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ROLE OF QUERCETIN IN OXIDATIVE STRESS-INDUCED GLIAL CELL INJURY

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ABSTRACT: Glial cells are crucial for maintaining the stability of the brain, providing support to neurons, and managing oxidative stress. This research examined the cytoprotective effects of the flavonoid Quercetin on C6 glial cells subjected to oxidative stress induced by the chemical hypoxic mimetic Cobalt-Chloride ($CoCl_2$). Treatment with cobalt chloride led to oxidative damage within the cells, indicated by increased levels of reactive oxygen species, lipid peroxidation, and a decrease in mitochondrial membrane potential. Pre-treatment with Quercetin offered significant protection by boosting antioxidant defences, lowering oxidative stress, and maintaining mitochondrial function, thereby reducing the number of apoptotic cells. The effectiveness of Quercetin was found to be dependent on concentration: lower doses were notably effective for certain measures, while higher doses displayed limited effects or, at times, exacerbated the damage, implying a biphasic response. These observations imply that quercetin's protective potential is determined not only by its dose but also by the particular type of cellular stress or injury encountered. Overall, these findings highlight Quercetin as a neuroprotective agent and underscore the need for its dose optimisation and further studies in physiologically relevant models.

INTRODUCTION: Quercetin is a pentahydroxy flavone having hydroxy group at 3-,3'-,4'-,5- and 7- positions [1, 2]. It is a natural flavonoid found in a variety of fruits, vegetables and grains like red onion, capers, berries, apples *etc*. It has radical scavenging properties which makes it a potent antioxidant. It also exerts anti-inflammatory, antihypertensive, gastroprotective, immuno-modulatory effects [1, 3, 4]. Three structural groups that are mainly responsible for free radical reactivity and stability of quercetin are: the B ring *o*-dihydroxyl groups, the 4-oxo group in conjugation with the 2,3-alkene, and the 3- and 5-hydroxyl groups [1, 5]. Quercetin helps in modulating cell signalling pathways and inhibit the activation of pro-inflammatory cytokines which makes it a potential compound in mitigating inflammatory responses in the body [6, 7, 8]. Its antioxidant activity protects the cell from oxidative damage caused by various factors including neurodegenerative disorders, cancers or hypoxia. Additionally, quercetin is also known to reduce viral replication and improve immune functions [7, 9, 10].

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The pathogenesis of hypoxia, ageing, and various neurodegenerative disorders like Alzheimer's, Parkinson's is often associated with oxidative stress. Oxidative stress arises when the endogenous antioxidant defence system fails to neutralise harmful free radicals. This impairment results in an imbalance between antioxidants and reactive oxygen species, causing the accumulation of ROS, which triggers oxidative damage to the biomolecules, resulting in damage at the cellular level and ultimately compromising organ integrity and function [11, 12, 13, 14]. The brain is an extraordinary organ of the Nervous system, highly intricate and remarkable in its structure and

function. It consumes approximately 20 percent of the total oxygen consumed by the body [15]. It has abundant lipid content and has high energy demands which makes it more vulnerable to oxidative insults. Besides this, the brain has relatively low antioxidant enzymes to combat oxidative stress [16, 17]. The effect of oxidative stress is not same on all the types of brain cells. Among the brain cells, neurons and glial cells respond differently to the oxidative insults [18, 19]. Neurons have long been the primary focus of study due to their crucial role in information transmission and signalling. They are susceptible to the risk posed by oxidative stress. However, Glial cells which were traditionally considered merely supporting cells to the neurons, are now considered very important component of nervous system playing pivotal multifaceted role in neuronal signalling, regulating brain homeostasis, forming myelin sheath and contributing to the dynamic functions of the nervous system. These cells have more robust antioxidant defence system and can help protect the neurons against the damage caused by Reactive oxygen species (ROS), however in some cases they become overactivated in response to oxidative stress signals generating more ROS [20, 21].

Astrocytes, the most abundant type of glial cells perform variety of functions like neurotransmitter uptake and recycling, regulating blood brain barrier, supplying antioxidants to the surrounding neuronal cells *etc*. Microglia, are the resident immune cells of brain which helps in removing dead and decaying neurons and maintain the overall health of the brain. They are also known as Brain's macrophage. Oligodendrocytes are the type of glial cells which helps in formation of lipid rich myelin around the neurons. As glial cells perform various important functions, any damage to these would eventually lead to adverse effect on neuronal cells [21, 22, 23, 24, 25].

Quercetin plays a neuroprotective role by modulating mitochondrial dynamics and neuronal function. While its potential in neuronal protection is well-documented, its impact on glial cells remains relatively underexplored. The present study aims to study the antioxidant and anti-inflammatory properties of quercetin on glial cells subjected to oxidative insults.

MATERIALS AND METHODS:

Cell Culture: Glial cell line, C6 were cultured in DMEM supplemented with 10% FBS and 1% Antibiotic-Antimycotic solution and maintained in 37˚C humidified CO_2 incubator.

Hypoxic Exposure and Quercetin Treatment: Oxidative stress was induced in the cells by using Cobalt- chloride ($CoCl_2$), a hypoxic mimetic. For inducing hypoxia, the culture media was removed and cultured cells were washed with PBS 2-3 times and supplemented with fresh medium containing desired concentration of $CoCl_2$. The 10mM stock solution was made by using $CoCl_2.6H_2O$ (Sigma) dissolved in water. Similarly, quercetin treatment was done by removing culture media, washing with PBS and replacing it with fresh medium containing the desired concentrations of Quercetin. Quercetin was used in the form of Quercetin dihydrate 98% HPLC grade (Sigma). The quercetin was dissolved in Dimethyl sulfoxide (DMSO) to make a 10mM stock. To assess the effect of Quercetin on hypoxia-exposed glial cells, cultured cells were washed with PBS 2-3 times, pre-treated with the desired concentration of Quercetin for 1 hour before treating it with $CoCl_2$ for 24 hours.

Determination of $CoCl_2$ and Quercetin Concentrations:

$CoCl_2$ -Induced Cytotoxicity and Dose Selection using Cell Viability Assay: Cell viability assay was done using MTT for optimising the desired concentrations of $CoCl_2$ and Quercetin. 3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) (Sigma Aldrich, India) stock solution was prepared by dissolving 5mg MTT in 1 ml PBS solution and then sterile filtered. Cells were seeded at a density of 2×10^4 cells per well in a 96-well plate, incubated overnight in a CO_2 incubator and then treated in triplicate with $CoCl_2$ at various concentrations (50µM, 100µM, 150µM, 200µM, 250µM, 300µM, 350µM) for 24 hours. Blank wells with only media were also included. Cells were then washed with PBS and MTT solution was added at a concentration of 0.5 mg/ml per well. After the cells were incubated at 37°C in 5% CO_2 incubator for 4h, the MTT solution was removed and 150ul of HCl- isopropanol solution (0.01 N HCl in Isopropanol) was added overnight to dissolve the formazan crystals.

The amount of formazan product formed was determined by measuring absorbance at 570 nm using microplate reader.

Quercetin Dose Selection: The dose optimisation for quercetin was also done in a similar way. Preliminary experiments were conducted to ensure that quercetin alone at different concentrations did not exhibit cytotoxicity. C6 cells were pre-treated with the selected Quercetin concentration, and then treated with determined optimal concentration of $CoCl_2$. Apoptosis assays (as described below) were performed to identify the concentrations of quercetin that most effectively attenuated $CoCl_2$-induced apoptotic cell death without causing toxicity themselves.

Apoptosis Assay: C6 glial cells were treated with different concentrations of Quercetin, $CoCl_2$ (300µM) alone or together at 37°C. After the treatment, the cells were washed, harvested, and resuspended in PBS. Apoptosis was determined using the Annexin V-FITC/PI Apoptosis kit (Elabscience) as per the manufacturer's protocol. Briefly cells were washed with PBS and supernatant was discarded. 100µl 1x Annexin V binding buffer containing 2.5 µl 1X Annexin V and 2.5µl Propidium Iodide (PI) was added to each tube containing cell pellet, mixed and incubated in dark for 30 minutes. After the incubation, 400µl 1X Annexin V binding buffer was added, mixed and cells were analysed using flow cytometer.

Experimental Design and Treatments: C6 cells were divided into following experimental groups:

Control: Cells treated with fresh culture medium.

$CoCl_2$: Cells treated with the selected $CoCl_2$ concentration.

5µM Quercetin+ $CoCl_2$: Cells pre-treated with 5µMquercetin for 1 hour followed by co-treatment with CoCl2 for 24 hours.

10µM Quercetin+ $CoCl_2$: Cells pre-treated with 10µMquercetin for 1 hour followed by co-treatment with CoCl2 for 24 hours.

ROS Estimation: Reactive oxygen species were estimated by using fluorescent dye 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA). Cells were seeded at a density of 2×10^4cells per well in a black 96-well plate. After the desired treatment, culture media was removed and cells were washed with PBS. 100 µl 20 µM H_2DCFDA (10mM stock solution prepared in DMSO) to each well was added and incubated at 37° C for 30-45 min. The DCFH-DA working solution was removed and washed with PBS 2-3 times. 100 µl PBS was added to each well and the fluorescence was acquired at excitation 495 nm/emission at 530 nm [26].

Lipid Peroxidation: Lipid peroxidation levels, measured as malondialdehyde (MDA), were assessed using the CheKine™ Micro Lipid Peroxidation (MDA) Assay Kit (Abbkine) according to the manufacturer's instructions. Briefly, cell lysates were prepared as described in the kit protocol. The reaction mixture, consisting of Reagent I and Reagent II, was combined with the lysate and deionized water in Eppendorf tubes and incubated at 95 °C for 30 minutes. After cooling to room temperature, the samples were centrifuged, and the supernatant was transferred to a 96-well plate. Absorbance was measured at 532 nm and 600 nm using a microplate reader.

Catalase: Catalase activity in cell lysates was measured using the CheKine™ Catalase Activity Assay Kit (Abbkine, Cat. No. KTB1040) according to the manufacturer's instructions. Briefly, cells were washed with phosphate-buffered saline (PBS), lysed in the kit-provided Sample Diluent, and centrifuged to obtain the supernatant. A formaldehyde standard curve (0–75 µM) was prepared using the supplied 4.25 M stock solution. Reactions were performed in duplicate as described in the manual, and absorbance was measured at 540 nm using a microplate reader. Catalase activity (U/mL) was calculated based on the amount of formaldehyde generated per minute (nmol/min).

Measurement of GSH/GSSG Ratio: Intracellular levels of reduced (GSH) and oxidized (GSSG) glutathione were estimated using the methods described by Beutler *et al.* (1963) and Hissin and Hilf (1976) respectively, with slight modifications [27, 28]. For GSH estimation, cell lysates were prepared in 5% metaphosphoric acid (MPA) to stabilize glutathione.

The supernatant was mixed with 0.01 M DTNB prepared in 0.3 M phosphate buffer (pH 7.4). The absorbance was measured at 412 nm, and GSH concentration was determined using a standard curve generated from reduced glutathione standards which were prepared in 1% MPA. For GSSG estimation, the cell lysate was treated with N-ethylmaleimide (NEM) to block free GSH. The mixture was then mixed with 0.1 N NaOH and reacted with o-phthalaldehyde (OPT). Fluorescence was measured using a microplate reader at excitation 350 nm and emission 420 nm. GSSG concentration was determined from a standard curve prepared using oxidized glutathione dissolved in 0.1 N NaOH. The GSH/GSSG ratio was calculated by dividing the concentration of reduced glutathione by that of oxidized glutathione, and was used as an indicator of cellular redox status.

Superoxide Dismutase (SOD): Superoxide dismutase (SOD) activity in cell lysates was measured using the CheKine™ Micro Superoxide Dismutase (SOD) Activity Assay Kit (Abbkine Scientific Co., Ltd.) according to the manufacturer's instructions. Briefly, cells were lysed using the lysis buffer provided in the kit, and the resulting lysates were collected for analysis. Each sample was mixed with the prepared working solution, including xanthine oxidase, in designated wells. For the corresponding sample control wells, xanthine oxidase was replaced with sample diluent as specified in the protocol. The reaction mixtures were incubated at 37 °C for 30 minutes, and absorbance was measured at 450 nm using a microplate reader. SOD activity was calculated using the formula provided in the assay manual.

Mitochondrial Membrane Potential (MMP): Cells were seeded in 24 well plate at a density of 1×10^5 cells per well. After the subsequent treatments, cells were incubated with Rhodamine123 (10µg/ml in DMSO) for 30-45min. After washing with PBS, lysis buffer (10mM tris, 0.25% Triton X-100, 20mM EDTA; pH 8.0) was added to the cells and the fluorescence was measured at excitation wavelength of 485 nm and emission at 530nm.

Quantification of 8-Hydroxy-2'-Deoxyguanosine (8-OHdG): Levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of oxidative DNA damage, were quantified in cell lysates using a Rat 8-OHdG ELISA Kit (ELK Biotechnology), following the manufacturer's protocol. This assay is based on a competitive inhibition enzyme immunoassay. Briefly, standards and samples were added to 8-OHdG pre-coated wells, followed immediately by 1X biotinylated conjugate solution, and incubated at 37 °C for 1 hour. After incubation, the wells were washed thoroughly with 1X wash buffer to remove unbound components. Subsequently, 1X streptavidin–horseradish peroxidase (HRP) working solution was added and incubated for one hour at 37 °C. After multiple washes, TMB substrate was added, and the plate was incubated in the dark at 37 °C for 20–30 minutes. The reaction was terminated by adding stop solution, and absorbance was measured at 450 nm using a microplate reader.

Statistical Analysis: Data are presented as mean ± SD from three independent experiments unless stated otherwise. Statistical analysis was performed using one-way ANOVA followed by Dunnett's, Tukey's, or Šidák post hoc tests, as appropriate to the experimental design. $p< 0.05$ was considered statistically significant.

RESULTS:

Determination of $CoCl_2$ and Quercetin Concentrations: To induce the appropriate cellular oxidative insult, C6 glial cells were treated with various concentrations of $CoCl_2$ for 24 hours. As shown in **Fig. 1A**, $CoCl_2$ induced a dose-dependent reduction in cell viability. Based on the dose-response curve, **Fig. 1B** a concentration of 300 µM $CoCl_2$ was chosen for subsequent experiments, as the calculated IC_{50} value was 285.1 µM, and the concentration closest to the IC_{50} in our experiment was 300 µM, providing an optimal concentration of $CoCl_2$ for assessing its cytotoxic effects. To evaluate the protective effect of quercetin against $CoCl_2$ stress, it is first needed to determine the cytotoxicity of quercetin alone on C6 cells, as the effect of quercetin is dose and time dependent. The cell viability was determined using the MTT test. The calculated IC_{50} of quercetin was 62.94 µM so, the doses below the IC_{50} value i.e 5µM to 50µM quercetin were selected for evaluating the effect of quercetin on $CoCl_2$ induced glial cells. (**Fig. 2A, 2B).**

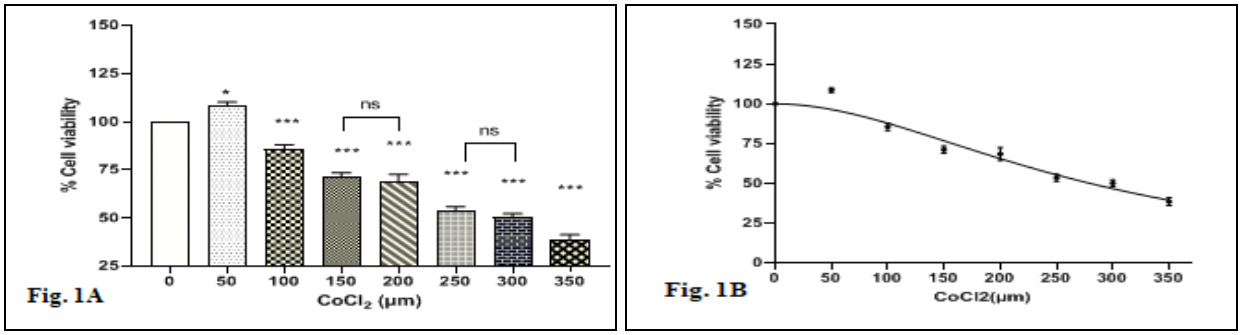


FIG. 1: DOSE OPTIMISATION OF $COCL_2$ VIA CELL VIABILITY ASSAY. (A) C6 Cells were treated with varying concentrations of $CoCl_2$ for 24 hours. Cell viability was assessed by MTT assay. **(B)** The dose- response curveof $CoCl_2$ on C6 cells evaluated the IC_{50} value of 285.1µM (95%CI: 261.1 to 303.5µM). Data are presented as mean% of viable cells± SD.Statistical analysis revealed that mean cell viability at different concentration of $CoCl_2$ to be significantly different as compared to control. ($p<0.001$, ***) ($p<0.05$, *).

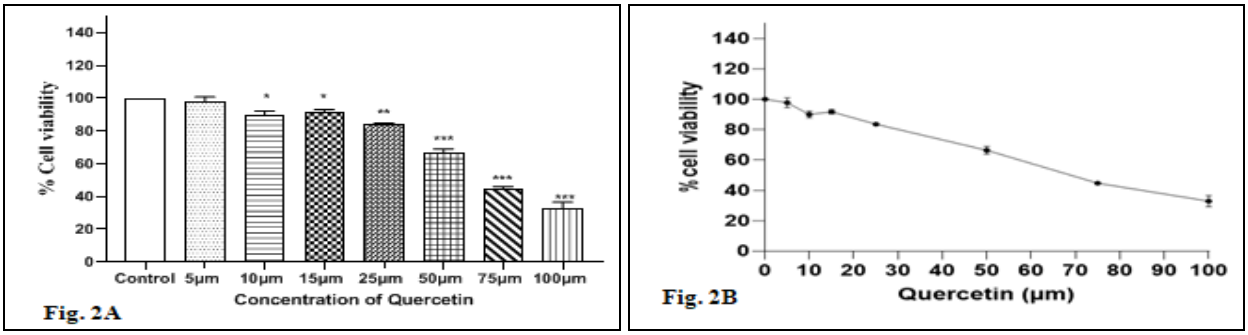
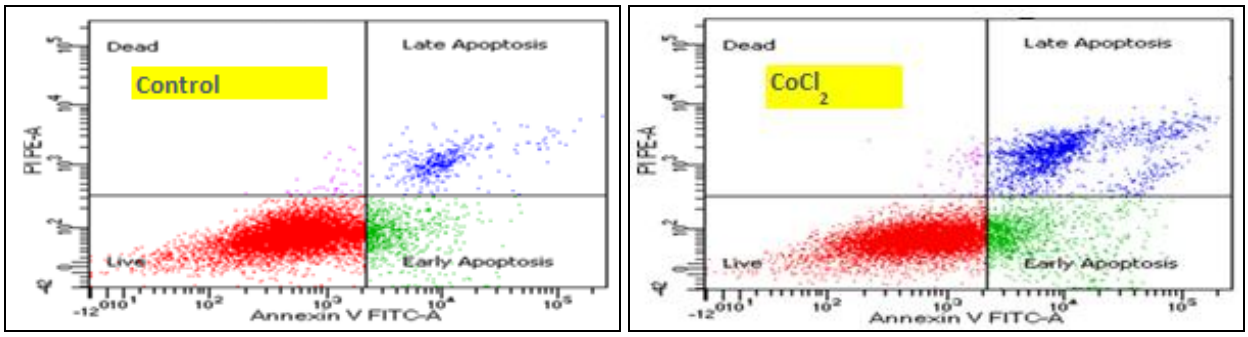


FIG. 2: DOSE OPTIMISATION OF QUERCETIN VIA CELL VIABILITY ASSAY. (A) C6 cells were treated with different concentrations of quercetin alone for 1 hour. Cell viability was assessed by MTT assay. **(B)** The curve resulted from dose response studies of quercetin resulted in an IC_{50} of is 62.94µM (95% CI:60.34 to 73.14µM); revealing its potential cytoprotective or cytotoxic effect across different concentrations. Data represented as mean% of viable cells ±SD. Statistical analysis revealed that mean percentage of cell viability at different concentrations of quercetin significantly different as compared to control. ($p<0.05$, *) ($p<0.01$, **) ($p<0.001$, ***).

Apoptosis assay Flow cytometry analysis using Annexin V-FITC/PI staining demonstrated that exposure to $CoCl_2$ markedly increased the percentage of apoptotic cells (including both early and late apoptosis) from 9.8% in the control group to 26.03% **Fig. 3**. Pre-treatment with quercetin significantly attenuated this effect in a concentration-dependent manner. Specifically, 5 µM and 10 µM quercetin reduced the apoptotic cell population to 17.9% and 12.6%, respectively, indicating a pronounced anti-apoptotic effect **Fig. 3**. However, higher concentrations of quercetin did not produce a similar protective response. Treatment with 15 µM and 25 µM resulted in 21.2% and 24% apoptotic cells, respectively, both not significantly different from the $CoCl_2$-only group **Fig. 3**. Notably, pre-treatment with 50 µM quercetin led to a significant increase in apoptosis (34.2%) compared to $CoCl_2$ treatment alone **Fig. 3**, suggesting potential cytotoxicity at this concentration. Based on these findings, 5 µM and 10 µM quercetin were selected for subsequent experiments.



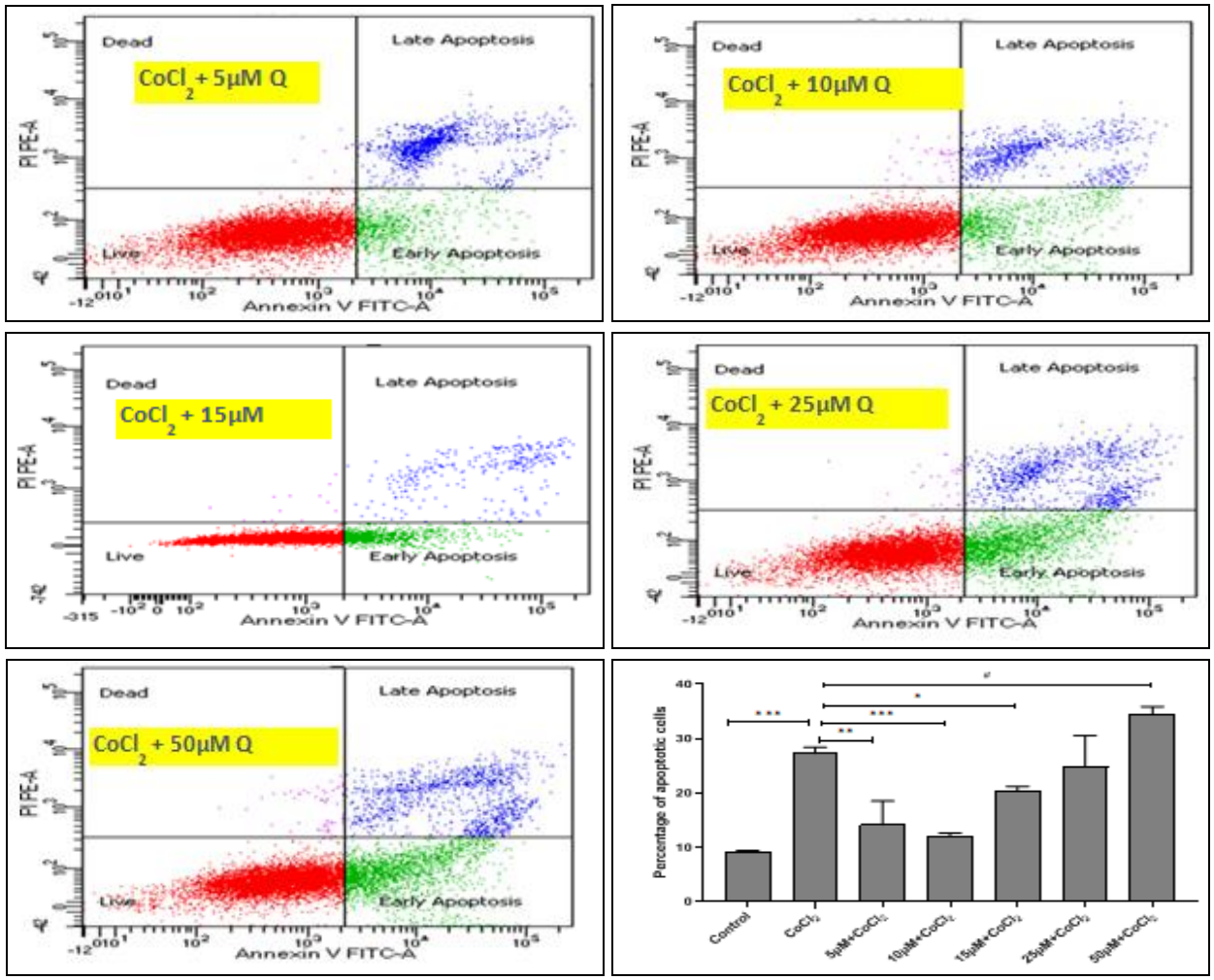


FIG. 3: ANALYSIS OF APOPTOSIS BY ANNEXIN V/PI. Representative dot plots of C6 cells stained with Annexin V-FITC and PI after $CoCl_2$ and Quercetin treatment. Quantified percentages of apoptotic cell population are summarised in the bar graph. Datain the bar graph are represented as mean % ±SD.Statistical analysis revealed the significant difference between various groups ($p<0.05$,*,#) ($p<0.01$,**) ($p<0.001$,***).

ROS Generation: Intracellular ROS production in $CoCl_2$-exposed cells showed a significant increase in DCFH-DA fluorescence intensity by 62% (1.62-fold increase) compared to control cells. However, pre-treatment with quercetin markedly reduced the accumulation of ROS induced by $CoCl_2$ stress. The 5 µM quercetin-treated group showed a significant 38.97% decrease (1.64-fold reduction) in ROS levels compared to the $CoCl_2$ group, bringing the values close to those of control cells. Similarly, the 10 µM quercetin group showed a significant 31.51% reduction (1.46-fold decrease) in ROS production relative to the $CoCl_2$-treated group. **Fig. 4A.**

Lipid Peroxidation: Consistent with the ROS results, Lipid peroxidation levels, measured by malondialdehyde (MDA) content, were significantly elevated in cells exposed to cobalt chloride ($CoCl_2$), indicating increased oxidative stress. The MDA level in the $CoCl_2$-treated group (0.1285 nmol/10^6 cells) was significantly higher as compared to the control group (0.0741 nmol/10^6 cells). However, pre-treatment with quercetin significantly decreased the stress caused due to $CoCl_2$. At 5 µM quercetin group, the MDA level decreased to 0.0779 nmol/10^6 cells, and at 10 µM, it was further reduced to 0.0744 nmol/10^6 cells, which was close to the control group **Fig. 4B.**

Cellular Antioxidant Status: Exposure to $CoCl_2$ treatment markedly reduced the antioxidant levels in the cells. Normal (untreated) cells maintained the mean catalase activity at 7.150 ± 0.312 nmol/min/mL which was decreased in the $CoCl_2$ treated group to5.865 ± 0.456 nmol/min/ml. Pre-treatment with quercetin before $COCl_2$ exposure enhanced catalase activity to levels even

above those of normal cells: at 5 µM the activity level increased to 8.525 ± 0.467 nmol/min/mL, and at 10 µM the activity dramatically continued to rise to 9.127 ± 0.190 nmol/min/mL. The quantitative determination of SOD activity in experimental groups confirmed the findings that exposure to $CoCl_2$ reduces SOD activity (0.174 ± 0.016 U/10^4 cells) compared to untreated control group (0.393 ± 0.015 U/10^4 cells). As shown in the figure, quercetin at the aforementioned 5 µM dose significantly increased SOD activity in both the $CoCl_2$ group and the untreated controls to 0.516 ± 0.036 U/10^4 cells. A 10 µM dose also induced an increase in SOD activity to 0.417 ± 0.020 U/10^4 cells. The cellular redox status was estimated by the ratio between reduced gluthathione (GSH) and oxidised Glutathione (GSSG) levels. In alignment with the other antioxidant result, GSH/GSSG also exhibited a similar trend in which the exposure to $CoCl_2$ resulted in decreased GSH/GSSG of 0.7 compared to untreated control group with GSH/GSSG ratio of 0.87. However, pre-treatment with 5 µM Quercetin resulted in an improvement of GSH/GSSG ratio to 0.91, which was slightly higher than the control cells. Furthermore, pre-treatment with 10um quercetin resulted in GSH/GSSG ratio of 0.84, which was very similar to the control group **Fig. 4C.**

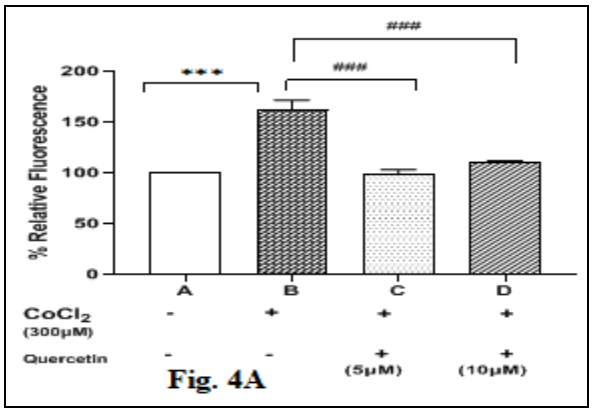


Fig. 4A

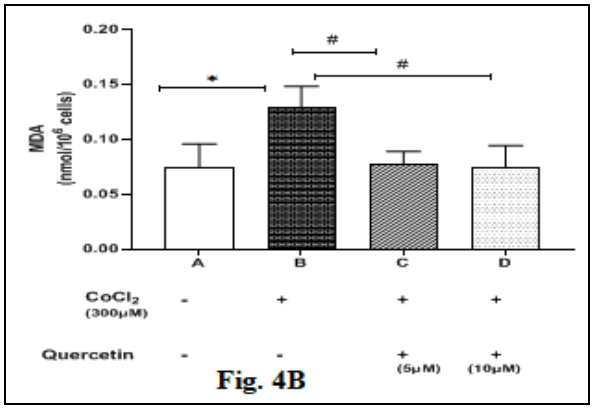


Fig. 4B

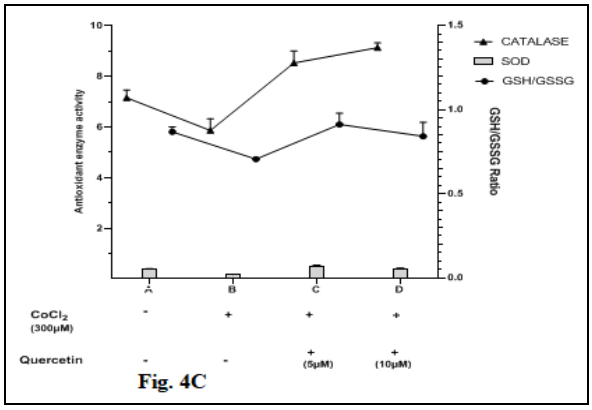


Fig. 4C

FIG. 4: ASSESSMENT OF OXIDATIVE STRESS AND ANTIOXIDANT STATUS. C6 cells were pre-treated with the optimised doses of quercetin following exposure to $CoCl_2$. **(A)** Assessment of reactive oxygen species (ROS) by DCFH-DA. Data is represented as mean percentage of fluorescence relative to control group ± SD. **(B)** Measurement of lipid peroxidation levels. Data is represented as mean± SD. Statistical analysis revealed the significant difference between various groups.(p<0.05,*,#)(p<0.01,**)(p<0.001,***,###). **(C)** Measurement of cellular antioixidant status across different groups. The left Y-axis indicates the catalase activityin nmol/min/mL and SOD activity in U/10^4 cells. The right Y-axis represents GSH/GSSG ratio. Data are presented as mean± SD. Although not shown in the graph, statistically significant difference was observed: Catalase activity was significantly decreased in $CoCl_2$ treated group as compared to control (*p<0.05)*; significant increase in catalase activity was found in 5µM and 10µMquercetin pre-treated groups as compared to $CoCl_2$ group (*p<0.001).* A significant increase was also found in 5µM pre-treated group (*p<0.01)* and 10µM pre-treated group (*p<0.001)* as compared to the control group. The SOD activity was also significantly decreased in $CoCl_2$ group with respect to control group (*p<0.001).* Both quercetin pre-treated groups showed significant increase in SOD as compared to $CoCl_2$ group(*p<0.001).* The 5µM pre-treated group also showed significant increase in SOD activity with respect to control (*p<0.01).* The ratio of reduced glutathione to oxidised glutathione (GSH/GSSG) was found to decrease in $CoCl_2$ group as compared to control (*p< 0.05),* the 5µM quercetin pre-treated group showed significant rise in GSH/GSSG ratio *(p<0.01)* and the 10µM quercetin pre-treated group also showed increase in GSH/GSSG ratio (*p<0.05)* as compared to $CoCl_2$ treated group.

Mitochondrial Membrane Potential: One crucial indicator of cellular metabolic activity is the mitochondrial membrane potential (MMP). The cells exposed to $CoCl_2$ had a 17% lower mitochondrial membrane potential than the control group. This loss was greatly lessened by the quercetin pretreatment. After the pre-treatment of 5µm dose of quercetin, the loss was 15.09%; however, a 10 µm dose of quercetin significantly decreased this loss to 4.14%.

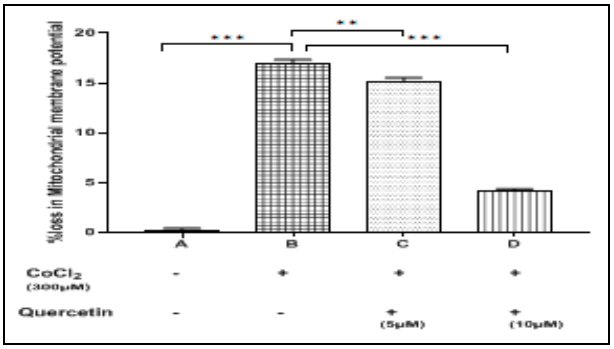


FIG. 5: MEASUREMENT OF MITOCHONDRIAL MEMBRANE POTENTIAL (MMP). MMP was measured by using Rhodamine 123 dye. Data are expressed as mean % loss in MMP with respect to control ±SD. Statistical analysis revealed significant difference across various groups (*p<0.01,**; p<0.001,***).*

Oxidative DNA Damage: The quantitative analysis of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker for oxidative DNA damage, revealed a 20.23% increase in $CoCl_2$ treated group relative to the control cells. **Fig. 6** illustrates that pre-treatment with quercetin at a concentration of 5 µM tended to reduce the oxidative damage induced by $CoCl_2$ exposure. However, the observed reduction was statistically nonsignificant. The 10µM dose of quercetin significantly reduced the oxidative damage by 14.9% as compared to the cells treated with $CoCl_2$ alone.

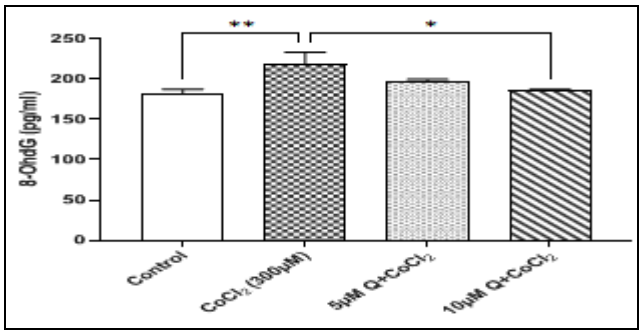


FIG. 6: MEASUREMENT OF 8-OHdG. Data are represented as mean 8-OHdG level±SD. Statistical analsysis showed significant difference across the groups. (*p<0.05,*;p<0.01,**).*

DISCUSSION: Glial cells are an integral part of our central nervous system as it helps in regulating oxidative stress, maintaining brain homeostatsis and providing structural and metabolic support to neuronal cells [20, 21, 22]. The present study examined the effect of Quercetin against $CoCl_2$ induced oxidative stress in C6 glial cells. $CoCl_2$ acts as a hypoxic mimetic as it stabilises Hypoxia inducible factors (Hif 1α and Hif 2α) under normoxic conditions and widely used chemical to induce hypoxia like conditions [29, 30]. Our findings revealed that $CoCl_2$ exerts a dose- dependent cytotoxic effect on C6 glial cells as indicated by the cell viability test with an IC_{50} value of 285.1 µM. Our findings aligned with the findings of Elkashef *et al.* [31] and in our experiment, 300µM of $CoCl_2$ was used to induce oxidative stress as it was close to IC_{50} value.

Quercetin is known to possess antioxidant and anti-inflammatory properties. To evaluate its protective ability on C6 cells, cells were first treated with quercetin alone to identify its non-cytotoxic concentrations. Cell viability test resulted in an IC_{50} of 62.94µM. Quercetin at doses of 5-50µM was selected for the determination of apoptosis induced by $CoCl_2$. Flow cytometric assessment by Annexin V/PI revealed that pre-treatment with lower doses of 5µM and 10µM quercetin before exposure to $CoCl_2$ significantly reduced the apoptosis in C6 cells. However higher doses did not provide a significant protective effect and 50µM of quercetin further exacerbated the rate of apoptosis caused due to $CoCl_2$, suggesting quercetin's biphasic effect where at lower doses it acts as an antioxidant and exerts pro-oxidant effect at higher concentrations. Hence dose -optimisation studies with the flavonoids are very crucial. Notably, a previous study by Gitika *et al.*[32] reported the dose of 75µM quercetin as cytoprotective, against t-BOOH induced oxidative stress in C6 cells. This difference in doses highlights that, the nature of chemical used to induce the stress and the duration of exposure are the decisive factors that influence the dose required and the resulting response to the stress.

Oxidative stress results from an imbalance between antioxidants and free radicals, resulting in cellular injury. There can be numerous factors which can result in Oxidative stress, such as hypoxia, neurodegenerative disorders, cancer, inflammation, smoking, drinking *etc* [33].

Our research shows that $CoCl_2$ profoundly increases oxidative stress by enhancing the generation of ROS in cells. This finding is in support of previous studies by He *et al.* [34], Gitika *et al.*[32], and Isik *et al.*[35]; which stated that$CoCl_2$ causes cellular dysfunction, thus increasing oxidative stress and cellular apoptosis. Furthermore, the high levels of lipid peroxidation in our experiments also strongly supported that oxidative stress was induced in $CoCl_2$-treated cells. The protective roles of quercetin against cytotoxicity, ROS formation, and lipid peroxidation are well-documented in previous literature, as emphasized by Gitika *et al.*[32]; Fang *et al.*[36]; Zhang *et al.*[37]. Our results showed that pre-treatment with quercetin significantly decreased the levels of malondialdehyde (MDA) to baseline values, demonstrating the potential of enhancing membrane integrity by antioxidative processes.

In addition, pre-treatment with quercetin resulted in enhanced activity of catalase and superoxide dismutase (SOD), as well as improved ratio of reduced to oxidized glutathione (GSH/GSSG).The decreased mitochondrial membrane potential indicated that $CoCl_2$ compromised the cellular mitochondrial integrity. The pre-treatment with quercetin effectively mitigated the stress. However, 5 μM quercetin offered only limited improvement in MMP loss as compared to 10μM dose which effectively restored MMP. Similar trend was found in Oxidative DNA damage, measured by 8-OHdG levels, where 10 μM dose of quercetin effectively reduced the levels of 8-OHdG but 5 μM dose could not produce statistically significant reduction. This suggests a possible threshold-dependent response, where lower doses may not effectively activate the pathways involved in mitochondrial and DNA protection.

However, both doses were found to be effective in assays evaluating general oxidative stress such as ROS, malondialdehyde (MDA) levels and antioxidant studies and in some cases, the 5 μM dose showed greater antioxidant and protective effect. In MMP and oxidative DNA damage studies, higher dose of 10μM was comparatively more effective. These outcomes indicate that quercetin's protective effect may vary depending on the pathway and the type of damage being assessed. Different doses may be needed to mitigate the stress, depending upon how severe the damage is and which molecular markers are being targeted [38, 39]. While the final doses selected in our experiments were based on the apoptotic response, using them for further experiment gave us important insights as to how different types of oxidative damage respond to the treatment. As evident by our studies, same dose might have variable efficacy depending on the stress parameters measured. This shows that antioxidant effects of quercetin cannot be confined to a specific dosage and it's crucial to examine several different markers to fully harness beneficial effects of quercetin.

While this study provides compelling evidence that pre-treatment with quercetin effectively mitigated the $CoCl_2$ induced stress. It is very important to consider the possible limitations of this study. It is an *in-vitro* study in which C6 glial cells were used, further research may be needed to elucidate the quercetin's response in other glial cells and *in-vivo*; as *in-vitro* studies, useful for molecular studies, lack the complex interactions present in a whole organism. Therefore, the effects of quercetin observed in C6 glial cells may not fully reflect its activity *in-vivo*. Secondly, $CoCl_2$ is being used to induce oxidative stress; it may not fully replicate the complex mechanism of oxidative stress induced in the brain. The present study did not study the underlying mechanism behind the protective effects of quercetin, therefore, to better understand these results, further studies are needed to explore the specific cellular mechanisms involved. However, our findings align with existing literatures showing that flavonoids including quercetin may activate endogenous antioxidant enzymes through Nrf2 pathways, hence enhancing cellular antioxidant protection [40, 41, 42]. Identifying the direct molecular targets of quercetin and the signalling pathways will provide a more detailed understanding behind its protective effect.

CONCLUSION: We demonstrated that quercetin pre-treatment helps in mitigating the oxidative injury caused by Cobalt-Chloride ($CoCl_2$) in C6 glial cell line. Our findings also suggested that the effect of quercetin is dose dependent and may vary across different oxidative parameters involved. The lower doses of quercetin were found to be beneficial in our study indicating the quercetin's

role as a potent antioxidant. These results may contribute further to exploring the therapeutic potential of quercetin. Further research is also needed to understand its underlying mechanism of protection, ultimately enabling better therapeutic utilization of quercetin.

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REFERENCES:

1. Vollmannova A, Bojnaska T, Musilova J, Lidikova J and Cifrova M: Quercetin as one of the most abundant represented biological valuable plant components with remarkable chemoprotective effects - A review. Heliyon 2024; 10(12): 33342.
2. National Center for Biotechnology Information: PubChem Compound Summary for CID 5280343, Quercetin [Internet]. 2025 [cited 2025 Jul 20]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Quercetin
3. Crnivec IGO, Skrt M, Polak T, Seremet D, Mrak P, Komes D, Vrhovsek U and Ulrih NP: Aspects of quercetin stability and its liposomal enhancement in yellow onion extracts. Food Chemistry 2024; 459: 140347.
4. Bai C, Hu X, Lu S, Xu B, Ma T and Song Y: Recent advances and application challenges of quercetin-loaded delivery systems: from material selection to bioavailability. Journal of Future Foods 2025.
5. Hollman PC, Van Trijp JM, Buysman MN, Van der Gaag MS, Mengelers MJ, De Vries JH and Katan MB: Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. FEBS Letters 1997; 418(1-2): 152–156.
6. Jiang M, Lv Z, Huang Y, Cheng Z, Meng Z, Yang T, Yan Q, Lin M, Zhan K and Zhao G: Quercetin alleviates lipopolysaccharide-induced inflammatory response in bovine mammary epithelial cells by suppressing TLR4/NF-κB signalling pathway. Frontiers in Veterinary Science 2022; 9: 915726.
7. Aghababaei F and Hadidi M: Recent advances in potential health benefits of quercetin. Pharmaceuticals (Basel) 2023; 16: 1020.
8. Aggarwal D, Chaudhary M, Mandotra SK, Tuli HS, Chauhan R, Joshi NC, Kaur D, Dufosse L and Chauhan A: Anti-inflammatory potential of quercetin: from chemistry and mechanistic insight to nanoformulations. Current Research in Pharmacology and Drug Discovery 2025; 8: 100321.
9. Islam MR, Al-Imran MIK, Zehravi M, Sweilam SH, Mortuza MR, Gupta JK, Shanmugarajan TS, Devi K, Tummala T, Alshehri MA, Rajagopal K, Asiri M, Ahmad I and Emran TB: Targeting signaling pathways in neurodegenerative diseases: Quercetin's cellular and molecular mechanisms for neuroprotection. Animal models and experimental medicine 2025; 8(5): 798–818.
10. Kaur K, Kulkarni YA and Wairkar S: Exploring the potential of quercetin in Alzheimer's Disease: Pharmacodynamics, Pharmacokinetics, and Nanodelivery systems. Brain research 2024; 1837: 149824.
11. Adnan M, Siddiqui AJ, Bardakci F, Surti M, Badraoui R and Patel M: Neuroprotective potential of quercetin in Alzheimer's disease: targeting oxidative stress, mitochondrial dysfunction, and amyloid-β aggregation. Frontiers in Pharmacology 2025; 16: 1593264.
12. Dash UC, Bhol NK, Swain SK, Samal RR, Nayak PK, Raina V, Panda SK, Kerry RG, Duttaroy AK and Jena AB: Oxidative stress and inflammation in the pathogenesis of neurological disorders: mechanisms and implications. Pharmacology & Therapeutics 2025; 267: 108805.
13. Houldsworth A: Role of oxidative stress in neurodegenerative disorders: a review of reactive oxygen species and prevention by antioxidants. Brain Communications 2024; 6 (1): 356.
14. Magistretti PJ and Allaman I: A cellular perspective on brain energy metabolism and functional imaging. Neuron 2015; 86(4): 883–901.
15. Lee KH, Cha M and Lee BH: Neuroprotective effect of antioxidants in the brain. International Journal of Molecular Sciences 2020; 21(19): 7152.
16. Jové M, Mota-Martorell N, Obis È, Sol J, Martín-Garí M, Ferrer I, Portero-Otín Mand Pamplona R: Lipid adaptations against oxidative challenge in the healthy adult human brain. Antioxidants (Basel) 2023; 12(1): 177.
17. Vishwas S, Kumar R, Khursheed R, Ramanunny AK, Kumar R, Awasthi A, Corrie L, Porwal O, Arshad MF, Alshammari MK, Alghitran AA, Qumayri AN, Alkhaldi SM, Alshammari AK, Chellappan DK, Gupta G, Collet T, Adams J, Dua K, Gulati M and Singh SK: Expanding arsenal against neurodegenerative diseases using quercetin based nanoformulations: Breakthroughs and Bottlenecks. Current Neuropharmacology 2023; 21(7): 1558–1574.
18. Lee KH, Cha M and Lee BH: Crosstalk between neuron and glial cells in oxidative injury and neuroprotection. International Journal of Molecular Sciences 2021; 22(24): 13315.
19. Lassmann H and Van Horssen J: Oxidative stress and its impact on neurons and glia in multiple sclerosis lesions. Biochimica et Biophysica Acta 2016; 1862(3): 506–510.
20. Hughes AN: Glial cells promote myelin formation and elimination. Frontiers in Cell and Developmental Biology 2021; 9: 661486.
21. Chen Y, Qin C, Huang J, Tang X, Liu C, Huang K, Xu J, Guo G, Tong A and Zhou L: The role of astrocytes in oxidative stress of central nervous system: a mixed blessing. Cell Proliferation 2020; 53(3): 12781.
22. Lenz KM and Nelson LH: Microglia and beyond: innate immune cells as regulators of brain development and behavioral function. Frontiers in Immunology 2018; 9:698.
23. Simons M and Nave KA: Oligodendrocytes: myelination and axonal support. Cold Spring Harbor Perspectives in Biology 2015; 8(1):a020479.
24. Chiang MC, Tsai TY and Wang CJ: The potential benefits of quercetin for brain health: A Review of Anti-Inflammatory and Neuroprotective Mechanisms. International Journal of Molecular Sciences 2023; 24(7): 6328.
25. Reynolds A, Laurie C, Mosley RL and Gendelman HE: Oxidative stress and the pathogenesis of neurodegenerative disorders. International Review of Neurobiology 2007; 82: 297–325.
26. Kim H and Xue X: Detection of total reactive oxygen species in adherent cells by 2',7'-dichlorodihydrofluorescein diacetate staining. Journal of Visualized Experiments 2020; (160): 10.3791/60682.

27. Beutler E, Duron Oand Kelly BM: Improved method for the determination of blood glutathione. The Journal of laboratory and clinical medicine 1963; 61: 882–888.
28. Hissin PJ and Hilf R: A fluorometric method for determination of oxidized and reduced glutathione in tissues. Analytical biochemistry 1976; 74(1): 214–226.
29. Muñoz-Sánchez J and Chánez-Cárdenas ME: The use of cobalt chloride as a chemical hypoxia model. Journal of Applied Toxicology 2019; 39(4): 556–570.
30. Tripathi VK, Subramaniyan SA and Hwang I: Molecular and cellular response of co-cultured cells toward cobalt chloride ($CoCl_2$)-induced hypoxia. ACS Omega 2019; 4(26): 22344–22354.
31. Elkashef SM, Allison SJ, SadiqM, Basheer HA, Ribeiro Morais G, Loadman PM, Pors K and Falconer RA: Polysialic acid sustains cancer cell survival and migratory capacity in a hypoxic environment. Scientific Reports 2016; 6: 33026.
32. Gitika B, Sai Ram M, Sharma SK, Ilavazhagan G and Banerjee PK: Quercetin protects C6 glial cells from oxidative stress induced by tertiary-butylhydroperoxide. Free Radical Research 2006; 40(1): 95–102.
33. Sharifi-Rad M, Anil Kumar NV, Zucca, P, Varoni EM, Dini L, Panzarini E, Rajkovic J, Tsouh Fokou PV, Azzini E, Peluso I, Prakash Mishra A, Nigam M, El Rayess Y, Beyrouthy ME, Polito L, Iriti M, Martins N, Martorell M, Docea AO, Setzer WN, Calina D, Cho CW and Sharifi-Rad J: Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. Frontiers in physiology 2020; 11: 694.
34. He Y, Gan X, Zhang L, Liu B, Zhu Z, Li T, Zhu J, Chen J, and Yu H: $CoCl_2$ induces apoptosis *via* a ROS-dependent pathway and Drp1-mediated mitochondria fission in periodontal ligament stem cells. American Journal of Physiology-Cell Physiology 2018; 315(3): 389-397.
35. Isik B, Suleyman B, Mammadov R, Bulut S, Yavuzer B, Altuner D, Coban TA and Suleyman H: Protective effect of cinnamon extract against cobalt-induced multiple organ damage in rats. Frontiers in Pharmacology 2024; 15: 1384181.
36. Fang P, Dou B, Liang J, Hou W, Ma C and Zhang Q: Quercetin reduces oxidative stress and apoptosis by inhibiting hmgb1 and its translocation, thereby alleviating liver injury in ACLF Rats. Evidence-based Complementary and Alternative Medicine 2021; 2021: 2898995.
37. Zhang W, Zheng Y, Yan F, Dong M and Ren Y: Research progress of quercetin in cardiovascular disease. Frontiers in Cardiovascular Medicine 2023; 10: 1203713.
38. Mirza MA, Mahmood S, Hilles AR, Ali A, Khan MZ, Zaidi SAA, Iqbal Z and Ge Y: Quercetin as a Therapeutic Product: Evaluation of Its Pharmacological Action and Clinical Applications-A Review. Pharmaceuticals (Basel) 2023; 16(11): 1631.
39. JoshiH, Gupta DS, Kaur G, Singh T, Ramniwas S, Sak K, Aggarwal D, Chhabra RS, Gupta M, Saini AK and Tuli HS: Nanoformulations of quercetin for controlled delivery: a review of preclinical anticancer studies. Naunyn-Schmiedeberg's Archives of Pharmacology 2023; 396(12): 3443–3458.
40. Li YR, Li GH, Zhou MX, Xiang L, Ren DM, Lou HX, Wang XN and Shen T: Discovery of natural flavonoids as activators of Nrf2-mediated defense system: Structure-activity relationship and inhibition of intracellular oxidative insults. Bioorganic & Medicinal Chemistry 2018; 26(18): 5140–5150.
41. Cheng M, Yuan C, Ju Y, Liu Y, Shi B, Yang Y, Jin S, He X, Zhang L and Min D: Quercetin attenuates oxidative stress and apoptosis in brain tissue of APP/PS1 Double Transgenic AD Mice by Regulating Keap1/Nrf2/HO-1 Pathway to Improve Cognitive Impairment. Behavioural Neurology 2024; 2024: 5698119.
42. Dinkova-Kostova AT and Talalay P: Direct and indirect antioxidant properties of inducers of cytoprotective proteins. Molecular Nutrition and Food Research 2008; 52(1): 128–138.

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