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Novel Neem leaves extract mouthwash therapy for Oral Lichen Planus

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Running title: Neem therapy for oral lichen planus.

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Abstract:

Oral lichen planus (OLP) is a potentially malignant, chronic inflammatory condition affecting the oral cavity. Most of the patients have associated systemic conditions like hypertension, diabetes mellitus and hyperthyroidism. Patients experience severe burning pain in the oral cavity. Although, the standard treatment for this condition is a corticosteroid, the associated side effects limit its prolonged use. Chronic topical use can lead to mucosal atrophy, secondary candidal infection and burning pain which could ultimately lead to dietary disturbance, psychological problems and negative impact on the quality of life. Therefore, an alternative approach like herbal therapy including neem tree leaves was tried in OLP patients. Aqueous neem leaves extract has anti-inflammatory, immunomodulatory, antifungal and anticancer agents in addition to various antioxidants, which could prove to be beneficial in treating OLP. This paper presents an herbal therapeutics case series of a novel aqueous neem leaves extract mouthwash therapy which was successful in resolving the signs and symptoms of OLP in four patients.

Key words: Anti-inflammatory agents, antioxidants, burning pain, neem tree, oral lichen planus.

1. Introduction:

Oral lichen planus (OLP) is a chronic T-cell mediated inflammatory condition of oral mucosa with an unknown predisposing factor. Affecting 0.5% to 2% of the general population worldwide, the usual complaint by the patient is severe oral discomfort in the form of pain or burning sensation. The lesions present as erosive, atrophic, bullous, reticular, plaque, papular or annular type, either singly or in combinations (Edwards et al., 2002, Roopashree et al., 2010). There could be periods of remission, in which the patients are symptom free (reticular, plaque, papular or annular type) and periods of exacerbations (erosive, atrophic or bullous type) in which the patients experience severe pain or burning which might be associated with periods of stress, exposure to allergen or medications, trauma or immunodeficiency (Roopashree et al., 2010).

A recent review on treatment modalities of OLP has shown that none of the available treatments are adequate (Thongprasom et al., 2011). Yet the standard care and first line of treatment for OLP has always been corticosteroids. Prolonged use of systemic corticosteroids can lead to hypertension, diabetes, osteoporosis, weight gain and muscle weakness. Topical preparations can cause burning pain, mucosal atrophy and secondary candidiasis in addition to systemic complications (Thongprasom et al., 2008, Dhar et al., 2014). Therefore, excessive use of steroids in these patients is limited mainly because of the triad of chronicity of the disease, old age and associated systemic diseases. Thus, an alternative medication which would be safe and effective in long terms should be identified, encouraged and studied. Natural therapies have gained lots of momentum in treating many diseases. In addition to being freely available, many of them are also safe. One such herb known for more than 2000 years for its wide range of biological activities is *Azadirachta indica*. It belongs to Meliaceae family and is considered to be a boon for most diseases and hence called “Sarvaroga Nivarini” which means “the curer of all ailments” (Girish et al., 2008). It is an important ethnomedicinal herb and is also used extensively by Ayurvedic, Unani and Homoeopathic practitioners for various diseases (Girish et al., 2008). All parts of neem tree namely leaf, bark, fruit, seeds, flowers, stems and twigs possess variable medicinal properties. Of all these, neem leaves are the most preferred for

medicine preparation because of their availability throughout the year, the ease of extracting its phytoconstituents and the wide array of properties they possess like anti-inflammatory, antifungal, immunomodulatory, antimicrobial, antiviral, antihypertensive, antidiabetic, antiulcerogenic, antioxidant, antipyretic, analgesic and anticarcinogenic (Girish et al., 2008).

In health and disease, clinical trials including neem leaves have been successfully carried out in diabetic (Shukla et al., 1973), malarial, ringworm, eczema, scabies and other dermatological diseases (Kanungo et al., 1996), and also in vaginosis to check spermicidal and antimicrobial activity (Raghuvanshi et al., 2001). In a study involving uncomplicated psoriasis patients, aqueous extract of neem leaves when taken in addition to coal tar showed better response in comparison to placebo group (Pandey et al., 1994). In dentistry, neem leaves in mouthwash (Chatterjee et al., 2011) and gel (Pai et al., 2004), forms have been tried in periodontal diseases for their antimicrobial effect with significant reduction in plaque and gingival scores. They are also important ingredients of herbal tooth powder and toothpaste. Neem's antimicrobial property has also proved it to be an effective endodontic irrigant (Dutta et al., 2014).

The possible role of neem leaves phytoconstituents as therapeutic modality in OLP has not been explored earlier. But with its well established anti-inflammatory, antioxidant, immunomodulating, antiproliferative and antifungal properties, its probable beneficial role in OLP could not be denied. This paper presents a case series of novel herbal therapeutic in the form of neem leaves extract mouthwash therapy in alleviating the signs and symptoms of four OLP patients.

2. Materials and methods:

2.1. Patient details:

Most of the OLP patients had associated systemic conditions like hypertension, diabetes mellitus, chronic liver diseases, endocrinopathies, additionally oral lesions similar to OLP could be present in patients having tobacco addictions or patients on chronic drug therapies for any of their systemic problems. Therefore, a

detailed case history of the OLP patients was taken with respect to their medical history, drug history and tobacco addiction. Those who had negative history for all of the above-mentioned parameters were planned to receive the herbal therapeutics. Also, only those patients were included for the herbal therapeutics who had not taken any medications for OLP for a minimum of two weeks and were willing to undergo the therapy. Pregnant or lactating females, OLP patients with associated cutaneous lesions and OLP lesions showing signs of malignant conversion were excluded.

2.2 Preparation of neem leaves extract (NLE) mouthwash (modified decoction method) (Sithisarn et al., 2006):

2.2.1. Plant sample:

Neem leaves were collected from the herbal garden of Mahatma Gandhi Ayurved college and hospital and research centre, Salod, Wardha on date 04/02/2019 at 11am. The plant material was authenticated from an expert botanist as *Azadirachta Indica* A. Juss (Herbarium reference number:10338). Neem leaves were first washed with water and then with distilled water to remove dust. Later they were shade dried at room temperature for 4 days. The neem leaves samples were ground in a grinder machine (Jaipan, Supper Deluxe) and pulverized to a fine powder.

2.2.2. Extraction procedure:

The neem leaves powder sample was boiled with distilled water in distillation assembly (Alka glassware, Nagpur) in proportion 1:10 for 6 hours. The collected condensed vapors were again distilled in the distillation assembly to collect the final distillate. This distillate was evaporated on a hot water bath to get aqueous leaf extract. 10 grams of extract was added to 100ml of distilled water to get a concentration of 10%. To this 0.001% of neotem (Nutrasweet Co supplier Kawalal Co Pvt limited, supplied by Sun pharma,

Varodara, Gujarat) syrup was added to mask the bitter taste. The prepared NLE mouthwash was stored in amber colored container.

2.3 Therapy protocol:

All the patients were informed about the therapy, method of administration and reporting schedule. The patients had to rinse with 5ml of NLE mouthwash thrice daily, each time holding the mouthwash for 15 min and circulating it evenly along the entire oral mucosa. They had to report weekly for assessing the effect of the therapy or possible side effects. Assessment was done by two parameters; (a) Visual analogue scale (VAS) was used for scoring burning sensation. This is a 10cm scale with 0 being no pain and 10cm which is the last limit of scale means severe unbearable pain. The patient had to mark the appropriate score on the scale. (b) Thongprasom scoring criteria for clinical scoring of the lesions. This is a 5 scale scoring system; 0= no lesions, 1=mild white striae, no erythematous area, 2=white striae with atrophic area less than 1cm², 3 = white striae with atrophic area more than 1cm², 4=white striae with ulcerative area less than 1cm² and 5=white striae with ulcerative area more than 1cm² (Wang et al., 2015).

All the following reported cases complied regarding the inclusion criteria and also followed the therapy protocol. Informed consent was taken from each participant.

3. Case reports:

3.1. Case 1:

A sixty five year old female patient reported with the chief complaint of a severe burning sensation of the oral cavity and discomfort in eating food for a week. She was quite distressed by the oral pain and was in severe stress as she had had a similar complaint in the past 2 years and the most recent one was approximately 5 months previously. She was earlier histopathologically diagnosed as having oral lichen planus but had no records of medicinal treatment taken earlier. She had no history of skin lesions and had a

non-significant medical history. Extraoral examination revealed no cutaneous lesions. The initial VAS score was 9. On clinical examination, intraorally erosive areas with fine reticular striations were noted bilaterally on the buccal mucosa near the retromolar areas (Fig. 1 a, c) accounting for an initial clinical score to be 4. The patient's blood profile along with the blood sugar levels were within normal limits. She was advised to rinse with the aqueous neem leaf extract mouthwash three times daily, each time holding the mouthwash for a maximum of 15 minutes. The patient was evaluated weekly and each time VAS and Thongprasom scores were recorded. During the treatment period the patient did not complain of any adverse effects. She had significant relief from the burning sensation (VAS score: 0) as could be evaluated from the complete resolution of the lesions (Thongprasom score: 1) after 4 weeks (Fig. 1 b,d). The mouthwash was discontinued. The patient has been kept under follow up. One month follow-up showed no signs of recurrence of oral lesions.

3.2.Case 2:

A forty two year old male patient reported with severe ulcerations and burning sensation for 4 days. The patient's mouth opening was restricted due to the severe discomfort. There was history of similar complaint one year previously. At that time, he was histopathologically confirmed as having OLP and was treated by topical steroids which resolved the lesions. His dental, medical and family histories were non-contributory. Further history revealed that for one month the patient's financial condition was not stable, due to which he was under severe stress. On examination cutaneous lesions were absent. Intraorally extensive erosive ulcerative lesions with reticular striae were evident on bilateral buccal mucosa (Fig. 2 a, c). The VAS score and clinical score on first visit were 9 and 5, respectively. The patient was informed about the NLE mouthwash therapy and advised to use it three times daily. Additionally, counselling was carried out at each visit to reduce his stress. The patient was recalled weekly. At the end of 5 weeks complete remission of

lesions were seen (Fig. 2 b, d). VAS (0) and clinical scores (1) were also reduced to minimum with the patient having adequate mouth opening. The patient is kept under follow-up.

3.3 Case 3:

A 53 years old female patient with history of OLP (for 2years), reported with recurrence of a burning sensation in her oral cavity for one week. The patient had the first episode of lesions two years back. At that time she had cutaneous lesions together with oral lesions. She was diagnosed as having lichen planus and was treated by a dermatologist and a dentist with corticosteroids. The patient achieved complete relief. The lesions reappeared 8 months ago for which she sought treatment and had relief. For the past week the patient had ulcerations and oral burning due to which she again reported to the hospital. Medical and family histories were non-contributory. There was an absence of cutaneous lesions. Intraorally ulcerative lesion with peripheral white striae and hyperpigmentation was present on left buccal mucosa (Fig. 3 a). Adjacent molars were having sharp buccal cusps. On right buccal mucosa post-inflammatory hyperpigmented areas were present. Coronoplasty was carried out with the buccal cusps of molars. This prevented further trauma to the ulcerative lesions. The VAS and clinical scores recorded on the first visit was 7 and 5 respectively. The patient was started with neem extract mouthwash therapy three times daily and recalled weekly. Subsequently the scores reduced each visit. By the end of 5th week (final VAS score: 0 and Thongprasom score: 1) the lesions regressed (Fig. 3 b) and the patient was referred for prosthesis planning to department of prosthodontia. No adverse effects were reported by the patient.

3.4 Case 4:

A thirty four year old female with a nine month history of successfully treated OLP lesions reported with the recurrence of ulcers in her oral cavity with severe burning for two days. Medical, family and drug history were non-contributory. On examination there was absence of cutaneous lesions. Intraorally upper and lower

gingiva showed erosive areas suggestive of desquamative gingivitis (Fig. 4 a). The patient was prescribed neem leaves extract mouthwash three times daily and recalled weekly. By the end of 5 weeks, complete remission was noted. The VAS score reduced from 7 (first visit) to 0 (post 5 weeks) and Thongprasom score reduced from 5 (first visit) to 0 (post 5 weeks) as seen in Fig. 4 b. The patient did not complain of any adverse effects.

4. Discussion:

A proper extraction method is very important to get the desired properties from any plant extract. Neem leaves extract exhibits its anti-inflammatory and immunomodulating properties primarily by the prevention of lipid peroxidation and free radical scavenging ability of its constituents like flavonoids. Therefore, aqueous neem leaf extract was obtained from the decoction method which has been reported to yield maximum antioxidant activity (Sithisarn et al., 2006). Alcoholic extract was avoided as it might have a local dehydrating effect in addition to being a risk factor for malignant transformation of the lesions.

As the OLP lesions are mostly generalized, involving multiple areas of the oral cavity, a mouthwash or a mouth rinse ensures application of medication to all areas of oral cavity. The mouthwash could be freshly prepared and stored in cool and dry place away from sunlight. For the same reason it could be stored in amber colored containers with a measuring cap. Neem leaves contain the maximum number of phytoconstituents, mainly saponins, flavonoids, tannins, glycosides and alkaloids. The active constituents are nimbin, nimbidine, isomeldenin, β -sitosterol, quercetin and azadirachtin (Biswas et al., 2002). These phytoconstituents present exert the various mechanisms of actions mainly anti-inflammatory, immunomodulatory, antioxidant, antifungal and anti-proliferative in view of the therapeutic role in OLP.

Oral lichen planus is a T-cell mediated autoimmune condition, the antigen for which is unknown. Cytotoxic T cells are activated by the cytokines released by T helper cells, Interleukin 2 (IL 2) and Interferon γ ($IFN\gamma$) and bind to the antigen presented at the basal keratinocytes. They release Tumor Necrosis Factor α ($TNF\alpha$)

and granzyme B which potentiates apoptosis of basal keratinocytes. Also there is mast cell degranulation and subsequent release of chymase and TNF- α which releases more T lymphocytes from the blood capillaries to further cause apoptosis of keratinocyte. RANTES (regulated on activation, normal T cell expressed and secreted) are released by T lymphocytes which degranulate more mast cells and the process further continues to undergo apoptosis of more keratinocytes. The inflammatory process sets in an environment of oxidative stress which has a synergistic role in further aggravating the condition (Roopashree et al., 2010). Fig. 5 shows a schematic representation of etiopathogenesis of OLP and the probable areas of action of NLE are highlighted by the numerics 1, 2 and 3. Whereas Fig. 6 explains the mechanism of action of NLE at the areas highlighted by the numerics 1,2 and 3 in Fig.5.

Oral lichen planus lesions could be superinfected or associated with *Candida albicans* (Hatchuel et al., 1990). Thus the antifungal action of neem leaves due to the presence of cyclic trisulphide and cyclic tetrasulphide compounds could be beneficial in these lesions (Pant et al., 1986).

Having a malignant transformation rate of 1-2%, OLP lesions probably could be affected by the increased oxidative stress and the cytokine-based microenvironment arising from chronic inflammation which may lead to genetic changes in the epithelial cells (Shirasuna, 2014; Mignogna et al., 2004). The anticancer property of neem could be beneficial in prevention and progression of malignancy in OLP. Its mechanism could be due to its well established antiproliferative property which is via modulation of apoptosis, tumor suppressor gene and various other molecular pathways (Paul et al., 2011).

OLP is common in females and later ages of life. This is evident from the cases reported; three out of the four patients were females in 3rd to 6th decades of life. All the patients had only oral lesions due to which topical therapy could be provided as against presence of cutaneous lesions which would warrant systemic drug administration. All patients had recurrent history of ulcerations in oral cavity. Such exacerbations are common in oral lichen planus and the factors responsible in these cases could be probably stress, exposure to any allergen, drug or trauma (Koebner phenomenon). Identifying such factors and correcting them should be

the priority of any treatment plan for OLP. Contrary to this, in few patients none of these factors are identifiable and still they suffer from recurrent episodes of ulcerations or erosions. Natural therapy, a safe and effective alternative could then be considered a boon in such cases.

In the present cases, NLE mouthwash was effective in relieving the burning and reducing the size of the lesions. This is similar to the findings of the previous studies conducted to evaluate the effect of natural plant product mouthwashes in OLP. Aloe vera (Mansourian A et al., 2011) and anthocyanins (Rivarola de Gutierrez E et al., 2014) were used in purely mouthwash forms that is rinse and spit, whereas cedar honey (Sanatkhani Met al., 2014) was administered via the swish and swallow techniques. All these natural mouthwashes were equally effective in reducing the burning pain and promoting healing of lesions as compared to topical steroids (Aloe vera compared with triamcnenolone acetonide 0.01%, anthocyanins compared with clobetasole propionate 0.05% and cedar honey compared with dexamethazone 0.5mg). This further proves the healing ability of natural mouthwashes as compared to medications (steroids) which are equally effective but incur adverse side effects after some period.

This case series is a preliminary research wherein few cases of OLP were evaluated to assess the effect of NLE mouthwash. OLP patients often have associated systemic problems and cutaneous lesions, thus reducing the number of patients fulfilling the selection criteria for the research to a minimum. For the same reason this case series had no comparative group which would have enabled us to compare the effectiveness of this mouthwash to a standard treatment like steroid therapy. *Azadirachta indica* has an inherent bitter taste and to mask this taste artificial sweetner, neotem was used. Neotem is 7000 times sweeter than sucrose and has also proved to be safer (Nofre C et.al., 2000).

All the four cases reported showed good acceptability to the NLE mouthwash which is further proved by their continuation of use of the same until complete relief. Thus, in routine clinical settings taste of the mouthwash would not be of much issue but regular fresh preparation of the mouthwash would be a concern for the clinicians as freshly prepared mouthwash ensures better results.

Neem leaf extract therapy could prove as an efficient and safe alternative to steroids in OLP, particularly in the vulnerable population, most of whom are the older females and often associated with systemic problems. To the best of our knowledge this is the first herbal therapeutics report where in cases of OLP are treated with novel NLE mouthwash therapy. Nevertheless, a randomized clinical trial of NLE mouthwash in larger number of OLP patients and comparing it to group of OLP patients receiving steroid as standard therapy would give a better evidence in this regard.

Conclusion: The novel concept of NLE mouthwash therapy shows promising results. With this natural herb available freely and also safe with no apparent side effects at the minimum dose prescribed, it should be tried for chronic conditions like OLP. Well planned clinical trials with different concentration, dosing patterns and formulations should be carried out to further evaluate the efficacy of neem in OLP.

Credit author's statement

Name	Contribution
Dr. Ashita Kalaskar	Conceptualization and main investigator
DR. Rahul Bhowate	Validation
DR. Ritesh Kalaskar	Resource availability
Dr. Sumeet Ghonmode	Writing - review & editing

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Fig. 1. Pre treatment clinical photographs of case 1: Erosive areas with reticular striations in oral cavity on right buccal mucosa (a) and left buccal mocosa (c). Post treatment clinical photographs showingxs complete resolution (b and d)

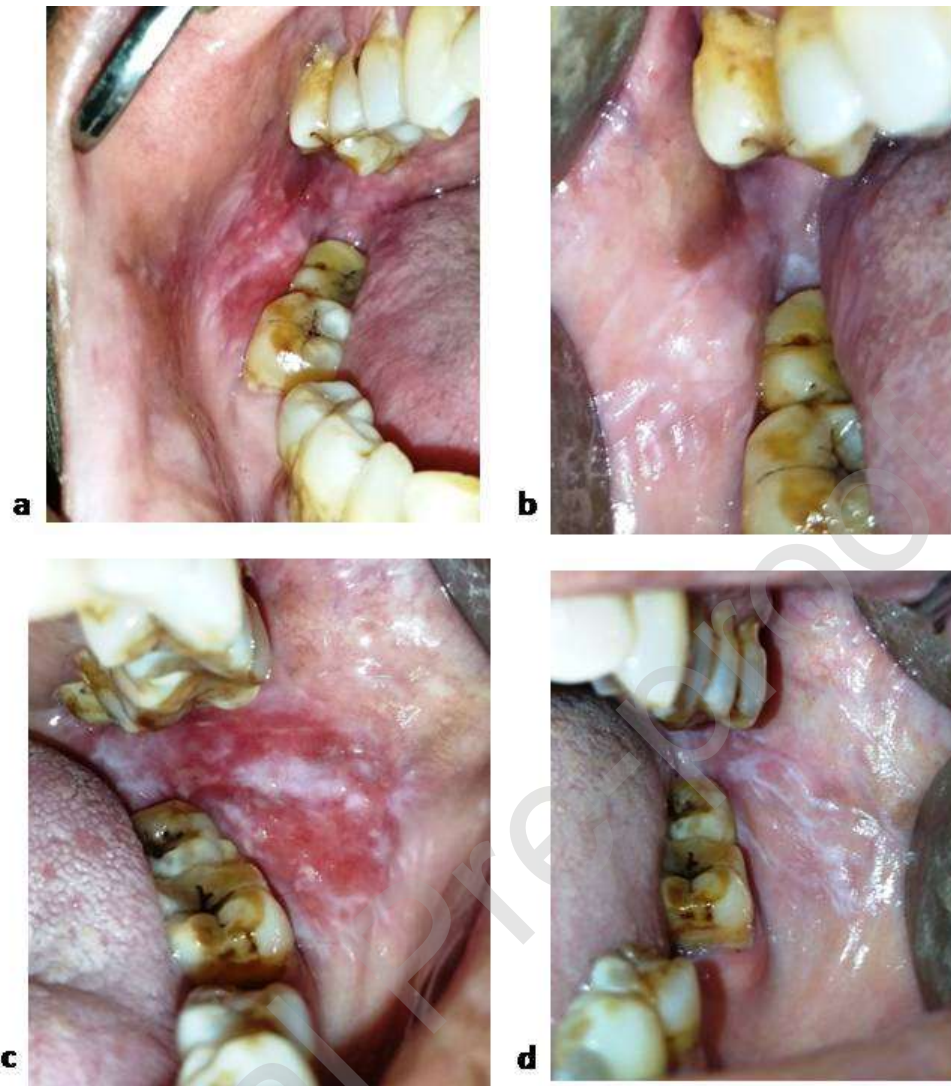


Fig. 2. Pre treatment clinical photographs of case 2: Erosive areas with reticular striations in oral cavity on right buccal mucosa (a) and left buccal mucosa (c). Post treatment clinical photographs showing complete resolution (b and d)

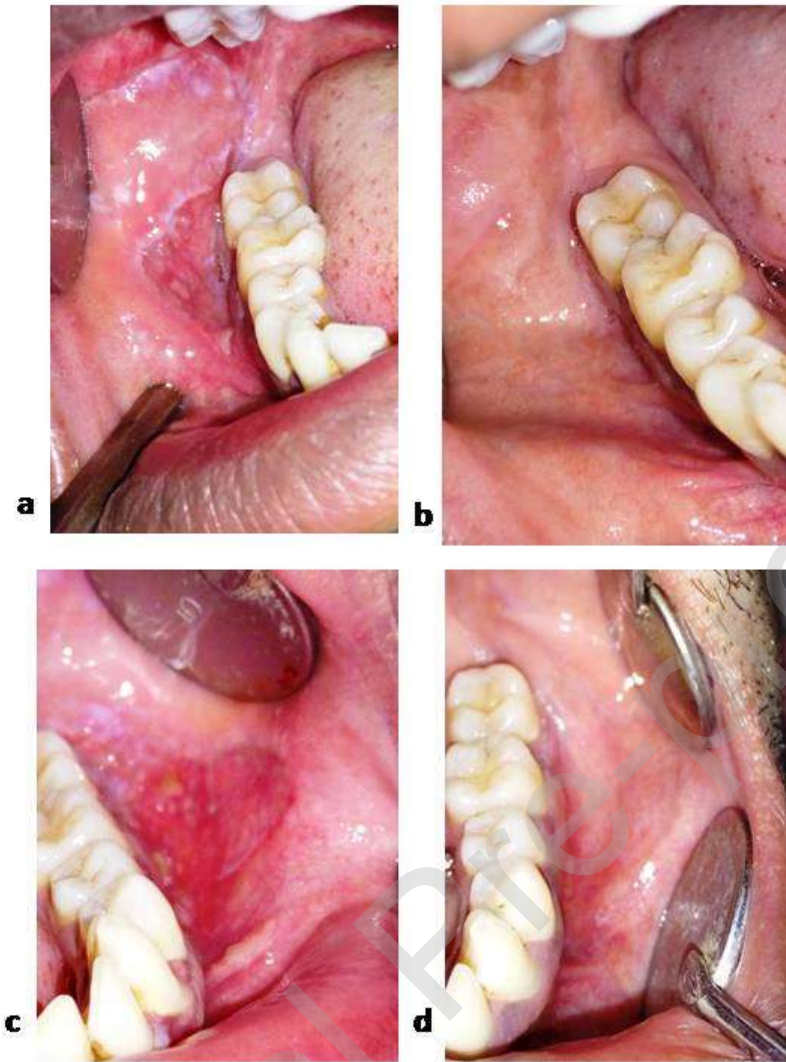


Fig. 3. Pre treatment clinical photograph of case 3: Erosive areas with reticular striations in oral cavity on right buccal mucosa (a). Post treatment clinical photograph showing complete resolution (b)

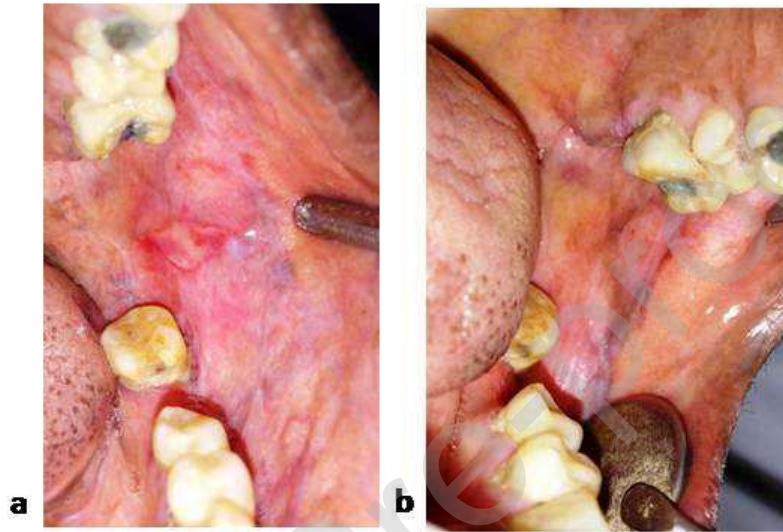


Fig. 4. Pre treatment clinical photograph of case 4: Erosive areas in oral cavity involving gingiva (a). Post treatment clinical photograph showing complete resolution (b)

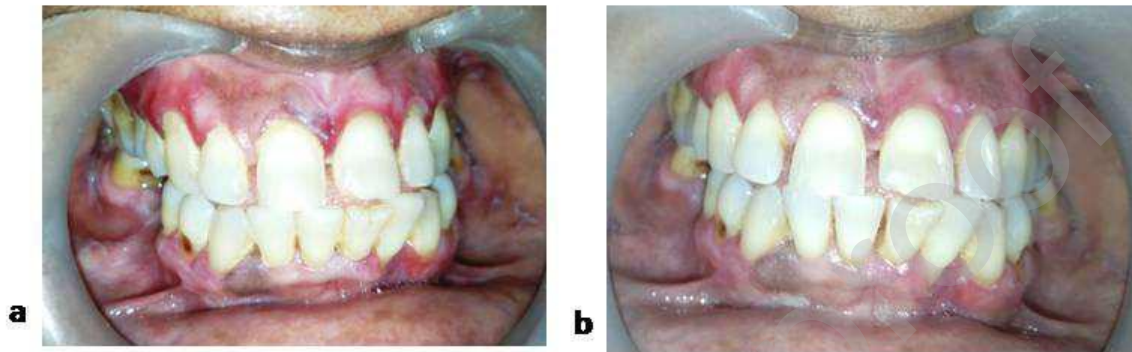


Fig. 5. Schematic representation of etiopathogenesis of OLP. The probable areas of action of NLE are highlighted by the numeric 1,2 and 3.

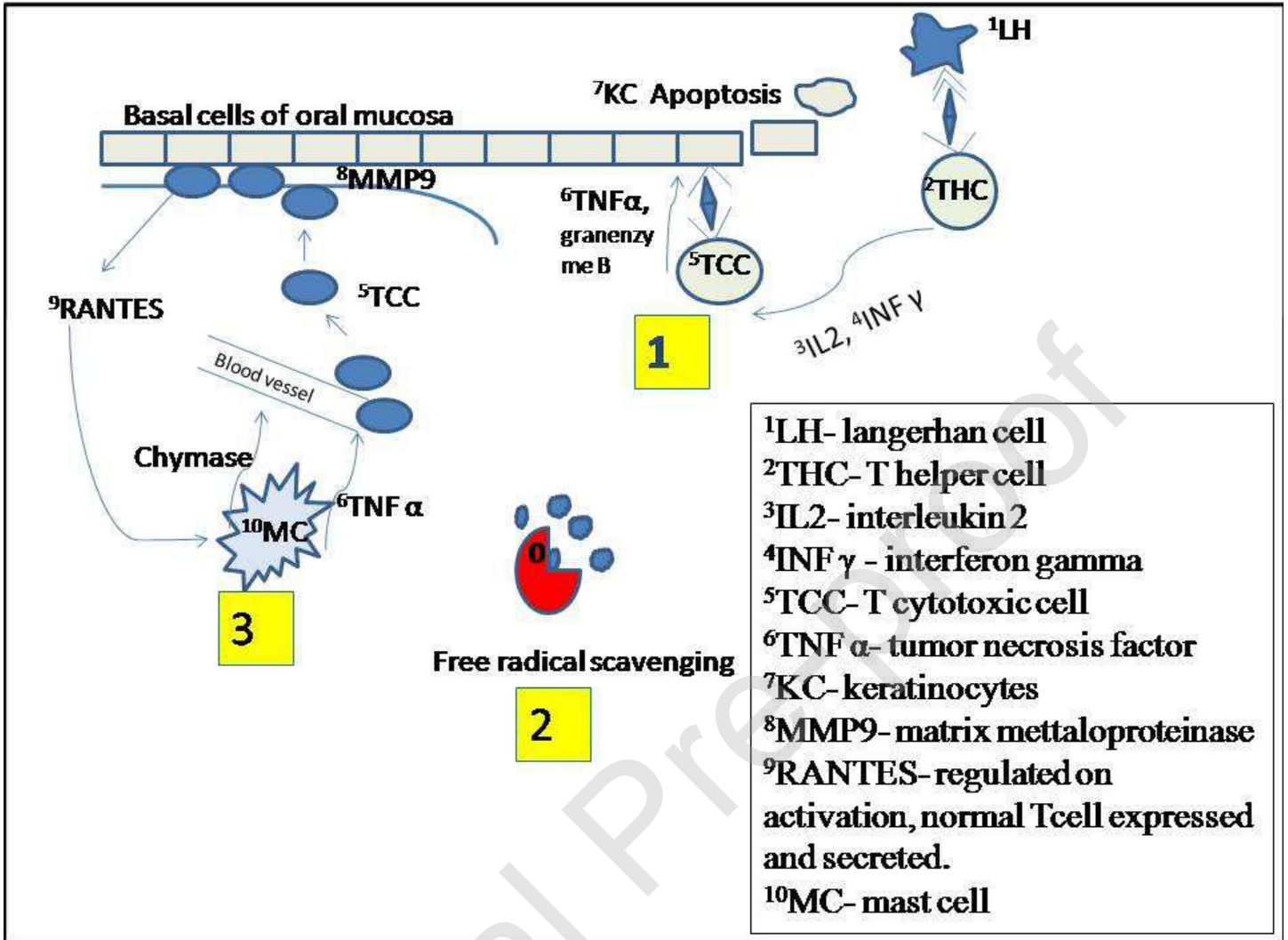


Fig. 6. Mechanism of action of NLE at the areas highlighted by the numeric 1, 2 and 3 in Fig. 5.

Probable mechanism of action of neem leaves extract (NLE) mouthwash

- 1** The activated cytotoxic T lymphocytes (activated to an unknown antigen on basal keratinocytes) are inhibited by the inhibitory action of NLE constituents on the release and action of the pro-inflammatory cytokines; TNF, IL 2, INF γ .
- 2** Antioxidant action of the NLE constituents further reduce the oxidative stress and prevent T cell hyperactivation .
- 3** Mast cell degranulation is prevented by NLE constituents thus cutting off the vicious cycle of release of chemokines (TNF α and chymase) \rightarrow release of T lymphocytes from vessels \rightarrow binding by MMP9 (matrix metalloproteinase) to basal keratinocytes \rightarrow apoptosis of keratinocytes \rightarrow release of RANTES by T lymphocytes \rightarrow further activation of more mast cells to degranulate.