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Dentistry Section

# Evaluating the Efficacy of Tulsi and Aloe Vera in the Management of Oral Submucous Fibrosis and their Correlation with Salivary Levels of Iron and Lactate Dehydrogenase: A Prospective Study

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## ABSTRACT

**Introduction:** Oral Submucous Fibrosis (OSMF) is a chronic, potentially malignant condition marked by fibrous bands in the oral cavity. It causes burning sensation with spicy foods, blanching of the oral mucosa, and progressive stiffness, leading to restricted mouth opening. Natural remedies like Tulsi (*Ocimum sanctum* Linn), and aloe vera are known for their antioxidant, anti-inflammatory, and healing properties. However, clinical evidence evaluating their efficacy in OSMF management remains limited.

**Aim:** This study Aimed to evaluate the effectiveness of natural antioxidants, "Tulsi" and "aloe vera", in managing OSMF and their association with changes in salivary levels of iron and Lactate Dehydrogenase (LDH).

**Materials and Methods:** The present prospective study was conducted from January 2020 to September 2021 at Drs Sudha & Nageswara Rao Siddhartha Institute of Dental Sciences, Andhra Pradesh, India. Sixty patients were divided into two groups: Group A received topical Tulsi paste, while Group B received aloe vera gel. Both groups included 28 males (93.3%) and 2 females (6.7%) with mean ages of 40.0 and 42.7 years, respectively. Salivary iron and LDH levels were measured before and after treatment. Clinical parameters such as mouth opening, burning sensation, and tongue protrusion were assessed bi-weekly over six weeks. Data was analysed using IBM Statistical Package for Social Sciences (SPSS) Version 20 software with descriptive statistics, Chi-square tests, t-tests, and Analysis of Variance (ANOVA). Statistical significance was set at $p \leq 0.05$.

**Results:** Significant improvements in clinical parameters were observed in both groups ($p<0.001$). The Tulsi group showed more pronounced enhancements in mouth opening ($p<0.001$) and tongue protrusion compared to the aloe vera group. Salivary iron levels increased significantly in both groups ($p<0.001$), with no significant intergroup difference ($p=0.39$). However, the Tulsi group exhibited greater reductions in salivary LDH levels ($p=0.001$) compared to the aloe vera group ($p=0.009$).

**Conclusion:** Topical application of Tulsi paste and aloe vera gel effectively reduced burning sensation while improving mouth opening and tongue protrusion. Tulsi demonstrated superior results compared to aloe vera in clinical outcomes and salivary LDH reduction, highlighting its potential for OSMF management.

**Keywords:** Atrophy, Herbal treatment, *Ocimum sanctum*, Premalignancy

## INTRODUCTION

The OSMF is a premalignant, progressive disease causing reduced mouth opening, first described by Susrutha in 600 BC and later termed by Joshi in 1953 [1,2]. OSMF is triggered by areca nut, tobacco use, and nutritional deficiencies, it affects 0.2-0.5% of Indians with a 7-30% malignancy rate [2]. In 1996, Pindborg and Sirsat defined OSMF as "an insidious, chronic disease affecting any part of the oral cavity and sometimes pharynx. Although occasionally preceded by and associated with vesicle formation, it is always associated with a juxta-epithelial inflammatory reaction followed by fibroelastic change of the lamina propria with epithelial atrophy leading to stiffness of the mucosa causing trismus and inability to eat" [1-3].

Arecoline in areca nut is considered to be the primary aetiological factor in the pathogenesis of OSMF [4]. The nitrosamine alkylates in arecoline lead to the formation of cyanoethyl, which further binds DNA to stimulate fibroblastic proliferation and increase collagen formation. These Alkaloids show their impact on collagen metabolism by enhancing the cross-linkage of collagen fibers. Fibrogenesis is produced in the cells by copper due to the upregulation of the Lysyl oxidase pathway, Prolonged exposure to chemical irritants leads to malignant transformation [5-7].

Different authors give several classifications of OSMF based on clinical and histopathological features. In the present study, OSMF patients were taken based on Khanna JN and Andrade NN's clinical classification. According to it, Grade-1 is considered a very early stage for which treatment is not required and Grade-4 is considered very advanced and cannot be treated by medical management alone. So, Grade-2 and Grade-3 OSMF patients were included in the present study [8].

Ayurveda is an Indian traditional natural system of medicine that today has become one of the emerging treatment modalities worldwide for treating and preventing various oral and other diseases [9]. Different natural herbal ingredients like aloe vera Tulsi have evolved in the last decade for the treatment of OSMF.

Aloe vera is a plant that belongs to the Liliaceae family, of which there are about 360 species. Only two commercially available species are Aloe barbadensis Miller and Aloe arborescence. Aloe vera contains several vitamins that include vitamin A, C, E, which have antioxidant properties [10], and help to combat destructive free radicals. Fatty acids, salicylic acid, and hormones such as auxins and gibberellins are also found in A. Vera, which help to reduce inflammation. Aloe vera contains lupeol, a sterol that functions as an antibacterial, analgesic, and anti-inflammatory.

Aloesin derivatives such as isorabaichromone, feruoylaloesin, and p-coumaroylaloesin have high free radical and superoxide anion activity [11].

Aloe vera is a mannoprotein that contains 'wound-healing hormones, which are a group of amino acids. The polysaccharides in the gels of leaves also have gastroprotective and anticancer properties. A. Vera has been helpful in OSMF, oral lichen planus, and burning mouth syndrome [12]. Tulsi (basil or *Ocimum sanctum* Linn), within the Ayurveda, is known as "The Incomparable One" and The Queen of Herbs". It also aids in the prevention of malignancies produced by harmful substances by lowering DNA damage and activating apoptosis in precancerous and cancerous cells, minimising the growth of experimental tumours and improving survival. Tulsi also protects against toxic substance harm and helps the body transform and eliminate them more efficiently by increasing the activity of liver detoxification enzymes such as the cytochrome P450 enzymes, which deactivate toxic chemicals and allow them to be safely expelled [13,14].

The aetiology of OSMF has been linked to nutritional inadequacies, particularly iron and vitamin deficits. Iron is necessary for the overall integrity and health of the digestive tract epithelium and appropriate enzymatic processes. Haemoglobin levels, and in particular, serum and serum irons levels, are considered biological indicators for nutritional assessment. Deficiencies in iron, vitamin B12, and folate can impair the integrity of the oral mucosa.

"LDH is a cytoplasmic enzyme found in almost all bodily tissues, and its levels rise significantly during tissue damage." Its main job is to catalyse the conversion of lactate into pyruvate [15]. LDH levels in tissues are 500 times higher than in serum. Leakage of the LDH enzyme from even a small quantity of injured tissue can raise the level of LDH to a large level, demonstrating its utility as a tissue damage indicator. Salivary glands produce salivary LDH oral epithelial cells are considered the primary source. Saliva diagnostics has ability to detect cancer based on the close contact between saliva and the mucosa where cancer develops [16] Treatments include iron, vitamins, corticosteroids, and Ayurvedic remedies like Tulsi and aloe vera, which possess anti-inflammatory and antioxidant properties [17,18]. OSMF is associated with reduced salivary iron levels, which are linked to impaired collagen synthesis. LDH is a key biomarker of tissue damage, often used to assess cellular injury in OSMF [19]. Many treatment protocols have been proposed to alleviate the signs and symptoms of OSMF. Conventional therapies, including corticosteroids and other pharmacological therapies, have shown limited success, particularly in early stages. Consequently, there is growing interest in alternative natural treatments that are cost-effective and minimal side-effects. This study aims to evaluate the effectiveness of tulsi paste and aloe vera gel in managing OSMF by assessing clinical outcomes and biochemical markers. While previous studies have explored these herbs in other oral conditions, this research uniquely investigates their comparative efficacy in OSMF.

The primary objectives of this study were to assess the clinical improvement in burning sensation, mouth opening, and tongue protrusion in OSMF patients treated with Tulsi paste and aloe vera gel. The secondary objectives include evaluating changes in salivary iron and LDH levels before and after treatment with these herbal treatments.

**Null hypothesis ($H_2$):** There is no significant difference in clinical outcomes (burning sensation, mouth opening, tongue protrusion) and biochemical markers (salivary iron and LDH levels) between the Tulsi and aloe vera treatment groups in patients with OSMF.

**Alternate hypothesis ($H_2$):** There is a significant difference in clinical outcomes and biochemical markers between the Tulsi and aloe vera treatment groups, indicating that one treatment is more effective than the other in managing OSMF symptoms.

## MATERIALS AND METHODS

The present prospective study was conducted in Department of Oral Medicine and Radiology at Drs Sudha & Nageswara Rao Siddhartha Institute of Dental Sciences, Chinnaoutpalli, Andhra Pradesh, India during the period commencing from January 2020 to September 2021. The study was ethically approved by the institution's Ethical Review Committee with number O.C.No./IEC/10/2019.

**Sample size calculation:** Calculation to determine the sample size was performed using G* power 3.1.9.7 software. The calculations were based on correlation value of 0.519 based on the results of my pilot study, an alpha level of 0.05, and the desired power of 80%. The total sample size was sixty divided into two groups 30 in each. Patients were informed about the procedure and asked for their consent to participate in the study and only those who agreed were included in the study.

**Inclusion criteria:**

- Subjects who have clinical signs and symptoms of OSMF, stage 2 and stage 3 (classification according to Khanna and Andrade) [8].
- Patients who have not used any other medication for OSMF previously.
- Patients who gave a positive history of areca nut intake in the form of pan, gutkha.

**Exclusion criteria:**

- Patients with stage 1 and stage 4 OSMF were excluded.
- Patients who gave a positive history of being treated for the same complaint, in the form of local applications, intraoral injections, or any systemic administration of drugs.
- Patients with underlying systemic diseases like diabetes, hypertension, bleeding, and cardiovascular diseases.
- Patients with a history of tobacco consumption in any other form were excluded from the study.

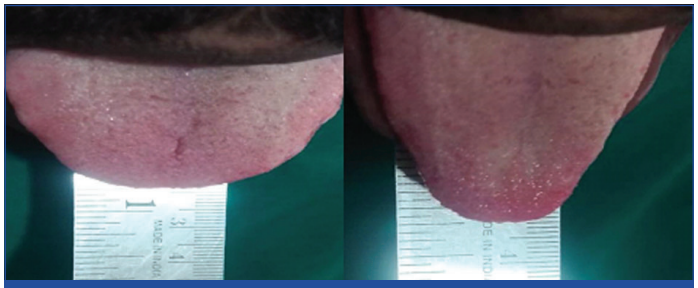
**Sample distribution:**

- A total of sixty patients were randomly assigned into two groups:
- Group A (Tulsi group): 30 patients were given Tulsi paste (prepared by mixing two scoops of Tulsi powder with one scoop of glycerine) for topical application.
- Group B (aloe vera group): 30 patients were given 95% pure aloe vera gel for topical application.

**Outcome measures:**

- Primary outcome: The primary outcomes of this study include clinical improvements in burning sensation, mouth opening, tongue protrusion, and. These parameters were assessed at baseline and at bi-weekly intervals for six weeks.
- Burning sensation: It has been assessed with the help of a Visual Analogue Scale (VAS), a psychometric response scale used to record patients subjective characteristics that cannot be measured directly [2]. It allows patients to rate their burning sensation level ranging from 0 (no sensation) to 10 (worst sensation).
- Mouth opening: It is recorded in millimeters by measuring the distance between the right upper incisor's incisal edge and the lower incisal edge [Table/Fig-1] [20].
- Tongue protrusion: It is assessed by measuring the distance from the tip of the incisal edge of the lower incisors to the tip of the tongue when the tongue is in a wholly protruded position, Nagesh and Bailoor described this method in 1993 [Table/Fig-2] [20].
- Secondary outcome measures: The secondary outcomes involved assessing changes in salivary iron and LDH levels. Salivary samples were collected pre- and post-treatment

**[Table/Fig-1]:** Pre and post-treatment mouth opening.



**[Table/Fig-2]:** Pre- and post-treatment tongue protrusion.

using the unstimulated whole saliva method. These samples were analysed for iron and LDH concentrations using atomic absorption spectrometry. Salivary iron levels were measured to assess changes in iron metabolism, while LDH levels were evaluated as an indicator of tissue damage and oxidative stress. The changes in these biochemical parameters were correlated with clinical improvements in burning sensation, mouth opening, and tongue protrusion to understand the potential biochemical effects of the treatments on OSMF.

### Study Procedure

After assessing and recording the pretreatment parameters, patients of both Group A (Tulsi) and Group B (aloe vera) self-applied their respective treatments topically thrice daily for six weeks. Both medications Tulsi paste and aloe vera gel are manufactured by Sahasra Yogam Health Sciences Pvt., Ltd.

**Method of saliva sample collection:** The saliva samples were collected from all sixty patients before and after treatment. For each collection, 5ml of unstimulated whole saliva was collected using spit method in a sterile container over a period of five minutes. The collected salivary samples were subjected to analysis of iron and lactose dehydrogenase levels by using atomic absorption spectrometry. Studies shows that the mean normal salivary iron level is 94.18±62.90 µg/dL [21], and salivary LDH level is 201.3700±89.1439 IU/L [22].

The measuring parameters were assessed bi-weekly and were recorded over a period of six weeks. Thus, post-treatment values were obtained at the end of the 6th week.

## STATISTICAL ANALYSIS

Data were analysed using IBM SPSS Version 20 software (IBM SPSS, IBM Corp., Armonk, NY, USA). Descriptive statistics, chi-square tests, independent samples t-tests, paired t-tests, and repeated measures analyses of variance were done to analyse the study data. Bar charts and line diagrams were used for the presentation of data. A p-value of ≤0.05 was considered statistically significant.

## RESULTS

The mean age of the study sample in group A was 40±11.32 years and in group B was 42.7±11.46 years; this difference was not statistically significant. This is illustrated in [Table/Fig-3].

Out of 30 subjects in group A, 28 (93.33%) were males, and 2 (6.67%) were females. In group B, 28 (93.33%) were males, and 2 (6.77%) were females (Chi-square test value=0, p-value=1). There was no significant variation in gender distribution across the study groups, with 93.3% of the sample being males and 6.77% females in both the study groups. This is illustrated in [Table/Fig-4].

**Stage-wise distribution of the data:** Out of 30 subjects in group A, 20 (66.7%) of patients suffer from OSMF stage II, and 10 (33.3%) suffer from OSMF stage III. In group B, 16 (53.3%) of patients suffered from OSMF stage II, and 14 (47.7%) suffered from OSMF stage III. In comparison of the OSMF stage between the two study groups; no significant difference was noted with the majority of subjects identified as having stage II OSMF in both group A (66.7%) and group B (53.3%). This is illustrated in [Table/Fig-5].

**Comparison of vas scores:** In the present study, all the patients presented with the burning sensation with mean values 6.53±1.69 in group A, 6.23±1.35 in group B. At the end of the treatment median scores of both groups was zero. About VAS scores, while there were no significant differences between the groups at baseline, first, and second subsequent observations, there was a considerable difference in the third assessment with lesser mean scores in group A (0.1±0.3) compared to group B (0.4±0.6). This is illustrated in [Table/Fig-6].

Pair-wise comparison in both groups showed a significant decline in the mean VAS scores from baseline (6.53±1.69 in group A and 6.23±1.35 in group B) to the third assessment (0.1±0.305 in group A and 0.47±0.62 in group B). The repeated measures analysis of variance (ANOVA) test showed a statistically significant reduction in both groups (p<0.001). This is illustrated in [Table/Fig-7].

**Comparison of the mouth opening:** Regarding mouth opening (in mm), at baseline, for group A mean±SD value was 30.0±5.6 and for group B mean±SD value was 25.9±4.6. At the end of treatment, group A mean±SD value was 35.0±4.9 and group B mean±SD was 31.4±4.6. group A demonstrated significantly higher values for mouth opening at all-time points than group B. This is illustrated in [Table/Fig-8].

| | Group A (n= 30) | | | | | Group B (n=30) | | | | | T-value | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age in years | Min | Max | Mean±SD | Median | IQR | Min | Max | Mean±SD | Median | IQR | -0.916 | 0.362 |
| | 23.0 | 62.0 | 40±11.32 | 39.0 | 19.0 | 22.0 | 65.0 | 42.7±11.46 | 44.0 | 20.0 | | |

**[Table/Fig-3]:** Comparison of mean age between the study groups, mean, median and Interquartile Range (IQR).
Independent samples t-test; p≤0.05 considered statistically significant

| Group | Gender | N (%) | Chi² test value | p-value |
|---|---|---|---|---|
| A | Male | 28 (93.33) | 0.0 | 1.00 |
| | Female | 2 (6.67) | | |
| B | Male | 28 (93.33) | | |
| | Female | 2 (6.67) | | |

**[Table/Fig-4]:** Comparison of gender distribution between the study groups.
Chi-square test; p≤0.05 considered statistically significant
Stage-wise distribution of the data:

| Stage | Group A | | Group B | | $\chi^2$ test value | p-value |
|---|---|---|---|---|---|---|
| | Count | % | Count | % | | |
| II | 20 | 66.7% | 16 | 53.3% | 1.11 | 0.29 |
| III | 10 | 33.3% | 14 | 47.7% | | |
| Total | 30 | 100% | 30 | 100% | | |

**[Table/Fig-5]:** Comparison of OSMF stage between the study groups.
Chi-square test; p ≤0.05 considered statistically significant.

Pair-wise analyses revealed a significant increase in mouth opening from baseline to the third assessment in group A compared to group B. [Table/Fig-9].

| Time | Group | N | Mean±SD | SE | Mini-mum | Maxi-mum | T-value | p-value |
|---|---|---|---|---|---|---|---|---|
| VAS Baseline | A | 30 | 6.53±1.69 | 0.31 | 4 | 9 | 0.759 | 0.452 |
| | B | 30 | 6.23±1.35 | 0.24 | 4 | 9 | | |
| VAS 1 (1st visit) | A | 30 | 4.27±1.48 | 0.27 | 2 | 7 | 0.148 | 0.927 |
| | B | 30 | 4.23±1.3 | 0.23 | 2 | 7 | | |
| VAS 2 (2nd visit) | A | 30 | 2.33±1.12 | 0.205 | 0 | 4 | -0.698 | 0.49 |
| | B | 30 | 2.53±1.1 | 0.202 | 0 | 5 | | |
| VAS 3 (3rd visit) | A | 30 | 0.1±0.305 | 0.056 | 0 | 1 | -2.94 | 0.006* |
| | B | 30 | 0.47±0.62 | 0.115 | 0 | 1 | | |

**[Table/Fig-6]:** Comparison of VAS scores between the study groups assessed at bi-weekly interval.
Independent samples t-test; p≤0.05 considered statistically significant; Asterisk (*) denotes statistical significance. VAS: Visual analogue scale; SD: Standard deviation; SE: Standard error

| Comparison with baseline values | Group A (F value = 24.6) | Group B (F value = 18.9) |
|---|---|---|
| | p-value | p-value |
| TP Baseline - TP1(1st visit) | <0.001* | <0.086 |
| TP Baseline - TP2 (2nd visit) | <0.001* | <0.086 |
| TP Baseline - TP3 (3rd visit) | <0.001* | <0.086 |

**[Table/Fig-7]:** Pairwise comparison of VAS scores.
Repeated measures ANOVA; p ≤ 0.05 considered statistically significant; Asterisk (*) denotes statistical significance. VAS: Visual analogue scale.

**Comparison of tongue protrusion:** About tongue protrusion (in mm), while there were no significant differences between groups at baseline (p-value-0.21) and the first subsequent observation (p-value-0.13), significant differences were noted in the second (p-value-0.03) and third observations (p-value-0.009) with higher mean values in group A. This is illustrated in [Table/Fig-10]. On pairwise comparison of tongue protrusion with change in time, significant improvement was noted in group A when compared to group B. This is illustrated in [Table/Fig-11].

**Salivary values:** There was no difference in both groups' pre, and post-salivary iron mean values (p-value-0.74 and 0.39). Whereas for salivary LDH, group A demonstrated significant changes in pre 563.4±186.39 (Mean±SD) and post treatment values 387.85±173.03 (Mean±SD). This is illustrated in [Table/Fig-12].

In intra-group comparisons, it was observed that the salivary iron values increased significantly with time in both the study groups; at the same time, there was a significant decline in salivary LDH values in both groups. This is illustrated in [Table/Fig-13].

## DISCUSSION

Among the study participants, the mean age of group A is 40.0, and group B is 42.7. [Table/Fig-3]. According to the study done by Shah PH et al., the mean age of study participants is 40.13±12.78 years [23].

In the present study, 93.3% were males and 6.7% were females. There was no significant difference in gender distribution between the two study groups (Chi-square test value=0, p-value=1). This indicates that gender was equally distributed in both groups, ensuring that any observed effects in the study are unlikely to be influenced by gender as a confounding factor [Table/Fig-4]. It is usual to find male predominance in OSMF due to the habit of tobacco and betel nut chewing. The current study results are somewhat similar to the study of Shah PH et al., where the male: female ratio is 7.33:1 [23].

| Variables | Group A | | | | Group B | | | | T-value | p-value |
|---|---|---|---|---|---|---|---|---|---|---|
| | Min | Max | Mean±SD | IQR | Min | Max | Mean±SD | IQR | | |
| MO at Base line (mm) | 18.0 | 37.0 | 30.0±5.6 | 9.3 | 16.0 | 33.0 | 25.9±4.6 | 7.5 | -2.759 | 0.008* |
| MO1 (1st visit) (mm) | 22.0 | 39.0 | 32.0±5.4 | 10.0 | 18.0 | 35.0 | 27.9±4.5 | 8.0 | -2.95 | 0.005* |
| MO2 (2nd visit) (mm) | 24.0 | 40.0 | 33.0±5.2 | 9.3 | 20.5 | 37.0 | 29.7±4.5 | 8.0 | -2.91 | 0.005* |
| MO3 (3rd visit) (mm) | 24.0 | 41.0 | 35.0±4.9 | 7.3 | 21.5 | 39.0 | 31.4±4.6 | 7.6 | -2.59 | 0.012* |

**[Table/Fig-8]:** Comparison of mean distribution of Mouth opening (MO) (in mm) between the study groups.
Independent samples t-test; p≤0.05 considered statistically significant; Asterisk (*) denotes statistical significance. MO: Mouth opening; SD: Standard deviation; IQR: Interquartile range; Min: Minimum; Max: Maximum; mm: Millimeters.

| Comparison with baseline values | Group A (F value = 6.2) | Group B (F value = 5.7) |
|---|---|---|
| | p-value | p-value |
| MO Baseline - MO1 (1st visit) (mm) | <0.001* | <0.062 |
| MO Baseline - MO2 (2nd visit) (mm) | <0.001* | <0.062 |
| MO Baseline - MO3 (3rd visit) (mm) | <0.001* | <0.062 |

**[Table/Fig-9]:** Comparison of Mouth-opening (MO) (in mm).
Repeated measures ANOVA; p ≤ 0.05 considered statistically significant; Asterisk (*) denotes statistical significance. MO: Mouth opening; BL: Baseline; mm: Millimeters.

On evaluating the clinical parameters, all the study subjects reported burning sensation, which can be due to atrophy of the epithelium. In the present study, Both group A and group B individuals has significant reduction in burning sensation from baseline to end of the treatment. This shows no statistical difference between the two groups in reducing the burning sensation in OSMF patients [Table/Fig-6].

The minimum and maximum Vas score was 4.0 and 9.0 in both groups before initiation of treatment procedure. After treatment (at the end of 6 weeks) the minimum and maximum Vas score was 0.0 and 1.0 in both groups, which shows a highly significant reduction in burning sensation at the end of treatment [Table/Fig-6].

The mean Vas scores were found to be 6.5 and 6.2 in both groups at baseline, which were reduced to 0.1 and 0.4 at the end of six weeks in both groups suggesting that there is almost 100% reduction in burning sensation in both groups at the end of treatment. The studies conducted by Alam S et al., and Srivastava A et al., shows

| Variables | Group A | | | | | Group B | | | | | T-value | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Min | Max | Mean | SD | IQR | Min | Max | Mean | SD | IQR | | |
| TP at Base line (mm) | 10.0 | 26.0 | 18.8 | 4.8 | 7.6 | 12.0 | 24.0 | 17.4 | 3.8 | 8.0 | 1.27 | 0.212 |
| TP1(1st visit) (mm) | 11.0 | 27.0 | 20.2 | 4.8 | 8.0 | 13.0 | 25.0 | 18.4 | 3.7 | 7.1 | 1.52 | 0.133 |
| TP2 (2nd visit) (mm) | 12.0 | 28.0 | 21.8 | 4.8 | 8.4 | 14.0 | 26.0 | 19.3 | 3.7 | 7.1 | 2.18 | 0.034* |
| TP3 (3rd visit) (mm) | 13.0 | 30.0 | 23.2 | 4.7 | 6.9 | 15.0 | 27.0 | 20.1 | 3.8 | 7.0 | 2.73 | 0.009* |

**[Table/Fig-10]:** Comparison of mean distribution of Tongue protrusion (TP) (in mm).
Independent samples t-test; p≤0.05 considered statistically significant; Asterisk (*) denotes statistical significance. TP: Tongue protrusion; SD: Standard deviation; IQR: Interquartile range; Min: Minimum; Max: Maximum; mm: Millimeters.

| Comparison with baseline values | Group A (F value = 24.6) p-value | Group B (F value = 18.9) p-value |
|---|---|---|
| TP Baseline - TP1(1st visit) | <0.001* | <0.086 |
| TP Baseline - TP2 (2nd visit) | <0.001* | <0.086 |
| TP Baseline - TP3 (3rd visit) | <0.001* | <0.086 |

**[Table/Fig-11]:** Pair wise comparison of Tongue Protrusion (TP).
Repeated measures ANOVA; p≤0.05 considered statistically significant; Asterisk (*) denotes statistical significance. TP: Tongue protrusion.

| Parameters | Group | Mean±SD | T-value | p-value |
|---|---|---|---|---|
| Pretreatment Iron | A | 43.48±12.65 | 0.32 | 0.748 |
| | B | 42.48±11.12 | | |
| Post-treatment | A | 71.98±17.91 | 0.85 | 0.398 |
| | B | 68.51±13.32 | | |
| Pretreatment LDH | A | 563.4±186.39 | 4.5 | 0.001* |
| | B | 349.23±183.02 | | |
| Post-treatment LDH | A | 387.85±173.03 | 2.68 | 0.009* |
| | B | 285.09±118.08 | | |

**[Table/Fig-12]:** Comparison of salivary parameters (Salivary Iron and LDH Levels) between the study groups.
Independent samples t-test; p≤0.05 considered statistically significant; Asterisk (*) denotes statistical significance. LDH: Lactate dehydrogenase; SD: Standard deviation

| Parameters | Group | Time | Mean±SD | T-value | p-value |
|---|---|---|---|---|---|
| Salivary Iron (µg/dL) | 1 | Pre | 43.48±12.65 | 7.125 | 0.001* |
| | | Post | 71.98±17.91 | | |
| | 2 | Pre | 42.48±11.12 | 8.07 | 0.001* |
| | | Post | 68.51±13.32 | | |
| Salivary LDH (IU/L) | 1 | Pre | 563.4±186.39 | -3.78 | 0.001* |
| | | Post | 387.85±173.03 | | |
| | 2 | Pre | 349.23±183.02 | -1.61 | 0.009* |
| | | Post | 285.09±118.08 | | |

**[Table/Fig-13]:** Within-group comparisons of Salivary parameters (Salivary Iron and LDH Levels).
Paired t-test; p≤0.05 considered statistically significant; Asterisk (*) denotes statistical significance; LDH: Lactate dehydrogenase; µg/dL: micrograms per deciliter; IU/L: International units per liter; SD: Standard Deviation

similar results compared to the present study where tulsi and aloe vera are compared to other treatment procedures [24,25].

In a study done by Anuradha A et al., the post-treatment mean value of Vas score was 1.80±0.65 (46%) in patients treated with aloe vera gel, but the results of this study revealed that it to be 0.4±0.6. Hence, the present study has been found to show better results [26].

Trismus in OSMF patients is due to the presence of fibrous bands. In this study, all patients has decreased mouth opening. In group A, minimum and maximum mouth opening is 18.0 mm & 37.0 mm, respectively. At baseline and at the end of the treatment, minimum and maximum mouth opening was 24.0 mm and 41.0 mm, respectively. In group B, minimum and maximum mouth opening were 16.0 mm, 33.0 mm, respectively; at baseline and the end of the treatment, the minimum and maximum mouth opening were 21.5, 39.0, respectively. This revealed increased mouth opening in both groups, but group A was more significant than group B.

The mean value of the mouth opening at baseline in group A and group B was found to be 30.0±5.6 and 25.9±4.6. At the end of six weeks, the mean value of mouth opening in group A and group B was 35.0±4.9 and 31.4±4.6 [Table/Fig-8]. Significant results were obtained in both groups. group A was statistically more substantial than group B in comparison between the two groups [Table/Fig-9]. Improvement is due to the antioxidant, anti-inflammatory, immunomodulatory, and anticancer properties of aloe vera.

Khabiya P et al., conducted a study to evaluate the efficacy of turmeric and Tulsi in OSMF patients; in this study, the mean value of mouth opening at pretreatment was 28.80±3.12, and at post-treatment, it was 33.30±3.16, which shows that this analysis is almost similar to the current research [27].

Tongue protrusion usually gets affected in OSMF patients. This can be due to the fibrosis of the muscles of the tongue. Almost all the patients included in the present study presented decreased tongue protrusion. The mean values of tongue protrusion of group A and group B at baseline were 18.8±4.8 and 17.4±3.8, respectively. At the end of six weeks, the mean values of group A and group B were found to be 23.2±4 and 20.1±3.8, respectively. This shows increased tongue protrusion in both groups, but group A shows more statistical significance than group B. In a study conducted by Rizvi D et al., the mean values of tongue protrusion in the group in which patients were treated with topical application of Tulsi gel at pretreatment were 24.9±5.03 [28]. At the end of treatment, it was 28.2±4.43, which is superior to the present study. This is the first study to assess and compare salivary levels of Iron and LDH in patients treated with Tulsi and aloe vera. In this study, salivary biomarkers, Iron and LDH, are used for the early detection of OSMF.

In group A and group B, pretreatment salivary mean values of iron were found to be 43.48±12.65 and 42.48±11.12, respectively, and post-treatment salivary Iron values in group A and group B were found to be 71.98±17.91 and 68.51±13.32, respectively. This shows increased iron values in both groups. In the comparison of the two groups, no statistical difference was observed. In group A and group B, pretreatment salivary mean values of LDH were found to be 563.4±186.39 and 349.23±183.02, respectively, and post-treatment salivary LDH values in group A and group B were found to be 387.85±173.03 and 285.09±118.08 respectively, this shows decreased salivary LDH values in both groups [Table/Fig-12].

In intra-group comparisons, it was observed that the salivary iron values increased significantly with time in both the study groups; at the same time, there was a significant decline in salivary LDH values in both groups. Basavaraj N et al., conducted a study on LDH as a biomarker in oral cancer and OSMF [29]; in this study, mean salivary LDH levels in OSMF patients were 608.28±30.22, which is superior to the present study. In a study conducted by Shetty SR et al., the mean salivary iron levels in the OSMF group were 35.96±11.31; this shows that present study results were better compared to this study [30]. In the present study comparing two groups group A (Tulsi Group), showed better results in vas score, mouth opening, tongue protrusion, and salivary levels of Iron and LDH compared to group B (aloe vera Group).

### Limitation(s)

The present study achieved good results, but follow-up is necessary to evaluate the long-term effects of the drug and its recurrence after discontinuation of topical application. Additionally, various confounding factors may have impacted the outcomes, including individual differences in healing responses, dietary habits, genetic factors, oral hygiene practices, and levels of areca nut exposure. Psychological aspects such as stress and pain tolerance could also influence subjective measures like Visual Analog Scale (VAS) scores. Future research should focus on controlling these variables and involve a larger sample size with a more diverse demographic to improve the generalisability of the findings.

## CONCLUSION(S)

In the present study on comparing two groups, group A (Tulsi), showed better results in vas score, mouth opening, tongue protrusion, and salivary levels of Iron and LDH compared to group B (aloe vera). The present study achieved good results, but follow-up is necessary to evaluate the long-term effects of the drug and its recurrence.

## REFERENCES


[1] Prabhu SR, Wilson DF, Daftary DK, Johnson NW. Oral Diseases in the Tropics. 3rd ed. Oxford: Oxford University Press; 1992. p. 460-461.
[2] Sharma SR, Chavan S, Karjodkar FR, Sansare K, Bharathi S, Singh S. Correlation of clinical features in oral submucous fibrosis: A 9-year retrospective study. Ethiop J Health Sci. 2022;32(1):137-44. Doi: 10.4314/ejhs.v32i1.15. PMID: 35250225; PMCID: PMC8864384.
[3] Patil A, Gunjal S, Abdul Latif AA. Tulsi: A medicinal herb for oral health. A review. Galore Int J Health Sci Res. 2018;3(4):37-39.
[4] Devi MS, Manju J, Rajpurohit R, Sophia K, Sorokhaibam B. Adjuvant therapy for oral submucous fibrosis. Int J Oral Health Dent. 2020;62:78-80.
[5] Yadav S, Verma A, Sachdeva A, Virdi M. Etiopathogenesis and management of oral submucous fibrosis. Internet J Bioengineering. 2010;5(1).
[6] Ali FM, Patil A, Patil K, Prasant MC. Oral submucous fibrosis and its dermatological relation. Indian Dermatol Online J. 2014;5(3):260-65.
[7] Rajendran R. Oral submucous fibrosis: Etiology, pathogenesis, and future research. Bull World Health Organ. 1994;72(6):985-96.
[8] Shivakumar GC, Sahana S. Clinical staging of oral submucous fibrosis: A review. Int J Oral Med Sci. 2011;10(3):216-19. Doi: 10.5466/ijoms.10.216.
[9] Yadav S, Ahuja A. Garlic: A miraculous herb for oral diseases- A review. J Oral Health Commun Dent. 2014;8(1):51-54.
[10] Zagórska-Dziok M, Furman-Toczek D, Dudra-Jastrzębska M, Zygo K, Stanisławek A, Kapka-Skrzypczak L. Evaluation of clinical effectiveness of Aloe Vera- a review. J Pre Clin Clin Res. 2017;11(1):86-93.
[11] Mangaiyarkarasi SP, Manigandan T, Elumalai M, Cholan PK, Kaur RP. Benefits of Aloe Vera in dentistry. J Pharm Bio Allied Sci. 2015;7(Suppl 1):S255-S259.
[12] Sudarshan R, Annigeri RG, Vijayabala GS. Aloe Vera in the treatment for oral submucous fibrosis – a preliminary study. J Oral Pathol Med. 2012;41:755-61.
[13] Ahmed WA, Om-Hashem MA, Elhefny MA, Abo Elnasr AA, El Syed N. The first molecular insights to the effect of Ashwagandha and Propolis on cancer cells. Int Conf Bioengineering. 2015; Doi: 10.15242/IICBE.C0715115.
[14] Manikandan P, Vijaya Letchoumy P, Prathiba D, Nagini S. Combinatorial chemopreventive effect of Azadirachta indica and Ocimum sanctum on oxidant-antioxidant status, cell proliferation, apoptosis and angiogenesis in a rat forestomach carcinogenesis model. Singapore Med J. 2008;49(10):814-22.
[15] Gupta GS. The lactate and the lactate dehydrogenase in inflammatory diseases and major risk factors in COVID-19 patients. Inflammation. 2022;45(6):2091-123. Doi: 10.1007/s10753-022-01680-7. Epub 2022 May 19. PMID: 35588340; PMCID: PMC9117991.
[16] Mishra S, Kritika C, Bajoria AA, Choudhury P, Sahoo SK, Sangamesh NC. Estimation of salivary and serum lactate dehydrogenase in oral submucous fibrosis. J Int Soc Prevent Communit Dent. 2018;8:289-95.
[17] Dwivedi P, Majumdar A, Thakkar B, Saxena S, Tripathi V. Tulsi (Ocimum sanctum): Health Benefits and Nutritional Wonders. Acta Scientific Nutritional Health. 2023;7(9):16-21.
[18] Catalano A, Ceramella J, Iacopetta D, Marra M, Conforti F, Lupi FR, et al. Aloe vera—an extensive review focused on recent studies. Foods. 2024;13(13):2155. Doi: 10.3390/foods13132155.
[19] Chen X, Chen T, Xie H, Guo J. Salivary and serum levels of lactate dehydrogenase in oral submucous fibrosis: A meta-analysis. Medicine (Baltimore). 2024;103(15):e37788. Doi: 10.1097/MD.0000000000037788. PMID: 38608075; PMCID: PMC11018194.
[20] Chandramani G, Gupta S, Joshi J, Verma S. Classification system for oral submucous fibrosis. J Indian Acad Oral Med Radiol. 2012;24:24-29. Doi: 10.5005/jp-journals-10011-1254.
[21] Lokesh Sundaram B, Rathnavelu V, Sabesan M, Ganesh A, Anandan S. A study to assess the levels of salivary ferritin in iron deficiency anemia subjects and healthy subjects. Cureus. 2021;13(8):e17241. Doi: 10.7759/cureus.17241. PMID: 34540467; PMCID: PMC8444485.
[22] Rao K, Babu SG, Shetty SR, Castelino RL. Evaluation of serum and salivary lactate dehydrogenase levels in patients with oral potentially malignant conditions/lesions: A clinical and biochemical study. Indian J Community Med. 2024;49(2):316-21. Doi: 10.4103/ijcm.ijcm_612_22. Epub 2024 Mar 7. PMID: 38665466; PMCID: PMC11042121.
[23] Shah PH. Comparison of therapeutic efficacy of placental extract with dexamethasone and hyaluronic acid with dexamethasone for oral submucous fibrosis- A retrospective analysis. J Clin Diagn Res. 2016;10(4):ZE01-ZE07.
[24] Alam S, Ali I, Giri KY, Gokkulakrishnan S, Natu SS, Faisal M, et al. Efficacy of aloe vera gel as an adjuvant treatment of oral submucous fibrosis. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013;116(6):717-24.
[25] Srivastava A, Agarwal R, Chaturvedi TP, Chandra A, Singh OP. Clinical evaluation of the role of Tulasi and turmeric in the management of oral submucous fibrosis: A pilot, prospective observational study. J Ayurveda Integr Med. 2015;6(1):45-49.
[26] Anuradha A, Patil B, Asha VR. Evaluation of efficacy of aloe vera in the treatment of oral submucous fibrosis – a clinical study. J Oral Pathol Med. 2017;46(1):50-55.
[27] Khabiya P, Maity P, Halli R, Pawar S, Hebbale M. Efficacy of turmeric and tulsi in management of oral submucous fibrosis. J Dent Orofac Res. 2019;15(2):36-39.
[28] Rizvi D, Abidi A, Faiz SM, Hasan R, Thadani A. Efficacy of curcumin gel and tulsi gel in oral submucous fibrosis. Natl J Physiol Pharm Pharmacol. 2019;9(4):343-48.
[29] Kallalli BN, Rawson K, Muzammil, Singh A, Awati MA, Shivhare P. Lactate dehydrogenase as a biomarker in oral cancer and oral submucous fibrosis. J Oral Pathol Med. 2016;45(9):687-90.
[30] Shetty SR, Babu S, Kumari S, Shetty P, Hegde S, Castelino R. Status of salivary iron in oral cancer and oral potentially malignant disorders. J Cranio Max Dis. 2014;3:17-20.



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