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ORIGINAL ARTICLE

Comparison of flax seeds extract and fenugreek seed extract rinse against *streptococcus mutans* colonies

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ABSTRACT

Objective: To compare flax seeds extract and fenugreek seed extractrinse against streptococcus mutans colonies

Study setting: The bench work of this preclinical experimental study was carried out at Baqai Medical and Dental College Karachi., from December 2021 to January 2022.

Materials and Methods: It was a preclinical experimental study. Participants were divided into 4 groups randomization was performed in allocating the groups of participants. Film of plaque from labial surface of teeth of study participants was collected on sterile strips before and after the 2 hours of intervention. The extract preparation of both the plants and culture of *S. mutant*colonies were performed as per standard protocol. The extract was diluted at 1:4 for rinsing and study participants were given the rinses according to their groups. Data was analyzed by SPSS v. 22

Results: The growth of colonies was calculated on growth media plates in samples prior to rinsing and samples that were taken after the rinsing. The pre and post samples showed significant (p-value <0.05) decrease in number of colonies in positive control group (conventional rinse), flax seed extract group and fenugreek seed extract group as shown in table 1. The intra group comparison of negative and positive control showed significant difference in number of colonies and same was observed with the both the extract rinses. However, the positive control and flax seed extract and fenugreek seed extract comparison was insignificant that highlighted the equal potential of herbal extracts in reducing *the streptococcus mutans* colonies.

Conclusion: Flax seeds extract and fenugreek seed extract mouth rinses reduced the number of colonies equally as that of commercially available mouth rinse.

Keywords: Flax seed extract, Fenugreek Seed Extract, Rinse, Antibacterial, *streptococcus mutans*

INTRODUCTION

Trigonellafoenum-graceum L., also known as fenugreek, is an annual herbaceous plant that grows in multiple regions of the world including middle east, Iran, Pakistan, India and China[1]. Fenugreek belongs to *Fabaceae* family and different parts of this plant, specially seeds, are being utilized as condiment due to its aroma and flavor[2]. Its use in Chinese and Indian traditional medicine practices is also significant against various ailments[3]. *Linumusitatissimum,* commonly known as flax seeds or linseeds, belong to *Linnaceae* family[4]. Flax seeds are being cultivated widely in Australian, Asian and Chinese region[5]. Flax seeds are largely used for commercial linseed oil and fiber in more than 30 countries[6]. Like fenugreek, Flax seeds also has various bioactive constituents that have important pharmacological properties[7].

In the past few years, multiple phytonutrients of fenugreek with vast pharmacological effects are being researched and been proven for producing various therapeutic effects[8]. Furthermore, literature has reported fenugreek seeds as an antidiabetic, antibacterial, anti-lithogenic, antiulcer and anthelmintic agent. Reportedly, Fenugreek has also been in use as immunomodulatory agent, enzymatic pathway modifier, gastric stimulant, antifungal, hypocholesterolemic, hypoglycemic, antioxidant agent[9]. The presence of biologically active constituents in flax seeds, made it a topic of research that proved flax seeds to be beneficial and pharmacologically active against various diseases[10]. Literature has reported flaxseeds as an antioxidant, anti-inflammatory, antimicrobial, antiprotozoal, analgesic, anti-hyperlipidemic, anti-hyperglycemic and anti-tumor agent. Prophylactic and wound healing properties of flax seeds have also been reported. Traditionally flax seeds are used to treat multiple diseases like gastrointestinal, cardiovascular, urogenital, and respiratory disorders along with some neurological syndromes[11].

Along with major other pharmacological properties of fenugreek and flax seeds, antibacterial properties of these herbs against oral pathogens have also been reported[12,13]. Dental caries is one of the most prevalent chronic disease in humans worldwide and is also the most common disease of oral cavity in any age group[14]. *Streptococcus mutans* (*S. mutans*) is an anaerobic gram-positive bacterium that is acid tolerant and is most commonly associated with tooth caries disease which may further lead to various periodontal diseases[15]. Although, there is not enough literature reporting antibacterial activity of these herbs specifically against *Streptococcus mutans*but there are few studies which evaluated the antimicrobial activity of both fenugreek and flax seeds against *Streptococcus mutans*and compared it with the activity of commonly available mouth washes[13,16]. This will pave the way for using these natural herbs extracts in mouth rinses against oral pathogens specifically *Streptococcus mutans*.

MATERIALS AND METHODS

The bench work of this preclinical experimental study was carried out at Baqai Medical and Dental College Karachi., from December 2021 to January 2022. Sample size was calculated of 80 cases. Participants' plaque films were collected from the labial surfaces of their posterior teeth (Molars) and transported to a microbiology lab for cultivation in sterile containers. Flax seeds (1000-gram) and Fenugreek Seeds (1000-gram) were bought from local market of Karachi and verification number i.e. Specimen voucher 911 and 53 was allotted. The seeds of both plants were washed and shed dried and lastly were grinded to powder form. Ethanol 90% (2500ml) was used for the soaking of the grinded seeds and the soaking was donefor 15 days with intermittent shaking. The filtration of the mixture was performed with Whatman filter paper (number 1). Water bath method was used for processing at 60ºC. The well concentrated mixtures were obtained by drying them at 50ºC on rotary evaporator. The extracts were kept at 0-4ºC in an airtight container till usage. Concentrated extracts were diluted in distilled water at a 1:4 ratio for the dilution of extracts (Extract: Distilled water). Participants were not allowed to brush their teeth prior to taking the test. There were four groups of participants (negative control, positive control, flax seed extract group, and Fenugreek seed extract group), each with a random number of individuals (20 per group). A commercially available mouth rinse and distilled water were given to the negative control group and a commercially available flaxseed extract was given to the positive

control group. For post interventional analysis plaque filmwas obtained after two hours to record the pre and post interventional difference among all groups. The data was analyzed by SPSS v. 22, the inter and intra group comparison was done by applying ANOVA followed by post hoc tukey`s test. The pre and post experimental comparison was done by applying Paired t test, p-value<0.05 considered as significant and the data was analyzed at 95% confidence interval.

RESULTS

Out of eighty recruited participants males were 34 (42.5%) and female were 46 (57.5%), 29 ± 5.8 was the mean age of participants. Figure 1.Highlights the demographics of study participants. The pre and post samples showed significant (p-value <0.05) decrease in count of the colonies in positive control group (conventional rinse), flax seed extract group and fenugreek seed extract group as shown in table 1. The intra group comparison of negative and positive control showed significant difference in number of colonies and same was observed with the both the extract rinses. Table 2 shows the intra group comparison of experiment.

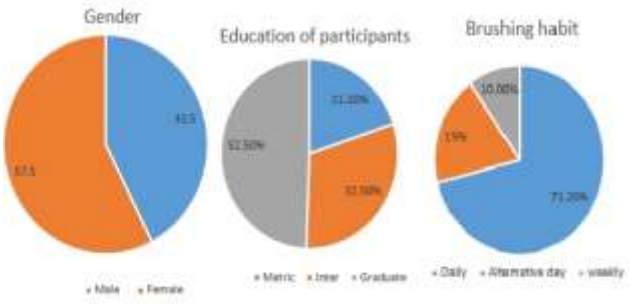

Figure 1. Demographic representation of participants

Table 1. Number of colonies in the negative control, positive control, and experimental groups before and after rinses

	Negative Control	Positive control	Flax seed extract	Fenugreek seed extract
Before	9×10^4	9×10^4	10×10^4	11×10^4
After	10×10^4	5×10^4	4×10^4	5×10^4
P value	0.417	0.001*	0.002*	0.001*

Table 2: Post hoc analysis among groups

Groups wise comparison				p- value
Negative control	10×10^4	Positive control	5×10^4	0.005*
Negative control	10×10^4	Flax seed extract	4×10^4	0.010*
Positive control	5×10^4	Flax seed extract	4×10^4	0.092
Negative control	10×10^4	Fenugreek seed extract	5×10^4	0.003*
Positive control	5×10^4	Fenugreek seed extract	5×10^4	0.143*
Flax seed extract	4×10^4	Fenugreek seed extract	5×10^4	0.193

DISCUSSION

Tooth decay or tooth cavity is one of the most widespread disease affecting people worldwide[17,18]. About 2 billion people are suffering from painful and destructive consequences of this highly neglected oral disease[19,20].

The number of colonies before and after rinse in positive control group treated with commercially available mouth wash was reduced remarkably and the p-value recorded was significant. A study comparing Spreng leaves ethanolic extract with conventional mouth wash also reported significant zone of inhibition that was 9.5mm against *Streptococcus mutans*colonies[21]. The number of *Streptococcus mutans*colonies were not reduced rather increased in the negative group treated with distilled water showing no bactericidal activity same as the results shown in another study done by Cristian Covarrubias[22].

Another research reported flax seeds extract as a potent antibacterial agent against different bacterial colonies including *Streptococcus mutans*, *Streptococcus Pyogenes*, *PseudomonaAerigenosa* and was comparable in efficacy to that of antibiotic *Streptomycin*[12]. The second herbal extract of our study, fenugreek extract, also showed positive antibacterial activity even slightly better than flaxseeds reporting p-value of 0.001* that is much remarkable. A research done to assess bactericidal activity of about 25 plants have reported antimicrobial effects of fenugreek against *Streptococcus mutans*[23].

At the end, antimicrobial activities of all the groups were compared with each other. Commercial mouth wash (positive group) reported remarkable antibacterial activity when compared with the distilled water (negative group). A study mentioned previously also compared distilled water with chlorhexidine (mouth wash) and did not find any bactericidal activity of distilled water when compared with mouth wash[21]. Comparison between flax seeds and negative group also reported significant antibacterial activity of flax seeds and zero inhibitory effects of distilled water. Almost same results were obtained when fenugreek was compared with the distilled water having no antibacterial activity. Our study did not report any significant difference in bactericidal activity of flax seeds extract and the conventional mouth wash with a non-remarkable p-value of 0.092. A study done by Yousefimanesh also reports that the antibacterial activity of flax seeds against *Streptococcus mutans* is comparable and almost equal to that of commercially available mouth wash[24]. Also the difference in antibacterial activity of fenugreek extract and mouth wash was not remarkable with a non-significant p-value of 0.143. Similar antimicrobial activity was recorded in between a herbal mouth wash containing fenugreek and a conventional mouth wash containing chlorhexidine when compared, in another research[13]. The flax seeds group and the fenugreek groups, both were having remarkable antibacterial activity. The flax seeds extract showed slightly more inhibitory effects against *Streptococcus mutans*when compared with fenugreek extract, showing a non-significant p-value of 0.193.

Now we can conclude that the fenugreek and flax seeds showed similar bactericidal activity when compared with conventional synthetic mouth wash. Chlorhexidine present in commercially available mouth washes have multiple reported adverse effects like dry mouth, tooth discoloration, mucosal irritation and cytotoxic effects[25].

CONCLUSION

Flax seeds extract and fenugreek seed extractmouth rinses reduced the number of colonies equally as that of commercially available mouth rinse. Their role should be validated further so that these herbal extracts can be commercialized as potential antibacterial mouth rinses.

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