

PREPARATION OF FLAXSEED MOUTHWASH AND EVALUATION OF IT'S ANTI-INFLAMMATORY PROPERTIES

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Abstract:

Introduction: Periodontitis is the inflammation of the periodontal tissues that can lead to gingival recession and destruction of alveolar bone. In the recent trends, periodontitis has become one of the leading dental problems worldwide. The effectiveness of mouthwashes in controlling plaque accumulation and their extraordinary antibacterial properties has led to the rise of use of mouthwashes. On the other hand, Flax seeds are fiber crops that are known to contain Omega 3,6 polyunsaturated fatty acids, Alpha linolenic acid, lignans and fiber which can alleviate the inflammatory process. They have pro-resolving properties which can inhibit the recruitment of TNF- α and neutrophils to the inflamed periodontium.

Aim: The current study aims at the preparation of Flaxseed mouthwash and assessing its anti-inflammatory properties.

Materials and method: The methodology includes the preparation of flax seed mouthwash and evaluation of its anti-inflammatory properties.

Results: The flax seed mouthwash is shown to have good anti-inflammatory properties and has shown a proportionate increase in activity with the increase in μ l concentration.

Conclusion: Flax seed mouthwash has shown significant anti-inflammatory activity and hence is considered as a potent anti-inflammatory agent.

Keywords: Periodontitis, Inflammation, Flax seeds, Mouthwash, innovative technique , anti inflammatory activity

Introduction:

Periodontitis is one of the leading dental problems affecting about 20 to 50% of the world's population. Periodontium has become the nidus of infection and inflammation which may lead to causation of various diseases[1]. It has been defined as an inflammatory disease of supporting tissues of teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal tissues[2]. Inflammation is a normal defense mechanism that

protects the host from infection and other noxious challenges. It is a crucial activity to initiate pathogen killing and tissue repair and to restore tissue homeostasis. The initial immuno inflammatory process of periodontium against the harmful microbial pathogens is very vital in the maintenance of a holistic oral environment [3]. There are high chances of resolution of tissues to homeostasis at this stage. But, A dysregulation in this well controlled process can lead to pathological inflammation. It often occurs as a result of pro inflammatory cells that activate host inflammation, such as IL-1, IL-6 and TNF- α . When the disease progresses, the metabolites of the bacteria damage the junctional epithelium causing the release of cytokines [4]. The pathophysiological situation persists through bouts of activity and quiescence, until the affected tooth is extracted or the microbial biofilm is therapeutically removed and the inflammation subsides [5]. Earlier intervention of the disease can control the destruction of the tissue and the alveolar bone. On that note, Mouthwashes are found to be so effective in controlling plaque accumulation as well as reducing inflammation. A mouthwash is a medicated liquid which is held in the mouth and swished by the action of perioral musculature to eliminate the oral pathogens [6]. Mouthwashes can reduce the build-up of dental plaque, gingivitis and halitosis. The number of mouthwash variants in the world has grown from 15 in 1970 to nearly 113 in 2012 [7]. In recent times the use of herbal mouthwashes is on the rise due to the spread in the awareness of the effect of complementary and alternative medicine. It is also due to the much stronger belief that the alternative therapy is with less side effects. Research has shown that herbal components or ingredients are varied with respect to composition and chemical structure. Mouthwashes of herbal origin prove to be worth consideration in recompensation of chemical ones [8]. Resolution of inflammation is essential to restore health. In that way, Flax seeds are found to have good anti-inflammatory properties. Nowadays, flax seeds are emerging as a “super food” as more scientific research points to their health benefits [9]. *Linum usitatissimum* is basically a fibre crop found to have abundant contents of Omega 3,6 polyunsaturated fatty acids, Alpha linolenic acid (ALA), lignans and fiber. It is mostly cultivated in the Middle East countries. Flax seeds are found to be a very good source of plant based Omega 3 fatty acids [10]. Animal studies have shown that the ALA in flax seeds prevented cholesterol from being deposited in the blood vessels of the heart, reduced inflammation in the arteries and reduced tumor growth [11]. Furthermore, a recent review of observational data concluded that ALA have great heart health benefits [12]. Further Resolvins derived from omega-3 fatty acids, primarily eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), as well as docosapentaenoic acid (DPA) and clupanodonic acid are specialized pro-resolving mediators [12, 13]. Resolvins and protectins are lipid-derived mediators of inflammation. They possess anti-inflammatory and proresolving properties by inhibiting the production of proinflammatory compounds such as TNF- α and reduce the recruitment of immune cells such as neutrophils to sites of inflammation. They serve as important precursors for lipid derived modulators of cell signaling, gene expression and inflammatory processes. Studies show that the consumption of Omega 3 polyunsaturated fatty acids on a regular basis can down regulate tissue inflammation [14]. As well as Lignans in flax seeds have got exemplary antioxidant and estrogen properties, both of which can help lower the risk of cancer and improve health [15]. Our team has extensive knowledge and research experience that has translate into high quality publications. [16–28], [29–33] [34] [35]. Therefore keeping in mind the extraordinary Anti inflammatory properties of Flax seeds, the current study aims at the preparation of Flaxseed mouthwash and evaluation of its anti inflammatory properties.

Methodology:

Preparation of Flax seed mouthwash:

The Flax seeds were powdered mechanically and 10g of the mesh powder was soaked in 100ml of distilled water at 55 - 60 degree Celsius in the heater. The solution was kept in the shaker for 1 hour for complete extraction. The extract was filtered and was then refrigerated. Other chemicals such as 15 grams of sucrose, 0.05 grams of sodium benzoate, the preservative, 0.5 grams of Sodium lauryl sulfate, 5 ml of peppermint oil were added to the mouthwash preparation (Figure 1). Sucrose acts as the sweetening agent, Sodium benzoate is the preservative, Sodium lauryl sulfate is the foaming agent and Peppermint oil is the flavouring agent. 50 ml of Flax seed extract had been added to the above listed chemicals for the mouthwash preparation [36].

Anti-inflammatory activity:

To check the Anti inflammatory activity of the mouthwash 2ml of 1% bovine albumin was mixed with the prepared Flax seed mouthwash of different concentrations (10-50 µg/ ml). Then distilled water (390-350l) was added to the mouthwash solution of varying concentrations(10-50 µg/ ml) and it is incubated at room temperature for 10 minutes [37]. Then the mixture was heated at 55 degree Celsius for 20 min in a water bath and was cooled to room temperature. The colour changes were absorbed (Figure 2) and the absorbance values were recorded at 660 nm. Diclofenac sodium was used as an anti-inflammatory pharmacological control drug. Concentrations ranging from 10µg/ml to 50µg/ml of the control drug were tested against 10-50µg/ml concentrations of flaxseed mouthwash. All the tests were done in triplicate.

Results:

From the Figure 3, We can infer that the Flax seed mouthwash has showed about 40% protein denaturation inhibition at 10µl concentration, 51% inhibition at 20µl concentration, 69% inhibition at 30µl concentration, 78% inhibition at 40µl concentration and 88% inhibition at 50µl concentration. Similarly. The standard has shown 47% protein denaturation inhibition at 10µl concentration, 60% inhibition at 20µl concentration, 72% inhibition at 30µl concentration, 78% inhibition at 40µl concentration and 84% inhibition at 50µl concentration.

Discussion:

Inflammation is a defensive process carried by the hosts to maintain homeostasis. There are various pro-inflammatory and inflammatory mediators produced in the process. Any imbalance in the process can even damage the host's own cells and tissues. Here, the Omega 3,6 polyunsaturated fatty acids come into play. Omega 3 poly unsaturated compounds are compounds that can resolve inflammation. They can affect the lipoxygenase pathway of inflammation. Omega 3 polyunsaturated fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are known to produce many specialised proresolving mediators such as resolvins, protectins, and maresins that act as anti inflammatory agents. Resolvins act by downregulating the recruitment of Polymorphonuclear leukocytes and by decreasing the synthesis of pro inflammatory mediators. They can inhibit the trans endothelial migration of the Polymorphonuclear cells. It is based on a receptor mediated mechanism of inflammation resolution. They inhibit the chemotaxis of leukocytes, adhesion molecule expression and interactions and the inflammatory cytokine production and stimulate the phases of inflammation resolution, speeding up recovery from critical illnesses. There are many studies across the globe that demonstrate the anti-inflammatory and antioxidant properties of trans animal sources of Omega 3 polyunsaturated fatty acids whereas only a handful studies demonstrate their plant sources. One such

great plant source of Omega 3 polyunsaturated fatty acids is Flax seeds. It is shown that one ounce of Flax seeds contain about 6388 mg of Omega 3,6 polyunsaturated fatty acids which surpasses the daily requirement quantity[38]

Several studies have analysed the various pharmacological properties of flax seeds. An in vitro study by Geetika pant et al., has evaluated the anti cholesterol and antioxidant activity of the plant. A methanolic preparation of flax seeds has been tested for their anti cholesterol and antioxidant properties against simvastatin as positive control. The results show that flaxseeds could control cholesterol levels and can reduce oxidative damage, showing that it could contribute to new formulations with great anti cholesterol and antioxidant effects.[39]. A similar study has demonstrated the antioxidant properties of ethanolic preparations of flax seeds against the standard anti oxidant drug α -tocopherol. It is seen that flax seeds show a dose dependent antioxidant activity, with maximum effects at 500 μ g/ml concentration[40]. From these we could say that flax seeds are potent antioxidant agents. Though these studies have demonstrated the anti cholesterol and antioxidant properties of alcoholic extracts of flax seeds, Our study is the first one to evaluate and demonstrate the anti inflammatory properties of aqueous extracts of flaxseeds. Similarly, A study by Apoorva B Badiger et al., also demonstrates the anti microbial properties of flax seeds. The ethanolic extracts of the plant were tested against key periodontal pathogens such as Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans and Tannerella forsythia. The study shows that the flax seeds have shown a great bactericidal activity against Porphyromonas gingivalis at about 100 μ l/ml concentration and has exhibited bacteriostatic activity against Aggregatibacter actinomycetemcomitans and Tannerella forsythia [41, 42]. From this we could also understand the great anti microbial properties of the plant. Taking these into consideration, the current study has evaluated the in vitro antiinflammatory properties of aqueous preparation of flax seed mouthwash against the standard drug, diclofenac sodium. From our study results, we can infer that the Anti inflammatory activity of Flaxseed mouthwash is proportionately increasing with the increase in the concentration of the mouthwash. The Anti inflammatory activity of the mouthwash is found to be greater than the standard in all concentrations except at 50 μ l concentration. The margin of difference between the Flax seed mouthwash and the standard is found to be 17% higher in flaxseed mouthwash at 10 μ l, 7% higher in flaxseed mouthwash at 20 μ l, 5% higher in flaxseed mouthwash at 30 μ l, 2% higher in flaxseed mouthwash at 40 μ l and 2% higher in the standard at 50 μ l respectively for the increase in concentration of the solutions between 10 μ l to 50 μ l concentrations. At the 50 μ l concentration i.e the highest concentration used in our study, the margin of difference of protein denaturation between the Flax seed mouthwash and the standard is found to be about only 2% and the average difference is found to be 5.8% higher in Flaxseed mouthwash than the standard. From this we can say that Flax seeds have got extraordinary anti-inflammatory properties that can be used for various therapeutic purposes. Apart from the pharmacological properties, several studies had also studied different formulations of flax seeds. A study by Priyanka Mariam George et al., evaluated the use of flaxseeds as a daily dietary supplement in patients with gingival inflammation and had proved the significant effects of flax seeds on serum CRP levels [43]. A study by Pappu R et al., evaluated the biodegradable gel containing flax seed extract as a targeted drug delivery for management of chronic periodontitis. The results of the study demonstrated that the flax seed extract gel could be equally associated to the effectiveness of flurbiprofen gel in reducing inflammation [44]. A study by Deepika A et al., has evaluated the oil formulation of flax seeds against chlorhexidine mouthwash and concluded that flaxseed oil is an effective adjuvant in reducing plaque-induced

gingivitis[45]. From our study, it is shown that flax seeds are potent anti-inflammatory agents that could act better than the standard drug, diclofenac sodium. Future studies are to be undertaken for formulation of flaxseed mouthwash.

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Conflicts of interest:

The Authors declare no conflicts of interest.

Author contributions:

Anu Iswarya Jaisankar, contributed to the data acquisition, statistical analysis and drafting of the manuscript. Dr.Sankari Malaiappan, contributed to the design, editing and critical revision of the manuscript. Dr.S.Rajeshkumar, contributed to the supervision and proof reading of the manuscript.

Figures:

Figure 1: Preparation of Flaxseed Mouthwash

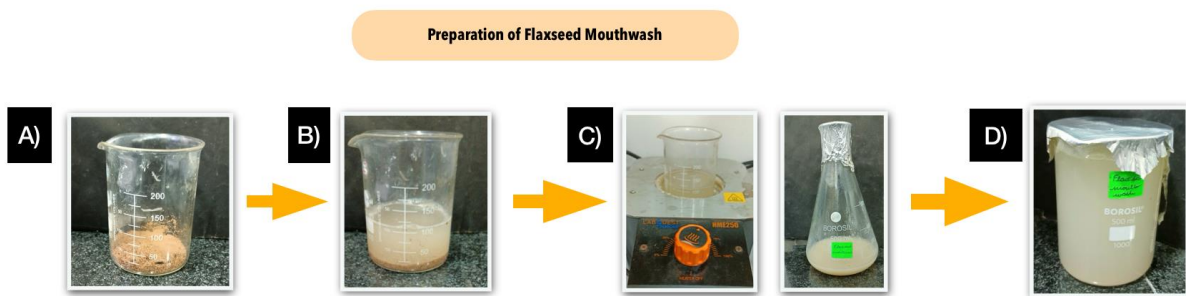


Figure 1: shows the steps involved in the preparation of flax seed mouthwash A) shows the beaker containing 10 grams of flaxseed powder B) shows the solution containing 10 gram of flaxseed powder and 100ml of distilled water C) shows the filtrate of flaxseed extract obtained after boiling D) shows the prepared flaxseed mouthwash

Figure 2: Anti inflammatory activity

Anti inflammatory activity

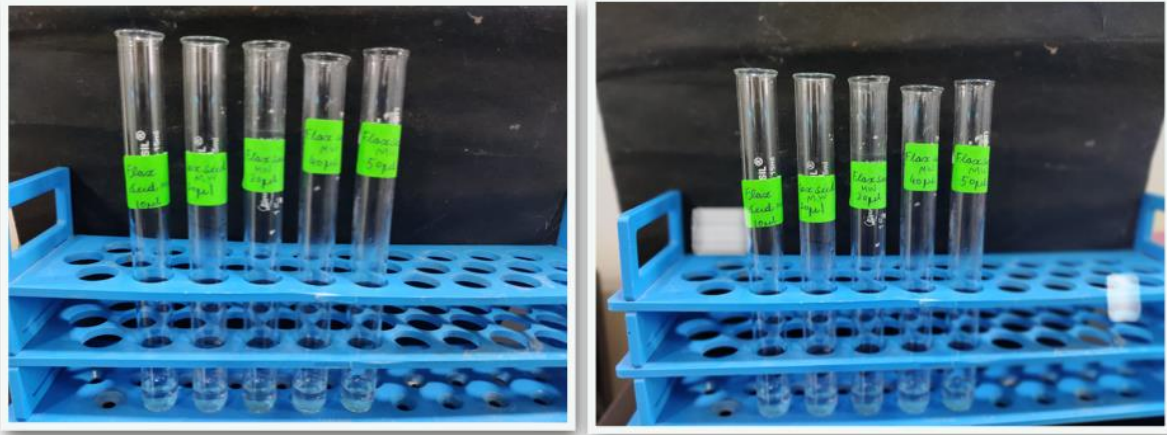


Figure 2: shows the color change observed in the anti inflammatory Activity (Before and After heating)

Figure 3: Comparison of Anti inflammatory activity of Flaxseed mouthwash against the standard drug, Diclofenac sodium

Comparison of Anti inflammatory activity of Flax seed mouthwash against the standard drug, Diclofenac sodium

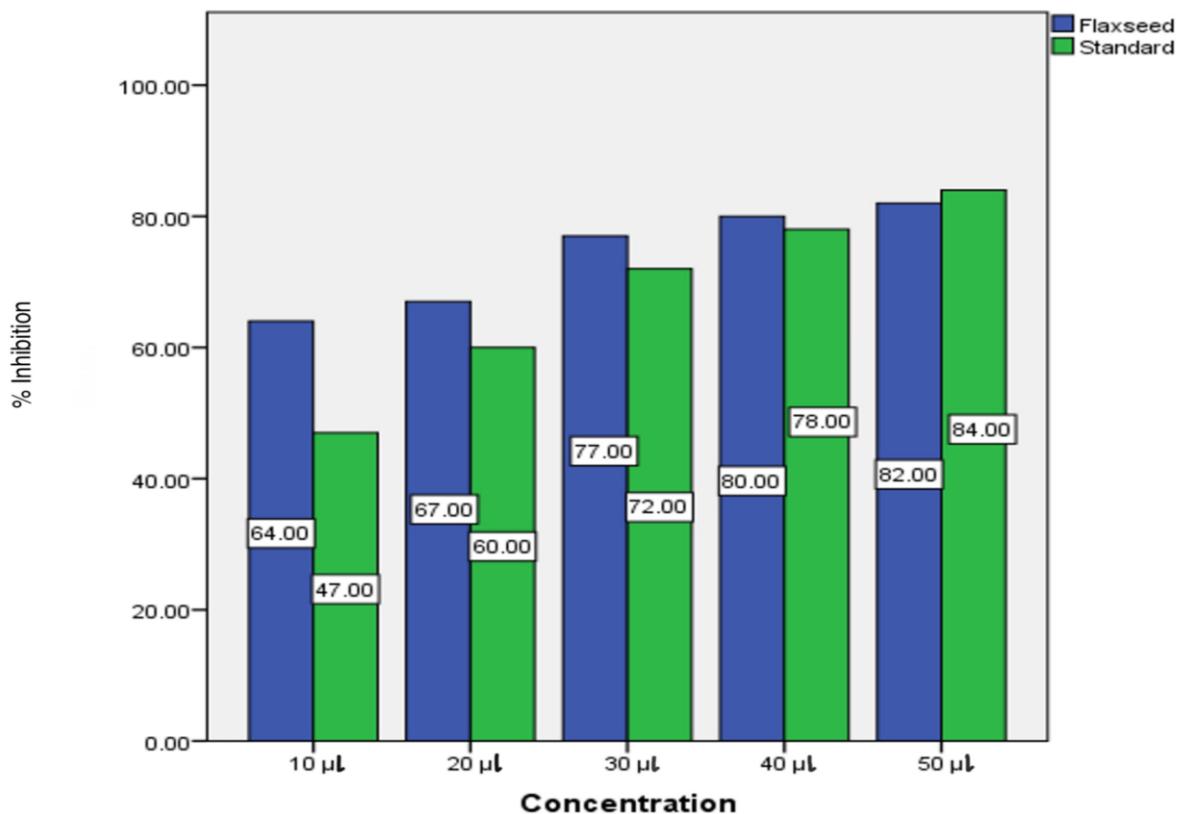


Figure 3: Comparison of Anti inflammatory activity of Flaxseed mouthwash against the standard drug, Diclofenac sodium. The blue zone shows the percentage protein denaturation of Flaxseed mouthwash and the Green zone shows the percentage protein denaturation of the standard. The x axis shows the concentration while the y axis shows the percent protein denaturation. Flax seeds show about 82% protein denaturation which is very close to the anti-inflammatory activity of the standard Diclofenac sodium, which is about 84%. From the chart, we infer that the anti-inflammatory activity of the Flax seed mouthwash increases proportionately with the increase in concentration and is greater than that of standard in the initial concentrations.

Conclusion:

From our study, we could conclude that Flax seeds have exemplary anti-inflammatory properties in comparison to the standard drug, Diclofenac sodium. They have shown a proportionate increase in the anti-inflammatory activity with the increase in concentration. They have shown to be a rich plant source of Omega 3,6 polyunsaturated fatty acids that could aid in resolution of localised inflammation. Our study forms the basis for future studies on clinical trial and evaluation of anti-inflammatory and antioxidant properties of flaxseed mouthwash. Further, it forms the basis for futuristic uses of flax seeds as periodontal dressings, sub gingival irrigants and as anti-inflammatory irrigants in flap surgeries.

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