2014). Furthermore, an inducer of proteasome expression, SFN, has been shown to increase cell viability of murine neuroblastoma cells when exposed to H_2O_2 (Kwak et al., 2007). Hence, it can be suggested that the observed enhancing effects of querectin on proteasomal activity and NS/PCs

proliferation may be related. Certainly, the use of a proteasome inhibitor such as GM123 in combination with quercetin in NS/PCs can further explain this relationship.

Quercetin increased Nrf2 protein levels and enhanced 20S proteasome activity in NS/PCs in this study. These findings are in agreement with a cross-talk between Nrf2 and proteasome (Chapple et al., 2012; Pickering et al., 2012; Jang et al., 2014). It has been reported that 26S proteasome degrades Nrf2 and regulates NRf2 activation. Under basal condition, inhibition of proteasome results in Nrf2 accumulation and activation of Nrf2-mediated gene expression (Dreger et al., 2009). Conversely, Nrf2 activation increases 20S proteasome expression to maintain redox homeostasis (Pickering et al., 2012) by enhancing degradation of oxidized protein (Dreger et al., 2009; Itoh et al., 2010) and switching off 26S proteasome degradation of ubiqutinated proteins (Grune et al., 2011). Therefore, it can be suggested that quercetin increased Nrf2-mediated 20S proteasome activity. Increased Nrf2-dependent 20S proteasomal activity has been also reported for other natural compounds such as SFN (Chapple et al., 2012). Future studies should evaluate 20S proteasome gene expression and 26S proteasome-dependent Nrf2 degradation in NS/PCs exposed to quercetin.

The enhancing effects of querectin on NS/PCs proliferation, Nrf2 levels and proteasomal activity reported for the first time in this study is very intriguing. These findings support a link between Nrf2, proteasome and cell proliferation in NS/PCs which was influenced by quercetin. Notably, increased Nrf2-mediated proteasome activity and 20S proteasomal gene expression have influenced the proliferation of human embryonic stem cells (Jang et al., 2014) and survival and growth of murine embryonic fibroblast cells (Pickering et al., 2012), respectively. Furthermore, pharmacological approaches or natural compounds can promote cell survival, growth and proliferation via Nrf2-proteasomal pathway. Hence, tBHQ increased Nrf2 level, 20S proteasome activity and cell survival in human neural stem cells (Li et al., 2005). In addition, SFN, an activator of Nrf2, has enhanced proteasomal activity leading to an increased neuroblastoma cell viability in response to H_2O_2 exposure (Kwak et al., 2007) and to a reduced cigarette smoke-induced Beas2B cell death (Malhotra et al., 2009). Other Nrf2 activators such as curcumin and lipoic acid have been also reported to increase 20S proteasome and cell growth (Pickering et al., 2012). Taken together, it can be suggested that quercetin increased NS/PCs proliferation and viability through Nrf2-mediated 20S proteasome activity in the current study. Equally, additional studies are required to further verify the stimulatory effect of quercetin on NS/PCs proliferation through Nrf2-mediated 20S proteasome activity.

Conclusion

In summary, this study for the first time revealed that quercetin concentration-dependently increased NS/PCs cell proliferation, Nrf2 protein levels and 20S proteasome activity in parallel. These findings suggest that quercetin effect on NS/PCs proliferation may be mediated through Nrf2-proteasome pathway. As a result, identifications of natural compounds that activate Nrf2-proteasome pathway may provide suitable basis for novel drug development strategies in regeneration medicine to promote endogenous cell proliferation.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

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