Attenuation of oxidative injury by quercetin from *Psidium guajava* and its therapeutic potential study against periodontitis on *in vitro* model system

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Abstract

The current research focuses on the biological potential of quercetin-rich fraction from Psidium guajava (QRF PG) leaf extract, emphasizing its role decreasing oxidative stress. Our study evaluated the antioxidant potential of an ethanolic extract derived from the leaves of Psidium guajava on a specific KB cell line. Cell viability was initially evaluated by directly observing the cells via phase contrast microscope. Following which a dose optimization of QRF PG on KB cell lines was analysed by MTT assay. Our study reported nearly 70% cell viability at 25 µg QRF PG. Cytoprotective activity of QRFPG on H_2O_2 induced oxidative stress was analysed by MTT assay. The oxidative state of the physiologic system in vitro was analysed by lipid peroxidation with a value of the satisfying reduction in the level of lipid peroxidation to 0.429 µg/MDA units, an increase in catalase level in cells treated with QRFPG as 0.5254 enzyme units, elevation in glutathione reductase to 0.5922 enzyme units and superoxide dismutase at 1.09 enzyme units.

Our study noted a steady reduction in fluorescence intensity in oral KB cells treated with QRFPG with a value of 17526.10 +/- 2586.9 AU indicating the ability of QRFPG in scavenging reactive oxygen species. The impact of QRF on the face of the Nrf2 gene was determined in KB cells subjected to oxidative stress by free radicals. The immune modulatory potential of quercetin was checked by evaluating the inflammatory mediators like cyclooxygenase 2, tumor necrosis factor-alpha and interleukins 6. The antimicrobial activity of the plant extract against the oral pathogens Streptococcus mutans and Enterococcus faecalis was done using the agar diffusion method. The present study highlights the role of QRFPG as an antioxidant, anti-inflammatory and antibacterial bioactive compound.

Keywords: Quercetin, Lipid Peroxidation, Catalase, Nrf 2, TNF ALPHA, Interleukin 6, Immunomodulatory.

Introduction

Quercetin is a plant flavonoid present in many of the tropical plants. Recent studies proved the efficacy of quercetin as an antibacterial[52], anti-inflammatory[72], anti-oxidant[88], anti-cancer[10], anti-biofilm agent[94] and antidiabetic[29]. Due to these properties, quercetin gained global attention to be exploited as a nutraceutical and was used in line with modern treatment strategies. Recently, quercetin gained GRAS (Generally Recognized as Safe) status[38]. Due to its ability to disrupt biofilm formation and to minimize the extent of bacterial aggregation and biofilm formation, it can be exploited as a medical adjuvant in treating oro dental infections[65,100]. The flavonoid antioxidant quercetin's ability to scavenge reactive oxygen species[23] is of greater significance in controlling periodontal disease[94].

Interestingly, the findings of a recent study demonstrated quercetin's ability to inhibit the release of inflammatory mediators, particularly cytokines, in gingival fibroblasts in an *in vitro* setting. This effect is reported to help reduce alveolar bone resorption[63]. Quercetin's molecular structure contains a keto carbonyl group, a dihydroxy group between the A ring, *o*-dihydroxy group B, C ring C2, C3 double bond and 4-carbonyl as the active groups[6,8,96]. The bioactive potential of quercetin is mainly due to the functional phenolic hydroxyl groups and double bonds[60]. Figure 1 depicts the structure of quercetin. The antioxidant mechanism of quercetin[77] is by regulating the enzyme GSH level, downregulating the MDA level and upregulating the activity of enzyme superoxide dismutase (SOD)[97].

Quercetin promotes interferon gamma cell expression and downregulates Interleukin -6 positive cell expression, hence its role is observed in the anti-inflammatory mechanism. This modulation of cytokine expression is one of the mechanisms by which quercetin is believed to exert anti-inflammatory effects[21,48].

By shifting the balance of cytokines towards a more pro-inflammatory (IFN-γ) response and away from a potentially allergy-promoting (IL-4) response, quercetin can contribute to an overall anti-inflammatory effect[13]. The anticancer potential of quercetin owes its ability to induce extrinsic and intrinsic apoptosis pathways[93]. It may help to lower the severity of hypertension and may act as an antihypertensive compound[95].

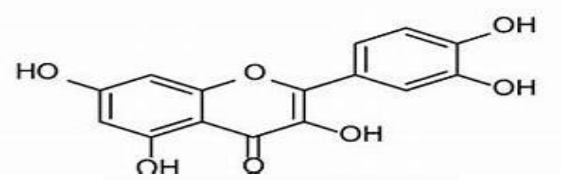

Fig. 1: Structure of Quercetin

Periodontitis is a dental burden affecting the global population[43]. It is a chronic inflammatory disease that has the potential to damage irreversibly the supporting tissues of the tooth like the periodontal ligament, cementum and alveolar bone, resulting in eventual tooth loss[53]. On exposure to bacteria, neutrophils and other immune cells release ROS, especially H_2O_2 and superoxide dismutase[31]. As the periodontal disease progresses, the ROS level in the tissues increases, resulting in higher lipid peroxidation levels. The elevated level of ROS in gingival crevicular fluid indicates progressing periodontitis[25]. The ROS triggers the NF-κB signaling pathway, resulting in the upregulation of the expression of inflammatory mediators[55]. This sequence of events leads to the activation and differentiation of osteoclasts, eventually progressing to alveolar bone loss and periodontal diseases[12,41,45].

The nuclear factor kappa B (NFkβ), a transcription factor, modulates cellular genes by regulating the inflammatory response[92]. Quercetin may suppress the production of inflammatory cytokines in LPS-induced inflammation in human gingival fibroblast cells. This suppression is achieved, in part, by upregulating Toll-like receptor 4 (TLR4) and peroxisome proliferator-activated receptor gamma (PPAR-γ)[26,78].

The link between chronic inflammation and cancer is a complex and multifaceted process involving various molecular and cellular mechanisms[73]. Khandia and Munjal[47] in their recent investigation, showed that chronic inflammation creates a microenvironment that can contribute to the initiation, promotion and progression of cancer. One of the key players in this connection is the upregulation of inflammatory mediators including cytokines and enzymes. Cyclooxygenase-2 (COX-2) is an enzyme that converts arachidonic acid into prostaglandins during the inflammatory response. Pro-inflammatory cytokines induce the expression of COX-2 at the site of inflammation[64]. COX-2 has been linked to inhibiting apoptosis (programmed cell death), allowing cancer cells to evade the standard regulatory mechanisms that control cell survival and death[103].

Interleukin-6 (IL-6) is a vital member of the pro-inflammatory cytokine family and plays a multifaceted role in the immune system and various physiological processes. IL-6 is produced by multiple cells including immune cells such as macrophages and T cells, as well as non-immune cells like fibroblasts and endothelial cells[89]. Several studies pointed to the inevitable role of IL-6 as a major inducer of the acute phase response, a series of systemic changes that occur in response to inflammation or infection.

An exciting study by Jones and Jenkins[44] proposed that IL 6 stimulates the liver to produce acute-phase proteins such as C-reactive protein (CRP), fibrinogen and serum amyloid A, which are involved in the inflammatory process. IL-6 can act as a pro-inflammatory and anti-inflammatory cytokine depending on the context and the presence of other signaling molecules.

TNF alpha is yet another inflammatory mediator that has a crucial role in regulating and altering the immune homeostasis of the system. TNF alpha is a multifunctional cytokine initially identified for its ability to induce tumor necrosis, hence its name. TNF-α binds to two cell surface receptors, TNF receptor 1 (TNFR1) and TNF receptor 2 (TNFR2)[51]. Upon binding, these receptors initiate signal transduction pathways within the cells. Inappropriate or excessive activation of TNF-α signaling is associated with chronic inflammation[98]. Understanding the TNF-α signaling mechanism has led to the development of therapeutic tools, including TNF-α inhibitors. These inhibitors treat autoimmune diseases to dampen the excessive immune response and alleviate symptoms[39].

Common examples of TNF-α inhibitors include drugs like infliximab, adalimumab and etanercept [68]. TNF-α inhibitors have effectively managed symptoms and improved the quality of life for individuals with autoimmune diseases. Despite all the suggested advantages, several studies pointed out the side effects of TNF alpha inhibitors including headaches, rashes, anemia etc. and worsening of cardiovascular diseases[62]. Cases of TB in patients with autoimmune disorders being cause of worry in using these therapeutics[40]. The present study emphasizes the potential of developing plant-based phytochemicals as therapeutics for diseases involving inflammatory mediators, aiming to improve the overall well-being of patients with these conditions while minimizing side effects. This approach aligns with the growing interest in natural compounds and their potential health benefits.

The study explicitly addresses diseases involving inflammatory mediators and free radicals like periodontal diseases, suggesting that targeting these pathways with plant-based compounds may offer a viable therapeutic approach. Inflammation is a critical component of various chronic diseases including autoimmune disorders. Modulating inflammatory responses can be crucial for disease management.

The bioactive pharmaceutical potential of quercetin as an anti-bacterial, anti-inflammatory, immune-modulatory, antioxidant[1] and wound healing[34] in managing periodontal disease is gaining global interest among researchers. The bacterial invasion progresses through the destructive interaction of inflammatory response and oxidative stress, culminating in tissue injury and bone loss[20,75,90]. The ability of quercetin to induce the synthesis of reduced glutathione (GSH) minimizes the risk of oxidative stress. The enzyme superoxide dismutase rapidly captures oxygen-free radicals produced in the body and converts them into hydrogen peroxide H_2O_2. H_2O_2 is then decomposed into nontoxic H_2O, requiring the presence of GSH as a hydrogen donor[57,69].

Quercetin controls reactive oxygen species by increasing total GSH levels 55[99]. The main aim of our present study was to analyze the extent of the potential of quercetin to attenuate oxidative stress injury in oral periodontal tissue. This is achieved by enhancing free radical scavenging and regulating various enzymes and inflammatory mediators to alleviate periodontal tissue destruction and promote the regeneration and rejuvenation of cells and tissues.

Material and Methods

Extraction of *Psidium guajava* leaves: The present study was conducted on the *Psidium guajava* tree leaf extract. The species identification of the plant was done by the Botany Department of Kerala University, Kariavattom, Trivandrum, India. The collected leaves were thoroughly washed and shade-dried before processing. The leaves were dried in the open air, protecting the area from direct exposure to sunlight. Further processing and extraction were done at the Centre for Research and Molecular Applied Sciences, Trivandrum, Kerala. The shade-dried *Psidium guajava* leaves were made into powder before extraction. Ethanolic extract was prepared using the cold percolation method[70]. 100-gram dried plant leaves powder was suspended in 250 ml of 80% ethanol each time for the study.

The suspension was kept overnight in a rotary shaker, maintaining aseptic conditions. The suspended plant leaf extract was then filtered using Whatmann filter paper no.1. Prepared crude extract was used for the preliminary screening and analysis. The solvent extract from the *Psidium guajava* leaves was partially purified by column chromatography[5]. After complete fractionation, the compound was isolated, purified and characterized using analytical chromatographic techniques. The purified and indicated quercetin-rich fractions were further used to study the compound's bioactive potential.

Dose Optimization of Quercetin Rich Fraction in KB Cell Lines: The study used the human mouth epidermal cell line, KB cells. KB cell lines were used since the present study concerns *in vitro* experimental periodontitis. KB cell lines were purchased from the NCCS, Pune, India and kept under controlled conditions in Dulbecco's modified eagle medium[30]. An antibiotic solution containing penicillin (100U/ml), streptomycin (100µg/ml) and amphotericin B (2.5µg/ml) was added to the cell culture. The cell culture was suspended in 10% growth medium and 100µl cell suspension ($5x10^4$ cells/well) was seeded in 96 well tissue culture plates and incubated at 37ºC in a humidified 5% CO_2 incubator. A cyclomixer dissolved 1mg of quercetin-rich fraction in 1mL 0.1% DMSO. The quercetin solution was filtered through a 0.22 µm Millipore syringe filter to ensure sterility.

The growth medium was removed after 24 hours and quercetin-rich fractions in varying concentrations (100µg, 50µg, 25µg, 12.5µg and 6.25µg in 500µl of DMEM) at 100µl each were added in triplicate to respective wells. Non-treated cells were maintained as control. Both treated and non-treated cells were incubated at 37ºC in a humidified 5% CO_2 incubator. Cell viability was evaluated by directly observing the cells using an inverted phase contrast microscope followed by the MTT assay method[49]. The percentage of viability was calculated.

Cytotoxicity evaluation by inverted phase contrast tissue culture microscope: After 24 hours of incubation, the treated and non-treated cells were observed under an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera). The result of direct observation was recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells, were considered indicators of cytotoxicity.

Cytotoxicity Assay by MTT Method: 15 mg of MTT (Sigma, M-5655) was reconstituted and dissolved in 3 ml PBS and filter sterilized. Cells were incubated for 24 hours, followed by removing the sample content from the wells. 30 µl of the reconstituted MTT solution was added to each well including control. The cell culture plate was incubated at 37°C for 4 hours in a humidified 5% CO_2 incubator. After incubation, the supernatant was removed and 100 µl of DMSO (Sigma Aldrich, USA) was added to each well to solubilize the formazan crystals formed by viable cells. Absorbance values were measured at 540 nm using a microplate reader[86]. The percentage of growth inhibition was calculated using the formula 1:

$$\% \text{ of viability} = \frac{\text{Mean OD Samples} \times 100}{\text{Mean OD of the control group}} \quad (1)$$

Non-toxic concentrations were selected for further biological studies based on cytotoxicity analysis. Dose optimization of quercetin-rich fractions in KB cell lines was done based on MTT assay.

Quercetin-Rich Fractions protecting KB Cells against H_2O_2-Induced Oxidative Injury: The study used the human mouth epidermal cell line, KB cells. KB cells were cultured and maintained aseptically using the standard protocol. Serially diluted 100µl plant extract (QRFPG) in concentrations 100µg, 50µg, 25µg, 12.5µg and 6.25µg in 500µl of 5% DMEM and 20mM H_2O_2 was co-administered to cell culture and incubated at 37ºC in a humidified 5% CO_2 incubator**.** Entire culture plates were observed and changes were noted every 24 to 72 hours in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera)[84].

The cytoprotective activity of QRF PG against the KB cell line induced with H_2O_2 was determined by MTT assay. The QRF PG extract was dissolved in dimethyl sulfoxide (DMSO) and diluted appropriately using a DMEM medium. The cells were washed with 1X PBS and 30 µl MTT

(5mg/ml dissolved in PBS) solution and incubated at 37ºC for 3 hours. Unreacted MTT was removed by washing with 1XPBS and solubilized by adding 200 µl of DMSO and incubated for 30 minutes at room temperature until the cells were lysed and color obtained. H_2O_2 induced cells without QRFPG were used as controls. Absorbance was measured in a microplate reader at 540 nm. The viability percentage was calculated[54,86].

Effect of Quercetin-Rich Fraction on Antioxidant Enzymes and Markers in H_2O_2-Induced Toxicity - Preparation of cell lysate: KB Cells were treated with QRFPG and then induced with H_2O_2, followed by trypsin-EDTA solution. After treatment with Trypsin-EDTA, the cells were detached and collected into Eppendorf tubes. The cells were then centrifuged at 5000 rpm for 5 minutes. The pellet formed after centrifugation was suspended in 200µL of lysis buffer (0.1M Tris, 0.2M EDTA, 2M NaCl, 0.5% Triton) which was then incubated at 40C for 20 minutes[16]. The prepared cell lysate was used for experiments relevant to the study.

Assessment of Lipid Peroxidation Level: Initiation of lipid peroxidation (LPO) is one of the prompting mechanisms of oxidative stress damage, culminating in altered cell metabolism and resultant changes. The body's antioxidant defense mechanism is designed to neutralize the effect of accumulating LPO and intracellular free radicals[19]. 500 µl of 70% alcohol and 1 ml of 1% TBA were added to 50µlof cell lysate and kept in a boiling water bath for 20 minutes, followed by adding 50 µl of acetone to all the test tubes at room temperature. The results were read spectrophotometrically at 535 nm[36].

The activity of the Catalase Enzyme: This enzymatic assay measures catalase's enzyme activity, catalyzing the decomposition of hydrogen peroxide (H_2O_2). This assay determines the rate at which the enzyme decomposes hydrogen peroxide and the action is expressed as moles of hydrogen peroxide decomposed per minute (usually defined as units per minute, U/min)[67]. To 0.5 mL of the cell lysate pretreated with QRFPG, 1.2 mL of 0.01 M phosphate buffer (pH 7.0) was added. The enzyme reaction, initiated by adding 1.0 mL of a 0.2 mM hydrogen peroxide solution that serves as the enzyme substrate, will be decomposed during the response. A spectrophotometer measures the decrease in absorbance at 240 nm. The decline in absorbance indicates the consumption of hydrogen peroxide during the enzymatic reaction which is estimated at regular intervals of 30 seconds up to 3 minutes. The enzyme activity is calculated based on the hydrogen peroxide decomposition rate and expressed as moles of hydrogen peroxide decomposed per minute. The decrease in absorbance at 240 nm is a direct measure of hydrogen peroxide consumption, reflecting the enzyme's activity in the cell lysate[66].

Estimation of Glutathione: Glutathione is an essential cellular antioxidant that helps to protect cells from oxidative stress. In this protocol, the levels of GSH were determined by a chemical reaction that led to the formation of a colored product and the absorbance of the product was measured at 420 nm[4]. Accumulation of oxidized glutathione is an indication of the presence of ROS and associated oxidative injury[35]. In healthy active cells, glutathione is seen in the reduced form. A steady reduction in the level of GSH provides a relevant and accurate measure of the oxidative state of the cell[87]. 0.5mL phosphate buffer (0.2M pH 8), 1.3mL distilled water and 0.2mL of DTNB (0.6mM) were added to 1 ml cell lysate and mixed well. The results were read spectrophotometrically at 420nm. The GSH levels were compared with a standard reduced glutathione graph.

Estimation of Superoxide Dismutase: (SOD) is a critical enzyme in the body's antioxidant defense system. Its primary function is to catalyze the dismutation (conversion) of the superoxide radical ($O_2^{\bullet-}$) into oxygen (O_2) and hydrogen peroxide (H_2O_2)[102]. This reaction is vital to the body's defense against oxidative stress. 50µl of cell lysate was added to the reaction mixture containing 0.1M NBT, 5.3mM riboflavin, 84µM potassium ferric cyanide and 45µM methionine in 50mM phosphate buffer. Following 20 minutes of incubation, the absorbance was read spectrophotometrically at 600nm[28]. The formula 2 calculated the percentage of inhibition:

Percentage of Inhibition = ((Control-Test)/Control) X 100
Enzyme Units = Percentage Inhibition/ 50 (2)

Evaluation of ROS Scavenging Potential of Quercetin Rich Fraction by DCFDA Method: 2',7' – dichlorofluorescein diacetate (DCFDA) assay is valuable for assessing intracellular ROS levels and oxidative stress[85]. It provides a means to visualize and quantify the presence of ROS in cultured cells, making it an essential technique in cellular and molecular biology research. Cultured cells are washed with PBS to remove any culture medium or extraneous compounds. After washing, the cells are treated with 50 µL of DCFDA. The cells are then incubated for 30 minutes. After the incubation, the excess DCFDA and any unreacted dye are removed by washing the cells with PBS. Fluorescence imaging uses a fluorescent microscope (Olympus CKX41 with Optika pro5 CCD camera). The fluorimeter excites the sample at 470 nm and measures the emitted fluorescence at 635 nm. This measurement indicates the level of ROS within the cells[95].

Effect of QRFPG in NRF2 Signalling Pathway: NRF2 is a transcription factor crucial in regulating the expression of various antioxidant and detoxification genes. It is involved in the cellular defense against oxidative stress. The effect of QRF on the face of the Nrf2 gene was determined in KB cells subjected to oxidative stress by free radicals. qPCR was performed. Real-time qRT-PCR analysis was carried out using SYBR Green Master Mix (G BIOSCIENCES, Product code-786-5062) using Lightcycler 96 (Roche). Overall, qRT-PCR analysis measured gene expression levels in

response to oxidative stress[93]. The ΔΔCt method was used to compare the expression of target genes with a control or reference sample. It quantifies the relative change in gene expression which can provide insights into how the expression of specific genes is affected by various experimental conditions such as exposure to H_2O_2 in KB cells *in vitro*.

Immune modulatory potential of QRFPG by Indirect ELISA: The cell line RAW 264.7(macrophage) was purchased from the National Centre for Cell Sciences (NCCS), Pune, India and was maintained in Dulbecco's modified Eagles medium from Sigma Aldrich, USA. The cell culture with DMEM was supplemented with growth factors including 10% FBS, L-glutamine and antibiotic solution and maintained at 37ºC in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany).A study conducted by Sul and Ra[85] stated that quercetin can significantly reduce the production of TNF-α, IL-1β and IL-6 cytokines released by macrophages stimulated with LPS. LPS is the cell wall component present in most Gram-negative bacteria. Cultured cell lines were induced with 1 µg/ml lipopolysaccharide (LPS) and incubated for 1 hour.

QRF (64.074835µg/mL) from 1mg/ml stock was added to LPS-induced cell lines and incubated for 24 hours at 37ºC in a humidified 5% CO_2 incubator. Non-LPS-treated cells were maintained as control. After incubation, 100µL of the supernatant was added to 96 well plates and incubated overnight. 100µl of primary antibodies Cyclooxygenase 2 (COX 2), Interlukin 6 (IL 6), Tumor Necrosis Factor (TNF) were added at room temperature and washed twice. 100µL of secondary antibody (HRP Conjugate, Santa Cruz, USA) was added and incubated for 1 hour at room temperature and washed twice.

200µL of dianizdine hydrochloride (1mg/100ml methanol + 21ml citrate buffer pH 5 + 60ml H_2O_2: Sigma Aldrich, USA) was added to the solution and incubated for 30 minutes at room temperature. 5N HCl (50µl) was added to stop the reaction. OD was read at 415 nm in an Elisa reader. The concentration of protein was also estimated. The following formula calculated the activity of the antibody (formula 3):

Activity of Antibody = OD Value / Protein Concentration (3)

Antibacterial effect by agar well diffusion method: Muller Hinton Agar Medium (HImedia) was purchased and prepared under standard conditions in an aseptic environment. The autoclaved medium was mixed well and poured onto 100mm Petri plates while still molten. One litre of commercially purchased nutrient broth was prepared sterilely and autoclaved for culturing the test organisms. *Streptococcus mutans* (MTCC 890) and *Enterococcus faecalis* (ATCC 29212) were test organisms. The growth of culture organisms was adjusted according to the McFarland standard, which is 0.5%. The standard antibacterial agent, streptomycin, was used as the positive control at 10mg/ml concentration. Petri plates containing 20ml Muller Hinton agar medium were seeded with the bacterial cultures of Streptococcus mutants and *Enterococcus faecalis*.

Using a good cutter, wells of approximately 10mm were bored and different concentrations of quercetin-rich fractions such as 250µg, 500µg and 1000µg were added, followed by incubation at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). The diameter of the zone of inhibition was measured in millimeters.

Results and Discussion

Prolonged oxidative stress results in tissue injury and progress towards periodontitis and similar oral manifestations. Reactive oxygen species affect the oral mucosa and lead to inflammation and aggressive infection. Orodental infection and inflammation are always linked with oxidative stress. In the present study, we focus on the biological properties of QRF PG with emphasis on the role of QRF PG to decrease oxidative stress. Phytochemical-based plant products are an alternative treatment in managing oxidative stress-related oral diseases. This property of plant extracts is attributed to their natural anti-oxidant and anti-inflammatory properties. Oxidative stress is mainly due to the imbalance between ROS and the body's antioxidant defense mechanisms. Our study focused on evaluating the antioxidant potential of an ethanolic extract derived from the leaves of *Psidium guajava* on a specific human cell line known as KB. This study employs an *in vitro* assay.

In the present study, we analyzed the extent of oxidative stress injury by inducing H_2O_2 to KB cell lines which were then treated with QRF PG. The oxidative state of the physiologic system *in vitro* was analysed by lipid peroxidation, glutathione reductase, superoxide dismutase and catalase[37]. The effect of QRF on the face of the Nrf2 gene was determined in KB cells subjected to oxidative stress by free radicals.

Dose Optimization of Quercetin Rich Fraction in KB Cell Lines: Cell viability was initially evaluated by directly observing the cells using an inverted phase contrast microscope. The purpose of this observation was to assess noticeable changes in the morphology of the cells which can be considered as indicators of cytotoxicity.

This includes rounding or shrinking of cells, blebbing, which refers to the formation of bulges or blisters on the cell surface, granulation within the cytoplasm of the cells and vacuolization which involves the construction of vacuoles within the cell cytoplasm[24,58]. After the initial observation, an MTT assay was performed to analyze the dose optimization of the quercetin-rich fraction on the KB cell line.

The present study reported a percentage of cell viability of nearly 70% at a concentration of 25 µg QRFPG. This can be considered the optimum or minimum concentration effective in minimizing oxidative injury. We checked the cell viability in a varied concentration from 6.25 µg to 100 µg. Of all the concentrations, 25 µg showed the optimum activity with maximum cell viability. LC50 Value of quercetin was 70.365062 µg/ml calculated using ED50 PLUS V1.0 software. The studies have confirmed that quercetin's antioxidant potential is due to a phenolic group and a double bond[82]. This result is in agreement with the study of Bao et al[9]. Their analysis showed that at the concentration of 25 µg, quercetin increased cell viability to 70.12 ± 2.58 %. This substantiates data proving quercetin's non-toxic potential in minimizing stress injury.

Among the flavonoids, quercetin is identified as the most potent anti-oxidant. Due to its antioxidant and prooxidant properties, quercetin balances the cell's redox potential by enhancing the expression of antioxidant enzymes like SOD, CAT and GSH[61]. Non-toxic concentrations were selected for further biological studies based on cytotoxicity analysis. Dose optimization of quercetin-rich fractions in KB cell lines was done based on MTT assay. Figure 2 depicts the graphical representation of cytotoxic activity by MTT assay. Figure 3 shows the change in cell viability on H_2O_2-induced KB cells treated with QRFPG viability assay.

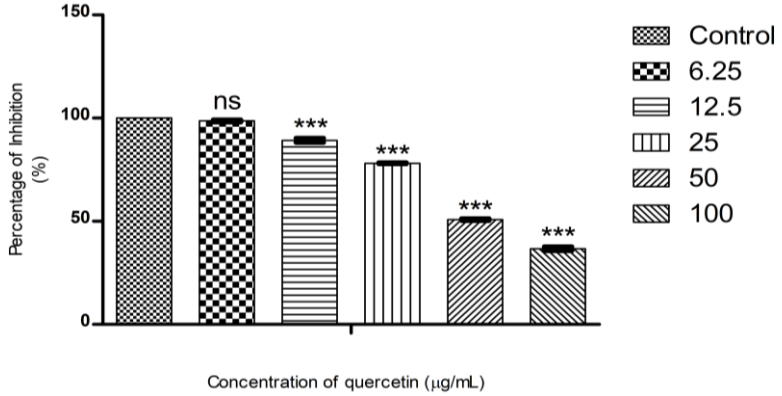

Fig. 2: Graphical representation depicting the cytotoxic effect of quercetin by MTT assay- Along Y-axis percentage viability, along X-axis varied concentration of quercetin. All experiments were done in triplicate, representing the results as Mean+/- SE. One-way ANOVA and Dunnets test were performed to analyze data. ***p< 0.001 was compared to the control group, ns – nonsignificant compared to the control group.

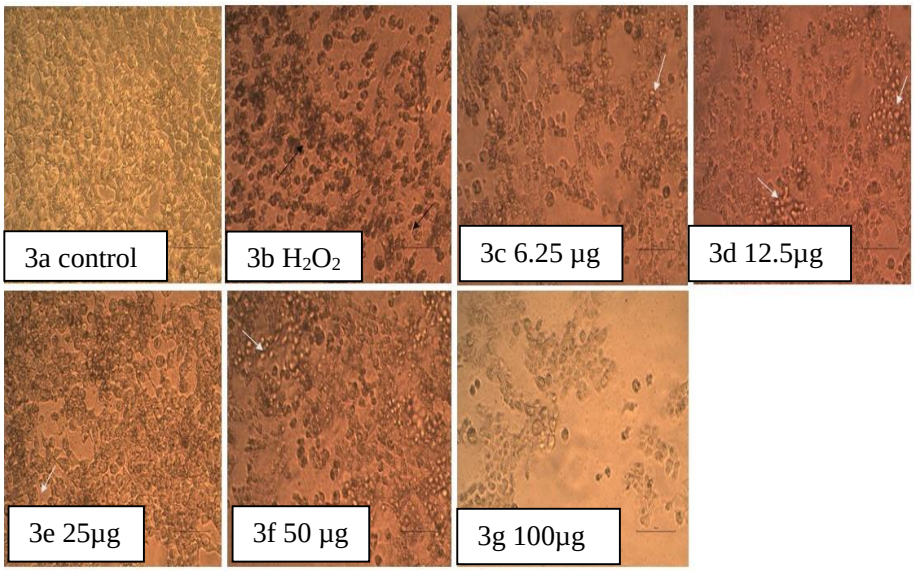

Fig. 3: Cell viability was initially evaluated by directly observing the cells using an inverted phase contrast microscope. The purpose of this observation was to assess any noticeable changes in the morphology of the cells, which can be considered indicators of cytotoxicity. This includes rounding or shrinking of cells, blebbing, which refers to the formation of bulges or blisters on the cell surface, granulation within the cytoplasm of the cells and vacuolization, which involves the construction of vacuoles within the cell cytoplasm. The figure depicts the changes in cell viability when treated with various concentrations of QRF PG in a dose-dependent manner. QRFPG reduced oxidative injury, evidenced by viable cells. The most viable cells are seen at a concentration of 25 µg, which can be considered the appropriate level of quercetin to prevent oxidative damage.

Cell viability was checked to evaluate how quercetin-rich fractions protect KB cells against H_2O_2-induced oxidative injury without being toxic to cells, followed by an MTT assay. The graph shows that quercetin protected KB cells against oxidative injury at various concentrations optimally in a range of 25 µg. Results of the MTT assay indicate quercetin's protective ability on H_2O_2-induced cells, which is comparable with the cells treated with H_2O_2 alone in a dose-dependent manner. Figure 4 shows the graphical representation depicting the *in vitro* cytoprotective effect of QRFPG in terms of percentage viability on - H_2O_2 induced KB cells with varying concentrations of QRFPG.

Figure 5 illustrates the changes in cell viability when treated with various concentrations of QRF PG in a dose-dependent manner. Table 2 shows the percentage viability of KB cells co-cultured with quercetin-rich fractions and H_2O_2. The most viable cells are seen at the concentration of 25 µg with a percentage viability more significant than 65%. Employing a standard cell viability and cytotoxicity assay i.e. MTT assay conducted by Sul and Ra[85] found that quercetin exhibited no toxicity at concentrations up to 50 µM. In contrast, a concentration of 100 µM resulted in reduced cell viability.

Effect of Quercetin-Rich Fraction on Antioxidant Enzymes and Markers in H_2O_2-Induced Toxicity: Prolonged oxidative stress results in tissue injury and progress towards periodontitis and similar oral manifestations. Reactive oxygen species affect the oral mucosa and lead to inflammation and aggressive infection. Orodental infection and inflammation are always linked with oxidative stress. In the present study, we focus on the biological properties of QRF PG with emphasis on the role of QRF PG to decrease oxidative stress. Phytochemical-based plant products are an alternative treatment in managing oxidative stress-related oral diseases. This property of plant extracts is attributed to their natural anti-oxidant and anti-inflammatory properties. Oxidative stress is mainly due to the imbalance between ROS and the body's antioxidant defense mechanisms.

Our study focused on evaluating the antioxidant potential of an ethanolic extract derived from the leaves of *Psidium guajava* on a specific human cell line known as KB. This study employs an in vitro assay. We used three sets of KB cell lines to investigate the effect of quercetin-rich fraction on antioxidant enzymes and markers in H_2O_2-induced toxicity. The first set is the control group without QRFPG and H_2O_2, the second group of cells treated with H_2O_2-alone and the third group co-administered with QRFPG and H_2O_2-*in vitro*. Dodda et al[22] reported from their investigation that cells treated with quercetin showed a significant reduction in LPO level and protection from oxidative tissue injury by hydrogen peroxide.

Table 1
Percentage of cell viability upon different concentrations of quercetin-rich fractions in MTT assay. Dose Optimization of Quercetin Rich Fraction in KB Cell Lines

Sample Concentration (µg/mL)	Percentage Viability	Standard error
Control	100.00	0
Quercetin Rich Fraction		
6.25	98.66	0.31021
12.5	89.24	0.614303
25	78.11	0.204874
50	50.81	0.261983
100	36.67	0.563822

Table 2
Shows the percentage viability of KB cells co-cultured with Quercetin-rich fractions and H_2O_2.

Sample Concentration (µg/ml)	Percentage Viability	Standard error
CONTROL	100.01	0
H2O2	47.16	0.568868
6.25	43.74	0.284599
12.5	52.83	1.64569
25	65.15	0.338382
50	52.03	0.459099
100	43.09	0.570771

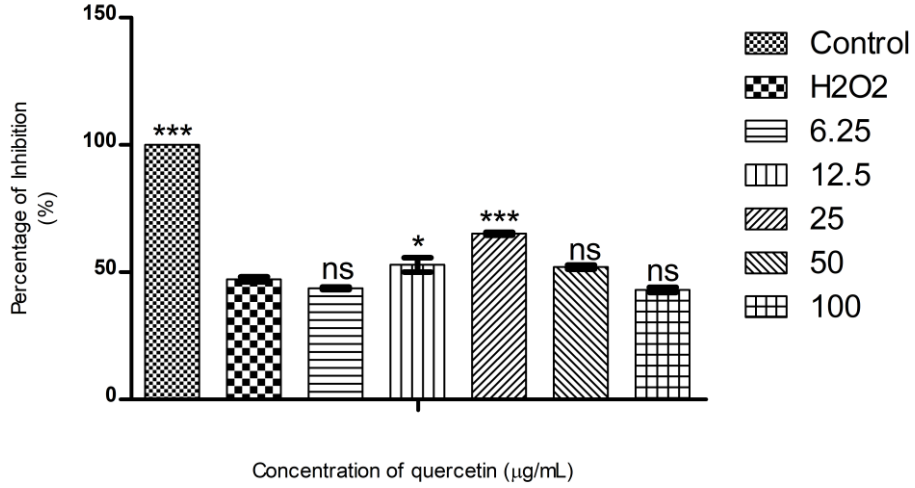

Fig. 4: Graphical representation depicting the cytoprotective effect of quercetin by MTT assay- along Y-axis Percentage viability, along X-axis varied concentration of quercetin. All experiments were done in triplicate, representing the results as Mean+/- SE. One-way ANOVA and Dunnets test were performed to analyze data. ***p< 0.001 compared to H_2O_2 exposed group, *p< 0.1 compared to H_2O_2 exposed group, ns – non significant compared to H_2O_2 exposed group

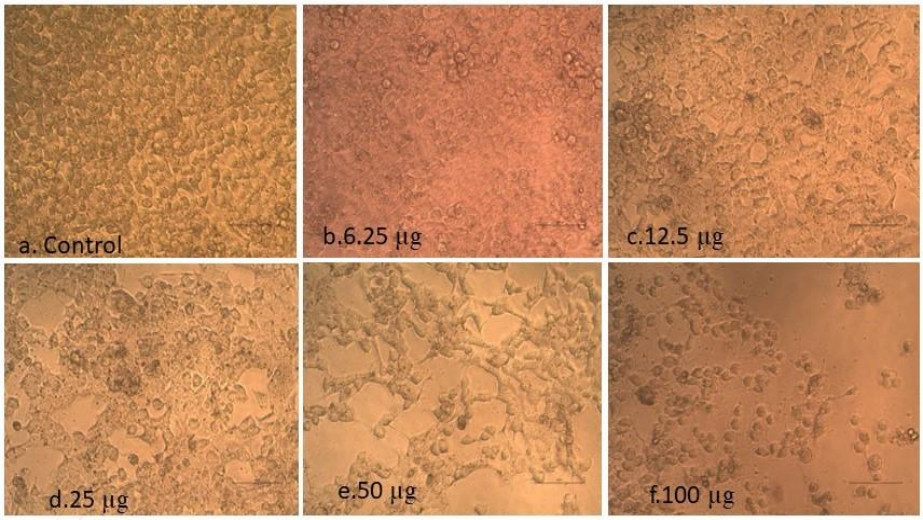

Fig. 5: The viability of cells was evaluated by the direct observation of cells using an inverted phase contrast microscope. The detectable changes in the morphology of the cells, like rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells, were considered indicators of cytotoxicity. The figure depicts the changes in cell viability when treated with various concentrations of QRF PG in a dose-dependent manner.

We treated H_2O_2-induced KB cells with QRF PG. We observed a satisfying reduction in the level of LPO to 0.429 µg/MDA units when compared with H_2O_2-induced KB cells not treated with QRF PG as 0.723 µg/MDA. The result indicates the ability of QRFPG to reduce lipid peroxidation and alleviate oxidative stress[27]. The effect of QRF on lipid peroxidation is depicted in figure 6.

Our study shows an increase in catalase level in cells treated with QRFPG as 0.5254 enzyme units compared with 0.2842 enzyme units in H_2O_2-treated cells alone. The increase in the level of catalase activity in cells treated with QRFPG supports our hypothesis, as QRFPG reduces oxidative stress by increasing catalase activity. The effect of the quercetin-rich fraction on antioxidant enzyme catalase in H_2O_2-induced toxicity is shown in figure 7.

A recent study revealed quercetin's ability to donate its H_2 atoms and reduce ROS activity[81]. A survey conducted by Srivastava et al[83] showed the antioxidant potential of quercetin. Quercetin directly participates in various intracellular signaling pathways, contributing to antioxidant efficacy. Eventually, several *in vivo* studies recommend quercetin's ability to inhibit xanthine oxidase, a potent antioxidant[18]. A recent investigation by Lyu et al[59] demonstrated the power of quercetin to defend cells from H_2O_2-induced oxidative injury by elevating the reduced glutathione content. The main principle stated was that

quercetin upregulates the expression of the first rate-limiting enzyme in GSH synthesis i.e. glutamate-cysteine ligase catalytic subunit (GCLC), culminating in the removal of accumulated ROS. The study of supported our results, proving that quercetin increases the levels of GSH and reduces oxidative stress associated with tissue injury. In our experiment with QRF PG on oral KB cells induced with H_2O_2, an elevated level of 0.5922 enzyme units of GSH was obtained when compared with cells not treated with QRF-PG with 0.3114 enzyme units of GSH, indicating a higher activity of quercetin rich fraction to stimulate the synthesis of GSH and alleviating oxidative stress. The effect of quercetin-rich fraction on the enzyme GSH on H_2O_2 induced KB cells is depicted in figure 8.

The enzyme GSH tends to react with free oxygen radicals. Continuous production of reactive oxygen species, such as hydrogen peroxide (H_2O_2), hydroxyl radicals (OH) and lipid peroxides, can occur in cells. ROS are highly reactive and can cause oxidative damage to various cellular components, leading to various health issues. As ROS accumulate, they can oxidize glutathione (GSH) molecules within the cell, converting them into their oxidized form GSSG. This conversion reflects the cell's response to oxidative stress. GSH, in its reduced form, plays a critical role in the body's antioxidant defense system. It is involved in neutralizing ROS and maintaining cellular redox balance. When GSH is oxidized to GSSG, its levels decrease, indicating that it has been used to counteract oxidative stress[3].

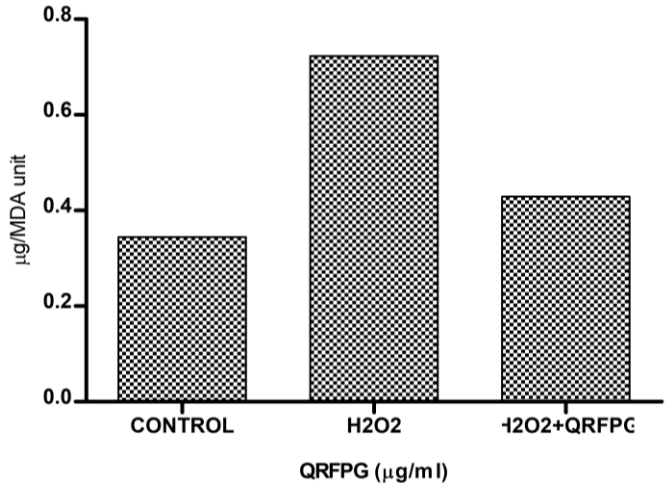

Fig. 6: Effect of QRF PG on H_2O_2-induced oxidative stress on oral KB cells *in vitro*. The graph shows the action of QRFPG on lipid peroxidation, which is expressed as µg/MDA. QRFPG reduced lipid peroxidation, substantiating its antioxidant potential. The control group constitutes the cell line alone; the sample mentioned in the graph is QRFPG.

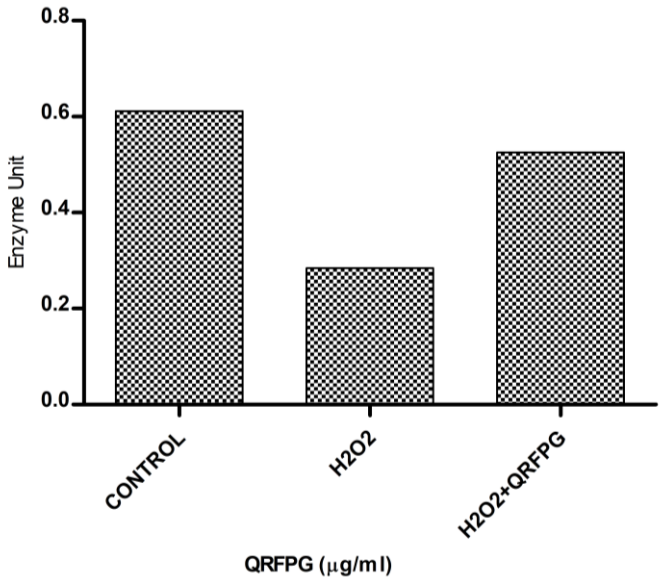

Fig. 7: Effect of QRF PG on H_2O_2-induced oxidative stress on oral KB cells *in vitro*. The graph shows the action of QRFPG on catalase activity, which is expressed as enzyme units. QRFPG enhanced the activities of Catalase, substantiating its antioxidant potential. The control group constitutes the cell line alone; the sample mentioned in the graph is QRFPG

Therefore, a concomitant reduction in the level of GSH and an increase in GSSG provide an accurate measure of the oxidative state of the cell. This GSH/GSSG ratio shift indicates the cell's response to oxidative stress and ability to maintain redox homeostasis[42]. Measuring the GSH/GSSG ratio or total glutathione levels is valuable for assessing oxidative stress and redox status in cells and tissues[80]. It helps researchers and clinicians to understand the impact of oxidative stress on various physiological processes and provides insights into potential interventions to mitigate the effects of oxidative damage[50,56]. In the present study, we investigated 3 KB cell lines: one treated with H_2O_2, the second control group without H_2O_2 and the third group treated with H_2O_2 and the phytochemical QRFPG.

The results showed that the cell lines treated with QRF PG showed increased enzymatic activity of 1.09 enzyme units of SOD compared to those treated with only H_2O_2, which was 0.729 enzyme units of SOD. The percentage inhibition of free radicals was in an appreciable amount of 54.89 units on cells treated with QRF PG. These results suggest the role of QRF PG in minimizing oxidative stress by increased SOD and catalase (CAT) activity. In short, quercetin attenuates the periodontal oxidative stress level and inflammatory response in oral KB cells[33]. The result of the study supports our suggestion of QRF PG as an excellent anti-oxidant scavenger. Another study conducted by Chen et al[14] demonstrated quercetin's efficacy in enhancing glutathione peroxidase (GPx) and catalase (CAT) activities in serum.

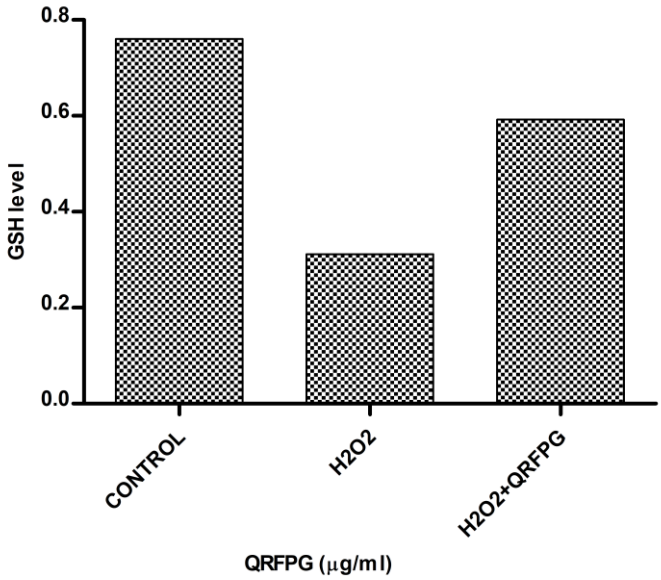

Fig. 8: Effect of QRF PG on H_2O_2-induced oxidative stress on oral KB cells in vitro. The graph shows the action of QRFPG on the GSH level. QRFPG upregulated the activities of GSH, substantiating its antioxidant potential. The control group constitutes the cell line alone; the sample mentioned in the graph is QRFPG.

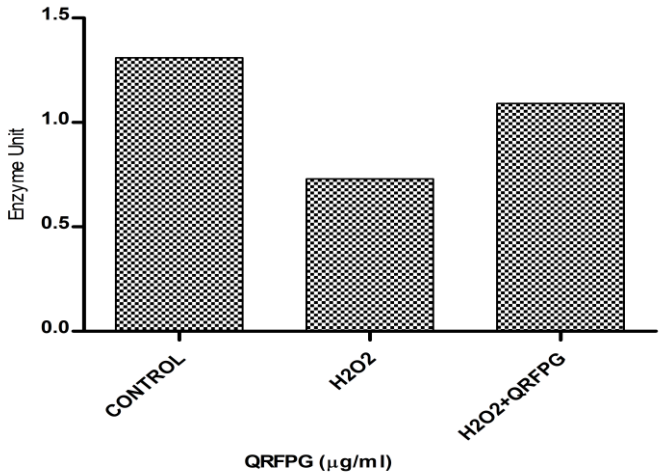

Fig. 9: The graph depicts an increase in the activity of SOD in QRFPG-treated cells when compared with H_2O_2 alone. SOD level is expressed in enzyme units. The control group constitutes the cell line alone; the sample mentioned in the graph is QRFPG

Additionally, quercetin increased the total superoxide dismutase (T-SOD) activity while reducing the levels of malondialdehyde (MDA) and reactive oxygen species (ROS)[15]. Overall, the results of these studies suggest that quercetin, specifically in the form of QRF PG, has antioxidant properties and may help to reduce oxidative stress and inflammation. It appears to enhance the activities of key antioxidant enzymes (SOD, CAT, GPx), reduce oxidative damage (MDA) and lower the levels of ROS, contributing its role as an antioxidant scavenger[15]. The effect of the quercetin-rich fraction on superoxide dismutase (SOD) is depicted in figure 9.

Our study suggests that quercetin may have protective effects against oxidative stress by influencing the expression of necessary antioxidant enzymes, namely catalase (CAT), glutathione peroxidase (GSH) and superoxide dismutase (SOD), under various conditions. As shown in figure 7, figure 8 and figure 9, the expression levels of CAT, GSH and SOD were significantly higher in the control samples and lower in samples incubated with H_2O_2 alone. The CAT, GSH and SOD enzyme levels were significantly higher in quercetin-rich fraction-treated instances than those set with H_2O_2 alone. These results demonstrated that H2O2 significantly inhibited the levels of antioxidant enzymes, but treatment with quercetin-rich fraction significantly attenuated this inhibition effect.

CAT, GSH and SOD expression levels were relatively normal in the control sample, suggesting that antioxidant enzymes are usually present and functional without oxidative stress. Samples incubated with H_2O_2 alone showed a significant decrease in the expression levels of CAT, GSH and SOD, indicating that exposure to H_2O_2 led to a notable reduction in the expression of these enzymes which implies that oxidative stress can inhibit their production or function. The cells co-administered with H_2O_2 and quercetin-rich fraction showed a significantly higher expression of these anti-oxidant enzymes when compared to the sample incubated with H_2O_2 alone. This outcome suggests that the quercetin-rich fraction is protective by preventing the significant inhibition of these anti-oxidant enzymes induced by H_2O_2.

Quercetin promotes the antioxidant defense system and maintains oxidative homeostasis by regulating Mitogen-Activated Protein Kinase (MAPK), 5' AMP-activated protein kinase (AMPK) and other important signaling pathways induced by ROS. The critical regulation mechanisms are the enzyme-mediated anti-oxidant and non-enzyme-mediated anti-oxidant defense mechanisms[76]. Quercetin is a known compound that can scavenge ROS. Most oxidative damage caused by ROS *in vivo*, like skin damage by UV radiations, can be neutralized by quercetin. Quercetin regulates the endogenous redox equilibrium by elevating the GSH and SOD levels and directly reacting with ROS[10].

Our study noted a steady reduction in fluorescence intensity in oral KB cells treated with QRFPG with a value of 17526.10 +/- 2586.9 AU compared with a higher fluorescence intensity in cells not treated with QRFPG with a value of 44594.14 +/- 1235.1. The steady decrease in the fluorescence intensity in QRFPG treated H_2O_2 induced KB cells is attributed to the ability of QRF PG to scavenge the free radicals due to induced H_2O_2. The fluorescence intensity (AU) of the QRFPG in 3 different experimental parameters is shown in table 3. DCFDA is a non-fluorescent dye that can enter the cells. Once inside, it can be hydrolyzed by intracellular esterases to form DCF (dichlorofluorescein) which is fluorescent when it reacts with ROS. During this time, the non-fluorescent DCFDA is converted into the fluorescent DCF within cells[17].

Intracellular esterases trigger this conversion. This step allows for visualizing the fluorescence signal produced by converting DCFDA to DCF in the cells. A fluorimeter is used to quantify the fluorescence signal more precisely. Figure 10 shows that H_2O_2-induced cells treated with QRF /PG produce diminished fluorescence[71]. From the above stated results, we suggest the possible therapeutic application of quercetin in managing oxidative stress-related tissue injury, especially in oral diseases like periodontitis.

Our study suggests that quercetin may protect against oxidative stress by regulating the expression and activity of antioxidant enzymes like catalase, reduced glutathione and superoxide dismutase. These enzymes are crucial in neutralizing the harmful reactive oxygen species and preventing oxidative damage. Quercetin-rich fractions appear to counteract the inhibitory effect of H_2O_2 on these antioxidant enzymes, potentially explaining protective effect against oxidative stress. These results demonstrated that H_2O_2 significantly inhibited the levels of antioxidant enzymes, but treatment with quercetin-rich fractions significantly attenuated this inhibition effect.

Table 3
Fluorescence intensity was detected with various experimental parameters in response to reactive oxygen species. The table shows that H_2O_2-induced cells treated with QRF PG produce diminished fluorescence, substantiating the potential of QRF PG as an antioxidant

S.N.	Experiment Parameter	Fluorescence Intensity (AU)
1	Control	9262.21 +/- 821.4
2	H_2O_2	44594.14 +/- 1235.1
3	H_2O_2+QRFPG	17526.10 +/- 2586.9
4	QRFPG	12979.82 +/- 1388.98

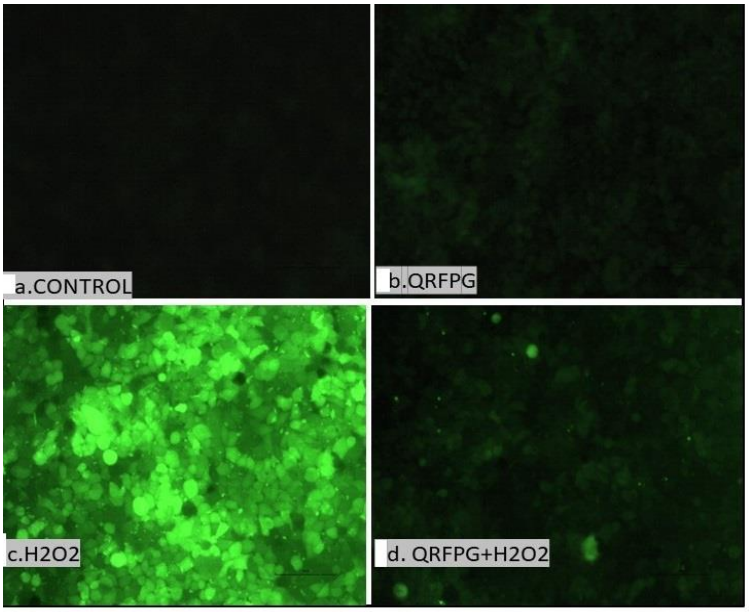

Fig. 10: H_2O_2-induced cells treated with QRF PG produce diminished fluorescence. Figure 10a: control with no fluorescence. Figure 10b: sample QRFPG alone. Figure 10c: Cells treated with H_2O_2 alone. The higher degree of fluorescence indicates the presence of reactive oxygen species. When the H_2O_2-induced cells were treated with QRFPG, the compound scavenged Reactive oxygen species and other free radicals. This is evident from the reduced fluorescence seen in figure 10d. Hence convincing the antioxidant potential of QRFPG. DCFDA is a non-fluorescent dye that can enter the cells. Once inside, it can be hydrolyzed by intracellular esterase to form DCF (dichloro fluorescein), which is fluorescent when it reacts with ROS. During this time, the non-fluorescent DCFDA is converted into the fluorescent DCF within the cells. Intracellular esterase triggers this conversion.

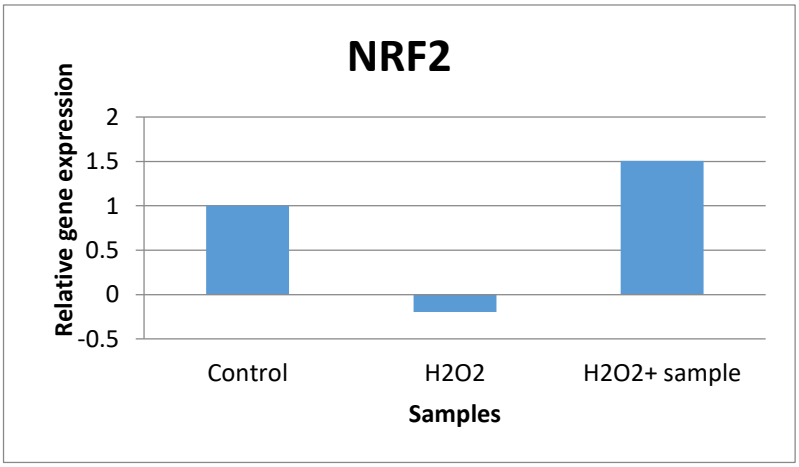

Fig. 11: The effect of QRFPG on the expression of the Nrf2 gene. The results suggest that exposure to reactive oxygen species (ROS) significantly decreased the face of the NRF2 gene in the model QRFPG.

The effect of QRFPG on the expression of the Nrf2 gene: Nrf2 is a transcription factor crucial in regulating the expression of various antioxidant and detoxification genes[32]. It is involved in the cellular defense against oxidative stress. The effect of QRF on the face of the NRF2 gene was determined in KB cells subjected to oxidative stress by free radicals. The nuclear factor erythroid 2–related factor 2 (NRF2) is an emerging regulator of cellular resistance to oxidants. Calabrese et al[11] proposed that NRF2 controls the basal and induced expression of various genes containing antioxidant response elements (AREs) in their promoter regions. From the results, it can be observed that ROS exposure significantly reduced the expression of NRF2, which QRF ameliorated. It has already been reported that an increase in NRF2 upregulates the oxidant-responsive elements, thereby increasing the antioxidant enzymes of the cells[10].

Our research seeks to determine if the QRF, rich in quercetin, can enhance the cellular resistance to oxidants by upregulating Nrf2 expression or activity. The results of our study suggest that exposure to reactive oxygen species (ROS) significantly decreased the face of the NRF2 gene. However, when treated with QRF (Quercetin-Rich Fraction), this reduction in NRF2 expression was mitigated or ameliorated. This finding is significant because it implies

that QRF may have a protective or ameliorating effect on the face of NRF2 when cells are exposed to ROS-induced oxidative stress[74]. Thus, NRF2 is a crucial regulator of the cellular defense mechanism against oxidative stress, so maintaining or increasing its expression protects cells from the damaging effects of ROS[46].

The study's results support the idea that QRF could be a potential therapeutic or protective agent against oxidative stress by helping to maintain or restore the expression of NRF2, which, in turn, promotes the cell's ability to resist and respond to ROS-induced damage. Figure 11 depicts the NRF2 expression in H_2O_2-induced KB cells treated with QRFPG. The present study investigated the anti-oxidant ability of the bioactive compounds in attenuating experimental periodontitis. We analyzed the efficacy of QRFPG in regulating different enzymes like SOD, GSH, Catalase, LPO etc.

Treatment with quercetin-rich fraction significantly increased the viability of KB cells exposed to H_2O_2, suggesting that it has a protective effect on cell survival. A substantial reduction in intracellular reactive oxygen species (ROS) levels was noted in KB cells treated with quercetin-rich fraction. Lower ROS levels are indicative of reduced oxidative stress. The free radical scavenging ability of the quercetin-rich fraction was suggestive of the anti-oxidant efficacy of quercetin. Quercetin treatment also reduced the production of malondialdehyde (MDA), a lipid peroxidation marker. Lower MDA levels suggest that quercetin helps to mitigate the damage to cell membranes caused by oxidative stress. Quercetin pretreatment alleviated the lipoperoxidation of cell membranes.

Lipoperoxidation is a process where cell membrane lipids are damaged by oxidative stress and quercetin appears to protect against this damage. Quercetin-rich fractions markedly reduced the inhibition effect of antioxidant enzymes such as CAT, GSH and SOD in KB cells exposed to H_2O_2. This suggests that quercetin helps to maintain or restore the activity of these enzymes in the face of oxidative stress. The results substantiate the potential of QRFPG in minimizing oxidative stress, claiming it to be a potent antioxidant. When Nrf2 is upregulated, it enhances the cell's ability to produce these antioxidant enzymes, bolstering its defense against oxidative damage. That is why Nrf2 is often considered a central player in the cellular response to oxidative stress and a potential therapeutic target for conditions involving oxidative stress and inflammation.

Immune modulatory action of quercetin: Epidemiological studies have suggested that diets rich in phytonutrients, which are bioactive compounds found in plants, may have potential benefits for cancer prevention. Many phytonutrients have been shown to modulate the activity of COX-2, IL6 and TNF alpha and possess anti-inflammatory properties. Various fruits, vegetables and other plant-based foods in the diet may contribute to a lower risk of certain cancers by influencing inflammatory pathways. In the present study, we evaluated the bio potency of quercetin-rich fractions from *Psidium Guajava* leaf extracts in modulating immune response, thereby alleviating conditions related to the cytokine mediators. We studied the effect of QRFPG on COX-2, IL6 and TNF alpha in LPS-stimulated RAW 246.7 macrophage cells.

Our study demonstrated a reduction in the COX 2 level in LPS-stimulated RAW 246.7 macrophage cells to 0.147489 enzyme units when compared with the cells treated with LPS alone with a value of 0.304102. Nearly a fifty percent reduction of COX 2 activity on QRFPG-treated cells was observed. Wang et al[91] demonstrated that Quercetin downregulated Cox 2 and attenuated neovascularization. This was achieved via inhibiting COX-2 by quercetin, which decreases HIF-1α/VEGF signaling-related angiogenesis. The result of Wang et al[91] substantiates our study that quercetin regulates COX 2 enzyme, thereby minimizing inflammatory diseases. Understanding these molecular connections provides insights into potential therapeutic strategies including the exploration of COX-2 inhibitors and the consideration of phytonutrient-rich diets for the prevention and control of various diseases including cancer.

Figure 12 depicts the action of the quercetin-rich fraction on LPS-treated RAW 246.7 macrophage cells on COX 2 action. COX-2 inhibitors have been investigated for their potential as cancer therapeutics. These inhibitors are designed to block the activity of COX-2, thereby reducing the production of prostaglandins and potentially slowing down cancer progression. However, the clinical use of COX-2 inhibitors in cancer treatment is still an area of active research and their efficacy and safety profiles continue to be explored. Understanding the intricate roles of cytokines like IL-6 in health and disease is crucial for developing targeted therapies and interventions that beneficially modulate immune responses.

TNF-α is a cytokine with complex roles in immune regulation and inflammation. While it is essential for normal immune responses, dysregulation of TNF-α signaling is implicated in many clinical conditions including autoimmune diseases. Our study demonstrated a steady reduction in the activity of the enzyme TNF alpha on LPS-treated RAW 246.7 macrophage cells *in vitro*. LPS-induced cell lines treated with QRF PG showed an excellent reduction in the activity of TNF alpha with a value of 0.149001 enzyme units/ mg of protein, compared with 0.317154 enzyme units/mg of protein in cells treated only with LPS. This is nearly fifty percent inhibiting enzyme activity, which can be considered a therapeutic target in managing inflammatory diseases.

Chen et al[14] proved the ability of quercetin to suppress TNF-α by blocking NF-κB and AP-1 signaling pathways which may contribute to the treatment of coronary heart disease. These results support our finding that quercetin down-

regulates the action of TNF alpha. Figure 13 depicts the activity of quercetin-rich fraction on LPS-treated RAW 246.7 macrophage cells on TNF alpha. Therapeutic interventions targeting TNF-α have proven valuable in managing various clinical conditions, demonstrating the practical application of our understanding of cytokine signaling in developing effective treatments. Therefore, it can be predicted that the knowledge of TNF-α signaling will be much more emphasized shortly to develop effective phytochemical remedies for treating a wide range of diseases involving TNF.

IL-6 plays a crucial role in the regulation of cell proliferation and differentiation. It is involved in the growth and development of various cell types including immune cells, hematopoietic cells and certain types of cancer cells. While IL-6 is essential for normal immune responses and tissue repair, dysregulation of IL-6 signaling is implicated in various diseases including chronic inflammatory conditions, autoimmune diseases and certain cancers. The current study investigated the action of QRFPG on LPS-treated RAW 246.7 macrophage cells *in vitro*. QRFPG reduced the enzyme activity of IL 6 *in vitro* on LPS-treated RAW 246.7 macrophage cells.

A reduction in 0.006113 enzyme units was noted in QRFPG-treated cells induced with LPS. This is comparable with 0.012097 enzyme units on LPS-induced cells not treated with QRFPG. Thus, a considerable reduction in enzyme activity is highly suggestive of the ability of quercetin to down-regulate IL6. In a current study conducted by Zhang et al[101] showed that LPS stimulation significantly elevated the mRNA levels of TNF-α, IL-6 and IL-1β in RAW264.7 cells compared to the control group. However, pretreatment with quercetin attenuated the LPS-induced upregulation of these pro-inflammatory cytokines especially IL 6 in a dose-dependent manner.

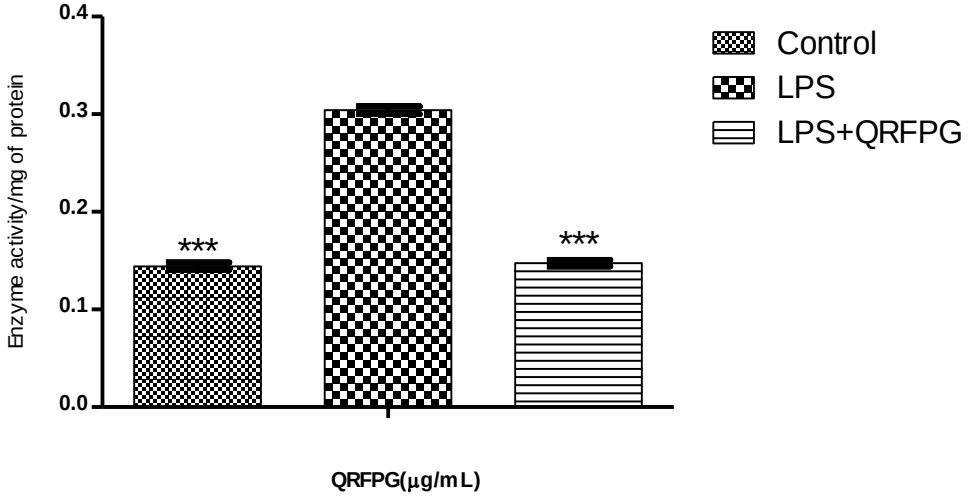

Fig. 12: The graphical representation depicts the downregulation of COX 2 activity on LPS-treated RAW 246.7 macrophage cells co-cultured with QRFPG *in vitro*.

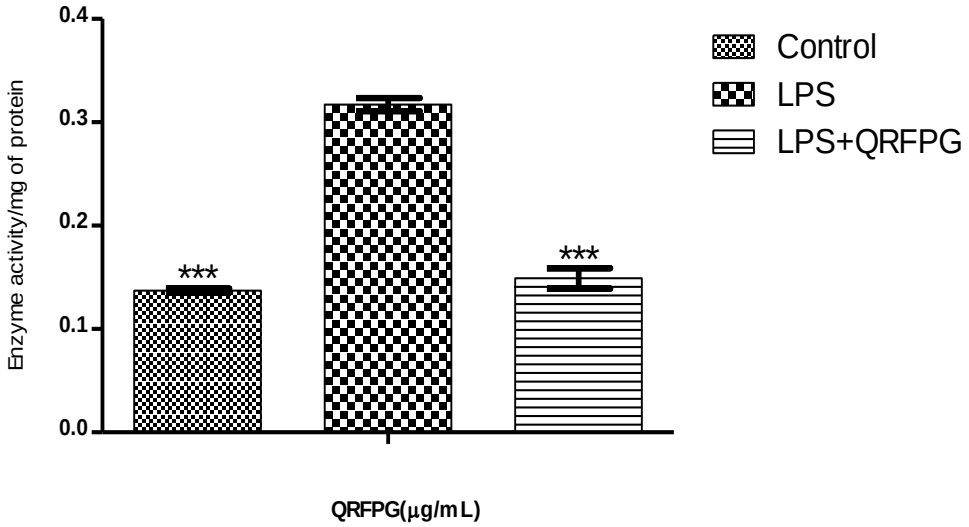

Fig. 13: The graphical representation depicts the downregulation of TNF alpha activity on LPS-treated RAW 246.7 macrophage cells upon treatment with QRFPG *in vitro*.

Antimicrobial activity of QRFPG by Agar- Well Diffusion Method: The antimicrobial activity of QRFPG was done using the agar well diffusion method. In the present study, we focused on the action of plant flavonoid quercetin against microorganisms involved in periodontal pathogenesis. The antimicrobial activity of quercetin against the test organisms was investigated and compared to that of the standard antibacterial agent streptomycin at a concentration of 10mg/ml (Table 4 and table 5). Our study showed that quercetin at 1000 µg concentration showed high antibacterial activity against *Enterococcus faecalis* with a zone diameter of 21 mm while quercetin was moderately active against *Streptococcus mutans* with a zone diameter of 14 mm at 1000 µg concentration. The antimicrobial activity of quercetin against two test organisms is depicted in figure 15.

Quercetin can be considered an antibacterial agent with the potential to reduce the growth of pathobionts, especially *Enterococcus faecalis*, in periodontal pockets, thereby minimizing periodontitis. Understanding how specific molecules exhibit antibacterial properties, can provide insights into designing new synthetic molecules to combat bacterial resistance to traditional antibiotics. Antibiotic resistance is a global health concern and developing new antibacterial molecules is crucial for maintaining effective treatment options. Our current study is vital in addressing the growing challenge of bacterial resistance and ensuring that effective treatment modalities like phytochemical-based molecules are available to combat bacterial infections and anti-microbial resistance. Several studies conducted recently suggested the potential of quercetin to inhibit the pathogenic species like *Enterococcus faecalis* and *Streptococcus mutans*[21,79].

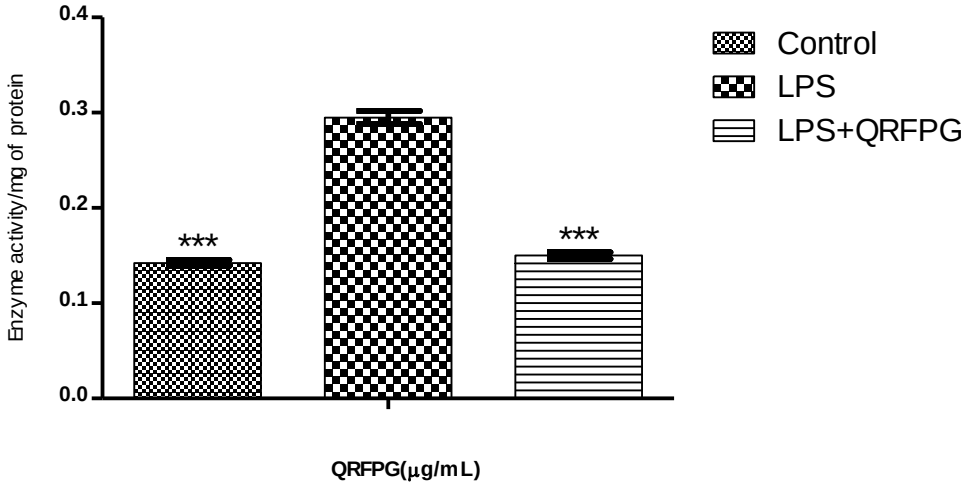

Fig. 14: The graphical representation depicts the downregulation of IL 6 activity on LPS-treated RAW 246.7 macrophage cells upon co cultured with QRFPG in vitro.

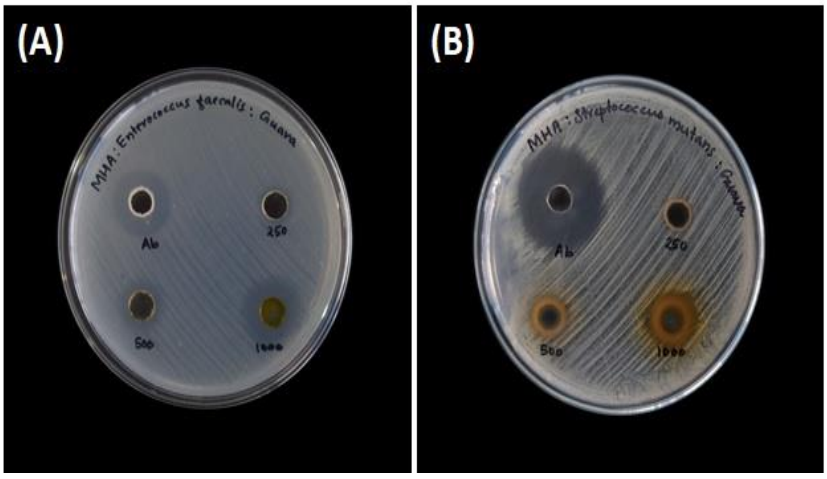

Fig. 15: Antimicrobial activity of Quercetin rich fractions against the test organisms *Enterococcus faecalis* and *Streptococcus mutans.* Quercetin shows more potent activity against *Enterococcus faecalis*, a powerful periodontal pathogen and moderate activity against *Streptococcus mutans*. Hence, quercetin can be considered an antibacterial agent with the potential to reduce the growth of pathobionts, especially *Enterococcus faecalis*, in periodontal pockets, thereby minimizing periodontitis. Figure 15 A shows the zone of inhibition of *Enterococcus faecalis* and figure 15 B shows the zone of inhibition of *Streptococcus mutans.*

Table 4
Antimicrobial activity of Quercetin against the test organism *Enterococcus faecalis* compared with the standard Streptomycin

Sample	Concentration(µg)	Zone of inhibition (mm)
Quercetin rich fractions of *Psidium guajava*	Streptomycin (100 µg)	20
	250	12
	500	14
	1000	21

Table 5
Antimicrobial activity of Quercetin against the test organism *Streptococcus mutans* compared with the standard Streptomycin

Sample	Concentration(µg)	Zone of inhibition (mm)
Quercetin rich fractions of *Psidium guajava*	Streptomycin (100µg)	26
	250	Nil
	500	12
	1000	14

Conclusion

Orodental infection and inflammation are always linked with oxidative stress. Most widely accepted therapeutic strategies focus on minimizing inflammation and microbial infection. These treatment methodologies do not give attention to oxidative stress and resultant medical conditions. The question to be considered is whether these therapeutic scenarios of orodental infections address issues related to oral diseases. The present study investigated the bioactive compound quercetin's antioxidant, anti-inflammatory and anti-bacterial ability to attenuate experimental periodontitis. The current research results prove that quercetin treatment effectively protects KB cells from H_2O_2-induced sustained oxidative stress. Plant-based phytochemical quercetin reduces ROS levels, MDA production and lipid peroxidation, maintaining important antioxidant enzyme activity.

In conclusion, the present study underscores the potential benefits of using plant-based phytochemicals to modulate inflammatory pathways in diseases, specifically focusing on minimizing side effects. These findings support the possible use of quercetin as a protective agent against oxidative stress-related cellular damage. Periodontitis is the outcome of multiple factors, among which microbial action, inflammation and oxidative injury play a significant role. Hence, the present study proposes using quercetin to attenuate periodontal diseases. This new strategy is a stepping stone in drug designing. Hence, we strongly recommend more investigation and analysis in this field to make quercetin an excellent pharmaceutical product against diseases like periodontitis and associated infections due to oxidative injury.

Acknowledgement

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References

1. Abdallah M., Neseem D., Elgazayerly O. and Abdelbary A., Topical delivery of quercetin loaded transfersomes for wound treatment: in vitro and in vivo evaluation, *Int J App Pharm*, **13**, 189–197 **(2021)**

2. Akshayaa L., Kumar J.K. and Shanmugam R., Formulation of Quercetin Mouthwash and Anti-microbial Potential Against Critical Pathogens: An In-Vitro Evaluation, *Cureus*, **16(1)**, e51688 **(2024)**

3. Ali S.S., Ahsan H., Zia M.K., Siddiqui T. and Khan F.H., Understanding oxidants and antioxidants: Classical team with new players, *J. Food Biochem*., **44(3)**, e13145 **(2020)**

4. Arif B., Arif Z., Ahmad J., Perveen K., Bukhari N.A., Ashraf J.M., Moinuddin and Alam K., Attenuation of hyperglycemia and amadori products by aminoguanidine in alloxan-diabetic rabbits occurs via enhancement in antioxidant defenses and control of stress, *PLoS One*, **17**, e0262233 **(2022)**

5. Arya S. and Usha R., Anti-Inflammatory Effect of Quercetin-Rich Fractions On Lps Stimulated Raw 264.7 Macrophage Cells, *J. Pharm. Negat. Results*, **13**, 10370–10383 **(2022)**

6. Asgharian P., Tazekand A.P., Hosseini K., Forouhandeh H., Ghasemnejad T., Ranjbar M., Hasan M., Kumar M., Beirami S.M., Tarhriz V., Soofiyani S.R., Kozhamzharova L., Sharifi-Rad J., Calina D. and Cho W.C., Potential mechanisms of quercetin in cancer prevention: focus on cellular and molecular targets, *Cancer Cell Int.*, **22,** 257 **(2022)**

7. Axelrod B., Cheesbrough T.M. and Laakso S., Lipoxygenase from soybeans: EC 1.13. 11.12 Linoleate: oxygen oxidoreductase, Methods Enzymol, Elsevier, 441–451 **(1981)**

8. Azeem M., Hanif M., Mahmood K., Ameer N., Chughtai F.R.S. and Abid U., An insight into anticancer, antioxidant, antimicrobial,

antidiabetic and anti-inflammatory effects of quercetin: a review, *Polym. Bull*., **80**, 241–262 **(2023)**

9. Bao D., Wang J., Pang X. and Liu H., Protective Effect of Quercetin against Oxidative Stress-Induced Cytotoxicity in Rat Pheochromocytoma (PC-12) Cells, *Molecules*, **22**, 1122 **(2017)**

10. Biswas P., Dey D., Biswas P.K., Rahaman T.I., Saha S., Parvez A., Khan D.A., Lily N.J., Saha K. and Sohel M., A comprehensive analysis and anti-cancer activities of quercetin in ROS-mediated cancer and cancer stem cells, *Int. J. Mol. Sci*., **23**, 11746 **(2022)**

11. Calabrese E.J., Pressman P., Hayes A.W., Dhawan G., Kapoor R., Agathokleous E., Manes P. and Calabrese V., Naringin commonly acts via hormesis, *Sci. Total Environ.*, **896**, 164728 **(2023)**

12. Chen E., Wang T., Tu Y., Sun Z., Ding Y., Gu Z. and Xiao S., ROS-scavenging biomaterials for periodontitis, *J. Mater. Chem.*, **B(11)**, 482–499 **(2023)**

13. Chen S., Jiang H., Wu X. and Fang J., Therapeutic effects of quercetin on inflammation, obesity and type 2 diabetes, *Mediators Inflamm*., **2016**, 1-5 **(2016)**

14. Chen T., Zhang X., Zhu G., Liu H., Chen J., Wang Y. and He X., Quercetin inhibits TNF-α induced HUVECs apoptosis and inflammation via downregulating NF-kB and AP-1 signaling pathway *in vitro*, *Medicine* (Baltimore), **99**, e22241 **(2020)**

15. Chen X., Liang D., Huang Z., Jia G., Zhao H. and Liu G., Anti-fatigue effect of quercetin on enhancing muscle function and antioxidant capacity, *J. Food Biochem.*, **45**, e13968 **(2021)**

16. Chen Z., Lu X., Mylarapu N., Kuthyar S., Sakhalkar O. and Watsky M.A., A Method for Eliminating Fibroblast Contamination in Mouse and Human Primary Corneal Epithelial Cell Cultures, *Curr. Eye Res.*, **48(11)**, 981–91 **(2023)**

17. Das S., Saha M., Mahata L.C., China A., Chatterjee N. and Das Saha K., Quercetin and 5-Fu Loaded Chitosan Nanoparticles Trigger Cell-Cycle Arrest and Induce Apoptosis in HCT116 Cells via Modulation of the p53/p21 Axis, *ACS Omega*, **8**, 36893–36905 **(2023)**

18. Deepika and Maurya P.K., Health Benefits of Quercetin in Age-Related Diseases, *Molecules*, **27**, 2498 **(2022)**

19. Demkovych A., Effects of flavonol quercetin on activity of lipid peroxide oxidation in experimental bacterial-immune periodontitis, *Interv. Med. Appl. Sci.*, **11**, 55–59 **(2019)**

20. Di Stefano M., Polizzi A., Santonocito S., Romano A., Lombardi T. and Isola G., Impact of oral microbiome in periodontal health and periodontitis: a critical review on prevention and treatment, *Int. J. Mol. Sci*., **23**, 5142 **(2022)**

21. Diao Y., Yu X., Zhang C. and Jing Y., Quercetin-grafted chitosan prepared by free radical grafting: characterization and evaluation of antioxidant and antibacterial properties, *J. Food Sci. Technol.*, **57**, 2259–2268 **(2020)**

22. Dodda D., Chhajed R., Mishra J. and Padhy M., Targeting oxidative stress attenuates trinitrobenzene sulphonic acid induced inflammatory bowel disease like symptoms in rats: role of quercetin, *Indian J. Pharmacol*., **46**, 286 **(2014)**

23. El-Khawagah A.R., Kandiel M.M. and Samir H., Effect of quercetin supplementation in extender on sperm kinematics, extracellular enzymes release and oxidative stress of egyptian buffalo bulls frozen–thawed semen, *Front. Vet. Sci.*, **7**, 604460 **(2020)**

24. Eshaghi M.M., Pourmadadi M., Rahdar A. and Diez-Pascual A.M., Improving quercetin anticancer activity through a novel polyvinylpyrrolidone/polyvinyl alcohol/TiO_2 nanocomposite, *J. Drug Deliv. Sci. Technol.*, **81**, 104304 **(2023)**

25. Georgiou A.C., Ulloa P.C., Van Kessel G.M.H., Crielaard W. and Van der Waal S.V., Reactive oxygen species can be traced locally and systemically in apical periodontitis: A systematic review, *Arch. Oral Biol.*, **129**, 105167 **(2021)**

26. Gong X., Huang Y., Ma Q., Jiang M., Zhan K. and Zhao G., Quercetin alleviates lipopolysaccharide-induced cell damage and inflammation via regulation of the TLR4/NF-κB pathway in bovine intestinal epithelial cells, *Curr. Issues Mol. Biol.*, **44**, 5234–5246 **(2022)**

27. Grewal A.K., Singh T.G., Sharma D., Sharma V., Singh M., Rahman M.H., Najda A., Walasek-Janusz M., Kamel M. and Albadrani G.M., Mechanistic insights and perspectives involved in neuroprotective action of quercetin, *Biomed. Pharmacother*., **140**, 111729 **(2021)**

28. Haida Z. and Hakiman M., A comprehensive review on the determination of enzymatic assay and nonenzymatic antioxidant activities, *Food Sci. Nutr.*, **7**, 1555–1563 **(2019)**

29. Hamid H.K. and Obaid M.A., Role of Quercetin Flavonoid as Antidiabetic: A Review, *Inter J Drug Deliv Tech*, **11**, 1495–1500 **(2021)**

30. Heidari M. and Doosti A., Staphylococcus aureus enterotoxin type B (SEB) and alpha-toxin induced apoptosis in KB cell line, **11**, 96-102 **(2023)**

31. Hirschfeld J., White P.C., Milward M.R., Cooper P.R and Chapple I.L.C., Modulation of Neutrophil Extracellular Trap and Reactive Oxygen Species Release by Periodontal Bacteria, *Infect. Immun*., **85**, e00297-17 **(2017)**

32. Hoang T., Johnson D.A., Raines R.T. and Johnson A.J., Angiogenin activates the astrocytic Nrf2/antioxidant-response element pathway and thereby protects murine neurons from oxidative stress, *J. Biol. Chem.*, **294**, 15095–15103 **(2019)**

33. Hu T., Yue J., Tang Q., Cheng K.W., Chen F., Peng M., Zhou Q. and Wang M., The effect of quercetin on diabetic nephropathy (DN): a systematic review and meta-analysis of animal studies, *Food Funct.*, **13**, 4789–4803 **(2022)**

34. Huang C.Y., Ng M.Y., Lin T., Liao Y.W., Huang W.S., Hsieh C.W., Yu C.C., and Chen C.J., Quercetin ameliorates advanced glycation end product-induced wound healing impairment and inflammaging in human gingival fibroblasts, *J. Dent. Sci.*, **19(1)**, 268–275 **(2023)**

35. Huchthausen J., Escher B.I., Grasse N., König M., Beil S. and Henneberger L., Reactivity of Acrylamides Causes Cytotoxicity and Activates Oxidative Stress Response, *Chem. Res. Toxicol.*, **36**, 1374–1385 **(2023)**

36. Ito F., Sono Y. and Ito T., Measurement and clinical significance of lipid peroxidation as a biomarker of oxidative stress: oxidative stress in diabetes, atherosclerosis and chronic inflammation, *Antioxidants*, **8**, 72 **(2019)**

37. Jabbar K., Biochemical evaluation of antioxidant enzyme activities and lipid peroxidation level associated with liver enzymes in patients with fascioliasis, *Arch. Razi Inst.*, **77**, 1067 **(2022)**

38. Jaisinghani R.N., Antibacterial properties of quercetin, *Microbiol. Res.*, **8**, 6877 **(2017)**

39. Jang D., Lee A.H., Shin H.Y., Song H.R., Park J.H., Kang T.B., Lee S.R. and Yang S.H., The role of tumor necrosis factor alpha (TNF-α) in autoimmune disease and current TNF-α inhibitors in therapeutics, *Int. J. Mol. Sci.*, **22**, 2719 **(2021)**

40. Jangir Rakesh, Kumawat Ketul, Bishnoi Pramila and Prakash Om, Structure-reactivity relation in the oxidation of some aliphatic aldehydes by Tripropylammonium chlorochromate, *Res. J. Chem. Environ.*, **27(3)**, 74 **(2023)**

41. Jayusman P.A., Nasruddin N.S., Mahamad Apandi N.I., Ibrahim N. and Budin S.B., Therapeutic potential of polyphenol and nanoparticles mediated delivery in periodontal inflammation: A review of current trends and future perspectives, *Front. Pharmacol.*, **13**, 847702 **(2022)**

42. Jia H., Zhang Y., Si X., Jin Y., Jiang D., Dai Z. and Wu Z., Quercetin alleviates oxidative damage by activating nuclear factor erythroid 2-related factor 2 signaling in porcine enterocytes, *Nutrients*, **13**, 375 **(2021)**

43. Jiménez C., Garrido M., Pussinen P., Bordagaray M.J., Fernández A., Vega C., Chaparro A., Hoare A. and Hernández M., Systemic burden and cardiovascular risk to Porphyromonas species in apical periodontitis, *Clin. Oral Investig.*, **26**, 993–1001 **(2022)**

44. Jones S.A. and Jenkins B.J., Recent insights into targeting the IL-6 cytokine family in inflammatory diseases and cancer, *Nat. Rev. Immunol.*, **18**, 773–789 **(2018)**

45. Kanzaki H., Wada S., Narimiya T., Yamaguchi Y., Katsumata Y., Itohiya K., Fukaya S., Miyamoto Y. and Nakamura Y., Pathways that regulate ROS scavenging enzymes and their role in defense against tissue destruction in periodontitis, *Front. Physiol.*, **8**, 351 **(2017)**

46. Khadrawy O., Gebremedhn S., Salilew-Wondim D., Rings F., Neuhoff C., Hoelker M., Schellander K. and Tesfaye D., Quercetin supports bovine preimplantation embryo development under oxidative stress condition via activation of the Nrf2 signalling pathway, *Reprod. Domest. Anim.*, **55**, 1275–1285 **(2020)**

47. Khandia R. and Munjal A., Interplay between inflammation and cancer, *Adv. Protein Chem. Struct. Biol.*, **119**, 199–245 **(2020)**

48. Khazdair M.R., Anaeigoudari A. and Agbor G.A., Anti-viral and anti-inflammatory effects of kaempferol and quercetin and COVID-2019: A scoping review, *Asian Pac. J. Trop. Biomed.*, **11**, 327–334 **(2021)**

49. Khongkaew P., Wattanaarsakit P., Papadopoulos K.I. and Chaemsawang W., Antioxidant Effects and in vitro Cytotoxicity on Human Cancer Cell Lines of Flavonoid-Rich Flamboyant (Delonix regia (Bojer) Raf.) Flower Extract, *Curr. Pharm. Biotechnol*., **22**, 1821–1831 **(2021)**

50. Khudiakova M. and Ryabokon E., Effect of periodontal treatment of patients with chronic generalized periodontitis **(2019)**

51. Kitaura H. et al, Mizoguchi, Role of the interaction of tumor necrosis factor-α and tumor necrosis factor receptors 1 and 2 in bone-related cells, *Int. J. Mol. Sci*., **23**, 1481 **(2022)**

52. Kost B., Svyntkivska M., Brzeziński M., Makowski T., Piorkowska E., Rajkowska K., Kunicka-Styczyńska A. and Biela T., PLA/β-CD-based fibres loaded with quercetin as potential antibacterial dressing materials, *Colloids Surf. B Biointerfaces*, **190**, 110949 **(2020)**

53. Kwon T., Lamster I.B. and Levin L., Current concepts in the management of periodontitis, *Int. Dent. J.*, **71**, 462–476 **(2021)**

54. Lim H.J., Kang S.H., Song Y.J., Jeon Y.D. and Jin J.S., Inhibitory effect of quercetin on Propionibacterium acnes-induced skin inflammation, *Int. Immunopharmacol.*, **96**, 107557 **(2021)**

55. Liu C., Mo L., Niu Y., Li X., Zhou X. and Xu X., The role of reactive oxygen species and autophagy in periodontitis and their potential linkage, *Front. Physiol.*, **8**, 439 **(2017)**

56. Liu X., Tao T., Yao H., Zheng H., Wang F. and Gao Y., Mechanism of action of quercetin in rheumatoid arthritis models: meta-analysis and systematic review of animal studies, *Inflammopharmacology*, **31**, 1629–1645 **(2023)**

57. Liu Y., Zheng Y., Ding G., Fang D., Zhang C., Bartold P.M., Gronthos S., Shi S. and Wang S., Periodontal ligament stem cell-mediated treatment for periodontitis in miniature swine, *Stem Cells*, **26**, 1065–1073 **(2008)**

58. Lombardini E.D. and Pacheco-Thompson M.E., Radiation and other physical agents, in: Haschek Rousseauxs Handb, *Toxicol. Pathol*., **3**, 839–927 **(2023)**

59. Lyu Y.L., Zhou H.F., Yang J., Wang F.X., Sun F. and Li J.Y., Biological Activities Underlying the Therapeutic Effect of Quercetin on Inflammatory Bowel Disease, *Mediators Inflamm*., **2022**, e5665778 **(2022)**

60. Magar R.T. and Sohng J.K., A review on structure, modifications and structure-activity relation of quercetin and its derivatives, *J. Microbiol. Biotechnol.*, **30**, 11–20 **(2020)**

61. Mahdi Eshaghi M., Pourmadadi M., Rahdar A. and Díez-Pascual A.M., Novel Carboxymethyl Cellulose-Based Hydrogel with Core–Shell Fe3O4@SiO2 Nanoparticles for Quercetin Delivery, *Materials*, **15**, 8711 **(2022)**

62. Mishra V., Chanda P., Tambuwala M.M. and Suttee A., Personalized medicine: An overview, *Int. J. Pharm. Qual. Assur.*, **10**, 290–294 **(2019)**

63. Napimoga M.H., Clemente-Napimoga J.T., Macedo C.G., Freitas F.F., Stipp R.N., Pinho-Ribeiro F.A., Casagrande R. and Verri W.A., Quercetin Inhibits Inflammatory Bone Resorption in a Mouse Periodontitis Model, *J. Nat. Prod.*, **76**, 2316–2321 **(2013)**

64. Neganova M., Liu J., Aleksandrova Y., Klochkov S. and Fan R., Therapeutic influence on important targets associated with chronic inflammation and oxidative stress in cancer treatment, *Cancers*, **13**, 6062 **(2021)**

65. Nguyen T.L.A. and Bhattacharya D., Antimicrobial activity of quercetin: an approach to its mechanistic principle, *Molecules*, **27**, 2494 **(2022)**

66. Ogata M. and Takahara S., Paper electrophoretic studies on the catalase protein in acatalasemic blood extract (stage 3 by Herbert-Pinsent), *Proc. Jpn. Acad.*, **38**, 361–364 **(1962)**

67. Onuche J.I. and Abu M.S., Assessment of the preventive effect of dietary inclusion of Cucurbita maxima (Duch) leaf on N-methyl-N-nitrosourea (MNU) induced colon carcinogenesis in Wistar rats, *Appl. Biol. Chem. J. TABCJ*, **2,** 93–101 **(2021)**

68. Owczarczyk-Saczonek A., Owczarek W., Osmola-Mańkowska A., Adamski Z., Placek W. and Rakowska A., Secondary failure of TNF-α inhibitors in clinical practice, *Dermatol. Ther.*, **32**, e12760 **(2019)**

69. Pal C., Molecular mechanism facets of Oxidative stress mediated pathogenesis, *J. Mol. Chem.*, **3**, 587–587 **(2023)**

70. Parekh J. and Chanda S., *In vitro* antibacterial activity of the crude methanol extract of Woodfordia fruticosa Kurz. flower (Lythraceae), *Braz. J. Microbiol.*, **38**, 204–207 **(2007)**

71. Pasciu V., Nieddu M., Sotgiu F.D., Baralla E. and Berlinguer F., An Overview on Assay Methods to Quantify ROS and Enzymatic Antioxidants in Erythrocytes and Spermatozoa of Small Domestic Ruminants, *Animals*, **13,** 2300 **(2023)**

72. Patel K., Patel K. and Zaveri M., Development, Optimization and Evaluation of Herbal Patch Formulation for Acne Treatment, *Int. J. Pharm. Investig.*, **13(1)**, 74-81 **(2023)**

73. Pazzaglia S. and Pioli C., Multifaceted role of PARP-1 in DNA repair and inflammation: pathological and therapeutic implications in cancer and non-cancer diseases, *Cells*, **9**, 41 **(2019)**

74. Peserico D., Stranieri C., Garbin U., Mozzini C.C., Danese E., Cominacini L. and Fratta Pasini A.M., Ezetimibe prevents ischemia/reperfusion-induced oxidative stress and up-regulates Nrf2/ARE and UPR signaling pathways, *Antioxidants*, **9**, 349 **(2020)**

75. Pisoschi A.M., Pop A., Iordache F., Stanca L., Predoi G. and Serban A.I., Oxidative stress mitigation by antioxidants-an overview on their chemistry and influences on health status, *Eur. J. Med. Chem.*, **209**, 112891 **(2021)**

76. Qi W., Wi Q., Xiong D. and Long M., Quercetin: Its Antioxidant Mechanism, Antibacterial Properties and Potential Application in Prevention and Control of Toxipathy, *Molecules*, **27,** 6545 **(2022)**

77. Rana K. and Gautam P., A review on antioxidants as therapeutic in use of oxidative stress and neurodegenerative disease, *Int J Pharm Qual Assur*, **13**, 77–82 **(2022)**

78. Shaker N.S., Sahib H.B., Hussein Z.A. and Tahseen N.J., Anti-cytokine storm activity of fraxin and quercetin, alone and in combination and their possible molecular mechanisms via TLR4 and PPAR-γ signaling pathways in lipopolysaccharide-induced RAW 264.7 cell line, *F1000Research*, **12,** 1120 **(2023)**

79. Sinha S., Parameswarappa P., Eregowda N.I., Kenchappa M. and Sasalawad S., Comparative Evaluation of Antimicrobial Activity of Quercetin against Endodontic Pathogens in Primary Molars, *CODS - J. Dent.*, **10**, 7–10 **(2018)**

80. Skibska B., Kochan E., Stanczak A., Lipert A. and Skibska A., Antioxidant and Anti-inflammatory Effects of α-Lipoic Acid on Lipopolysaccharide-induced Oxidative Stress in Rat Kidney, *Arch. Immunol. Ther. Exp. (Warsz.,)*, **71**, 16 **(2023)**

81. Speisky H., Shahidi F., Costa de Camargo A. and Fuentes J., Revisiting the oxidation of flavonoids: Loss, conservation or enhancement of their antioxidant properties, *Antioxidants*, **11**, 133 **(2022)**

82. Spiegel M., Cel K. and Sroka Z., The mechanistic insights into the role of pH and solvent on antiradical and prooxidant properties of polyphenols—Nine compounds case study, *Food Chem.*, **407**, 134677 **(2023)**

83. Srivastava T., Mishra S.K., Tiwari O.P., Nath Tiwari K., Kumar P., Kumar J., Kumar Singh A., Singh A. and Dwivedi A., Green synthesis, Characterization and *In vitro* Biological Studies of Quercetin complexes with Zn (II) Acetate and N^N Moiety, *Res. J. Pharm. Technol.*, **14(7)**, 3585–3590 **(2021)**

84. Sukumaran S.K., Vadakkekuttical R.J. and Kanakath H., Comparative evaluation of the effect of curcumin and chlorhexidine on human fibroblast viability and migration: An in vitro study, *J. Indian Soc. Periodontol.*, **24,** 109 **(2020)**

85. Sul O.J. and Ra S.W., Quercetin prevents LPS-induced oxidative stress and inflammation by modulating NOX2/ROS/NF-kB in lung epithelial cells, *Molecules*, **26**, 6949 **(2021)**

86. Talarico L.B., Zibetti R.G., Faria P.C., Scolaro L.A., Duarte M.E., Noseda M.D., Pujol C.A. and Damonte E.B., Anti-herpes simplex virus activity of sulfated galactans from the red seaweeds Gymnogongrus griffithsiae and Cryptonemia crenulata, *Int. J. Biol. Macromol.*, **34**, 63–71 **(2004)**

87. Thirupathi A., Pinho R.A., Ugbolue U.C., He Y., Meng Y. and Gu Y., Effect of running exercise on oxidative stress biomarkers: A systematic review, *Front. Physiol*., **11**, 1789 **(2021)**

88. Tian C., Liu X., Chang Y., Wang R., Lv T., Cui C. and Liu M., Investigation of the anti-inflammatory and antioxidant activities of luteolin, kaempferol, apigenin and quercetin, *South Afr. J. Bot*., **137,** 257–264 **(2021)**

89. Uciechowski P. and Dempke W., Interleukin-6: a masterplayer in the cytokine network, *Oncology*, **98**, 131–137 **(2020)**

90. Vo T.T.T., Chu P.M., Tuan V.P., Te J.S.L. and Lee I.T., The Promising Role of Antioxidant Phytochemicals in the Prevention and Treatment of Periodontal Disease via the Inhibition of Oxidative Stress Pathways: Updated Insights, *Antioxidants*, **9**, 1211 **(2020)**

91. Wang L., Wu H., Xiong L., Liu X., Yang N., Luo L., Qin T., Zhu X., Shen Z., Jing H. and Chen J., Quercetin Downregulates Cyclooxygenase-2 Expression and HIF-1α/VEGF Signaling-Related Angiogenesis in a Mouse Model of Abdominal Aortic Aneurysm, *BioMed Res. Int.*, **2020**, e9485398 **(2020)**

92. Wang Y., Tao B., Wan Y., Sun Y., Wang L., Sun J. and Li C., Drug delivery based pharmacological enhancement and current insights of quercetin with therapeutic potential against oral diseases, *Biomed. Pharmacother.*, **128**, 110372 **(2020)**

93. Wang Z. et al, Quercetin induces p53-independent cancer cell death through lysosome activation by the transcription factor EB and Reactive Oxygen Species-dependent ferroptosis, *Br. J. Pharmacol.*, **178**, 1133–1148 **(2021)**

94. Wei Y., Fu J., Wu W., Ma P., Ren L., Yi Z. and Wu J., Quercetin Prevents Oxidative Stress-Induced Injury of Periodontal Ligament Cells and Alveolar Bone Loss in Periodontitis, *Drug Des. Devel. Ther.*, **15**, 3509–3522 **(2021)**

95. Yamagata K., Onion quercetin inhibits vascular endothelial cell dysfunction and prevents hypertension, *Eur. Food Res. Technol*., https://doi.org/10.1007/s00217-023-04368-w **(2023)**

96. Yang D., Wang T., Long M. and Li P., Quercetin: its main pharmacological activity and potential application in clinical medicine, *Oxid. Med. Cell. Longev*., **2020**, 8825387 **(2020)**

97. Ying L., Chaudhry M.T., Xiao F., Mao Y., Wang M., Wang B., Wang S. and Li Y., The effects and mechanism of quercetin dietary supplementation in streptozotocin-induced hyperglycemic arbor acre broilers, *Oxid. Med. Cell. Longev*., **2020**, 9585047 **(2020)**

98. You K., Gu H., Yuan Z. and Xu X., Tumor necrosis factor alpha signaling and organogenesis, *Front. Cell Dev. Biol.*, **9**, 727075 **(2021)**

99. Zerin T., Kim Y.S., Hong S.Y. and Song H.Y., Quercetin reduces oxidative damage induced by paraquat via modulating expression of antioxidant genes in A549 cells: Quercetin reduces oxidative damage induced by paraquat in A549 cells, *J. Appl. Toxicol.*, **33**, 1460–1467 **(2013)**

100. Zhang H., Li S. and Cheng Y., Antibiofilm Activity of Allicin and Quercetin in Treating Biofilm-Associated Orthopaedics Infection, *Appl. Biochem. Biotechnol.*, DOI: 10.1007/s12010-022-03845-4 **(2022)**

101. Zhang J., Li H., Wang W. and Li H., Assessing the anti-inflammatory effects of quercetin using network pharmacology and in vitro experiments, *Exp. Ther. Med*., **23**, 1–8 **(2022)**

102. Zhao H., Zhang R., Yan X. and Fan K., Superoxide dismutase nanozymes: an emerging star for anti-oxidation, *J. Mater. Chem. B*, **9**, 6939–6957 **(2021)**

103. Zmigrodzka M., Rzepecka A., Krzyzowska M., Witkowska-Pilaszewicz O., Cywinska A. and Winnicka. A., The cyclooxygenase-2/prostaglandin E2 pathway and its role in the pathogenesis of human and dog hematological malignancies, *J. Physiol. Pharmacol.*, **69(5)**, 653–661 **(2018)**.

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