Potential Comparison of Anti-inflammatory Activities of Quercetin and Diclofenac Mouthwash Formulations

Comparación potencial de las actividades antiinflamatorias de las formulaciones de enjuague bucal de quercetina y diclofenaco

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ABSTRACT

Introduction: Periodontitis is a pandemic, with about 14% of people worldwide already suffering from severe periodontitis. Early intervention in the disease could probably reduce its progression and eliminate the need for the extraction of affected teeth. Quercetin is a probable candidate as it has exemplary anti-inflammatory properties. The presence of phenolic hydroxyl groups in them greatly contributes to their antioxidant and anti-inflammatory activities.

Objectives: The study introduces the formulation of Quercetin mouthwash and assesses its anti-inflammatory properties in comparison to Diclofenac sodium.

Methods: Quercetin mouthwash was prepared using a commercially procured bioactive agent. One standard nonsteroidal anti-inflammatory drug, Diclofenac was used as a reference drug. The percentage inhibition of protein denaturation was calculated and its anti-inflammatory properties were evaluated through Bovine Serum Albumin Assay and Egg Albumin Assay.

Results: Quercetin mouthwash showed parallel anti-inflammatory properties and showed a proportionate increase in anti-inflammatory properties with the increase in the concentration of the mouthwash. Comparable inhibition of protein denaturation at 10µl and 50µl concentrations with a proportionate variation of 1% (p>0.05) to the control in Egg Albumin Assay and 47% and 83% denaturation at 10µl and 50µl of Bovine Serum Albumin Assay were observed.

Conclusion: Quercetin mouthwash has shown significant anti-inflammatory activity and hence is considered a potent anti-inflammatory agent comparable to Diclofenac sodium. It is found to be a suitable agent as an oral formulation for reducing the progression of inflammatory conditions.

Keywords: quercetin; anti-inflammatory agents; oral health; antioxidants; mouthwashes.

INTRODUCTION

Dental caries and periodontal diseases are the most prevalent dental diseases globally. About 3.5 billion people worldwide are estimated to have been affected by oral diseases, according to “The Global Burden of Disease 2019”.

Quercetin and Diclofenac Mouthwash Formulations

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Resumen

Introducción: La periodontitis es una pandemia, ya que alrededor del 14% de las personas en todo el mundo padecen periodontitis grave. Una intervención precoz en la enfermedad podría, probablemente, reducir su progresión y eliminar la necesidad de extraer los dientes afectados. La quercetina es un candidato probable, ya que tiene propiedades antiinflamatorias ejemplares. Su presencia de grupos hidroxilos fenólicos contribuye en gran medida a sus actividades antioxidantes y antiinflamatorias.

Objetivos: El estudio presenta la formulación del colutorio de quercetina y evalúa sus propiedades antiinflamatorias en comparación con el diclofenaco sódico.

Métodos: Se preparó un colutorio de quercetina, utilizando un agente bioactivo obtenido comercialmente. Se utilizó un antiinflamatorio no esteroideo estándar, el diclofenaco. Se calculó el porcentaje de inhibición de la desnaturalización de proteínas y se evaluaron sus propiedades antiinflamatorias mediante ensayo con albúmina de suero bovino y con albúmina de huevo.

Resultados: El colutorio de quercetina mostró propiedades antiinflamatorias paralelas y mostró un aumento proporcional de las propiedades antiinflamatorias con el aumento de la concentración del colutorio. Se observó una inhibición comparable de la desnaturalización de proteínas a concentraciones de 10µl y 50µl con una variación proporcional del 1% (p>0.05), respecto al control en el ensayo de albúmina de huevo y una desnaturalización del 47% y 83% a 10µl y 50µl del ensayo de albúmina de suero bovino.

Conclusiones: El enjuague bucal de quercetina ha mostrado una actividad antiinflamatoria significativa, por lo que se considera un potente agente antiinflamatorio comparable al diclofenaco sódico. Se considera un agente adecuado como formulación oral para reducir la progresión de las afecciones inflamatorias.

Palabras clave: quercetina; agentes antiinflamatorios; salud bucal; antioxidantes; enjuagues bucales.
population is affected by severe periodontitis, which represents around one billion people worldwide. (2) Has-
turk H et al proposed that Periodontium has become the nidus of infection and inflammation, leading to the
activation of inflammatory pathways throughout the body. (3) Periodontitis is a chronic disease with a multifactor-
torial etiology that causes inflammation of the periodontium in the host associated with an imbalance in mi-
icrobiota, leading to progressive damage of the tooth-supporting apparatus and periodontal attachment loss. (4)

The main cause of the disease is microorganisms that live in the subgingival tooth region. Subgingival bio-
film formation, most commonly by anaerobic proteolytic bacteria, is well known to play a role in the induction of
inflammatory processes. (5) Microorganisms begin colonizing hard tooth surfaces, forming the dental biofilm,
because there is no shedding process as there is in the oral mucosa. (6) Porphyromonas Gingivalis and Treponema
denticola are the most important microorganisms in periodontal disease causation. (8)

The initial immunomodulatory process of the host tissue against these harmful pathogens attempts to main-
tain a holistic oral environment and promote resolution, which results in the production of pro-inflammatory
cells such as IL-1, IL-6, and TNF; inflammation is the body’s defense mechanism and is important in initiating
pathogen killing and restoring tissue homeostasis. (7) Larsson et al found that as the disease progresses, the
inflammatory process becomes dysregulated, and the production of cytokines increases. (8) This reciprocally
reinforced interaction between the altered microflora and the dysregulated inflammatory process leads to ging-
vivitis, ultimately leading to periodontitis if not treated.

The pathophysiological situation continues to exist through quiescence until the therapeutic removal of the
microbial biofilm or the extraction of the affected tooth. (8,9) As a result, intervention in the formation of dental
biofilms and the resolution of inflammation should be the primary goals of the treatment modality. Although
mechanical plaque control is arguably considered the gold standard, around half the biofilm remains even af-
after brushing for two minutes. In this scenario, chemical plaque control is used as an adjunct therapy to the
dexterous mechanical one. (10) Hence, mouthwashes are extremely beneficial in the early intervention of the
disease process. (11)

Mouthwash is a medicated formulation that aids in the removal of harmful periodontal pathogens. They are
retained in the mouth and swung around by the perioral muscle tissues. They help reduce bacterial load,
disrupt biofilm formation, eliminate halitosis, and provide anti-inflammatory, analgesic, anti-microbial, and
other pharmacological therapeutics. (12) Herbal types of mouthwash are becoming more popular as an alterna-
tive to chemical mouthwashes; because more people are becoming aware of safer alternatives to conventional
agents. (13) In that way, the quercetin compound can be a probable candidate, as it has exemplary pharmacolo-
gical properties.

Quercetin is a pentahydroxy flavone in high concentrations of edible fruits and vegetables. (14) They are found
in kale, apples, oranges, grapes, blueberries, olive oil, tea, and other cherries. (15) The presence of phenolic
hydroxyl groups in them greatly contributes to their antioxidant and anti-inflammatory properties. (16) They can
halt the lipid peroxidation process and inhibit platelet aggregation and capillary permeability. (17) They are highly
capable of down-regulating inflammation, inhibiting lipopolysaccharide-induced tumor necrosis factor alpha,
and preventing the production of cyclooxygenase and lipoxygenase enzymes. Their main target is leukocytes,
inhibiting many other signaling pathways. It also has potent anti-oxidant properties by upregulating the activi-
ty of glutathione, affecting the redox ratio and inducing flavonoid-like activity. Recently extensive research is
on the way to analyze various benefits of Quercetin and uncover its use for therapeutic purposes. Therefore,
keeping in mind, the anti-inflammatory activities of Quercetin and the paucity of literature, the current study
aims to explore the use of its mouthwash formulation for Oral health and assesses its anti-inflammatory pro-
erties in comparison to Diclofenac sodium.

METHODS

Sample details
Quercetin compound (USP1592409) and cyclooxygenase inhibitor, Diclofenac 2-[(2,6-Dichlorophenyl) amino]
benzene acetic acid sodium salt (D6899) at ≥ 99 % purity had been procured from the Sigma-Aldrich ©Merck KGaA.

Sampling strategy
The preparation of quercetin mouthwash from its ethanolic extracts and In-vitro analysis by Bovine serum albumin assay and Egg albumin assay. Both are regarded as standardized In-vitro anti-inflammatory tests.

Sample testing in material
Commercially procured Quercetin compound (USP1592409) Sigma-Aldrich ©Merck KGaA, 0.02mg has been
separated by weighing in a weighing machine and 10 ml of ethanol (≥ 99 % purity) has been pipetted and taken
separately.
Preparation of mouthwash

The weighed 0.02 mg of commercially obtained Quercetin (USP1592409) Sigma-Aldrich ©Merck KGaA was mixed with 10 ml of ethanol (≥ 99 % purity). About, 0.5ml of prepared quercetin solution is taken then, 0.3g of sucrose the sweetening agent, 0.001g of sodium benzoate the preservative, and 0.01g of sodium lauryl sulfate the foaming agent had been added to make the Quercetin mouthwash preparation. All were prepared and based on U.S. Food & Drug Administration (USFDA) approved standards. Diclofenac sodium or 2-[(2,6-Dichlorophenyl) amino] benzene acetic acid sodium salt had been used as the positive control for comparison and validation of the tests.

BSA assay

To check the anti-inflammatory activity of the mouthwash, 2 ml of 1% bovine albumin was added to the prepared quercetin mouthwash at different concentrations (10-50 g/ml). Following that, various concentrations of Quercetin mouthwash (10-50 g/mL) were mixed with distilled water. They were incubated at room temperature for about 10 minutes. The prepared solutions were then heated at 55 degrees Celsius for 20 minutes in a water bath and cooled to room temperature. The color changes were observed, and the absorbance values were recorded at 660 nm. Diclofenac sodium was used as an anti-inflammatory pharmacological control drug. Concentrations ranging from 10 g/ml to 50 g/ml of the control drug were tested against 10-50 g/ml concentrations of quercetin mouthwash. All the tests were done in triplicate.

EA assay

The solution comprised 2.8 mL of freshly prepared phosphate-buffered saline with a pH of 6.3 and 0.2 mL of egg albumin extracted from the hen's egg. Specific concentrations of 10 g/mL to 50 g/mL for Quercetin mouthwash were prepared separately. The solutions were then heated at 37 degrees Celsius for 15 minutes in a water bath. They were allowed to cool down to room temperature and the absorption values were then measured at 660 nm. All the tests were done in triplicate.

Statistical analysis

The results were tabulated in MS Excel, and Microsoft Corporation (2018), and sorted. IBM SPSS Statistics for Windows, version 23 (IBM Corp., Armonk, N.Y., USA) had been used to run independent sample t-tests and to perform inter-group comparisons. The values were tabulated as mean ± SD. The data were subjected to quantitative analysis and parametric tests were used, after estimating the normality of the available data. The results were represented in the form of pictorial graphs and tables. The p values of ≤ 0.05 were considered statistically significant.

Ethics approval

The protocol was reviewed and approved by the Ethical and Research Committee as a formally constituted Institutional Review Board (IRB) for human studies (number EC/NEW/INST/2021/1967 assigned), and it followed the ethical principles of the Declaration of Helsinki updated in 2013. The study was in-vitro in nature and was cleared, as it did not involve any foreseeable risks.

RESULTS

The anti-inflammatory property of the mouthwash has been evaluated by the BSA (Bovine Serum Albumin Assay) and the EA (Egg albumin) assay. Independent sample t-test has been performed with the SPSS statistics software version 23 (IBM Corp., Armonk, N.Y., USA) for intergroup comparison, tabulated in figure/table 1 and 2.

From the results of the BSA assay as from figure/table 1, we could infer that both the Quercetin mouthwash and the Diclofenac sodium have exhibited a proportionate increase in the percent protein anti-denaturation with the increase in the concentrations ranging from 10µl to 50µl.

The Quercetin mouthwash has shown 47% protein denaturation at 10µl concentration, 55% at 20µl concentration, 73% at 30µl concentration, 76% at 40µl concentration, and 83% at 50µl concentration. Similarly, the standard has shown 48% protein denaturation at 10µl concentration, 56% at 20µl concentration, 74% at 30µl concentration, 77% at 40µl concentration, and 84% at 50µl concentration. In the same way, figure/table 2, shows the results of the Egg albumin assay, where the Quercetin mouthwash has shown comparable denaturation at 10µl and 50µl concentrations with a proportionate variation of 1% to the control.
Table 1 - Bovine Serum Albumin Assay

<table>
<thead>
<tr>
<th>Control Group (Diclofenac Sodium)</th>
<th>Test Group (Quercetin)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>Standard Deviation</td>
<td>Mean</td>
</tr>
<tr>
<td>67.80</td>
<td>15.1398</td>
<td>66.80</td>
</tr>
</tbody>
</table>

Footnote: Shows the results of the Independent sample t-test run between the protein denaturation percentages of Quercetin mouthwash, and Diclofenac sodium from the BSA assay. The p-value is found to be 1.000 (> 0.05) which states no statistically significant difference between the two groups.

Source: The mean protein denaturation concentration of the experimental formulation (Quercetin mouthwash) and control formulation (Diclofenac mouthwash) had been estimated (table 1) using BSA assay and tested with SPSS version 23 (IBM Corp., Armonk, N.Y., USA).

An Independent sample t-test had been performed for protein denaturation percentages for Quercetin mouthwash and Diclofenac sodium from both BSA and EA assays. Table 2, presents the independent sample t-test results between the two groups from the BSA assay. The p-value is found to be 1.000 (> 0.05) which states no statistically significant difference between the two groups. Similarly, table 2 shows the p-value is found to be 0.954 (> 0.05) which states no statistically significant difference between the two groups of EA Assay.
**Table 2 - Egg Albumin Assay**

<table>
<thead>
<tr>
<th>Control Group (Diclofenac Sodium)</th>
<th>Test Group (Quercetin)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean 88.40</td>
<td>Mean 66.80</td>
<td></td>
</tr>
<tr>
<td>Standard Deviation 10.78425</td>
<td>Standard deviation 10.78425</td>
<td></td>
</tr>
<tr>
<td>0.954*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Legend:** *p* > 0.05

Footnote: Shows the results of the Independent sample t-test run between the protein denaturation percentages of Quercetin mouthwash and Diclofenac sodium from the EA assay. The p-value is found to be 0.954 (>0.05) which states no statistically significant difference between the two groups.

Source: The mean protein denaturation concentration of the experimental formulation (Quercetin mouthwash) and control formulation (Diclofenac mouthwash) had been estimated (table 1) using Egg Albumin assay and tested by SPSS version 23 (IBM Corp., Armonk, N.Y., USA)

**DISCUSSION**

Quercetin mouthwash has shown a potential increase in anti-inflammatory activity with a proportionate increase in its concentration. In both assays, the quercetin mouthwash has shown an anti-inflammatory activity that is comparable to that of the non-preferential cyclooxygenase inhibitor, Diclofenac sodium with a marginal difference. From the BSA assay results, we could analyze the difference in percent protein denaturation between the quercetin mouthwash and the Diclofenac sodium to be just 1% higher in the Diclofenac sodium that has been used at all varying concentrations. Similarly, from the EA assay, the difference in the percent protein denaturation is found to be 1% higher in the standard at 10μl concentration, 2% higher at 20μl concentration, 3% higher in the standard at 30μl concentration, 2% higher in the standard at 40μl concentration and 1% higher in the standard at 50μl concentration with the average percent difference being 1.8%. Therefore, the Quercetin compound has shown comparable anti-inflammatory properties to that of reference non-specific COX inhibitor Diclofenac sodium.

Flavonoids are found to be great anti-inflammatory agents; they inhibit key signaling pathways and downregulate inflammation. They also greatly reduce the production of reactive oxygen species. Flavanol is a type of flavonoid and is found in abundance in Quercetin compounds. Quercetin can inhibit the cyclooxygenase (COX) and lipoxygenase (LOX) enzymes that produce inflammation. Quercetin is found to inhibit IL-8 and IL-6 way better than Cromolin, an anti-allergic drug. Following our study, a study by Morikawa et al. shows that quercetin acts as an immune booster and is able to exhibit anti-inflammatory activity in both in vitro and animal studies. Quercetin glycoside extracted from fresh leaves of fenugreek has also shown potential anti-inflammatory and antioxidant properties. Similarly, in a murine model of airway allergic inflammation, a quercetin-loaded microemulsion demonstrated significant anti-inflammatory properties and has been shown to have significant therapeutic potential in the management of airway inflammatory diseases. Furthermore, Quercetin inhibited the activity of LPS-induced mRNA levels of TNF- and interleukin (IL)-1 and reduced neuronal cell death in glial cells.

A double-blind placebo-controlled study by Javadi F et al. tested the effects of Quercetin in reducing the clinical symptoms of Rheumatoid arthritis, especially in women. It was shown that the active disease significantly reduced in the test arm treated with Quercetin. Further, a dosage of 500mg per day for 8 weeks was suggested to gradually decrease the symptoms of Rheumatoid arthritis. Such evidence presents the study’s rationality with Quercetin’s potent anti-inflammatory properties. Further benefits were presented by Khan A et al. (2022), where Quercetin was found to be an adjuvant in treating mild to moderate symptoms of COVID-affected patients. It has been stated that the co-supplementation of Curcumin, Quercetin, and cholecalciferol-D3 combination therapy had greatly modulated the hyperinflammatory reactions and helped in the resolution of acute inflammatory disease. Another similar study also tested the synergistic effects of Quercetin, Vitamin C in SARS-COV2 patients; showed the anti-inflammatory action of the drug, and stated its use as a prophylactic agent for preventing COVID-19 in high-risk populations, and as a possible adjunct to the standard of care treatment.

**CONCLUSION**

Bioactive compounds can be sourced both naturally and synthetically. These indications elaborate on the flexible utilization of bio-active compounds such as Quercetin, in various formulations, as adjuvants, therapeutics, and for prophylactic purposes through various routes of administration. From this evidence, Quercetin has been proposed to show exemplary anti-inflammatory properties as shown by a significant reduction in C-reactive protein, and could effectively passivate oral biofilm, reduce inflammation, and intervene in the disease.
progress in periodontal diseases. When formulated as a mouthwash, it has potent anti-inflammatory properties and exhibits an efficiency that is almost equivalent to that of the reference drug, Diclofenac sodium. The current study supports the use of Quercetin mouthwash as it has exemplary properties as a therapeutic and a prophylactic agent. Our study forms the basis of futurist uses of Quercetin as periodontal dressings, subgingival irrigants, and anti-inflammatory irrigants in flap surgeries. Further comparative and elaborative clinical trials could provide a great resource in the implementation of its usage in clinical practice.

References


CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR’S CONTRIBUTIONS

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Formal analysis: Jishnu Krishna Kumar, Anu Iswarya Jaisankar.
Supervision: Jishnu Krishna Kumar.
Resources: Jishnu Krishna Kumar, Anu Iswarya Jaisankar.
Investigation: Jishnu Krishna Kumar, Anu Iswarya Jaisankar.
Methodology: Jishnu Krishna Kumar, Anu Iswarya Jaisankar.
Project administration: Jishnu Krishna Kumar.
Writing - original draft: Jishnu Krishna Kumar, Anu Iswarya Jaisankar.
Writing - review & editing: Jishnu Krishna Kumar, Anu Iswarya Jaisankar.

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