Journal of Pioneering Medical Sciences
Received July 11, 2023 Accept December 12, 2023 Publish December 23, 2023.
Volume 12, Issue 3, Pages 61-64

DOI https://doi.org/10.61091/jpms202312312

Comparative Anti-Inflammatory Activity of Rutin-Based Mouthwash and Diclofenac Sodium: An in-Vitro Evaluation

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Abstract **Background:** One of the most common oral inflammatory conditions is Gingivitis. It involves the inflammation of the gingiva supporting the tooth. The onset of Gingivitis is due to the recurrent deposition of plaque and its inefficient removal. Use the right oral dentifrice and mouthwash to keep the inflammation at bay. Rutin is a natural substance extracted from citrus fruits. This citrus flavonoid has antimicrobial, antioxidant, and cytotoxic properties. **Aim:** This study aims to formulate a mouthwash with rutin and compare its anti-inflammatory potential with an already commercially anti-inflammatory agent. **Materials and Methods:** For this in-vitro study, two different anti-inflammatory assays were performed using different substrates. The first test performed was the bovine serum albumin anti-inflammatory test, and the second was the egg albumin denaturation test. **Results:** The results suggest that the rutin mouthwash made in our study shows a similar anti-inflammatory activity as the Diclofenac sodium-based formulation. Rutin mouthwash and the Diclofenac sodium have exhibited a non-significance ($p>0.05$) in the percent protein anti-denaturation with the increase in the concentrations ranging from 10μl to 50μl. **Conclusion:** Rutin-based mouthwash possesses satisfactory anti-inflammatory activity compared to standard commercial mouthwash.

Key Words Rutin, Anti-inflammatory agents, Mouthwash, Oral health, Prevention

1. Introduction

In the era of switching to organic and natural forms of medicine, a dietary flavonoid called rutin is a center of attention among researchers. It is called rutoside,sophorin and quercetin-3-O-rutinoside [1]. Rutin is commonly found in citrus fruits and it is considered as a citrus flavonoid. Rutin is also abundantly found in plants like buckwheat, apple, tea, and passion flowers [2].

Rutin is a substance of keen interest due to the various pharmacological properties it is considered to exhibit. It is known to possess anti-microbial, anticancer, antidiabetic, and cytotoxic effects. Some studies have proven its effectiveness in controlling hormones and influencing the female reproductive processes [3]. Among the naturally derived substrates, rutin is considered to have the best anti-oxidant potential [4]. Animal studies on rats have suggested that the pre-treatment with rutoside is effective in the treatment of colitis in rats [5].

In the body, the inflammatory responses are stimulated by various intercellular protein complexes getting activated. These protein complexes are termed inflammasomes. The two main signals necessary for the activation of inflammasomes are the priming and triggering signals [6].

A large number of mechanism studies have successfully demonstrated that these functional foods exhibit anti-inflammatory effects by inhibiting the production of inflammatory mediators, such as Nitrous oxide (NO), Prostaglandin E2 (PGE2), COX-2, Tumor Necrosis Factor TNF, Interleukins-IL1, IL-6, IL-15, and interferon (IFN)- and suppressing the inflammatory signaling pathways, such as Toll-like receptors (TLRs), NF-B, AP-1, and IRFs as well as the activities of intracellular inflammatory signaling molecule.

Depending on the nutritional habits of an individual, for an average person, the normal daily intake of rutin will range between 1 to 1.5g and 70 mg/kg. The bioavailability of rutin dictates the amount of absorption in the body. Studies have suggested that there is minimal absorption of rutin via the intestines. This occurs because of the molecular structure glycosylated with a disaccharide.

Inflammation can be classified into acute and chronic based on the duration of onset and the cells actively participating in the inflammatory process. Acute inflammatory

processes are characterized by the emigration of leukocytes, mainly neutrophils, and exudation of fluid. Acute inflammation occurs over a short duration, unlike chronic inflammation which occurs for a longer duration of time. The characteristic features defining chronic inflammation are macrophage and lymphocyte infiltration, proliferation of blood vessels, and signs of fibrosis. Inflammation is primarily intended as a defence mechanism against foreign molecules. Hence, conventionally, the inflammation process is stopped after the invading molecule or species is eliminated from the body, the removal of all the secreted products is also an important step in the termination of inflammatory processes. There are proven interlinks between chronic inflammation that can affect the periodontium in the oral cavity. Such chronic inflammatory diseases include diabetes, heart disease, and arthritis to specify a common few. Similarly, it is suggested that the local periodontal disease has the potential to alter the factors controlling the severity and progression of any systemic inflammatory condition. Reciprocity of systemic and local inflammatory diseases is important to note here. This is thus one of the most important reasons to ensure the maintenance of good oral hygiene.

The anti-inflammatory mechanism of rutin at a molecular level involves the inhibition of tumor necrosis factor TNF alpha via a quercetin-mediated pathway [7]. In the mouth, the inflammatory responses are caused by plaque-producing bacteria or viruses present in the teeth and gums. With time, the plaque that is present calcifies and forms a scale on the surface of the tooth. With the formation of scales with a rough surface, it acts as a scaffold for the breeding of various types of oral bacteria. With the increase in the amount of oral microorganisms, there is also an increased risk for the onset of gingivitis and periodontitis. At the molecular level, there is the release of lipopolysaccharide which is known to cause various inflammatory reactions in the body. It leads to the release of pro-inflammatory cytokines like TNF alpha and interleukin 6 and 12 along with nitric oxide. The purpose of these substances is to provide an inflammatory response to protect the body and host against microbial invasion. When there is an overproduction of these products it leads to the perpetual damage of the host cells themselves [8].

Understanding the origins of rutin, its biochemical properties, and its anti-inflammatory potential allows us to choose rutin to make an anti-inflammatory mouthwash. Such a mouthwash with specific anti-inflammatory activities must be available as it can not only control the inflammatory processes occurring in the oral cavity, but it can help prevent the triggering of other systemic inflammatory conditions.

This study aims to formulate a mouthwash with rutin and compare its anti-inflammatory potential with an already proven potent anti-inflammatory agent.

2. Materials and Methods

Sampling Strategy

The preparation of rutin 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.

Preparation of Mouthwash

The weighed 0.02 mg of commercially obtained Rutin was mixed with 10 ml of ethanol ($\geq$99% purity). About, 0.5ml of prepared Rutin solution was 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 been added to make the Rutin 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 was used as the positive control for comparison and validation of the tests.

Anti-Inflammatory Activity

Albumin Denaturation Assay

The Muzushima and Kabayashi convention with specific alterations is used to assess the anti-inflammatory activity of Rutin mouthwash (Pratik Das et al.,2019). 0.45 ml of bovine serum albumin (1% aqueous solution) was added to 0.05 ml of the rutin mouthwash of various fixation (10μL,20μL,30μL,40μL,50μL) and the pH of the resultant mixture was adjusted to 6.3 with the addition of the required amount of 1N hydrochloric acid. All the samples are heated at $55^{\circ}C$ for half an hour after incubating them at room temperature for 20 minutes. The heated samples are then cooled down to room temperature before estimating the absorbance via spectrophotometry at 660 nm. The control used for the assay is DMSO and the standard used is diclofenac sodium. The percentage of protein denaturation was determined utilizing the following equation,

$$\%inhibition = \frac{AC - AS \times 100}{AC}$$

Where AC: Absorbance of control and AS: Absorbance of sample

Egg Albumin Denaturation Assay

Using 0.2 ml of egg albumin from a hen's egg and 2.8 ml of freshly prepared phosphate buffer saline with a pH of 6.3, a 5 ml mixture is prepared. Rutin mouthwash of specific concentrations such as 10 μL,20 μL,30 μL,40 μL, and 50 μL was prepared. The positive control used for this assay is Diclofenac sodium. Then the mixtures were heated in a water bath at 37° C for 15 minutes. After which the samples were allowed to cool down to room temperature and absorption was measured at 660 nm.

Statistical Analysis

The results were tabulated in MS Excel, Microsoft Corporation (2018), and sorted. IBM SPSS Statistics for Windows, version 23 (IBM Corp., Armonk, N.Y., USA) was used to run independent sample t-tests and to perform inter-group comparisons. The values were tabulated as mean $\pm$ SD. The

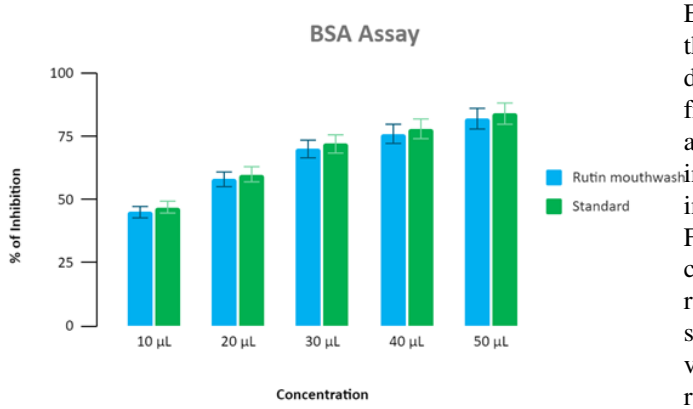

Figure 1: Graph represents the anti-inflammatory activity of the rutin mouthwash against commercial mouthwash using the BSA assay. Both the mouthwashes were tested in different concentrations - 10 μL,20 μL,30 μL,40μL,50μL The blue bar represents the anti-inflammatory potential of the rutin mouthwash and the green bar represents the anti-inflammatory activity of the Diclofenac sodium. [BSA-Bovine Serum Anti-inflammatory test]

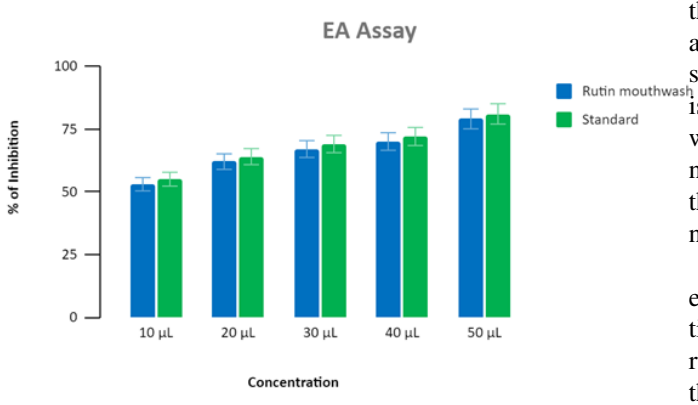

Figure 2: Graph represents the anti-inflammatory activity of the rutin mouthwash against commercial mouthwash using the EA assay. Both the mouthwashes were tested in different concentrations - 10μL,20μL,30μL,40μL,50μL The blue bar represents the anti-inflammatory potential of the rutin mouthwash and the green bar represents the anti-inflammatory activity of the Diclofenac formulation. [EA- Egg Albumin assay]

data were subjected to quantitative analysis and parametric tests were used, after estimating the normality of available data. The results were represented in the form of pictorial graphs and tables. The p values $\leq$0.05 were considered statistically significant.

3. Results

The anti-inflammatory activity of the Rutin mouthwash was tested using the BSA (Bovine Serum Albumin Assay) and the EA (Egg albumin) assay. Using the SPSS statistics software, the intergroup comparisons were made by performing an Independent sample-t test. From the results of the BSA assay as from Graph 1, we could infer that both the Rutin 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. From the results obtained from the EA assay(Graph 2), it can be inferred that the anti-inflammatory activity of the rutin mouthwash when compared to the standard showed similar anti-inflammatory activity to the standard. An in-vivo study done in rats suggested that copper coupled with rutin produces better anti-inflammatory activity than standard copper drugs.

4. Disscussion

In our study, a mouthwash was made with Rutin with the chief objective of creating an anti-inflammatory mouthwash. Two different assays were performed to check the anti-inflammatory potential of rutin against the Diclofenac sodium based formulation. The two assays were done to check anti-inflammatory response in two different methods and check if there are any differences. The results suggest that the rutin mouthwash made in our study shows a similar anti-inflammatory activity as the commercial agents. In a study done by Sandra Sagar et al, it is suggested that there is an anti-microbial and cytotoxic potential of herbal mouthwashes. This finding is significant as it forms the basis of the need to improve herbal mouthwash formulations such that they can replace conventional mouthwashes and provide the necessary protection against pathogens in the mouth [9].

Similar results were derived in a study done by Sekita et al. [10] analyzed the anti-inflammatory potential of multiple flavonoids like hyperin, isoquercitrin, quercetin, and rutin. The anti-inflammatory activity was checked against the lipopolysaccharide of P.gingivalis. Their study concluded that at 50μL, rutin showed the most anti-inflammatory activity compared to the other flavonoids. Suggesting that rutin possesses good anti-inflammatory activity among other types of commonly used flavonoids. A systematic review compared herbal extract dentifrices with the commercially available ones. The study suggested that the herbal counterparts perform similarly and the green tea extract dentifrices perform better than the store-bought conventional dentifrices and can be used to control plaque in the oral cavity [11]. additionally, a study conducted in Chennai suggested that the zone of inhibition of the oral microbiota was higher in the case of the herbal mouthwash made of green tea in comparison to the conventional mouthwash formulations. The results from their study reinforce the necessity of finding herbal alternatives to conventional mouthwashes [12]. A similar study was performed to check the antimicrobial activity of herbal formulation-mediated copper nanoparticles against clinical pathogens. The results from their study indicate that the zone of inhibition of the clinical pathogens increases with the increase in concentration of the herbal extracts [13].

A study by Shanmugasundaram D et al in 2022 examined the anti-inflammatory activity of a rutin-quercetin combination. The results of their study allowed them to conclude that the product - SophorOx possesses significant anti-inflammatory activity. It is known to have good suppressive action on TNF alpha and IL-6 [14]. Significant anti-inflammatory activity was also noticed using rosa and Jasminum extract in the manufacture of silver nanoparticles in a study conducted by Prithiksha et al [15].

A study conducted by Chen et al in 2001 suggested that products like rutin, and quercetin produce their anti-inflammatory characteristics by inhibiting the lipopolysaccharides induced nitric oxide in a dose-dependent manner. There is significant anti-inflammatory activity without any evident cytotoxic effects.

5. Conclusion

Rutin-based mouthwash possesses satisfactory anti-inflammatory activity compared to the standard commercial herbal mouthwash.

Conflict of Interest

The authors declare no conflict of interests. All authors read and approved final version of the paper.

Authors Contribution

All authors contributed equally in this paper.

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