Original Article

Comparison of the effectiveness of red ginseng herbal mouth rinse with chlorhexidine and saline in oral cancer patients: A pilot double-blinded randomized control trial

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Abstract

Background: Red ginseng is an herb with many medicinal properties and aids as a mouth rinse with fewer side effects than chlorhexidine.

Aim: The study aimed to compare the efficacy of red ginseng herbal mouth rinses with those of chlorhexidine and saline in oral cancer patients.

Materials and Methods: The present pilot study was a double-blinded randomized control trial with 45 histopathologically diagnosed oral squamous cell carcinoma patients divided into three groups: two intervention groups (herbal and chlorhexidine mouth rinse) and one control group (saline). Saliva samples for each patient were collected at baseline and after 14 days of using the mouth rinses. A microbiological examination of salivary samples was done by analysing total oral bacterial load along with specific counts for Porphyromonas gingivalis and Fusobacterium nucleatum at baseline and after the usage of mouth rinse.

Statistical Analysis: The data normality was analysed using the Shapiro–Wilk test, and following the normal distribution of data, parametric tests were employed. Paired t-test and one-way analysis of variance, followed by post hoc Bonferroni test, were used for inter-group and intra-group differences.

Result: There was a significant mean difference in total colony count, Fusobacterium nucleatum, and Porphyromonas gingivalis with oral hygiene index and gingival index improvement in the red ginseng herbal mouth rinse group when compared to the chlorhexidine and saline groups.

Conclusion: In this study, red ginseng mouth rinse exhibited an increased antibacterial effect compared to chlorhexidine and saline. Hence, red ginseng mouth rinse can be used in oral cancer patients to maintain oral health, thereby improving the prognosis of these patients.

Keywords: Chlorhexidine, ginseng, mouth rinse, oral squamous cell carcinoma, saline

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INTRODUCTION

The oral bacterial flora is essential in maintaining a normal oral microbial environment. Dysbiosis is a term used for microbial imbalance. It is characterised by the general loss of microbial diversity, loss of beneficial microbes, and the expansion of pathogenic microbes. Microbial dysbiosis leads to various diseases, such as dental caries and periodontal disease, and can eventually lead to oral cancer. The three most common pathogenetic processes involving microbiota in cancer development are the trigger of chronic inflammation and immune responses that can promote carcinogenesis, alteration of metabolic activity, which leads to the accelerated production of toxic metabolites, and virus latency abrogation.[1]

Vimal *et al*. and Hooper *et al* . suggested that certain microorganisms can cause carcinogenesis. They looked into the microbiome of the biofilm covering oral squamous cell carcinoma lesions and discovered that the tumour location had a higher anaerobic colony population. Porphyromonas, Actinomycosis, Fusobacterium, Prevotella, Veillonella, and Clostridium were among the anaerobe species that were more frequently isolated from tumour locations, while Enterobacteria, Haemophilus, and Streptococcus were among the aerobe species. These microbial mechanisms collectively contribute to the indirect induction of inflammation-mediated carcinogenesis and tumour progression.[1,2] Specifically, the presence of Fusobacterium nucleatum and Porphyromonas gingivalis is linked to the onset of cancer.[3]

According to Yao *et al*., Porphyromonas gingivalis deactivates the pro-apoptotic protein Bad through Akt while obstructing caspase-9 independently of Akt. Additionally, it generates cysteine proteinases that cleave the MMP-9 pro-enzyme, converting it into its fully active form.[4] This enzymatic process, which relies on NF-ϰB, triggers the degradation of the basement membrane structure, thus facilitating the migration and invasion of carcinoma cells, as reported by Inaba *et al*.[5]

Sakuri *et al*. findings indicate that Porphyromonas gingivalis amplifies the expression of prostaglandin-endoperoxide synthase through cyclooxygenase-2 gene expression, leading to the manifestation of inflammation symptoms by attracting pro-inflammatory mediators to the infection site.[6]

Moreover, Gallimidi *et al*. study on an animal model of chronic inflammation-associated tumourigenesis highlights how Fusobacterium nucleatum and Porphyromonas gingivalis manipulate the interleukin 6 signal transducer and transcription factor 3 axis of inflammatory signaling pathways. These bacteria additionally facilitate tumour progression by triggering an unconventional activation of immunocytes, resulting in the generation of reactive chemical species and subsequent DNA damage.[7]

Furthermore, Bashir *et al*. research reveals that Fusobacterium nucleatum not only promotes IL-6-mediated immune responses but also enhances the production of inflammatory cytokines like tumor necrosis factor α (TNF α), IL-1β, and IL-12, as well as IL-17 in response to lipopolysaccharide. The produced interleukins culminate in the up-regulation of inflammation-induced transcription factors, such as nuclear factor kappa beta (NF-ϰB), further fostering tumourigenesis at the infection site.[8] As per Cao *et al*. research, this microorganism triggers the activation of oncogenes, including cyclin D1 and myc, via the β-catenin pathway.[9]

To be more precise, Liu *et al*. uncovered that Fusobacterium nucleatum stimulates the release of TNF-alpha, IFN-gamma, IL-1beta, IL-6, and IL-17. In a separate study using a murine model, Kostic *et al*. observed that fusobacteria amplify tumour multiplicity and selectively attract tumour-infiltrating myeloid cells, known for their role in promoting tumour progression.[10]

Mouth rinses are solutions or liquids that reduce plaque accumulation and maintain oral hygiene. Most mouthwashes in the market are synthetic, including alcohol, and ingredients like chlorhexidine and triclosan that give users a burning feeling in their mouths have adverse effects if used for an extended period.[11] Alternatively, herbal mouth rinses are considered to overcome such side effects. Studies have reported that red ginseng herbal mouth rinses significantly reduced the total colony count compared to other commercially available synthetic mouth rinses in normal patients.[12] Jeddy *et al*. compared the efficacy of red ginseng herbal mouthwash and stated that the herbal mouth rinse exhibited maximal efficacy in reducing the bacterial load than chlorhexidine mouth rinse in healthy participants.[12]

Ginseng, categorised under the genus Panax and the family Araliaceae, is extensively employed in East Asia as an herbal medicinal plant owing to its exceptional medicinal attributes, as noted by Wang *et al*., and its recognised anti-tumour properties, as highlighted by Kang *et al*.[13,14] Natural dry ginseng is called white ginseng. Red ginseng is prepared by steaming fresh ginseng root to enhance its efficacy, safety, and preservation.[15] Red ginseng contains over 40

ginsenosides. The fundamental structure of ginsenosides is built around a steroidal core featuring various sugar moieties [such as glucose (glc), rhamnose (rha), xylose (xyl), and arabinose (ara)] linked to the C3, C6, and C20 positions. These ginsenosides are broadly categorised into two main groups, primarily distinguished by the functional group at the C6 position. The panaxadiol (PD) group (including Rb1, Rb2, Rc, Rd, Rg3, Rh2) comprises a hydrogen atom at C6, while the panaxatriol (PT) group (like Re, Rf, Rg1, Rh1) includes a C6 sugar side chain. Additionally, two minor classes of saponins exist, namely, the oleanolic acid group (e.g., Ro-C3: glc-glc and C28: glc) and the ocotillol group (e.g., pseudo ginsenoside F11-C6: glc-glc and C20/C24: epoxy). It is widely believed that the biological activities of each ginsenoside are closely linked to the type, position, and number of sugar moieties attached by the glycosidic bond at C3 and C6.[16]

Red ginseng, which is produced by steaming fresh ginseng without peeling the roots and subsequently drying it, can exhibit superior properties compared to white ginseng due to the presence of distinct ginsenosides (such as Rg3, Rg5, Rg6, Rh2, Rh3, Rh4, Rs3, and F4) generated during the steaming process.[17] These ginsenosides are essential for their medicinal value.[18] Ginseng is known for its anti-inflammatory, anti-carcinogenic, and anti-microbial properties. The anti-carcinogenic mechanism includes cell cycle arrest, induction of apoptosis, inhibition of proliferative signaling pathways, and angiogenesis.[19] The anti-microbial effect in oral microbes is because of their polysaccharide content and heat-transferred ginsenosides. They show anti-adhesive activity, anti-hemagglutination, and damage bacterial cell membrane integrity.[20]

PG-HMW and PG-F2, which are acidic polysaccharides derived from the roots of P. ginseng, were found to demonstrate the ability to hinder the attachment of Porphyromonas gingivalis to oral adenocarcinoma cells, including KB cells, as shown by Lee *et al*.[21] Additionally, Lee *et al*. observed that PG-F2 effectively inhibits Porphyromonas gingivalis-mediated hemagglutination.[22,23]

According to Sun *et al*., the administration of ginsenoside Rb3 effectively mitigated inflammation induced by Porphyromonas gingivalis lipopolysaccharide (LPS) by impeding the mitogen-activated protein kinase (MAPK)/AKT/nuclear factor (NF)-kB signaling pathway.[24] Furthermore, research has revealed that the steaming process of American ginseng leaves prompts the transformation of polar ginsenosides to less polar ones. The heat-induced altered saponins have been observed to disrupt cell integrity more readily and display enhanced antibacterial properties compared to their unprocessed counterparts. The fraction enriched with less polar ginsenosides (Rg2, Rg3, Rg6, F4, Rg5, and Rk1) resulting from the heat-induced transformation has demonstrated notable efficacy in inhibiting the growth of periodontal pathogens, including Fusobacterium nucleatum, Clostridium perfringens, and Porphyromonas gingivalis in studies, as highlighted by Xue *et al*.[25]

Furthermore, upon comparing and scrutinising the distinctions in the gut microbiome after the consumption of red ginseng extract, a notable reduction in Fusobacteria was observed ($P < 0.001$), as highlighted by Kim *et al*.[26] The study conducted by Cha in 2014 confirmed that sophoraflavaone G and RGE exhibit similar antibacterial activity, as evidenced by the MIC50 and MIC90 values determined for cariogenic and periodontal pathogenic bacteria.[27]

The present pilot study evaluated the efficacy of red ginseng herbal mouth rinse in improving gingival health and reducing microbial load in oral cancer patients and compared its efficacy with those of chlorhexidine and saline. The research conducted by Saito *et al*. illustrates that the pathogenic capability of Porphyromonas gingivalis experiences a synergistic increase when co-infected with Fusobacterium nucleatum.[28] Hence, this study focused on only two bacteria: Porphyromonas gingivalis and Fusobacterium nucleatum. This research project was original and innovative, and it helped determine the potential additive effect of red ginseng herbal mouth rinse as an adjunct therapy in oral cancer patients. Furthermore, this effort will contribute to a better understanding of the role of red ginseng in the prognosis of oral cancer patients.

The proposed research hypothesised that red ginseng herbal mouth rinses would be an effective and tolerable preventive treatment that will help maintain oral health in oral cancer patients compared to synthetic mouth rinses. This hypothesis was formulated based on the existing literature that a synthetic mouth rinse causes a burning sensation in the mouth and has side effects on long-term use.

MATERIALS AND METHODS

Study design

This study was a pilot double-blinded randomised controlled trial conducted on oral cancer patients with poor oral hygiene. The participants were recruited from various cancer centres in Chennai, and the study was conducted in a dental institution in Chennai, Tamil Nadu, India, in the

year 2022–2023. The study protocol was approved by the institutional ethical clearance board (Reference number: 230/2023/IEC/TMDCH, dated 07.08.2023). The study is registered in the Clinical Trial Registry-India with CTRI number CTRI/2023/09/057325. A block randomisation scheme randomised the eligible patients to the control or intervention arm. There was an initial assessment with a detailed case history and extra-oral and intra-oral examination. After the final histopathological diagnosis was obtained that the patient had oral squamous cell carcinoma, saliva samples for each participant were collected at baseline and after 14 days of using the mouth rinses. A microbiological examination of salivary samples was done by analysing the total oral bacterial load and specific counts for Porphyromonas gingivalis and Fusobacterium nucleatum at baseline and after using mouth rinse. All the data were collected and recorded.

Eligibility criteria

Patients who were histopathologically diagnosed with oral squamous cell carcinoma and over Stage 2 of TNM classification for oral cancer were included in this study. Patients were above the age of 40 years and included both males and females with any of the tobacco habits. All the patients were treated with modified radical neck dissection surgery, followed by radiotherapy. Pregnant or lactating women; patients with any systemic disease in the co-morbid conditions/on radiotherapy or chemotherapy; patients taking immunosuppressive agents or using systemic corticosteroids, antiresorptive drugs, anti-inflammatory drugs, or antibiotics; patients using any other mouth rinse; and patients with a history of allergies, human immunodeficiency virus (HIV), and metabolic and bone tissue disorders were excluded from the study.

Sample size and randomisation

This study involved 45 oral cancer patients, with 15 in each group. The patients were randomly disseminated into three groups using a block randomisation method. Group 1 (experimental) received red ginseng mouth rinse, group 2 (experimental) received chlorhexidine mouth rinse, and group 3 (control) received saline. The randomisation process involved five blocks, with nine participants in each block (herbal/chlorhexidine/saline). Eligible individuals were randomly assigned computer-generated numbers and then allocated to the treatment groups with equal probability by the clinical centre.

Blinding

This was a double-blinded study, meaning that the patients and the principal investigator were unaware of which group they belonged to. All the glass bottles of the mouth rinse were identical and without any labels to avoid potential bias.[5] All three bottles were marked with three different coloured crosses for identification by the second investigator. The red cross was for saline (Normal Saline 0.9% W/V Solution, Venus Remedies Ltd.), the green cross was for chlorhexidine mouth rinse (Chlorhexidine Gluconate (0.2% w/v), Icpa Health Products Ltd.), and the black cross was for red ginseng herbal mouth rinse (Dr. Dental care liquid, Jangin Pharm Co., Ltd.).

Methodology

At baseline (0 days), patients diagnosed with oral squamous cell carcinoma histopathologically were only included in the study. Every patient's case history was taken to collect information on name, age, gender, occupation, residence, chief complaint, medical history, personal habits, family history, dental history, and so on. A complete extra-oral and intra-oral examination was performed. The procedures were informed, and consent from the patients was obtained before the initiation of the study. Oral prophylaxis was performed for every patient before the beginning of the study. On the 0th day, the initial saliva sample was gathered, and the individual was instructed to use an 8 ml mouth rinse, gargling for 1 minute, twice daily for 14 days, as per the recommended instructions.[5] At the endpoint (15th day), saliva samples were collected after 14 days of mouth rinse use. The patient's follow-up was carried out only once because the oral cancer patients were not ready for a second follow-up as they had already undergone various treatments [Figure 1].

Saliva sample collection and processing

Two millilitres of unstimulated saliva samples were collected from the mouth directly into the Eppendorf on a single occasion by asking the patients to press their tongue against their palate so that freshly secreted saliva was collected in the sublingual region for 5 min. Saliva was collected from the Cancer Center and transported in a thermocol icebox to the microbiology lab within 2 hours. Saliva was centrifuged at 3500 rpm for 10 min to remove the excess mucus and unwanted particles. The samples were cultured in an anaerobic blood agar and incubated under vacuum jars at 37 degrees Celsius for 48–60 hours. At the end of 5 days, colony morphology and confirmation with gram stain, colony counting, and specific counts were done. The total colony count and bacterial count were counted before and after the mouth rinse use for Porphyromonas gingivalis and Fusobacterium nucleatum as these two bacteria were part of the bacterial spectrum that is involved in the prognosis of the oral squamous cell carcinoma. The gingival and oral hygiene indices were measured to assess gingival inflammation and oral hygiene before and after mouth rinse use.

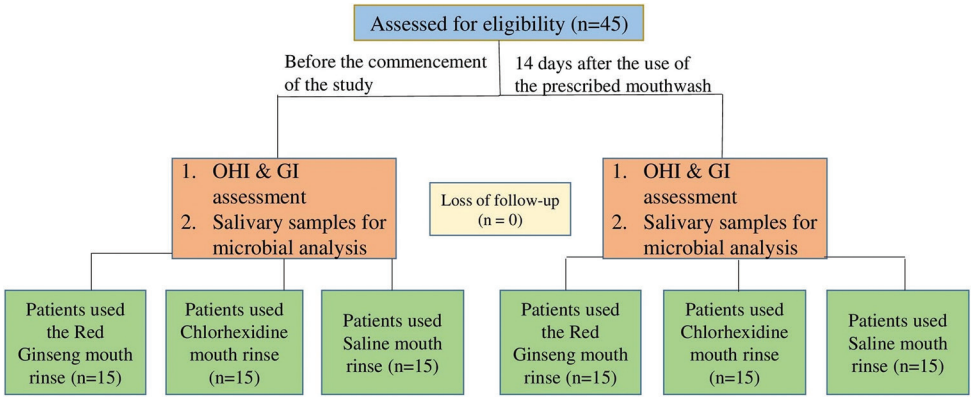

Figure 1: A consort flowchart

Primary outcomes

The outcomes measured include oral hygiene index (OHI), gingival index (GI), total bacterial colony count, and specific bacterial count for Porphyromonas gingivalis and Fusobacterium nucleatum.

Secondary outcomes

The outcomes measured include gingival inflammation, improvement in oral hygiene, reduction in the bacterial count, and any symptoms such as a burning sensation while using the mouth rinse.

Statistical analysis

Descriptive statistics were executed using SPSS version 27.0. The data normality was assessed utilising the Shapiro–Wilk test, and following the normal distribution of data, parametric tests were employed. Paired t-test and one-way ANOVA (analysis of variation), followed by post hoc Bonferroni test, were used for inter-group and intra-group differences.

RESULTS

Table 1 summarises the total colony counts before and after 14-day use of red ginseng, chlorhexidine and saline mouth rinses in patients with gingivitis. The summary statistics on the bacterial counts before and after 2-week use of mouth rinses were compared using paired t-tests. Mean and standard deviation were calculated for each group. ANOVA was used to analyse the inter-group differences in the reduction of bacterial counts after using red ginseng, chlorhexidine, and saline mouth rinses. The results showed that the mean bacterial count after using red ginseng for 2 weeks decreased by 6.52×10^3 CFU (colony forming unit). It was followed by chlorhexidine, with a mean CFU reduction of 4.18×10^3 CFU, while saline resulted in a decrease of 1.73×10^3 CFU. Subsequent ANOVA revealed that highly significant differences existed regarding the reduction capability of bacterial counts between the three mouth rinses (a *P*-value of 0.0001). The post hoc Bonferroni test was employed to determine the differences between the mouth rinses, and the results showed that the mean difference in the reduced bacterial count was the least between red ginseng and chlorhexidine (-2.20) in reducing bacterial counts after 2 weeks of use as a mouth rinse.

Tables 1 and 2 and Figure 2 show the difference in Porphyromonas gingivalis levels after using three different mouth rinses. The results showed that the mean bacterial count after using red ginseng for 2 weeks decreased by 5.30×10^3 CFU, which was followed by chlorhexidine, with a mean CFU reduction of 3.72×10^3 CFU, while saline resulted in a decrease of 1.43×10^3 CFU. One-way ANOVA revealed that highly significant differences existed regarding the reduction capability of bacterial counts between the three mouth rinses. The mean difference in reduced Porphyromonas gingivalis levels was the least between red ginseng and chlorhexidine (-1.48), and the maximum difference was seen between red ginseng and saline (-4.31×10^3 CFU).

They also show the difference in Fusobacterium nucleatum levels after using three different mouth rinses. The results showed that the mean bacterial count after using red ginseng for 2 weeks decreased by 1.06×10^3 CFU, which was followed by chlorhexidine, with a mean CFU reduction of 0.61×10^3 CFU, while saline resulted in a decrease of 0.34×10^3 CFU. One-way ANOVA revealed that highly significant differences existed regarding the reduction capability of bacterial counts between the three mouth rinses. The mean difference in reduced Fusobacterium nucleatum levels was the least between red ginseng and chlorhexidine (-0.24), and the maximum difference was seen between red ginseng and saline (-0.37×10^3 CFU). However, there was no significant difference in the reduction capacity of red ginseng and chlorhexidine ($P = 0.131$).

Table 1: Assessment of before and after use of selected mouth rinses for bacterial colony count and indices

Assessment	Herbal mouth rinse				Chlorhexidine mouth rinse				Saline mouth rinse			
	Before	After	MD (Before and After)	*P*[a]	Before	After	MD (Before and After)	*P*[a]	Before	After	MD (Before and After)	*P*[a]
Total Colony Count (* 10^3 CFU)	8.01±2.31	1.48±1.17	6.52	0.0001*	7.87±3.52	3.69±2.01	4.18	0.0001*	8.15±2.76	6.42±2.23	1.73	0.001*
Bacterial Count P. Gingivalis (* 103 CFU)	6.41±1.88	1.10±0.84	5.30	0.0001*	6.31±3.44	2.59±1.80	3.72	0.0001*	6.85±2.67	5.42±1.99	1.43	0.001*
Bacterial Count F. Nucleatum (* 10^3 CFU)	1.27+0.61	0.21+0.26	1.06	0.0001*	1.06±0.43	0.45±0.34	0.61	0.0001*	0.92±0.45	0.58±0.34	0.34	0.021*
Gingival Index	2.52±0.41	0.94±0.25	1.57	0.0001*	2.49±0.43	1.21±0.14	1.28	0.0001*	2.42±0.44	1.63±0.41	0.80	0.002*
Oral Hygiene Index	5.23±0.78	1.56±0.55	3.67	0.0001*	5.04±0.81	2.29±0.66	2.75	0.0001*	5.14±0.79	3.12±0.61	2.02	0.002*

[a]Paired *t*-test applied, [b]One-way ANOVA, Post hoc Bonferroni applied, **P*-value significant at $P<0.05$

Table 2: Inter-group comparison of before and after use of selected mouth rinses for bacterial colony count and indices

Bacterial Colonies (* 10^3 CFU)	Before	After	MD before and after use
	P[b]		*P*
Total colony count	0.965	0.0001*	Group 1 v/s 2 = -2.20 (0.007)* Group 1 v/s 3 = -4.93 (0.0001)* Group 2 v/s 3 = -2.73 (0.001)*
Bacterial count P. Gingivalis	0.849	0.0001*	Group 1 v/s 2 = -1.48 (0.049)* Group 1 v/s 3 = -4.31 (0.0001)* Group 2 v/s 3 = -2.82 (0.0001)*
Bacterial count F. Nucleatum	0.166	0.008*	Group 1 v/s 2 = -0.24 (0.131) Group 1 v/s 3 = -0.37 (0.007)* Group 2 v/s 3 = -0.13 (0.763)
Gingival index	0.829	0.0001*	Group 1 v/s 2 = -0.27 (0.042)* Group 1 v/s 3 = -0.68 (0.0001)* Group 2 v/s 3 = -0.41 (0.001)*
Oral hygiene index	0.813	0.0001*	Group 1 v/s 2 = -0.73 (0.006)* Group 1 v/s 3 = -1.56 (0.0001)* Group 2 v/s 3 = -0.83 (0.002)*

[a]Paired *t*-test applied, [b]One-way ANOVA, Post hoc Bonferroni applied, **P*-value significant at $P<0.05$

With regard to the difference in GI levels after the use of three different mouth rinses, the results displayed that there was a significant decrease in GI levels after the use of three mouth rinses. However, the reduction was found to be the highest in red ginseng (1.57), followed by chlorhexidine (1.28), and the least in the saline group (0.80). One-way ANOVA revealed that highly significant differences existed regarding the GI reduction capability between the three mouth rinses. The mean reduction was the least between red ginseng and chlorhexidine (-0.27), and the maximum difference was seen between red ginseng and saline (-0.68).

With regard to the difference in OHI levels after the use of three different types of mouth rinse, the results displayed that there was a significant decrease in OHI levels after the use of three mouth rinses. However, the reduction was found to be the highest in red ginseng (3.67), followed by chlorhexidine (2.75), and the least in the saline group (2.02). One-way ANOVA revealed that highly significant differences existed regarding the OHI reduction capability between the three mouth rinses. The mean reduction was the least between red ginseng and chlorhexidine (-0.73), and the maximum difference was seen between red ginseng and saline (-1.56).

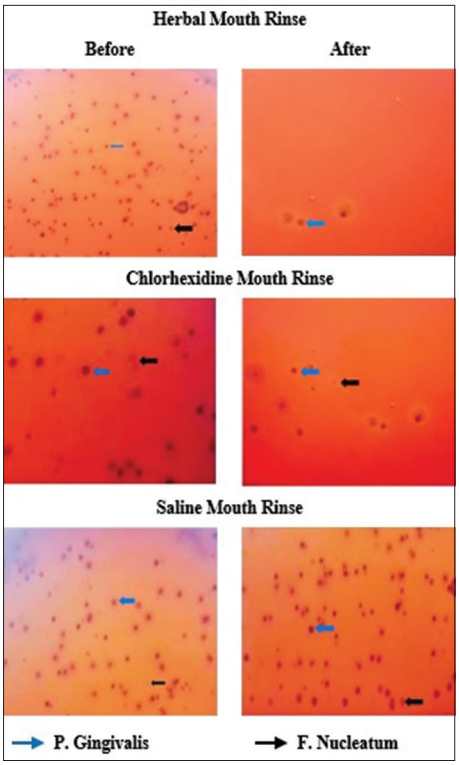

Figure 2: Bacterial colonies before and after using herbal, chlorhexidine and red ginseng mouth rinse

Figure 3 displays the maximum efficacy of red ginseng mouth rinse in reducing total bacterial load, specifically Porphyromonas gingivalis and Fusobacterium nucleatum, and improved the gingival and oral hygiene indexes.

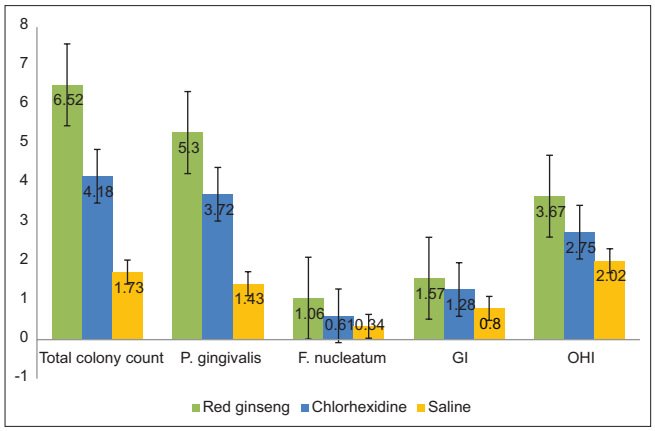

Figure 3: Mean differences of the total bacterial count, Porphyromonas gingivalis, Fusobacterium nucleatum, GI, and OHI before and after using mouth rinses

DISCUSSION

Oral squamous epithelial cell carcinoma (OSCC) is the most frequent malignant tumour type in the oral cavity. According to the World Health Organisation, there were 377,713 reported cases of oral and lip cancer in 2020. The primary risk factors for oral cancer initiation are the consumption of tobacco and alcohol, poor oral hygiene, and inappropriate dietary habits.[29]

According to Stasiewicz *et al*., oral squamous cell carcinoma (OSCC) has links to the presence of various oral bacteria, such as Porphyromonas gingivalis, Fusobacterium nucleatum, and Streptococcus sp., as well as specific viruses like human papillomavirus (HPV), human herpes virus 8 (HHV), herpes simplex virus 1 (HSV), and Epstein-Barr virus (EBV), along with yeast like Candida albicans. Additionally, the study suggests that certain members of the oral microbiota are connected with the occurrence of cancers in the oesophagus, stomach, pancreas, colon/rectum, and lung.[30]

Gingivitis, a prevalent inflammatory state of the gingiva, is often observed in patients with oral carcinoma who commonly experience compromised oral hygiene due to symptoms such as restricted mouth opening and pain. The decline in oral hygiene among these individuals frequently leads to the development of gingivitis or periodontitis, resulting in the loss of remaining teeth. For preventive and therapeutic purposes, mouthwashes may be prescribed to manage oral infections and mitigate inflammation. Notably, chlorhexidine, known for its broad-spectrum anti-microbial properties, demonstrates efficacy against various pathogens, encompassing Gram-positive and Gram-negative bacteria, aerobes and anaerobes, yeasts, fungi, and lipid-enveloped viruses, as highlighted by Parashar *et al*.[31]

The present pilot study evaluated the efficacy of red ginseng herbal mouth rinse in improving gingival health and reducing microbial load in oral cancer patients and compared its efficacy with those of chlorhexidine and saline. Red ginseng is a natural herb of high medicinal value native to China and Korea. Ginsenosides are bioactive chemical substances responsible for red ginseng's medicinal properties. According to Rokot *et al*., ginseng and its derivatives exhibit a spectrum of beneficial properties, including anti-inflammatory, antioxidative, anti-cancer, bacteriostatic, antiaging, antifatigue, antidiabetic, antistress, and antidepressant effects.[32] An essential property of this red ginseng is its anti-carcinogenicity. Red ginseng is part of the diet in Western countries as it can reduce cancer risk.[33] The major anti-cancer properties of red ginseng include cell cycle arrest, stimulation of apoptosis, and inhibition of angiogenesis.

In the present study, there was a statistically significant mean difference of 6.52×10^3 CFU in total bacterial count in the red ginseng group from the baseline and after 14 days of using the mouth rinse [Table 1]. The present study results were in accordance with the study done by Jeddy N *et al*. in normal patients.[12] The anti-microbial effect of ginseng in oral microbes is because of their polysaccharide content and heat-transferred ginsenoside. They showed anti-adhesive activity, anti-haemagglutination, and damage to bacterial cell membranes. The statistical study with ANOVA proved that highly significant differences existed regarding the reduction capability of bacterial counts between the three mouth rinses (*P* 0.0001).

The present study showed a significant mean difference of 5.30×10^3 CFU of Porphyromonas gingivalis count in patients who used red ginseng-containing herbal mouth rinses compared to other mouth rinses [Tables 1 and 2]. Porphyromonas gingivalis is a part of periodontal diseases and is also one of those bacteria that contribute to the prognosis of oral cancer.[18] In this study, ANOVA revealed that highly significant differences existed regarding the reduction capability of bacterial counts between the three mouth rinses.

Another specific bacterium that has been seen associated with oral squamous cell carcinoma is Fusobacterium nucleatum, and in our study, there was a mean difference of 1.06×10^3 CFU [Tables 1 and 2]. Recent evidence suggests that Fusobacterium nucleatum uses its trimeric autotransporter adhesin CbpF to inhibit T-cell function by activating CEACAM1.[29] CEACAM1 overexpression is associated with oral squamous cell carcinoma grade and inversely correlated with both overall and disease-specific

5-year survival. Therefore, Fusobacterium nucleatum may potentially drive the progression of oral cancer via multi-functional adhesion. ANOVA revealed that highly significant differences existed regarding the reduction capability of bacterial counts between the three mouth rinses. However, there was no significant difference in the reduction capacity of red ginseng and chlorhexidine ($P = 0.131$).

A gingival index is used to analyse the gingival health of a patient. There was a significant improvement in gingival health in all three groups [Tables 1 and 2]. The mean reduction was the least between red ginseng and chlorhexidine (-0.73). However, the mean difference and *P*-value convey that red ginseng-containing mouth rinses are more efficient than the other two mouth rinses. On the contrary, Subramaniam *et al*. study concluded red ginseng was comparable to chlorhexidine. As proved in this study, red ginseng effectively reduces total bacterial count and specific bacterial count of carcinogenic Porphyromonas gingivalis and Fusobacterium nucleatum compared to other mouth rinses. Their fewer adverse effect makes it a better choice of mouth rinse in case of oral cancer patients.[34]

The oral hygiene index was employed to analyse and rate a patient's oral hygiene. There was significant progress in oral hygiene in all three groups. However, the reduction was found to be the highest in red ginseng (3.67), followed by chlorhexidine (2.75), and the least in the saline group (2.02). One-way ANOVA revealed that highly significant differences existed regarding the OHI reduction capability between the three mouth rinses. So, looking at the mean difference and *P*-value [Tables 1 and 2], it can be concluded that red ginseng-containing mouth rinses effectively improve oral hygiene compared to the other two mouth rinses.[35]

Limitations of the study

- The study's sample size was small as it was a pilot study.
- Freshly diagnosed oral cancer patients were involved, and many patients were not ready to participate.
- Many oral cancer patients did not fulfill the inclusion and exclusion criteria, so they could not participate.

CONCLUSION

Be natural and be with nature! Modern day is an era where problems gain solutions from tradition and nature. Red ginseng is a natural and traditionally used herb that has a lot of medicinal value. Especially, their anti-inflammatory, anti-microbial, and anti-carcinogenic properties make them a key substitute for synthetic medicines. The study's results proved that the herbal mouth rinse effectively reduced the harmful bacteria in the oral cavity and reduced the colony count of certain bacteria, which promotes carcinogenesis. Hence, it can be used in oral cancer patients to prevent carcinogenesis progression, improving the prognosis. However, a bigger sample size and in-depth study are further required to prove the effects are specific as this was a pilot study performed on a few patients.

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Conflicts of interest

There are no conflicts of interest.

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