"COMPARATIVE EVALUATION OF THE EFFICACY OF *Nigella sativa* (BLACK SEED) (75 % W/V) CREAM AND CLOBETASOL PROPIONATE (0.05 % W/W) GEL FOR THE MANAGEMENT OF ORAL LICHEN PLANUS- A DOUBLE BLINDED RANDOMIZED CONTROL TRIAL"

By

Dr. LOKESH KUMAR S

REG NO. IG0219003

Dissertation

Submitted to KLE Academy of Higher Education and Research (KAHER), Belagavi in Partial Fulfillment of the Requirements for the Degree Of

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Under the Guidance of

Dr. ZAMEERA NAIK M.D.S., PGDHPE.,

DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY,

KAHER'S KLE VISHWANATH KATTI

INSTITUTE OF DENTAL SCIENCES, JNMC CAMPUS,

NEHRU NAGAR, BELAGAVI- 590010, KARNATAKA.

2019 - 2022

KLE Academy of Higher Education and Research, Belagavi, Karnataka.

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Date: 5/1/2022

Place: Belagavi

(Dr. LOKESH KUMAR S)

REG NO. IG0219003

KLE Academy of Higher Education and Research, Belagavi, Karnataka.

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Date : 5/1/22

Place : Belagavi

Guide

5/1/22

Dr. Zameera Naik
Consultant Reg No. 6911-A
Oral Medicine & Radiology
KLE VK IDS, Belgaum

Dr. ZAMEERA NAIK, M.D.S., PGDHPE.,
Professor,
Department of Oral Medicine and Radiology,
KAHER'S KLE Vishwanath Katti Institute of Dental Sciences, Belagavi.

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HOD

Kheluskar

Dr. VAISHALI KELUSKAR, M.D.S.,
Professor and Head,
Department of Oral Medicine and Radiology
KLE Vishwanth Katti Institute of Dental Sciences,
JNMC Campus, Nehru Nagar, Belagavi-590010.

Professor and Head
Department of Oral Medicine and Radiology
KLE VK Institute of Dental Sciences, Belagavi

Date: 5/1/22
Place: Belagavi

Principal

PRINCIPAL
KLE V. K. Institute of Dental Sciences,
Nehru Nagar, Belagavi

Dr. ALKA D KALE, M.D.S., Ph.D.,
Principal,
KLE Vishwanth Katti Institute of Dental Sciences, JNMC Campus,
Nehru Nagar, Belagavi- 590010

Date: 5/1/22
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FAX: 0831-2470640

Web: http://www.kledental-bgm.edu.in
E-mail:principal@kledental-bgm.edu.in

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: 0831-2470362
FAX: 0831-2470640
Web: http://www.kledental-bgm.edu.in
E-mail: principal@kledental-bgm.edu.in

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-Thomson S Monson

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Dr. Lokesh Kumar S.

LIST OF ABBREVIATIONS

ABBREVIATION	FULL FORM
OLP	Oral lichen planus
CD	Cluster of differentiation
MHC	Major histocompatibility complex
T cells	Thymus cells
INF- γ	Interferon- γ
IL	Interleukin
TNF- α	Tumor necrosis factor- α
HPLC	High performance liquid chromatography
API	Active pharmaceutical ingredient
ISI	Indian Standards Institute
GMP	Good manufacturing practice
pH	Potential of hydrogen
MTT	tetrazolium salt- 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
DMEM	Gibco Dulbecco's Modified Eagle medium
CO_2	Carbon dioxide
DMSO	Dimethyl sulfoxide
OD	Optical density

NRS	Numeric Pain Rating Scale
RECIST	Response evaluation criteria for solid tumors
SPSS	Statistical Package for the Social Sciences
WHO	World Health Organization
HLA	Human leukocyte antigen
DR1	Down-regulator of Transcription-1
HPV	Human papilloma virus
EBV	Epstein-Barr virus
HHV	Human herpes virus
HIV	Human immunodeficiency virus
HCV	Hepatitis-C virus
DM	Diabetes mellitus
LP	Lichen planus
PUVA	Psoralen Ultraviolet light A
UV	Ultraviolet
EBDM	Evidence-based decision making
VAS	Visual Analogue Scale
OH radical	Hydroxyl radical
SRB	Sulphordamine B
IC_{50}	Half maximal inhibitory concentration

DAS	Disease activity score
ACR	American College of Rheumatology
EULAR	European Alliance of Associations for Rheumatology
DMARD	Disease-modifying antirheumatic drugs
TAC	Total antioxidant capacity
MDA	Malondialdehyde
NO	Nitric oxide
SOD	Superoxide dismutase
KOOS	Knee injury and osteoarthritis outcome score
GC-MS	Gas chromatography-Mass spectrometry
TLC	Total leukocyte count
BAL	Bronchoalveolar lavage
PPD	Periodontal pocket depth
CAL	Clinical attachment loss
PI	Plaque index
BoP	Bleeding on probing
AGS	Adenocarcinoma gastric
PANC	Human pancreatic cancer cell
ANOVA	Analysis of Variance
TMQ	Thymoquinone

CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF FIGURES

LIST OF ANNEXURES

ABSTRACT

Background: Oral Lichen Planus (OLP) is a common potentially malignant mucocutaneous disorder associated with chronic inflammation and cell-mediated immunological dysfunction. The current main stay of treatment is the administration of topical or systemic corticosteroids which are known to cause certain side-effects. *Nigella sativa* oil that is traditionally known to have anti-inflammatory, analgesic, wound healing, and antioxidant properties is gaining attention due to its minimal or no side effects.

Aim: To evaluate and to compare the efficacy of *Nigella sativa* (75 % w/v) cream and Clobetasol propionate (0.05 % w/w) gel for the management of Oral Lichen Planus.

Study design: An Institutional-based double-blinded randomized control trial.

Clinical Trial Registration Reference No: REF/2019/11/029518.

Materials and Methods: Sixty clinically diagnosed cases of OLP were stratified into moderate cases and severe cases based on burning sensation before getting allocated to Group I receiving *Nigella sativa* (75 % w/v) cream and Group II receiving Clobetasol propionate (0.05 % w/w) gel, two times a day for 45 days. Patients in each group were examined every 15 days until 45 days for change in burning sensation and size of the lesion using Numeric Pain Rating Scale (NRS) and a standard Vernier caliper, respectively.

Statistical Analysis used: The recorded data were tabulated before subjecting for statistical analysis using Microsoft Excel 2019 and IBM-SPSS® 25 software package. Statistical tests including Shapiro-Wilk's test, Mann-Whitney U test, Wilcoxon signed rank test, Friedman's test, Dunn's Post-Hoc test, Unpaired *t*-test, Paired *t*-test, One-way repeated measures ANOVA, and Bonferroni's Post-Hoc test were done.

Results: By the end, 25 patients in Group I and 24 patients in Group II were remaining, excluding those who failed to report for the follow-up and those who had candida infection in Group II. There was statistically significant reduction in the burning sensation as well as in the size of the lesion in both the groups ($p \leq 0.05$). In Group I, there was 87.8 % (moderate cases) and 85.7 % (severe cases) of reduction in the mean NRS scores on 45^{th} day when compared to the 96.5 % (moderate cases) and 93.48 % (severe cases) of reduction in the Group II. In Group I, there was 92.9 % (moderate cases) and 90.7 % (severe cases) of reduction in the size of the lesion on 45^{th} day when compared to the 92.6 % (moderate cases) and 93.1 % (severe cases) of reduction in the Group II.

Conclusion: The study concluded that the topical application of *Nigella sativa* cream was effective and comparable to Clobetasol propionate 0.05 % gel in the management of Oral Lichen Planus. All the patients responded to *Nigella sativa* cream topical therapy without any side-effects or signs of toxicity during the follow-ups. Anti-inflammatory, analgesic, wound healing, and immunomodulatory properties of *Nigella sativa* cream could be the reason behind these effects. Hence, topical *Nigella sativa* cream therapy appears to be a safe and promising treatment modality in OLP.

Keywords (MeSH): Lichen Planus, Oral; Precancerous conditions; Pain; Clobetasol; Nigella sativa; Randomized Control Trial.

INTRODUCTION

Oral Lichen Planus (OLP) is a common potentially malignant disorder associated with chronic inflammation and cell-mediated immunological dysfunction [1,2]. It occurs on the skin and mucosa having a prevalence of 1 to 2 % [3]. It is commonly seen in the age of 30-60 years having a female predilection [4]. Several factors have been predisposed to this condition but the exact etiology is unknown. However, evidence suggests that it is an autoimmune disorder in which the auto-cytotoxic CD8+ T lymphocytes induce apoptosis causing damage to the oral basal epithelium [4,5].

In the first place, the disease mechanism includes the keratinocyte antigen expression or antigen unmasking that may be a heat shock protein or a self-peptide [6]. Following this, CD8+ and CD4+ T cells migrate toward basal keratinocytes into the epithelium owing to chemokines [7]. Also, there is increased Langerhans cell count in OLP along with the up-regulation of MHC-II expression and consequent antigen presentation to CD4+ cells, which are activated by Interleukin (IL)-12, that in turn activate CD8+ T cells via receptor interaction, interferon γ (INF – γ), and IL-2. Finally, CD8+ T cells activate and kill the basal keratinocytes via tumor necrosis factor-α [8].

Andreason clinically classified OLP into 6 types namely the reticular, papular, plaque, ulcerative, erosive, and bullous of which papular and plaque types rarely present with any symptoms [9]. However, reticular, erosive, atrophic, and bullous types clinically present with burning sensation and/ or pain showing rare remission [10,11]. Reticular OLP is the most common type and occurs as a network of

overlapping white striae followed by erosive (ulcerative), atrophic, and plaque type [12]. The malignant transformation rate is accounted to be 1 to 2% [6,13]. OLP presents with a wide spectrum of appearance, behavior, texture, degree of epithelial changes. This variation in the clinical presentation of OLP is so enormous that it creates a dilemma while diagnosing some cases.

Various therapeutic modalities have been used to improve the lesions and reduce the associated pain but a permanent cure has not been achieved yet, owing to its recalcitrant nature [13]. Topical corticosteroids namely betamethasone, clobetasol propionate (0.05 %), dexamethasone, and triamcinolone acetonide (0.1 %); systemic or topical calcineurin inhibitors namely pimecrolimus, tacrolimus (0.1 %) or cyclosporin (0.1 %); retinoids namely tretinoin; immunosuppressive drugs namely Mycophenolate-mycophenolic acid (500 mg); photochemotherapy, laser, and newer traditional medicines have been reported to treat symptomatic OLP [14].

Corticosteroids remain to be the gold standard for treating OLP and other alternatives have resorted only in severe OLP cases with failure, intolerance, and contraindication to corticosteroids. The rationale for their usage is their anti-inflammatory and immunomodulatory property [14]. Even though these drugs are prescribed routinely, they do have adverse effects like adrenal suppression on systemic use and atrophy of epithelium, immunosuppression, and candidiasis on topical use. Hence, almost all the above-mentioned modalities are associated with adverse effects like atrophy, ulceration, itching, stinging, feeling of warmth, etc; some of these effects are serious, which limits the use of these interventions [15]. Topical formulations are preferred mostly due to their reduced adverse effects [2,16,17]. Studies

have also reported that the primary goal of treatment of OLP is symptomatic i.e., reduction of burning sensation and improvement in the quality of life [13,18,19].

Currently, herbal medications such as propolis, lycopene, aloe vera, honey, curcumin, quercetin, etc., are gaining increased attention owing to their minimal or no side effects [20]. *Nigella sativa* Linn., that belongs to the family *Ranunculaceae* and commonly known as black seed or black cumin, is an annual plant that has been conventionally used in the Indian subcontinent [21], Arabian countries [22], and Europe [23] for cooking and medicinal purposes as a natural remedy for several illnesses and conditions such as bronchitis, asthma, headache, rheumatism, cough, fever, influenza, and eczema [24].

Many of the claimed medicinal uses and beneficial pharmacological actions of *Nigella sativa* including antioxidant property [24], analgesic action [25,26,27,28,29], anti-inflammatory [29], antimutagenic property [30,31,32,33,35], antiulcer action [35,36], anti-diabetic [24], antihistaminic [24], antimicrobial property [37,38,39] etc., have been scientifically tested. It contains thymol, dithymoquinone, thymohydroquinone, and thymoquinone [40], out of which thymoquinone has potent anti-inflammatory and immunomodulatory properties as it inhibits the activity of leukotrienes and prostaglandins [41] and improves cell-mediated immunity caused by T cells and natural killer cells [42]. The rationale for developing an alternative modality is strongly advocated considering the adverse effects of the prolonged use of corticosteroids. As *Nigella sativa* is known for its medicinal value with very minimal or no side effects, it can be an alternative promising therapeutic modality of OLP based on the current evidences [24,25, 26,27,28,29,35,36].

The efficacy of Nigella sativa in the management of OLP has never been evaluated in the past. Therefore, this is a preliminary study, conducted to evaluate the efficacy of *Nigella sativa* (Black seed) (75 % w/v) cream in the management of OLP as an alternative treatment modality in comparison with the gold standard- Clobetasol propionate (0.05 % w/w) gel.

HYPOTHESIS

NULL HYPOTHESIS:

There will be no change in the intensity of burning sensation and size of the lesion on application of *Nigella sativa* (75 % w/v) cream in comparison to Clobetasol propionate (0.05 % w/w) gel in Oral Lichen Planus patients.

ALTERNATE HYPOTHESIS:

There will be change in the intensity of burning sensation and size of the lesion on application of *Nigella sativa* (75 % w/v) cream in comparison to Clobetasol propionate (0.05 % w/w) gel in Oral Lichen Planus patients.

AIM OF THE STUDY

This study aims to evaluate and to compare the efficacy of *Nigella sativa* (75 % w/v) cream (75 % w/v) and Clobetasol propionate (0.05 % w/w) gel for the management of Oral Lichen Planus.

OBJECTIVES

1. To assess the efficacy of *Nigella sativa* (75 % w/v) cream and Clobetasol propionate (0.05 % w/w) gel in determining the change in size of the lesion in Oral Lichen Planus patients.
2. To assess the efficacy of *Nigella sativa* (75 % w/v) cream and Clobetasol propionate (0.05 % w/w) gel in determining the change in degree of burning sensation in Oral Lichen Planus patients.
3. To compare the efficacy of *Nigella sativa* (75 % w/v) cream and Clobetasol propionate (0.05 % w/w) gel in determining the change in size of the lesion and the intensity of burning sensation in Oral Lichen Planus patients.

REVIEW OF LITERATURE

OLP can be diagnosed based on the clinical as well as histopathological features. The World Health Organization (WHO) has proposed diagnostic criteria (1978) to diagnose Oral lichen planus as follows [43]:

Clinical criteria:

1. Presence of gray-white lines radiating from the papular, reticular, annular, plaque-type lesions.
2. Presence of gray-white lines with a lace-like network in a reticular form.
3. Presence of lesions with or without erosions or bullae formation.

Histopathologic criteria:

1. Presence of thickened ortho or parakeratinized layer in sites with normal keratinization, and if a site is normally non-keratinized, this layer may be very thin.
2. Presence of Civatte bodies in the epithelium and the connective tissue.
3. Presence of a well-defined band-like zone that is confined to the connective tissue, comprising mainly of lymphocytes.
4. Presence of the sign of 'liquefaction degeneration' in the basal cell layer.

Modified WHO diagnostic criteria of OLP (2003) [44]:

Clinical criteria:

1. Presence of bilateral lesions that are more or less symmetrical.
2. Presence of gray-white lines with a lace-like network in a reticular pattern.

3. Atrophic, erosive, bullous, and plaque-type patterns are subtypes of reticular lesions and are accepted only on the oral mucosa.

The term "clinically compatible with" should be used for all the other lesions resembling OLP, but do not fulfil the abovementioned criteria.

Histopathologic criteria:

1. Presence of a well-defined band-like zone that is confined to the connective tissue, comprising mainly of lymphocytes.
2. Presence of the sign of 'liquefaction degeneration' sign in the basal cell layer.
3. Absence of epithelial dysplasia.
4. The term "histopathologically compatible with" should be used when the histopathologic features are obvious.

OLP is known to be one among the oral potentially malignant disorder. The overall malignant transformation rate is accounted to be 1-2 % in the literature [43]. However, there is lack of sufficient evidence on malignant transformation, owing to which, a prospective long-term follow-up study with strict diagnostic criteria is essential to elucidate the malignant potential of OLP [45].

OLP presents with a wide spectrum of appearance, behavior, texture, and extent of the epithelial changes. This varied clinical presentation is so enormous that leads to a diagnostic dilemma in some cases.

Andreason has classified OLP into 6 types due to its varied clinical presentations:

1. Reticular- It is the most common type that presents with a fine, asymptomatic, interwind lace-like pattern known as "Wickham's striae" in a bilateral symmetrical form, mostly involving the posterior buccal mucosa. (Figure 1)
2. Erosive- It is a significant type of OLP as it presents with symptomatic lesions that are often surrounded by fine radiant keratinized striae with network-like appearance. (Figure 2)
3. Atrophic- It presents as diffuse red lesions resembling the presence of white lesions of the reticular type that are surrounded by an erythematous area. (Figure 3)
4. Plaque-like- It is characterized by white homogenous irregularities similar to leukoplakia, mainly involving the tongue dorsum and the buccal mucosa. (Figure 4)
5. Papular- It is a rare form of OLP and presents with small white papules showing fine striae in its periphery. (Figure 5)
6. Bullous- It is an uncommon and unusual clinical form of OLP presenting as blisters that increase in size and rupture, leaving a painful ulcerated surface. (Figure 6)

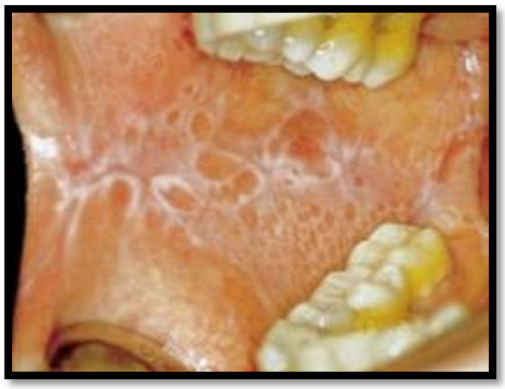


Figure 1: Reticular Lichen Planus

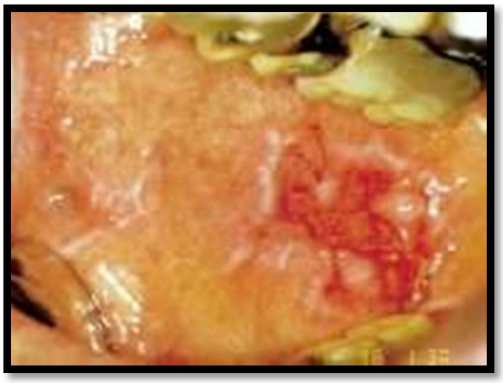


Figure 2: Erosive Lichen Planus

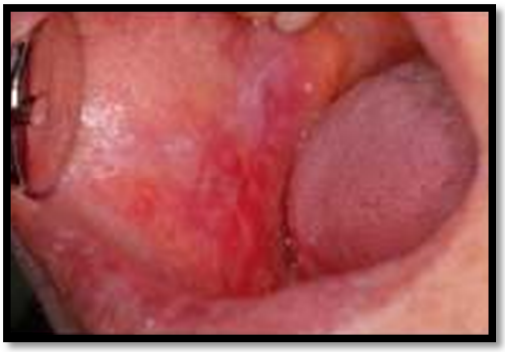


Figure 3: Atrophic Lichen Planus

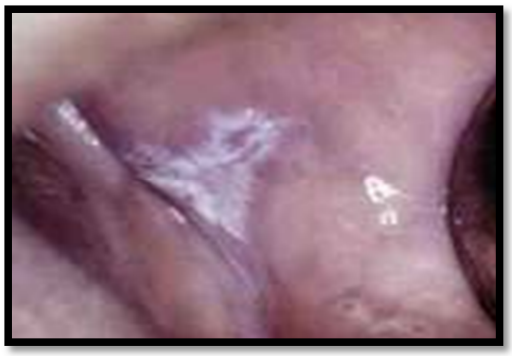


Figure 4: Plaque-like Lichen Planus

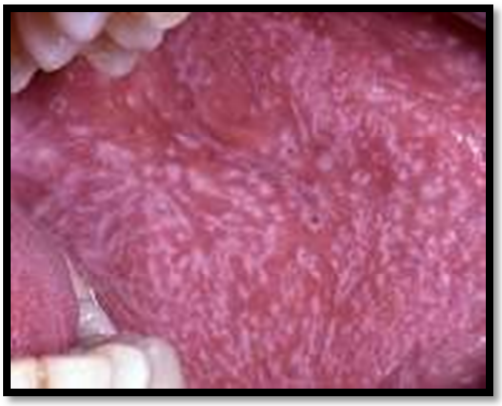


Figure 5: Papular Lichen Planus

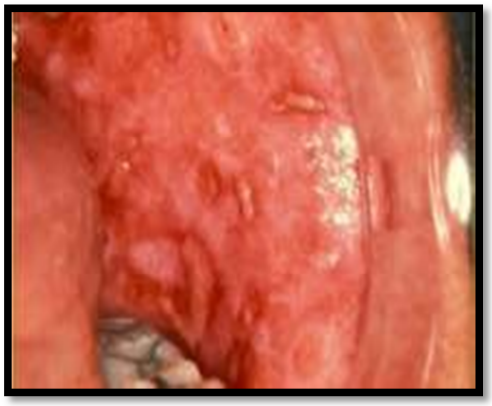


Figure 6: Bullous Lichen Planus

Etiology:

There are several etiologic factors that are associated with OLP which are as follows [45]:

Genetic background:

The history of familial occurrence in OLP cases is rare, but an interrelation has been identified with HLA-A3, HLA-A11, HLA-A26, HLA-A28, HLA-B3, HLA-B5, HLA-B7, HLA-B8, DR1, and DRW9 [46,47].

Dental Materials:

Various dental materials that are used for restoration of teeth such as silver amalgam, cobalt, chromium, palladium, gold, epoxy resins (composite), and denture use for a long period have been recognized as triggering factors for OLP [48,49].

Infectious agents:

Several bacterial agents like Gram negative anaerobic bacillus, spirochetes, and *Helicobacter pylori* etc., and viral agents like human papilloma virus (HPV), Epstein-Barr virus (EBV), Human Herpes Virus-6 (HHV-6), human immunodeficiency virus (HIV), & Hepatitis C virus (HCV) are known to have association with OLP [50,51,52,53,54,55]. Increased occurrence of *Candida* species has been observed in OLP patients although its role in the etiopathogenesis is controversial [50].

Autoimmunity:

Occasionally, OLP is found to be related with autoimmune disorders namely chronic active hepatitis, primary biliary cirrhosis, ulcerative colitis, thymoma, and myasthenia gravis [56].

Bowel disease:

OLP is found to be occasionally associated with Bowel diseases like coeliac disease, ulcerative colitis, and Crohn's disease [57].

Food allergies:

OLP is also known to be related with allergy to food additives such as cinnamon aldehyde [50].

Stress:

Stress is one of the major factors responsible for the development of OLP. The role of the psychological stress in the etiology of OLP is well described in the literature [58,59,60,61].

Diabetes and hypertension:

There is an association of OLP with diabetes mellitus (DM) and hypertension. Grinspan syndrome is characterized by the triad including OLP, diabetes mellitus, and hypertension [62,63,64]. However, this association is controversial.

Clinical features:

Cutaneous lichen planus (LP) is characterized by 5 P's namely purple colour, polygonal shape, pruritis, papules, and plaque [64]. Usually, LP commences as discrete, flat-topped papules having a diameter of 3-15 mm that may coalesce to form larger plaques. They appear red in the early stage but soon they become reddish purple or violaceous hue. The surface of the papule is covered by the characteristic, fine grayish-white lines with an umblicated center, which is referred to as Wickham's striae. Most commonly, these lesions occur on the flexor surface of limbs, inner aspects of thighs, knees, trunk, and may also appear on the lines of trauma, depicting the Koebner's phenomenon [65]. The face is usually not involved. Involvement of the genitals with features similar to the skin lesions [66], scalp (lichen planopilaris), and nail beds is reported in some patients [64].

Oral manifestations:

The presentation of the LP in oral cavity differs from that on the skin. It appears as typical radiating white and gray, lacy, reticular patches, rings, velvety, thread-like papules in an annular or linear form. Characteristic presence of a white elevated dot present at the intersection of the white lines called as Wickham's striae is seen. The lesions are asymptomatic or symptomatic, bilateral, affecting any region of the oral cavity. The most commonly involved sites are buccal mucosa, tongue, lips, gingiva, floor of the mouth, palate, and may occur weeks or months prior to the appearance of skin lesions [67].

Mucocutaneous Lichen Planus:

LP commonly affects the oral mucous membrane but can also involve the vulvovaginal area. However, it rarely manifests in the conjunctiva, esophagus, and larynx [68].

Treatment modalities for OLP:

Pharmacological modalities:

Several therapeutic modalities have been used, including systemic and topical medications like corticosteroids namely triamcinolone acetonide (0.1 %), clobetasol propionate (0.05 %); calcineurin inhibitors like cyclosporin (0.1 %), tacrolimus ointment (0.1 %); and immunosuppressive drugs namely mycophenolate-mycophenolic acid (mycophenolate mofetil- 500 mg) [14].

Corticosteroids:

Corticosteroids are the most commonly used interventions for the management of OLP. Clobetasol propionate is used based on the severity of the lesion [69]. The rationale behind their use is their anti-inflammatory and immunomodulatory property [69]. Despite being the standard drugs, they cause adverse effects of adrenal suppression on systemic use and atrophy & candidiasis on topical use. Additionally, there is a rise in the prevalence of recalcitrant cases of OLP [69].

Clobetasol propionate is a glucocorticoid, often used to treat several skin disorders such as eczema, lichen planus, psoriasis, herpes labialis, and graft Vs host disease of the skin [69]. It is available in many forms namely emollient, cream, ointment, gel, mousse, and shampoo.

Immunomodulators and Immunosuppressants:

Calcineurin inhibitors: Calcineurin is a protein phosphatase that activates the transcription of Interleukin-2 (IL-2), which is responsible for the stimulation of growth and differentiation of T-cell response [69]. Calcineurin inhibitors like cyclosporine and tacrolimus inhibit the calcineurin thereby modulating the immune response.

Cyclosporine: Cyclosporine is a lipophilic cyclic polypeptide that decreases the production of cytokines by inhibition of the activation and/or maturation of several cell types, especially those involved in cell-mediated immunity [70]. It is generally useful in organ transplant rejections, but has been used to manage OLP.

Tacrolimus: Tacrolimus has an immunosuppressive property similar to that of cyclosporine and also, has a greater ability to penetrate the mucosa. It hinders the first phase of T-cell activation thereby inhibiting the phosphatase activity of calcineurin [69].

Pimecrolimus: Pimecrolimus inhibits the activation of T-cell by preventing the production of and release of cytokines from T-cells and mast cells. It has a significant anti-inflammatory and immunomodulatory activity with low systemic immunosuppression [69].

A recent systematic review and meta-analysis reported that Tacrolimus 0.1 % and pimecrolimus 1 % showed similar clinical efficacy and reduction of symptoms when compared to the topical steroids. Pimecrolimus demonstrated superior clinical efficacy than placebo but not in terms of symptom resolution. However, both of these performed better in terms of clinical relapse prevention. Cyclosporine was better than

placebo, but topical steroids showed better clinical efficacy. Also, it was associated with adverse effects of higher frequency and variation [71].

Retinoids: Topical retinoids including tretinoin, fenretinide, and isotretinoin have been effectively used in OLP. Though, resolution of white striae can be achieved, there may be temporary effects only. Systemic retinoids have been used in severe OLP but with variable success. However, the benefit Vs risk factor is yet to be weighted as they have significant side effects [72].

Dapsone: Dapsone, as an antibacterial agent, inhibits the production of dihydro folic acid by bacteria when used in the management of leprosy. But it is also known to have anti-inflammatory properties by the inhibition of the release of mast-cell chemotactic factors. Hence, it has an application in OLP [73].

Mycophenolates: Mycophenolates are used to treat psoriasis originally. It is a well-tolerated drug used in the organ transplants for its immunosuppressive properties. It has been used to treat severe OLP cases successfully [74].

Low dose, low molecular weight heparin: Low dose heparin inhibits the T-lymphocyte heparanase activity that is essential in migration of T-cell to target tissues. It promises to be a safe, simple, and effective therapeutic option for OLP on subcutaneous injections with no side effects [75].

Efalizumab: Efalizumab is used to cure psoriasis for its immunosuppressive properties being a recombinant humanized monoclonal antibody. It binds to the adhesion molecule CD11a to decrease the activation and migration of T lymphocytes causing improvement in OLP. It is parenterally administered once a week subcutaneously [76].

Non-pharmacological treatment modalities:

PUVA therapy: PUVA therapy utilizes the photochemotherapy using 8-methoxypsolaren and long wave ultra-violet (UV) light. It involves oral administration of methoxypsolaren followed by irradiation of UV light intraorally for 2-hour duration. It has been used to manage severe OLP. The disadvantages of this non pharmacological modality include nausea, dizziness, and photosensitivity for 24 hours [77].

Photodynamic therapy: Photodynamic therapy is the technique of using a photosensitizing agent like methylene blue that can be activated at a definite wavelength of laser beam, to cause destruction of the target cells through strong oxidizers that results in cell damage, cell membrane lysis, and inactivation of protein. Owing to its immunomodulatory effects, it may cause apoptosis of the proliferating inflammatory cells in psoriasis and OLP thereby reversing hyperproliferation and inflammation [78].

Laser therapy: In recalcitrant cases of symptomatic erosive OLP even after use of super potent corticosteroids, surgical management by cryotherapy and different types of lasers including 980-nm Diode laser, CO_2 laser, pulse diode laser using 940-nm infrared rays, low dose excimer 208-nm laser with UV-B rays etc., have been tried. Laser destroys the superficial epithelium by protein denaturation targeting the keratinocytes. Deeper penetrating beam of lasers destroy the connective tissue along with the inflammatory component. Lasers have been proven to give promising results in OLP but their efficacy to yet to be elucidated [79,80].

Nigella sativa:

Nigella sativa is one among the top ranked herbal medicines recommended based on the evidence-based decision making (EBDM) that has been referred as the "miracle herb of the century" [81,82]. It is an annual flowering plant that belongs to the family *Ranunculacae* and also called as black cumin, black seed, or *Habbat al-barakah* in Arabic. The scientific classification of *Nigella sativa* is given in Table 1. It is a native of the South and South-west Asia being cultivated in several countries in the Southern Europe, Mediterranean region, Turkey, Syria, and Saudi Arabia [83,84]. It grows to a height of 7.9 to 11.8 inches (20-30 cm) having linear lanceolate leaves. The flowers are delicate with 5 to 10 finely divided sepals and yellow, pink, white, pale blue or pale violet in colour. The fruit is large with an inflated capsule having 3 to 7 united follicles having numerous seeds in each of them. The black seeds are tiny (2 to 4 mm long), flat, oblong, angular having a funnel or boat shape (2 mm long and 1mm wide) [85].

In history, ancient Greek and Egyptian physicians used the seeds of *Nigella sativa* to cure various diseases and ailments including headache, toothache, nasal congestion, intestinal worms, promotion of menstruation, and to promote milk secretion [86,87].

Table 1: Scientific classification of *Nigella sativa* [87]

Kingdom	*Plantae*
Subkingdom	*Tracheobionata*
Division	*Spermatophyte*
Order	*Ranunculales*
Family	*Ranunculaceae*
Genera	*Nigella*
Species	*sativa*

Chemical composition:

Nigella sativa has a diverse chemical composition containing proteins, carbohydrates, minerals amino acids, alkaloids, saponins, fixed oils, volatile oils, and many more compounds [88]. Several active therapeutic constituents have been isolated and identified from the *Nigella sativa* seeds, but the most reported ones are thymoquinone, thymol, thymohydroquinone, and dithymoquinone, especially in the oil [40,89]. The investigations on the chemical composition of *Nigella sativa* seeds commenced in the year 1880 when Greenish reported the presence of 37 % oil and 4.1 % ash (calcium salts in the seeds) [90]. The general composition of the *Nigella sativa* seeds is given in Table 2.

Table 2: Composition of *Nigella sativa* seeds [91]

Constituent	Quantity (% w/w)
Oil	31-35.3
Carbohydrates	16-19.9
Protein	33-34
Ash	4.5-6.5
Fiber	3.7-7
Saponins	0.013
Moisture	5-7

Nigella sativa total oil contains both fixed and volatile oil with the former being the major constituent whereas the essential oil ranges from 0.4 to 0.7 % of the seeds' weight [91]. The chemical composition of the fixed oil is outlined in Table 3.

Table 3: Chemical composition of *Nigella sativa* fixed oil [91]:

Constituent	Quantity (% w/w)
Linoleic acid	44.7-56
Oleic acid	20.7-24.6
Palmitic acid	12-14.3
Linolenic acid	0.6-1.8
Arachidic acid	2-3
Stearic acid	2-7.3
Palmitoleic acid	3
Eicosadienoic acid	2-2.5
Myristic acid	0.16
Sterols	0.5

Volatile oil contains various active constituents including thymoquinone (up to 27.8 %), carvacrol phenol (5.8-11.6 %), p-cymene (15.5-31.7 %), α-pinene (9.3 %), 4-terpineol (2-6.6 %), longifolene (1-8 %), t-anethole (0.25-2.3 %), reduction products of thymoquinone and thymohydroquinone along with esters of 16 % [91].

Several studies have extensively reviewed *Nigella sativa*, especially thymoquinone and reported their various pharmacological properties including antibacterial, antifungal, anthelminthic, antiviral, analgesic, anti-inflammatory,

antihistaminic, hypoglycemic, anticariogenic, antihypertensive, anticancer, antioxidant, hepatoprotective, nephroprotective, and wound healing properties [81,83,84,89].

Review of the studies involving the treatment for Oral Lichen Planus:

A randomized controlled longitudinal investigation was conducted by **Sardella A *et al* (1998)** to compare topical mesalazine (5-aminosalicylic acid) and Clobetasol propionate in the management of symptomatic OLP. Twenty-five patients of OLP were randomly allocated to Group A and B for the mesalazine 5 % or Clobetasol propionate 0.05 % therapy twice daily for 4 weeks. The patients were evaluated using Visual Analogue Scale (VAS) before and after treatment of OLP from zero (no pain) to 10 (extreme pain) for discomfort and pain. Both the interventions obtained either partial or complete resolution of symptoms. The mesalazine group showed complete absence of symptoms, partial response, and no response in 57 %, 21.3 %, and 9 % of the subjects, respectively. No statistically significant difference was seen between the two interventions. The results suggested that mesalazine may be an alternative to clobetasol propionate to treat symptomatic OLP [92].

A clinical study was done by **Chopra A *et al* (1999)** in which 75 patients of Lichen Planus (LP) were enrolled for evaluating the therapeutic effect of dapsone. Patients were randomly allocated to two groups. In regimen 1 (R1), topical corticosteroids and oral chlorpheniramine maleate were given to 25 patients. In regimen 2 (R2), oral dapsone, chlorpheniramine maleate and topical coconut oil were given to 50 patients. The results depicted a 18 % higher total efficacy of R2 than R1 [93].

A double blind randomized controlled trial was conducted by **Conrotto D *et al* (2006)** to compare the effectiveness of clobetasol and cyclosporin in the management of OLP, to evaluate the ability of longest remission of signs and symptoms. 40 patients were assigned to two groups for receiving clobetasol and cyclosporin for the period of 2 months. Both the medicines were incorporated in 4% hydroxyethyl cellulose bioadhesive gel. After the end of therapy patients were assessed for 2 months which resulted 18 out of 19 patients receiving clobetasol showed 95% improvement while 13 out of 20 patients receiving ciclosporin showed 65% clinical response. Eighteen clobetasol treated patients showed 95% reduction in symptoms and 17 cyclosporin treated patients showed 85% reduction in symptoms. Patients receiving clobetasol showed more side effects than cyclosporin. The study concluded clobetasol is more effective in clinical improvement than cyclosporin, with the latter showing more side effect [94].

Review of the studies involving *Nigella sativa* (Black seed) oil:

An *in-vivo* study done by **Khanna T *et al* (1993)** on rats and mice by three antinociceptive tests (hot plate test, tail-pinched test, acetic acid-induced writhing) concluded that the *Nigella sativa* fixed oil is capable of potent antinociceptive actions owing to an opioid principle present in the oil, as it was antagonized by naloxone. They also showed that the oil has significant CNS depressing properties [95].

An *in-vitro* study done by **Houghton PJ *et al* (1995)** on rat peritoneal leukocytes stimulated with calcium ionophore A23 187 showed that both crude fixed oil of *Nigella sativa* and pure thymoquinone inhibited the cyclo-oxygenase pathway and 5-lipoxygenase pathway of arachidonate metabolism, as depicted by dose dependent inhibition of thromboxane B2 and leukotriene B4, respectively. Among

the two, thymoquinone was more potent with IC_{50} value of <1 µg/ml against 5-lipooxygenase and 3.5 µg/ml against cyclo-oxygenase. Thymoquinone was 10 times potent than crude fixed oil in the inhibition of non-enzymatic peroxidation in ox brain phospholipid liposomes. The fixed oil of *Nigella sativa* showed greater inhibition of eicosanoid generation and lipid peroxidation than expected for its content of thymoquinone (0.2 % w/v) which may be contributed by other components present like unusual c20:2 unsaturated fatty acids. It was concluded that the aforesaid pharmacological properties of the oil support the therapeutic use of it in rheumatic arthritis and in other inflammatory conditions [96].

An *in-vitro* study done by **Burtis *et al* (2000)** for rapid evaluation of antioxidants, using two TLC screening methods, depicted that thymoquinone and the components carvacrol, 4-terpineol, and t-anethole had respectable radical scavenging property. These constituents and the essential oil had variable antioxidant activity when analyzed by diphenylpicrylhydracyl assay for non-specific hydrogen atom or electron donating activity. They were also effective OH radical scavenging agents in the assay for non-enzymatic lipid peroxidation in liposomes and the deoxyribose degradation assay [97].

A randomized control trial done by **Bashir MU *et al* (2010)** on 90 male albino mice with *Nigella sativa* seed extract (50 mg/kg) as study group, normal saline as control, and diclofenac sodium as positive reference using acetic acid induced writhing test depicted that *Nigella sativa* seed extract had significant analgesic effect ($p < 0.001$) having an inhibition of 41.9% on writhing although it was less as compared to diclofenac sodium which had 72.82% inhibition on number of writhing [98].

A single blinded randomized controlled trial done by **Al-Harchan NA (2010)** on 93 patients of age group 13-23 years with acne vulgaris who were randomized to apply *Nigella sativa* oil lotion and a control solution twice a day for 2 months reported a significant mean reduction (p= 0.0001) in the count of papular lesions and pustules in *Nigella sativa* oil lotion group with a good, moderate and no response in 58 %, 35 % and 7 % of the participants respectively. The control group showed no significant reduction (p= 0.352 for papules/ 0.248 for pustules) after 2 months of treatment and the treatment response was good, moderate and no response in 8 %, 34 % and 58 %. The patient satisfaction was full in 8 %, partial in 24 % and nil in 68 % and thus concluding *Nigella sativa* oil lotion has proved its efficacy in treating Acne vulgaris as a natural remedy without any side effects [99].

An experimental animal study was done by **Al-Douri AS and Al-Kazaz S Gh (2010)** on twelve rats divided into treatment and control groups who received injection of 0.3 ml of 1% formalin into cheek mucosa to induce oral ulcers. Subsequently, the rats in the treatment group were treated with the daily application of topical *Nigella sativa* oil twice on the site of ulcer for 3 days and sacrificed after 5 days. The results depicted that *Nigella sativa* oil showed a marked anti-inflammatory activity on chemically induced oral ulcers and histopathological examination revealed a difference in the epithelialization rate and in the healing process between the two groups. The treatment with *Nigella sativa* oil enhanced significant reduction in the ulcer size (p= 0.012) and healing (p= 0.002) when compared to the control group concluding that the *Nigella sativa* oil has obvious effect on the rate of healing of the oral ulcers [100].

An experimental study was done by **Khalil J *et al* (2010)** in 40 albino rats to evaluate the healing effects of *Nigella sativa* in experimentally produced gastric ulcers as compared to cimetidine. The rats were administered with aspirin 0.2 g/kg body weight to induce ulcers. Four rats were sacrificed by the end of 2 weeks to confirm the gastric ulcers by histopathological examination. The remaining 36 rats were equally allocated to two groups and three subgroups based on the duration (2, 4, & 6 weeks) of the therapy with *Nigella sativa* and cimetidine (30 and 15 mg/kg, respectively). The animals were anesthetized and sacrificed to remove their stomachs for gross and microscopic histopathological examination. The results depicted that there was no abnormality detected on gross examination in 14/18 (78 %) and 17/18 (94 %) of the rats in group A (*Nigella sativa*) and in group B (cimetidine) respectively due to complete response that was statistically not significant (p= 0.60). Similarly, a complete recovery was noted in 13/18 (72 %) and 16/18 (89 %) of the albino rats of groups A and B respectively that was statistically not significant (p= 0.40). The study concluded that *Nigella sativa* has equal efficacy to cimetidine in gastric ulcers and hence it was suggested for use in routine practice to treat gastric ulcers [101].

An animal experimental study was conducted by **Yaman I *et al* (2010)** to compare the wound healing activity of *Nigella sativa* and sulfadiazine on burn wounds in rats. Fifty-four adult Wistar-albino rats were allocated to three groups equally where the burn wounds were covered with daily cold cream (control), sulfadiazine cream (10 mg/g), and *Nigella sativa* oil cream (50% oil + 50% cold cream). The burn injury was created on the backs of the rats after prior anaesthetization, shaving, and 10 % povidine-iodine application using brass probes for 20 seconds to generate full thickness second degree burn wounds of 1 cm diameter. The animals were sacrificed at four, nine, and fourteen days from injury to

collect the tissue samples for histopathological evaluation. The wound healing was statistically different between the 4th, 9th and 14th day ($p< 0.001$). Hence, the authors concluded that the application of *Nigella sativa* cream and sulfadiazine cream are effective in the wound healing for burn-related skin wounds in the rats [101].

An *in-vitro* as well as *in-vivo* study done by **Dwarampudi LP *et al* (2012)** on HaCaT human keratinocyte cell lines using Sulphorhodamine B (SRB) Assay to evaluate the anti-psoriatic property and cytotoxicity of 95% of ethanolic extract of *Nigella sativa* seeds revealed that it produced a significant epithelial differentiation, from its degree of orthokeratosis (71.36±2.64) as compared to the negative control (17.30±4.09%) that was equivalent to the effect of standard positive control, tazarotene (0.1%) gel, that showed a (90.03±2.00%) degree of orthokeratosis on histometric analysis. When compared to the IC_{50} value of positive control of Asiaticoside (20.13 μg/ml), *Nigella sativa* extract showed an IC_{50} value of 239 μg/ml. Hence, *Nigella sativa* extract significantly increased the relative epidermal thickness when compared to the control affirming its traditional use in the treatment of psoriasis [102].

A placebo-controlled study was done by **Gheita TA and Kenawy SA (2012)** to assess the effectiveness of *Nigella sativa* oil in the treatment of Rheumatoid arthritis. Forty female rheumatoid arthritis patients were involved in this study. The patients consumed two starch filled placebo capsules per day for 1 month. Later, they took two capsules of *Nigella sativa* oil per day for 1 month. Disease activity scores (DAS-28) were recorded at day 0 and at the end of each treatment. The results revealed that the DAS significantly decreased after treatment with *Nigella sativa* oil capsules (4.55 ± 0.82) compared to those before (4.98 ± 0.79) and after (4.99 ± 0.72)

the placebo treatment having a p-value of 0.017. Likewise, there was an improvement in the number of inflamed joints and duration of morning stiffness with a significant improvement in the ACR20 and EULAR response criteria in 43.5 % and 30 % of the subjects, respectively after *Nigella sativa* oil treatment. Hence, it was concluded that *Nigella sativa* oil supplementation with DMARD therapy is effective for Rheumatoid arthritis [103].

An *in-vivo* study done by **Agah S *et al* (2013)** on irritable bowel syndrome patients (ROME II diagnostic criteria) by application of 20 drops of *Nigella sativa* essential oil per day through oral route reported significant decrease in abdominal pain, bloating, fecal urgency, incomplete defecation, and mucus discharge during and after the treatment [104].

An experimental animal study was done by **Üstün K *et al* (2014)** in tongue tissue of thirty-two Sprague-Dawley rats to evaluate the radio-protective effects of *Nigella sativa* oil against radiation-induced oxidative stress. The rats were assigned to 4 groups namely- Group 1: control group; Group 2: sham control group that received 1 ml of saline orally and sham-irradiation; Group 3: irradiation group that received irradiation and 1 ml saline orally; Group 4: the irradiation plus *Nigella sativa* oil group that received irradiation and 1g/ kg/ day of *Nigella sativa* oil through oral route for 10 days. The tongue tissues were excised from the animals, after euthanization, for evaluating the biochemical oxidative parameters. The oxidative stress index, lipid hydrogen peroxide levels, and total oxidant status in the Group 3 were statistically higher than those in Group 1, 2, and 4 ($p < 0.05$ (1 Vs 3), < 0.001 (2 Vs 3, 3 Vs 4). However, the paraoxonase levels in the Group 3 were significantly lower than the other three groups ($p < 0.001$). Hence, *Nigella sativa* oil may be a

beneficial agent having radio-protective effects against the ionizing radiation-related tissue injury [105].

A Triple-Blinded, Placebo-Controlled, Randomized Clinical Trial done by **Hasan Fallah Huseini (2016)** on women with cyclic mastalgia by *Nigella sativa* oil (30 % w/w) topical application in comparison to the topical diclofenac and placebo reported a significant decrease (82%) in VAS scores after 1- and 2-month interval [106].

A cross over clinical study with a one-month washout period done by **Akram Kooshki (2016)** on 40 elderly patients of knee osteoarthritis comparing the efficacy of *Nigella sativa* oil topical application (1cc 8 hourly for 3 weeks)- Group I and 325 mg of oral acetaminophen- Group II reported significant decrease in knee pain after 1 month with *Nigella sativa* oil group and advocated a safe use of it as a supplement. The pain was measured using the VAS during the first and second stages. Hence, *Nigella sativa* oil topical therapy was effective in reducing pain in knee osteoarthritis patients and therefore it is a recommended safe supplement for the elderly patients [107].

A double-blinded, randomized, placebo-controlled trial was conducted by **Kheirouri S *et al* (2016)** in order to assess the immunomodulatory effect of *Nigella sativa* oil on T-Lymphocytes of female patients with Rheumatoid arthritis. Forty-three female patients with mild to moderate rheumatoid arthritis were allocated to *Nigella sativa* group (n= 23) and placebo group (n= 20) to receive 1 g of *Nigella sativa* oil and starch capsule in two doses, respectively. The disease activity scores of 28 joints (DAS28) were calculated and the percentages of CD4+, CD8+, and CD4+CD25+ T cells were determined using flow cytometry. *Nigella sativa* oil

treatment significantly reduced the serum high-sensitivity C-reactive protein levels and DAS28 score improving the number of swollen joints when compared to the baseline and placebo groups. However, the CD4+ T percentage was comparable both in *Nigella sativa* group and placebo group at the baseline and end of the study. It was also noted that the *Nigella sativa* oil therapy reduced CD8+ and increased the CD4+CD25+ T cell percentages, CD4+/CD8+ ratio when compared to the day 0 and placebo. There was negative and positive correlation in the *Nigella sativa* oil group between, the changes in CD8+ and to that of CD4+CD25+ T cell percentage; and the changes in CD4+CD25+ T cell percentage and to that of CD4+/CD8+ ratio, respectively. Therefore, the findings of this study support the use of *Nigella sativa* oil in the clinical management of rheumatoid arthritis through the modulation of T-Lymphocytes [108].

An experimental study was done by **Pise HN and Jadhav SS (2016)** to assess the analgesic and anti-pyretic action of *Nigella sativa* fixed oil and to compare with that of aspirin and control. Eighteen Albino Wistar rats were included in this study with six animals in each group. The animals were allotted to three groups namely-control group that received 2 ml/kg (p.o) of normal saline, standard group that received 300 mg/kg (p.o) of aspirin, and test group that received 10 ml/kg (p.o) *Nigella sativa* oil. Tail flick method described by D'Amour and Smith and acetic acid induced writhing methods were used to evaluate the analgesic property. Baker's yeast induced pyrexia method was used to evaluate the antipyretic property. Reaction time (latency) was also recorded at each interval. In acetic acid writhing test, the Swiss Albino rats were injected with an irritant intraperitoneally to produce peritonitis-like situation. Later, 0.1 ml of 1 % acetic acid was injected intraperitoneally after 30 mins. The mice were observed for the duration of 10 mins after 5 mins elapse time to

check for the number of writhing. In Baker's yeast induced pyrexia method, 1 g/kg of 20 % suspension of freeze-dried Baker's yeast in 9.9 % saline subcutaneously, in nape of neck. The study groups were treated with the respective interventions after 4 h. The readings were measured at 0, 3, 4, and 6 h. The *Nigella sativa* oil showed a comparable analgesic activity compared to aspirin in tail flick method; a significantly higher analgesic activity than control group ($p< 0.001$) and a comparable activity as compared to aspirin in acetic acid writhing test. However, it did not show any significant decrease in the rectal temperature in any of the intervals during the Baker's yeast induced pyrexia method ($p > 0.05$). The rectal temperature changes were comparable to the control group. The study concluded that the *Nigella sativa* oil has a significant analgesic property in both of the above-mentioned methods of analgesia. But it does not show any significant antipyretic effect in Baker's yeast induced pyrexia method [109].

A doubled blinded randomized placebo-controlled trial was conducted by **Hadi V *et al* (2016)** to the anti-inflammatory and anti-oxidant properties of *Nigella sativa* oil in rheumatoid arthritis patients. Biomarkers of inflammatory, antioxidant, and oxidative stress involving tumour necrosis factor- α (TNF- α), Interleukin- 10 (IL-10), superoxide dismutase (SOD), Total antioxidant capacity (TAC), Catalase, Malondialdehyde (MDA), and Nitric oxide (NO) were assessed. Forty-two patients with rheumatoid arthritis were randomly allocated to 2 groups where the study group ($n= 23$) received two capsules of *Nigella sativa* oil (500 mg each) per day for 8 weeks and the placebo group ($n= 16$) received two empty capsules per day for the same duration. Serum TNF- α, IL-10, and levels of oxidative stress in whole blood were evaluated at baseline and at the end. *Nigella sativa* oil group had an increased serum level of IL-10 ($p= 0.001$). *Nigella sativa* oil treatment showed a significant decrease

in serum MDA and NO levels compared to baseline (p= 0.03, 0.013, respectively). However, there were no significant differences in the TNF- α, catalase, SOD, and TAS levels within or between the groups, before and after the intervention (p> 0.05). Therefore, the authors concluded that *Nigella sativa* reduces the inflammation and oxidative stress in rheumatoid arthritis. Also, it may be a useful adjunctive therapy for rheumatoid arthritis patients [110].

A double-blind, randomized clinical trial was done by **Azizi F *et al* (2017)** to compare the effect of topical gels of *Nigella sativa* oil and diclofenac for osteoarthritis pain in older people. This study involved a total of 52 participants, who were randomly divided into *Nigella sativa* oil and diclofenac gel groups. The respective interventions were applied topically twice daily for 21 days. Knee injury and osteoarthritis outcome score (KOOS) questionnaire was used to measure the pain at day 0, on the 10th day, and on the 21st day of topical applications. The results depicted that *Nigella sativa* oil group depicted a significantly lower pain than that of the diclofenac gel group on 21st day (p= 0.04). Also, it was shown that *Nigella sativa* oil demonstrated a better pain relief action than diclofenac gel. Before the treatment, the mean pain scores were 75 ± 16.29 in *Nigella sativa* oil group and 57.66 ± 19.66 in diclofenac gel group. This reduced to 38.88 ± 17.84 and 50.33 ± 20.38, respectively on the 21st day. Hence, *Nigella sativa* oil was more effective than diclofenac gel for alleviating pain in osteoarthritis [111].

An *in-vitro* study was done by **Sudhir SP *et al* (2017)** to evaluate the antimicrobial potential and free radical scavenging property of *Nigella sativa* cold pressed oil and n-Hexane extract from Tunisia, Oman, Pakistan, Egypt, Saudi Arabia, and Turkey. The different seeds were separately crushed and pressed to expel the oil.

N-Hexane was obtained by Soxhlet extraction for 4 hours. The cultures used were *Salmonella typhimurium, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans, Escherichia coli, Bacillus cereus,* and *Aspergillus brasiliensis.* The antibacterial property was assessed by disc diffusion method using Muller-Hinton broth. The results revealed that the *Nigella sativa* oil and n-Hexane had a strong antimicrobial property against Bacillus cereus and Staphylococcus aureus; low antimicrobial effect against *Salmonella, typhimurium, Pseudomonas aeruginosa, Escherichia coli,* and yeast, mold and *Candida albicans.* However, it did not demonstrate antimicrobial property against *Aspergillus brasiliensis*. It was also noted that the *Nigella sativa* seeds and n-Hexane from India, Pakistan, and Tunisia showed a strong antimicrobial property owing to the rich active constituents like thymoquinone and phenolic compounds as determined by GC-MS analysis. This was complemented by the DPPH Assay of the *Nigella sativa* oil. Also, it is noteworthy that the free radical scavenging property of the seeds from Indian subcontinent and Tunisia are superior than their Middle eastern varieties [112].

An animal experimental study was conducted by **Sari Y *et al* (2018)** to investigate and evaluate the comparative efficacy of *Nigella sativa* oil and Aloe vera gels on wound healing in the diabetic rats. Twelve Wistar rats were segregated into 3 groups to receive *Nigella sativa* gel, Aloe vera gel, and control interventions. Alloxan monohydrate (90 mg/kg body weight) was administered intraperitoneally, after a week of acclimatation and overnight fasting, to induce diabetes where the rats with blood glucose level of ≥ 250 mg/dL were deemed diabetic. After removing the hair 1-day priorly and anaesthetization with an intraperitoneal injection of ketamine hydrochloride (40 mg/kg body weight), a wound of 1 cm diameter was created on the back of the rats. In both the interventional groups, 100 µl of the respective gels were

applied and was later covered using a transparent film. In control group, the wound was covered using a plain transparent film without any gel application. The necrotic tissue and the inflammation reduced from day 5 in the Aloe vera gel group compared to the other groups. The wound areas on day 6 (p= 0.020) and day 7 (p= 0.021) were significantly smaller in Aloe vera gel group compared to the *Nigella sativa* gel group along with a better reepithelialization in the former. Thus, Aloe vera gel demonstrated better results than the *Nigella sativa* gel in the topical treatment of diabetic wounds and hence, clinicians can use Aloe vera gel instead of *Nigella sativa* gel for treating the diabetic wounds topically [113].

An animal experimental study was done by **Rafique MA *et al* (2018)** to investigate the effect of *Nigella sativa* essential oil in the guinea pig model of allergic airway inflammation. Eighteen guinea pigs were segregated into 3 groups- normal control, disease control, and *Nigella sativa* oil. The guinea pigs of disease control and *Nigella sativa* oil groups were sensitized priorly by ovalbumin (100 µg ovalbumin and 200 mg alum in phosphate buffer solution, intraperitoneally) on 0th and 14th day followed by challenging with 1 % ovalbumin inhalation on 22nd, 23rd and 24th day. Similarly, guinea pigs of normal control group were provided the equivalent treatment with phosphate buffer solution (1.5 ml, intraperitoneally). The results revealed that disease control group showed significantly higher blood TLC than normal control group (p=0.043). *Nigella sativa* oil treatment reduced blood TLC; however, the difference was insignificant as compared to both disease control and normal control groups. Also, it showed highly significant reduction of TLC in bronchoalveolar lavage (BAL) fluid. Likewise, both the sensitized groups had a significantly high eosinophil percentage (p=0.001) than normal control group with disease control group having the highest. *Nigella sativa* oil was capable to reduce the eosinophil percentage

in both blood and BAL fluid as compared to disease control group that was highly significant (p=0.001) but it was still significantly higher (p=0.001) than normal control group. Therefore, *Nigella sativa* oil treatment reduces the airway inflammation in experimental animals when used prior to an allergic airway challenge. Hence, *Nigella sativa* oil therapy can be considered as a prophylaxis in the treatment of asthma [114].

An experimental animal study was done by **Jerin IA and Begum N (2018)** to evaluate the effect of *Nigella sativa* oil after single administration of three increasing doses in Long Evans rats. Twenty Long Evans rats were equally (5 in each) segregated into four groups namely- control (10 ml/kg of 1 % solution of Tween 20) and experimental (NSO0.5- 0.5 ml/kg of *Nigella sativa* oil, NSO1- 1 ml/kg, NSO1.5- 1.5 ml/kg). Early, inter, and late phase of formalin test were observed within 1st to 5th min, 6th to 15th min, and 16th to 60th min, respectively to assess the nociceptive, central nervous system, and inflammatory pains. The test agents were administered intraperitoneally in a single dose just 1 h before the formalin test. Total frequency of jerking, total duration of flexing, and licking of right-hand paw were recorded post-administration of 50 µl, 2.5 %) formalin subcutaneously. *Nigella sativa* oil significantly the jerking in all doses ($p \leq 0.001$) and flexing, licking in the higher 2 doses ($p \leq 0.001$). Also, jerking was significantly reduced at 1.5 ml/kg dose ($p \leq 0.01$) but flexing and licking were significantly reduced by all the three doses ($p \leq 0.05$, 0.001, 0.001, respectively). Likewise, in late phase, jerking was lowered by higher 2 doses ($p \leq 0.001$) and flexing & licking were lowered by all the three doses ($p \leq 0.001$). Therefore, *Nigella sativa* oil has analgesic effect that is more effective in higher two doses than the lower dose [115].

A randomized controlled trial was done by **Ameen HA *et al* (2019)** to evaluate the efficacy and safety of *Nigella sativa* oil in chemoradiation induced oral mucositis in patients of Head and neck cancer (HNC). Forty head and neck cancer patients were randomized to two groups namely- Group I (*Nigella sativa* oil mouthwash 5 times a day/ 10ml each 6 hourly) and Group II (magic mouthwash with routinely followed protocol/ control group). All patients underwent Radiotherapy of 60-70 Gy in 30-35 fractions over 6-7 weeks with or without chemotherapy. *Nigella sativa* oil mouthwash significantly reduced Radiation Therapy Oncology Group grading in the last 3 weeks of radiotherapy and improved the reported outcomes (pain and swallowing, p= 0.001) during the next 6 weeks of radiotherapy compared to control. Hence, *Nigella sativa* oil decreases the duration and severity of oral mucositis with better pain control and patient reported outcome compared to the routine treatment in patients of HNC [116].

A parallel-arm triple-blinded placebo-controlled randomized control trial was done by **Hassan G *et al* (2020)** in 50 chronic periodontitis patients who were screened by baseline clinical periodontal parameters like clinical attachment loss (CAL), periodontal pocket depth (PPD), plaque index (PI), and bleeding on probing (BoP) to get categorized into two groups- control group (n= 25, normal saline/placebo group) and treatment group (n= 25, *Nigella sativa* oil). The interventions were given for two weeks and the salivary samples were collected on day 0 and day 15 for evaluation of the salivary interleukin-1β, which is crucial for the periodontitis, collagen degradation, and bone turn-over, by Enzyme Linked Immunosorbent Assay. The results depicted that there was no significant difference between the day 0 and day 15 salivary interleukin-1β in both control and treatment group with a p-value of 0.786 and 0.093 respectively. Hence, there is no correlation between the salivary

interleukin-1β and the use of *Nigella sativa* oil in chronic periodontitis patients as compared to the normal saline use, however, it is still an effective alternative herbal modality for other dental diseases. It was also stated that the above results may be due to the smaller sample size of 40 and may vary when evaluated in a larger sample size [117].

An *in-vitro* as well as *in-vivo* study was done by **Shehensha S and Jyothi V (2020)** to evaluate the anti-inflammatory effect of *Nigella sativa* oil mediated silver nanoparticles. The in-vitro anti-inflammatory activity was evaluated using inhibition of albumin denaturation method. The in-vivo anti-inflammatory activity was evaluated by carrageenan-induced inflammation method in male Wistar rats. The paw oedema was induced by the injection of 0.1 ml 1 % carrageenan in normal saline in the right hind foot pad. A dose of 500 mg/kg *Nigella sativa* seed extract was administered orally prior to the carrageenan injection. The animals were divided into 5 groups with 6 in each- group 1: control group; group 2: positive control group that was treated with indomethacin 10 mg/ b.w. intraperitoneally; group 3: the rats were pre-treated with carrageenan in the sub-plantar region, group 4: pre-treated with 500 mg/ b.w. *Nigella sativa* seed extract; group 5: pre-treated with silver nanoparticles 0.3 mg/b.w. (0.19 mg/ mL). The rat paw oedema was found by the volume displacement method using a plethysmometer prior to the administration of various agents and at 30 min, 1, 2, 3, 4, and 5 h post-carrageenan injection. The results revealed that the ethanolic seed extract of *Nigella sativa*, silver nanoparticles, and indomethacin showed the lowest activity and highest activity in the concentrations of 100 µg/ ml and 500 µg/ ml respectively. The significant effect of the silver nanoparticles was near to that of the 500 µg/ ml standard indomethacin. These results show that the silver nanoparticles showed significant anti-inflammatory property comparable to that

of standard against protein denaturation proving inhibition of thermally induced albumin denaturation at tested concentrations. *Nigella sativa* seed extract showed anti-inflammatory effect in the administered dose with the p value > 0.05. Also, there was a substantial reduction in the paw oedema on oral administration of silver nanoparticles compared to the control group. The maximum inhibition percentages were 54.4 % (1 h) and 60.3 % (5 h) at a dose of 0.3 mg/ kg of body weight. Hence, *Nigella sativa* oil mediated silver nanoparticles can be used as an effective anti-oxidant to treat inflammation in various medicinal applications [118].

An *in-vitro* study was conducted by **Manjunath NS *et al* (2020)** to evaluate the anticancer and anti-inflammatory activities of *Nigella sativa* oil. The cytotoxic activity against was assessed on AGS and PANC-1 cell lines, whereas the anti-inflammatory activity was tested on RAW264.1 cell line. *Nigella sativa* oil was extracted from the seeds using Soxhlet apparatus with petroleum ether solvent. Cytotoxicity was evaluated using MTT assay and RAW264.1 cell line activity to iNOS release after the stimulation of lipopolysaccharide was assessed colorimetrically. During cytotoxicity study, serially diluted concentrations of 10, 20, 40, 80, 160, 320 µg/ml of the *Nigella sativa* oil showed a % inhibition of 6.88, 19.50, 34.23, 55.26, 67.88, 76.10 %, respectively and an IC50 of 43.31 µg/ml against AGS cell line. Whereas, % inhibition of 9.21, 17.9, 22.51, 30.70, 55.99, 62.43 %, respectively and an IC50 of 144.4 µg/ml against PANC-1 cell line. *Nigella sativa* oil showed 35.08 % inhibition when tested on RAW264.7 cell line at the highest concentration of 325 µg/ml. *Nigella sativa* extract inhibits the cell growth by initiating apoptosis having an emphasis on its anticancer activity [119].

A double blinded randomized control trial was conducted by **Samadipour E *et al* (2020)** on 124 female students to determine the effect of *Nigella sativa* oil in the pain intensity of primary dysmenorrhea. The participants were randomly allocated to 2 groups to receive two drops of *Nigella sativa* oil (Group I) and liquid olive oil as a placebo (Group II) on the fontanel lobe 3 of the head at night starting from three days before the menstruation period up to five days after the menstruation period. A pain intensity form was filled by the students before and after the three menstrual cycles. Similarly, pain intensity was recorded using the VAS before and after the three menstrual cycles. The results depicted a highly significant decrease in the pain intensity in both the groups ($p< 0.001$). The ANOVA test revealed that the *Nigella sativa* oil group demonstrated a significant decrease in the pain intensity than that of the placebo group with a score of 0.6 and p-value of 0.006. Therefore, *Nigella sativa* could be an easily available, safe, and promising analgesic supplement for use in women with primary dysmenorrhea [120].

An *in-vitro* study was done by **Ciesielska-Figlon *et al* (2021)** to determine the influence of *Nigella sativa* oil on human lymphocytes. Monoclonal anti-CD3 antibody was used to stimulate the lymphocytes using serial dilutions of oil in ethanol. Later, the rates of proliferation and apoptosis were evaluated by flow cytometry. The lowest dilutions (1:1 and 1:10) of *Nigella sativa* oil inhibited the proliferation of lymphocytes. The number of cell divisions and percentage of proliferating cells after stimulation with anti-CD3 antibody or in combination with 1:1 & 1:10 serial dilutions were 8, 1.25, 1.88 and 92.48%, 8.75%, 24.3% respectively. The mean percentage (%) of living cells post-stimulation with anti-CD3 antibody in the presence of 1:1 & 1:10 serial dilutions was 81 %. Thus, preliminary studies show that the *Nigella sativa* oil

has a potent anti-proliferative and proapoptotic activity on human lymphocytes in *in-vitro* setting [121].

A quasi-experimental study was done by **Mardhiah (2021)** to assess the effects of *Nigella sativa* oil on 34 bedridden patients with decubitus risk. The application of oil was done to find the effects of it on pressure ulcers including prevention. Braden scale was utilized as the observation sheet, where the researcher recorded the observation once a day for 7 days, after each application. Before the application of *Nigella sativa* oil, 52.9 % (18), 41.2 % (14), and 5.9 % (2) had very high, high, and moderate risk of decubitus respectively. But after the application, 23.5 % (8), 50 % (17), and 26.5 % (9) had a high, moderate, and low risk of pressure ulcers. Paired t-test revealed a significant difference ($p=0.000 < 0.05$) before and after using *Nigella sativa* oil as a skin care regimen. Hence, this finding would be an additional information in the nursing field, especially for nurses who self-practice skin care using *Nigella sativa* oil [122].

METHODOLOGY

SOURCE OF DATA/ STUDY SETTING:

This study included the patients reporting to the Out Patient Department of Oral Medicine and Radiology, KLE Academy of Higher Education and Research's KLE Vishwanath Katti Institute of Dental Sciences, Belagavi. The thymoquinone (main therapeutic constituent) estimation by High Performance Liquid Chromatography (HPLC) method in the *Nigella sativa* oil was performed in BioQuest Research Pvt Ltd, Kolhapur. The phytochemical screening of the procured black seed oil was done in the Department of Pharmacognosy; formulation, organoleptic evaluation, pH determination, viscosity evaluation of the *Nigella sativa* (75 % w/v) cream and Clobetasol propionate (0.05 % w/w) gel were done in the Department of Pharmaceutics; and mucoadhesive property testing were done in the Department of Pharmacology, KLE Academy of Higher Education and Research's KLE College of Pharmacy, Belagavi. The cytotoxicity evaluation of the prepared formulations was done in KLE's Dr. Prabhakar Kore Basic Sciences Research Centre, Belagavi.

STUDY PERIOD:

August 2019 to November 2021.

STUDY DESIGN:

It is a double blinded randomized control trial.

PERMISSIONS OBTAINED:

1. This study was approved by the Institutional Research and Ethics Committee (Ethical clearance reference number: 1333) of KLE Academy of Higher Education and Research's KLE Vishwanath Katti Institute of Dental Sciences, Belagavi.

2. Written informed consent was obtained from the patients before recruitment into this study.

3. This study was conducted in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1964 and later versions.

4. This study was registered in the Clinical Trial Registry of India with the reference number- REF/2019/11/029518.

5. Permissions were obtained from the Department of Oral Medicine and Radiology, KLE Academy of Higher Education and Research's KLE Vishwanath Katti Institute of Dental Sciences; Department of Pharmaceutics/ Pharmacology, KLE Academy of Higher Education and Research's KLE College of Pharmacy; and Dr. Prabhakar Kore Basic Sciences Research Centre, Belagavi.

SAMPLE SIZE ESTIMATION:

The Sample size estimation was done based on the pilot study which compared the size of the lesion (mm) in Group I (*Nigella sativa* 75 % w/v cream) and Group II (Clobetasol propionate 0.05 % w/w gel) using the formula given below:

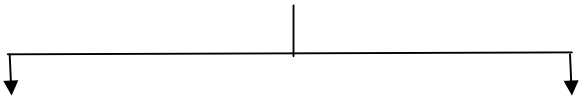
$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 (SD_1^{\,2} + SD_2^{\,2})}{(\bar{x}_1 - \bar{x}_2)^2}$$

where SD_1= 18.9, SD_2= 10.2, $\bar{x}_1$= 11.8, $\bar{x}_2$= 6.4 α= 5 %, 1-β= 85 % [123].

Estimated sample size for each group n= 23.6 (inclusive of 10 % attrition) which was standardized to 24 in each group.

TOTAL SAMPLE SIZE (n)

(48)



Group I	Group II
Nigella sativa (75 % w/v) cream	Clobetasol propionate (0.05 % w/w) gel
(24)	(24)

INCLUSION CRITERIA:

1. Patients clinically diagnosed with OLP (all variants according to Andreason [9]) with fine white radiating striae bilaterally along with burning sensation irrespective of mucocutaneous involvement.
2. Patients above 18 years of age and of either gender.
3. Patients who are willing to participate in this study.

EXCLUSION CRITERIA:

1. Patients with any other potentially malignant disorders other than OLP.
2. Patients who are allergic to *Nigella sativa* cream and Clobetasol propionate gel after oral mucosa patch test.
3. Patients with any other systemic disease.
4. Pregnant and lactating females.
5. Patients who have already undergone treatment for OLP (within the past 3 months).

MATERIALS AND ARMAMENTARIUM:

ANALYTICAL PROCEDURE (THYMOQUINONE ESTIMATION OF OIL):

1. High performance liquid chromatography (HPLC) system (Shimadzu Prominence – I series LC-2030c 3D plus, Kyoto, Japan).
2. Lab solutions Version 5.97 SP1 software.
3. Shim-pack GIST C18 column (250 x 4.5 mm i.d., 5 µm)
4. Guard column C18 (ODS; 4 x 3.0 mm i.d., Phenomax, USA)
5. HPLC grade methanol
6. HPLC grade distilled water
7. Vortex
8. Ultrasonic bath sonicator
9. 0.2 µm filter

PREPARATION OF THE *Nigella sativa* (75 % W/V) CREAM:

1. Preservative-free cold pressed *Nigella sativa* oil
2. Span 20 (Sorbitan monolaurate)
3. Span 60 (Sorbitan monostearate)
4. Tween 80 (Polysorbate 80)
5. Butylated hydroxytoluene
6. Glycerine
7. Distilled water
8. Boiling water bath
9. High speed propeller
10. Homogenizer
11. Mortar and pestle
12. Measuring cylinder
13. Electronic weighing balance
14. Digital pH meter
15. Brookfield CAP+ 2000 viscometer

PREPARATION OF THE CLOBETASOL PROPIONATE (0.05 % W/W) GEL:

1. Clobetasol propionate (API) drug
2. Carbopol 940
3. Distilled water
4. Methyl paraben
5. Propyl paraben
6. Glass rod
7. Glass beaker

8. Measuring cylinder
9. Spatula
10. Electronic weighing balance
11. Ultrasonic bath sonicator
12. Ultrasonic probe sonicator
13. Digital pH meter
14. Brookfield CAP+ 2000 viscometer

CLINICAL STUDY:

MATERIALS:

1. Case history proforma
2. Patient consent form
3. Patient information sheet

ARMAMENTARIUM:

1. *Nigella sativa* (75 % w/v) cream
2. Clobetasol propionate (0.05% w/w) gel
3. Analog Vernier caliper
4. Latex gloves
5. Mouth mask
6. Mouth mirror
7. Cotton tweezer
8. Kidney tray
9. Cheek retractor

10. Patient drape
11. Sterile cotton and guaze
12. Sterile cotton buds
13. Sterile plastic containers for dispensing the cream/ gel.
14. 3M tegaderm transparent adhesive film
15. 5-mm filter paper disk

ESTIMATION OF THYMOQUINONE PERCENTAGE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) [124]:

Commercially available preservative-free *Nigella sativa* oil from Greenish India Trades Pvt. Ltd, Chennai with all the certifications like ISI, GMP, GVCS certificate of compliance etc., was purchased online and subjected for Thymoquinone (major constituent responsible for medicinal properties) estimation through High Performance Liquid Chromatography (HPLC) method, (using standard reference marker of Thymoquinone from Sigma-Aldrich, India) in BioQuest Research Pvt Ltd, Kolhapur.

Chromatographic conditions:

The chromatographic separation of Thymoquinone (TMQ) was done using a Shim-pack GIST C18 column (250 x 4.5 mm i.d., 5µm,) fitted with guard column C18 (ODS; 4 x 3.0mm i.d., Phenomenex, USA). The mobile phase consisted of methanol-water combination in the proportions of 70:30 v/v, eluted at the flow rate of 1.0mL/min. The UV detection was done at 257 nm, maintaining the column temperature at 40 °C, and the injection volume of each sample was 10 µL. The total run time of the chromatography was 14 min.

Preparation of the standard and working solutions:

A stock solution of TMQ (1 mg/mL) was prepared by dissolving the pure drug in methanol. The standard working solutions of TMQ with concentrations of 5, 10, 15, 20, 25 and 30 μg/mL were obtained by diluting the stock solution with the definite volumes of the mobile phase.

Preparation of the sample solution:

The oil containing TMQ was vortex mixed with the methanol, subjected to sonication (60 min) for the complete extraction of the TMQ present in the oil. After sonication, the supernatant was separated and filtered using 0.2 μm filters. The filtrate was suitably diluted with methanol and analysed for TMQ content present in the sample.

PHYTOCHEMICAL SCREENING OF THE *Nigella sativa* OIL:

Phytochemical screening of the procured *Nigella sativa* oil was done for the presence of alkaloids, tannins & phenolic compounds, glycosides, steroids, flavonoids, protein, fats & oils, and volatile oil. Dragendoff's and Meyer's tests were done for screening the presence of alkaloids. The presence of tannins & phenolic compounds was screened using 5 % Ferric Chloride, acetic acid, and lead acetate solutions. Baljet's and Keller-Killiani tests were done for screening the presence of cardiac glycosides. Salkowski's and Liebermann's reactions were checked for the presence of steroids. Sulphuric acid/ lead acetate tests and Biuret test were performed for screening the presence of flavonoids and protein, respectively. Solubility test was done for checking the presence of fats and oil (fixed oil).

PREPARATION OF THE CREAM AND GEL FORMULATIONS:

The preparation of *Nigella sativa* (75 % w/v) cream and Clobetasol propionate (0.05 % w/w) gel were prepared in a laminar air flow chamber under sterile conditions.

PREPARATION OF *Nigella sativa* (75 % W/V) CREAM FORMULATION:

The dosage calculation was based on the amount of thymoquinone (active therapeutic constituent responsible for the medicinal/ therapeutic properties in *Nigella sativa* oil by HPLC. The LD_{50} (Lethal dose 50) of thymoquinone in the previous literature was determined to be 57.5 mg/kg and 794.3 mg/kg after intraperitoneal injection oral gavages, respectively [126].

Step 1: Weighed quantities of the three emulsifying agents namely span 20, span 60 and tween 80 were melted in a China dish at 55°C in water bath.

Step 2: The obtained molten mass of the emulsifying agents or surfactants from step 1 were mixed with the calculated quantity of *Nigella sativa* oil at room temperature using a high-speed propeller (2500 rpm) for 10 minutes.

Step 3: To the homogenized mixture of oil and surfactants obtained from step 2, calculated quantity of distilled water, glycerine (humectant) and butylated hydroxytoluene (preservative) were added slowly with high-speed stirring (2500 rpm) using a homogenizer for 30 minutes until a homogenous cream was formed. pH of the cream was adjusted to 6.8 to 7.2 by adding triethanolamine.

Step 4. The final product of cream obtained from the step 3, was stored in an air-tight amber coloured container at room temperature.

The ingredients and constitutes of the *Nigella sativa* (75 % w/v) cream formulation with their proportions per 100g are given in Table 4.

Table 4: Composition of *Nigella sativa* (Black seed) (75 % w/v) cream formulation

CONSTITUTES	ACTIVITY	QUANTITY
Nigella sativa (Black seed) oil	Main therapeutic ingredient (Antibacterial, antifungal, antinociceptive, antiulcer, analgesic agent)	75 ml
Span 20	Emulsifying agent	10 ml
Span 60	Emulsifying agent	5 g
Tween 80	Emulsifying agent	5 ml
Butylated hydroxytoluene	Antioxidant	25 mg
Glycerine	Humectant	10 ml
Distilled water	Vehicle	Quantity sufficient
Triethanolamine	For pH adjustment	Quantity sufficient

PREPARATION OF CLOBETASOL PROPIONATE (0.05%) GEL FORMULATION:

Step 1: Weighed quantity of Carbopol 940 was soaked in the calculated quantity of distilled water for 24 hours.

Step 2: Weighed quantities of the clobetasol propionate (API) pure drug powder and beta cyclodextrin were dissolved in the calculated quantity of distilled water with the help of ultrasonic probe sonicator.

Step 3: The solution obtained from Step 2 were added slowly to the Carbopol 940 mixture obtained from Step 1 with high-speed stirring using a homogenizer for 10 minutes until a homogenous mixture was formed.

Step 4: A few drops of triethanolamine were added to the homogenous mixture obtained from step 3 and mixed using a glass rod until a homogenous gel was formed.

Step 5: Calculated quantities of methyl paraben & propyl paraben (preservatives) and sufficient quantity of triethylamine (to adjust the pH to 6.8 to 7.2) was added to homogenous gel obtained from Step 4 and mixed using a glass rod.

Step 6: The final product of gel was subjected to homogenization using a homogenizer for 10 minutes and was stored in an air-tight container at room temperature.

The ingredients and constitutes of the Clobetasol propionate (0.05 % w/w) gel formulation with their proportions per 100g are given in Table 5.

Table 5: Composition of Clobetasol propionate (0.05 % w/w) gel

CONSTITUTES	ACTIVITY	QUANTITY
Clobetasol propionate (API) pure drug powder	Major therapeutic ingredient	50 mg
Beta cyclodextrin	Solubility enhancer	120 mg
Carbopol 940	Gelling agent	750 mg
Sodium benzoate 0.1 %	Preservative	100 mg
Methyl paraben	Preservative	0.01 mg
Propyl paraben	Preservative	0.05 mg
Distilled water	Vehicle	Quantity sufficient
Triethanolamine/ triethylamine	For pH adjustment	Quantity sufficient

ORGANOLEPTIC EVALUATION OF THE FORMULATIONS:

The changes in the organoleptic properties of the prepared *Nigella sativa* (75 % w/v) cream and Clobetasol propionate (0.05 % w/w) gel like colour, liquefaction, and phase separation was evaluated by the same examiner through visual examination, over a period of three months at 0 day, 15 days, 1 month, 2 months and 3 months of cream and gel preparation. Also, odour and texture were evaluated by a single pharmacist.

DETERMINATION OF pH OF THE FORMULATIONS [127]:

The determination of pH was done using a calibrated digital pH meter where the glass electrode was dipped completely into the cream and gel formulations, over a period of three months at 0 day, 15 days, 1 month, 2 months and 3 months of cream preparation.

DETERMINATION OF VISCOSITY OF THE FORMULATIONS [128]:

The viscosity of the prepared *Nigella sativa* cream was determined by Brookfield CAP+ 2000 viscometer and Capcalc version 3.0 build 20-0 software using a spindle No 1, following the standard operating procedure of the equipment. One gram of the *Nigella sativa* (Black seed) (75 % w/v) cream and Clobetasol propionate (0.05 % w/w) gel was placed on the centre of the viscometer table using a spatula. The readings were noted near to 100% torque, by rotating the spindle at the speed of 50 rpm at 25°C.

MUCOADHESIVE PROPERTY TESTING [126,129,130]:

The mucoadhesive property of the prepared *Nigella sativa* (75 % w/v) cream and Clobetasol propionate (0.05 % w/w) gel was evaluated using a fabricated modified physical balance apparatus, that consists of a horizontal self-cured acrylic rigid beam with pulleys on either side and two acrylic pans attached on either side with thread over the pulleys, which were held in place using a burette stand and aeration tube holder. Sheep buccal mucosa procured from the local slaughter house was used as the biological membrane and it was secured to an acrylic plate with board pins which in turn was stuck over an inverted 250 ml glass beaker with cyanoacrylate glue. The sheep buccal mucosa was hydrated using phosphate buffer of pH 6.8 and

maintained at 37 ± 1 °C. The height of the apparatus was adjusted at a height sufficient to accommodate the glass beaker on which the sheep buccal mucosa is secured. A weighed quantity of 1g of the preparations were placed at the centre of the sheep buccal mucosa corresponding to the centre of the tissue side pan. The tissue side pan of the apparatus was lowered to rest on the sheep buccal mucosal surface with the preparations and was loaded with a preload weight of 20 g for 3 minutes to facilitate for the formation of mucoadhesive joints. After 3 min, the preload weight was removed and the other side pan was added with weights gradually until the tissue side pan gets separated off the sheep buccal mucosa having the prepared *Nigella sativa* (75 % w/v) cream and Clobetasol propionate (0.05 % w/w) gel. The total weight required for the complete detachment of the tissue side pan from the sheep buccal mucosa with the *Nigella sativa* (75 % w/v) cream and Clobetasol propionate (0.05 % w/w) gel was recorded.

CYTOTOXICITY TESTING OF *Nigella sativa* (75 % W/V) CREAM AND CLOBETASOL PROPIONATE (0.05 % W/W) GEL BY MTT ASSAY ON L929 MOUSE FIBROBLAST CELLS:

In-vitro cytotoxicity or growth inhibition effect of the *Nigella sativa* (75 % w/v) cream and Clobetasol propionate (0.05 % w/w) gel was studied using MTT Assay on L929 mouse fibroblast cells by the colorimetric or spectrophotometric determination of the conversion of MTT into Formazan by the viable cells.

Cells: The L929 mouse fibroblast cells from Dr. Prabhakar Kore Basic Sciences Research Centre were used to determine the cytotoxicity of the *Nigella sativa* (75 % w/v) cream and Clobetasol propionate (0.05 % w/w) gel**.**

Methodology:

Preparation of the L929 cell suspension:

The L929 mouse fibroblasts cell suspension was prepared in the concentration of 1 x 10^5 cells/ ml.

Preparation of the MTT solution:

5 mg of the MTT (tetrazolium salt- 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) salt was added to 1 ml of the phosphate buffer solution with a pH of 7.4.

Procedure:

Day 1:

The prepared L929 mouse fibroblasts cell suspension was seeded into each well of a 96 well microtiter plate and the final volume was made up to 150 µl by adding Gibco Dulbecco's Modified Eagle medium (DMEM) media. Then the plate was incubated overnight at 37 °C.

Day 2:

The dilutions of the *Nigella sativa* (75 % w/v) cream were prepared in DMEM media whereas Clobetasol propionate (0.05 % w/w) gel was used without dilution. Equal volumes (100 µl) of different concentrations of the prepared *Nigella sativa* (75 % w/v) cream in DMEM were added to the wells of the 96 well microtiter plate. The untreated cell cultures were taken as control and 100 µl of DMEM will be added

to the same. Then, the microtiter plate was incubated into a CO_2 incubator at 37 °C for 24 h, in the presence of 5 % of CO_2 in air.

Day 3:

After 24 h of incubation, 20 µl of the prepared 5 mg/ml MTT reagent solution was added to the wells. Then the microtiter plate was covered with an aluminium foil and incubated in a dark place at room temperature for 4 hours as the MTT reagent is photosensitive. Later, the supernatant was removed carefully without disturbing the precipitated formazan crystals and 100 µl of dimethyl sulfoxide (DMSO) was added to dissolve the crystals. The optical density (OD) was measured at a wavelength of 492 nm. The percentage of the surviving cells was calculated using the formula given below:

$$\text{Percentage of surviving cells} = \frac{\text{Mean OD of the test compound}}{\text{Mean OD of the control (untreated cells)}} \times 100$$

The study was done in triplicate where the mean of the three readings was taken as the final result.

CLINICAL EVALUATION OF THE EFFICACY OF *Nigella sativa* (75% W/V) CREAM AND CLOBETASOL PROPIONATE (0.05 % W/W) GEL IN ORAL LICHEN PLANUS:

Clinically diagnosed cases of OLP (according to Andreason [9]) with bilateral white striations along with burning sensation were included in this study. The clinical evaluation of burning sensation was done using Numeric Pain Rating Scale (NRS) and the size of the lesion was measured by a standard Vernier caliper (according to

RECIST criteria [131]) by the first investigator. The second investigator categorized the OLP cases into moderate and severe cases using scoring of Malhotra *et al*, 2008 [132] following a stratified method of allocation. Following this, equal number of the patients from either category were allocated to Group I receiving *Nigella sativa* (75 % w/v) cream and Group II receiving Clobetasol propionate (0.05 % w/w) gel, using concealed chit method. The patients were subjected for an oral mucosa patch test and checked for any erythema, oedema, and/or vesicles as well as itching or dermatographia at the site of application after 2 hours, 24 hours and 72 hours by the second investigator. The application of the cream/ gel was demonstrated to the patients by the second investigator on no positive result on oral mucosal patch test. The patients were then advised for topical application of *Nigella sativa* (75 % w/v) cream and Clobetasol propionate (0.05 % w/w) gel respectively with sterile cotton buds twice a day after breakfast and dinner. Every patient was given a treatment card (Table 6) where he/she was asked to enter the daily application of cream/ gel. The patients were dispensed with 30 g of cream or gel during each visit in sterile, concealed plastic containers. Both the patient and the first investigator were blinded of the type of intervention. Patients were recalled after 15, 30, & 45 days for re-evaluation of burning sensation using Numeric Pain Rating Scale (NRS) and size of the lesion (RECIST criteria [131]) by the first investigator. The application of cream/ gel was advised until the healing of lesion. The allocation concealed chits and opaque sterile plastic containers are shown in Figure 7.

On each application, 1g of *Nigella sativa* (75 % w/v) cream and Clobetasol propionate (0.05 % w/w) gel was delivered. The patients were asked to refrain from drinking or eating for the next 1-hour post-application. The patient compliance *Nigella sativa* (75 % w/v) cream and Clobetasol propionate (0.05 % w/w) gel was

ensured by checking the used empty container brought during the follow-up. In case of any untoward reaction to the medications or any discomfort, the patients were asked to report immediately for documentation.

Additionally, stress and sleep history were elicited and the patients were counselled for the same if necessary.

Figure 7: CONSORT flow diagram:

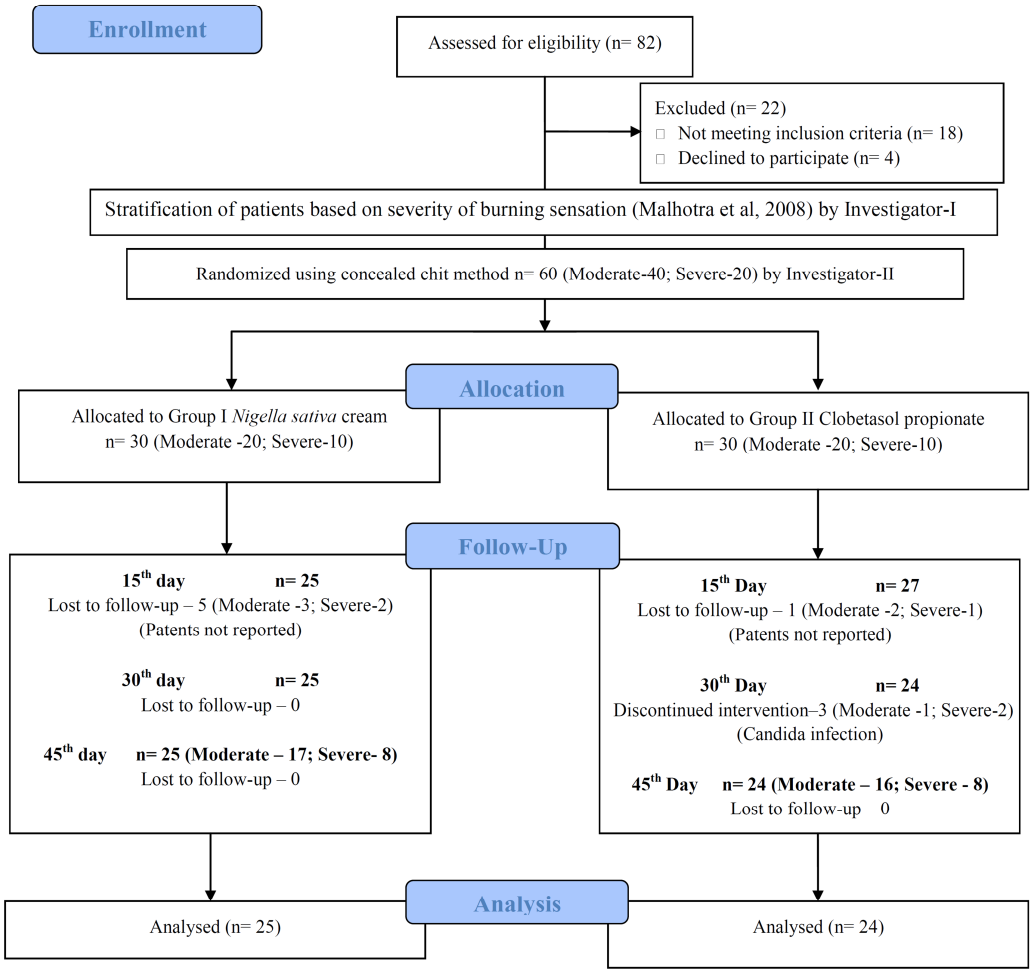


Table 6: Treatment card given for the patients to enter daily application of cream/ gel:

Name of the patient	Day 1	Day 2	Day 3					Day 45
Morning	√							
Night	√							

Randomization technique:

After stratification of the clinically diagnosed cases of OLP into moderate and severe cases according to Malhotra *et al* (2008) [132] scoring and clinical evaluation by the first investigator, the patients were asked to pick the chits in the concealed envelopes with the specific group name i.e., Group I- *Nigella sativa* (75 % w/v) cream group or Group II- Clobetasol propionate 0.05 % w/w gel by the second investigator. Thus, the patients were randomly assigned to two groups.

Oral Mucosa Patch test [133]:

The oral mucosa patch test was done in the upper labial mucosa after removing the excess saliva with sterile gauze. Briefly, a 5-mm filter paper disk saturated with the cream/ gel was applied on the test site and held in place by an adhesive transparent film (Tegaderm, 3M) that in turn, was covered with a small absorbent pad. After 2 hours, the patch was removed to closely observe the site of application for any lesion or reaction, repeating the evaluation at 24 and 48 hours. Even the occurrence of any general reaction was carefully evaluated. If the patients showed adverse effects before 2 hours, the patch was removed immediately and the reaction time was noted. Also, any local and/or general reaction was checked.

Specifically, the appearance of erythema, oedema, vesicles on the test site, as well as itching or dermographia, was considered as a positive result.

RECIST criteria:

The RECIST system is a simplified tumour measurement, where only the longest unidimensional measurement of a lesion, and the sum of the longest diameters for the multiple lesions should be considered [131].

Clinical estimation of the lesion:

1. Change in size of the lesion was assessed by a standard Vernier calliper.
2. Clinical evaluation of the burning sensation and size of the lesion was done by a single researcher i.e., the first investigator.
3. The longest unidirectional measurements were done at four different time points i.e., at the baseline (before the start of the topical cream/ gel therapy), at 15^{th} day, at 30^{th} day, and at 45^{th} day from the start of topical cream/ gel therapy.
4. Proper retraction methods with illumination were followed to visualize the lesion.
5. Care was taken, so as to not stretch the lesion while measuring the lesion.

STATISTICAL ANALYSIS:

The obtained results from the clinical study were tabulated and subjected to the statistical analysis using Microsoft Excel® 2019 and IBM-SPSS® 25.0 software package, USA.

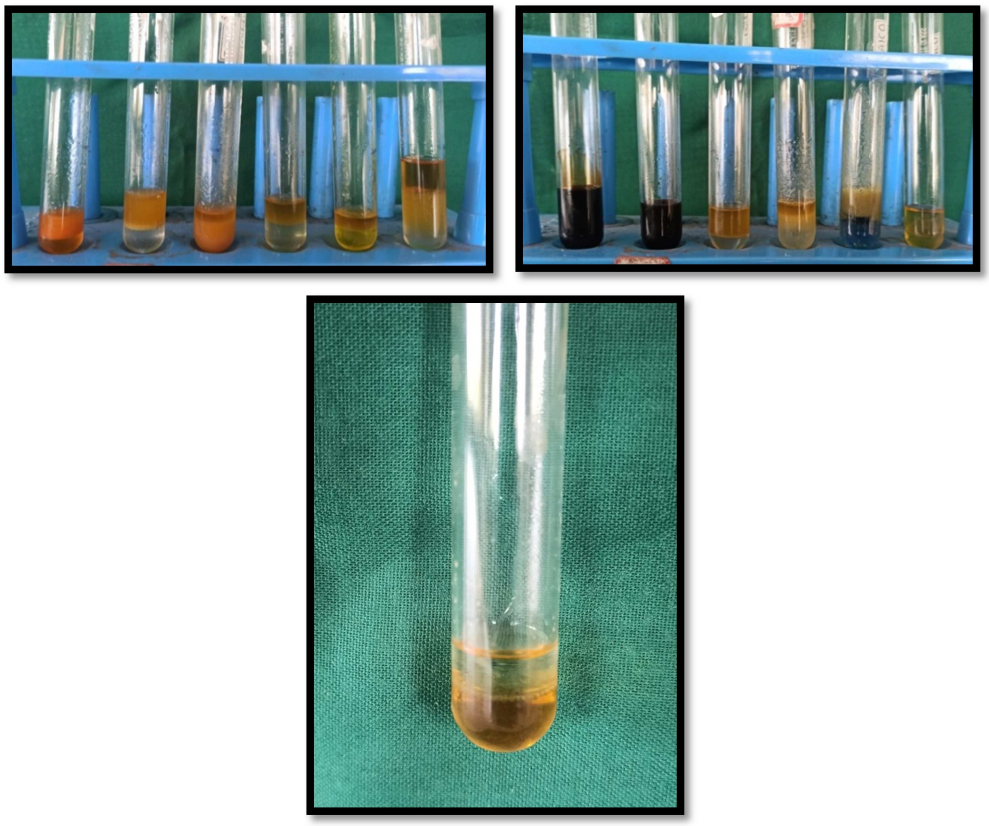


Figure 8: Phytochemical screening of *Nigella sativa* oil

Figure 9: Preparation of *Nigella sativa* (75 % w/v) cream

Figure 10: Preparation of Clobetasol propionate (0.05 % w/w) gel

Figure 11: *Nigella sativa* (75% w/v) cream

Figure 12: Clobetasol propionate (0.05% w/w) gel

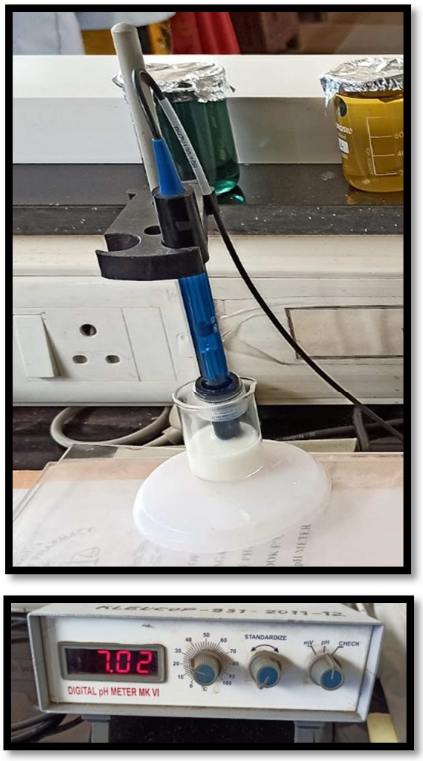


Figure 13: Determination of pH of the formulations

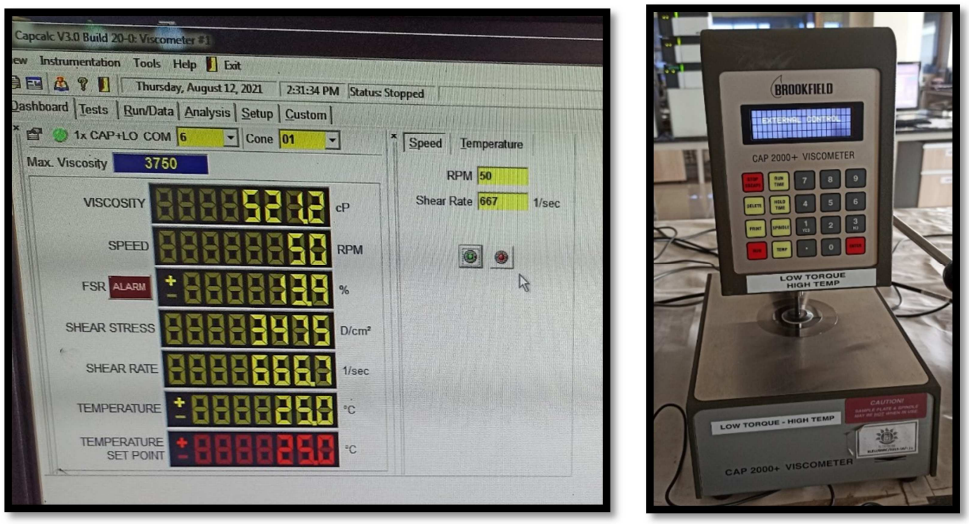


Figure 14: Determination of viscosity of the formulations

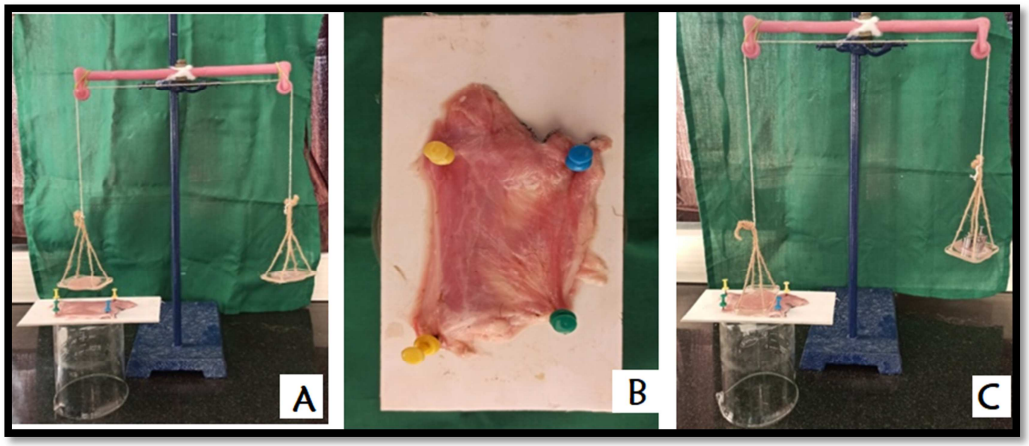


Figure 15: Mucoadhesive property testing- (A) Fabricated modified acrylic physical balance (B) Sheep buccal mucosa (C) Testing procedure

Figure 16: Armamentarium used for clinical examination

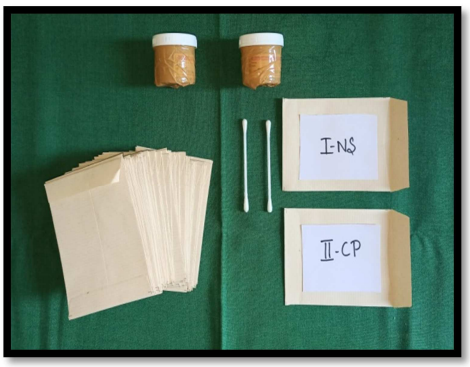


Figure 17: Randomization and allocation concealment

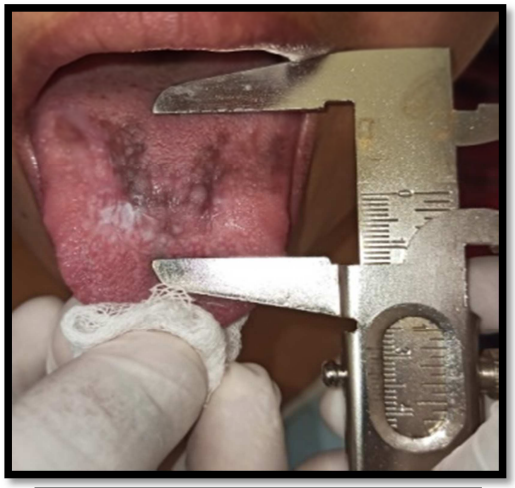


Figure 18: Measurement of size of the lesion using Vernier caliper

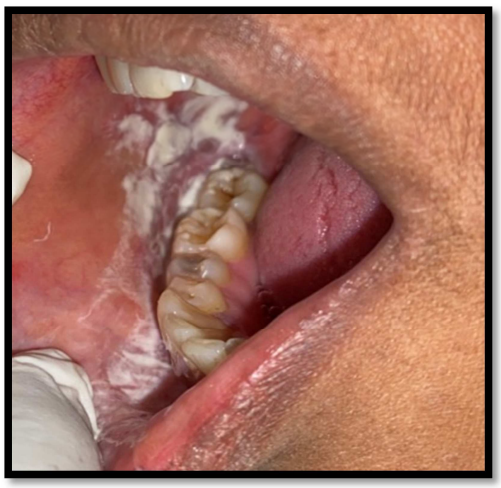


Figure 19: Application of *Nigella sativa* (75% w/v) cream

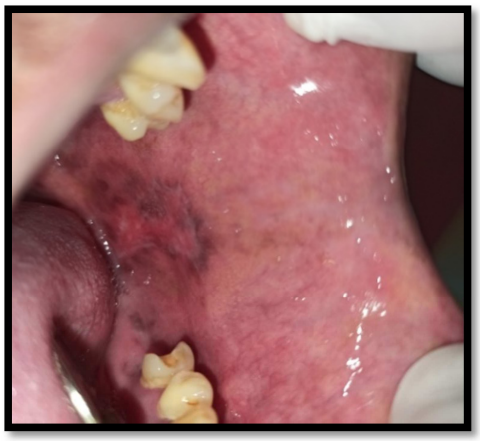


Figure 20: Clinical presentation of Oral lichen planus at baseline before *Nigella sativa* (75 % w/v) cream therapy

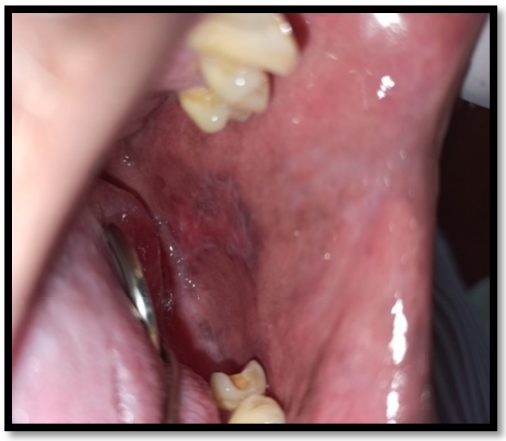


Figure 21: Clinical presentation after 15 days of *Nigella sativa* (75% w/v) cream therapy

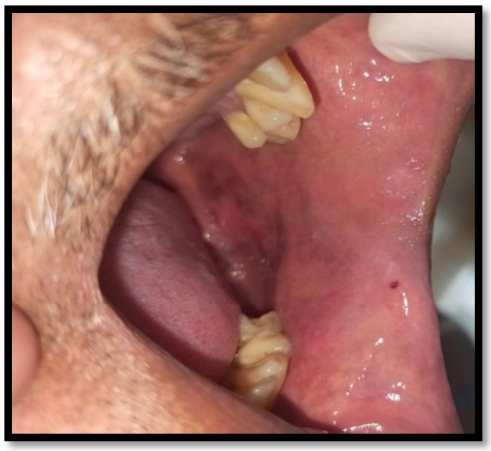


Figure 22: Clinical presentation after 30 days of *Nigella sativa* (75% w/v) cream therapy

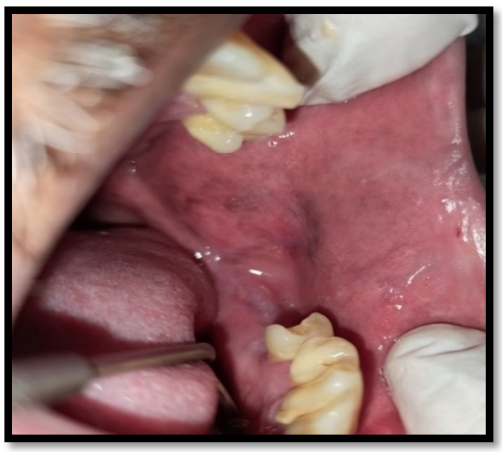


Figure 23: Clinical presentation after 45 days of *Nigella sativa* (75% w/v) cream therapy

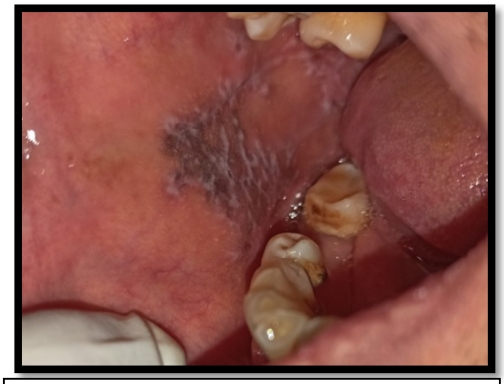


Figure 24: Clinical presentation of Oral lichen planus at baseline before Clobetasol propionate (0.05% w/w) gel therapy

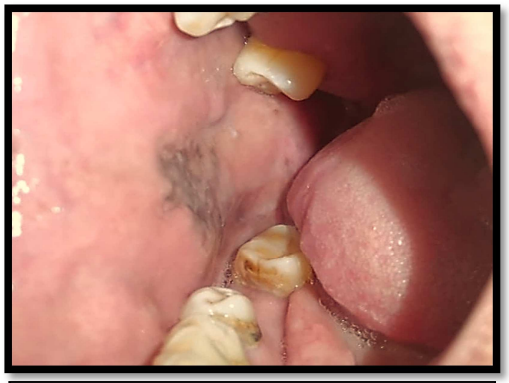


Figure 25: Clinical presentation after 15 days of Clobetasol propionate (0.05% w/w) gel therapy

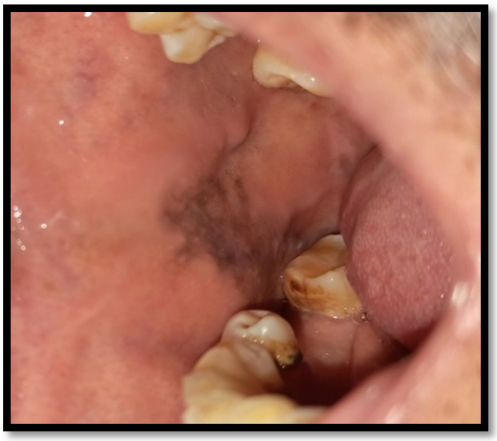


Figure 26: Clinical presentation after 30 days of Clobetasol propionate (0.05% w/w) gel therapy

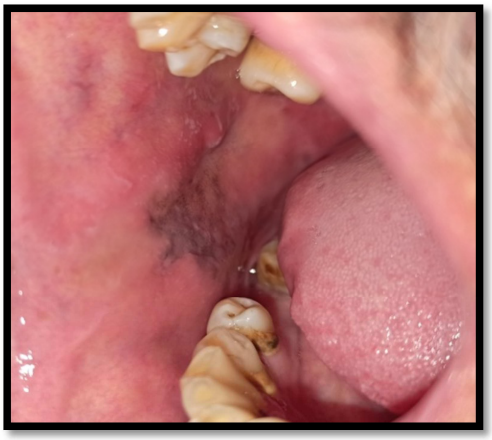


Figure 27: Clinical presentation after 45 days of Clobetasol propionate (0.05% w/w) gel therapy

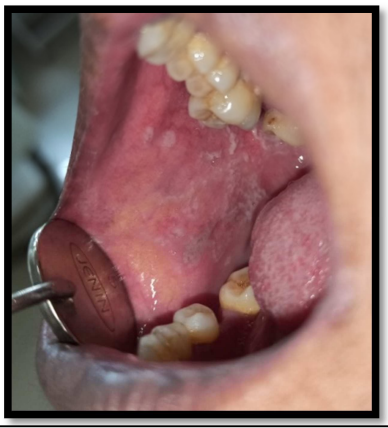


Figure 28: Candidiasis in a OLP patient of Group II- (Clobetasol propionate (0.05% w/w) gel) on 30th day follow-up

RESULTS AND OBSERVATION

THYMOQUINONE ESTIMATION IN THE *Nigella sativa* OIL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) METHOD:

The standard calibration curve for TMQ was found to be linear in the concentration range of 5-30 µg/mL with the regression equation of y = 70267x – 94410 and a correlation coefficient (r2) of 0.9997. The calibration curve of thymoquinone is given in Figure 29.

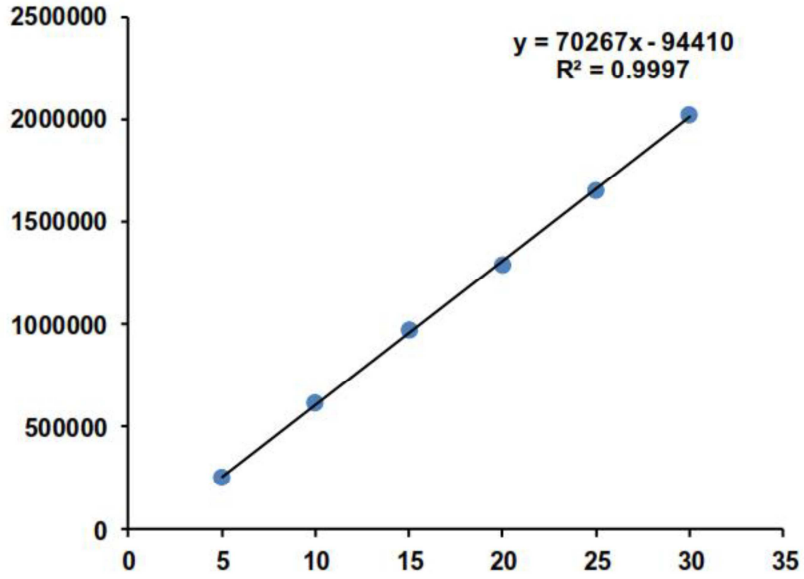


Figure 29: Calibration curve of thymoquinone

Under above mentioned conditions, TMQ eluted at a retention time of 8.41 ± 0.01 min, with a tailing factor of 1.10 ± 0.002 (Figure 30A). Similar retention times were observed for TMQ present in the oil (Figure 30B). No peaks were observed in the chromatograms of oil samples at the retention time of TMQ indicating the specificity of the developed method.

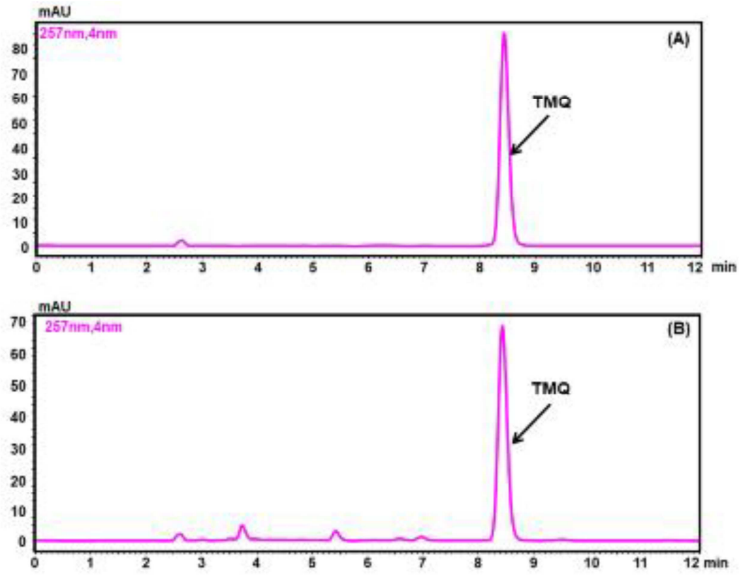


Figure 30: HPLC chromatograms of (A) standard thymoquinone (15 µg/ml) and (B) oil containing thymoquinone

Determination of Thymoquinone in oil:

Peak area = 767114.6667

Standard Calibration curve equation: y = 70267x – 94410 R^2 = 0.9997

Concentration = (767114.6667 +94410)/70267 = 12.26073

Concentration = 12.26073 x 50 (dilution factor) = 613.0365 µg in 500 µl

Total concentration= 613.0365 x 2 = 1226.073 µg/ml or 1.22 mg/ml or 0.122 w/v

One ml of oil contains 1.22 mg/ml (0.122 w/v) of thymoquinone

Table 7: PHYTOCHEMICAL SCREENING OF THE *Nigella sativa* OIL

Phytoconstituent	Test done	Observation	Inference
Alkaloids	Dragendoff's test	Orange brown precipitate observed	Positive
	Meyer's test	Precipitate observed	Positive
Tannins and phenolic compounds	5 % Ferric chloride	No deep blue-black colour observed	Negative
	Acetic acid solubility test	No red colour observed	Negative
	Lead acetate	White precipitate observed	Positive
Cardiac glycosides	Baljet's test	No yellow to orange colour observed	Negative
	Keller-Killiani test	No appearance of reddish brown colour at junction of two liquid layers or bluish green upper layer	Negative
Steroids	Salkowski's reaction	No appearance of red chloroform layer or greenish yellow fluorescence in acid layer	Negative
	Liebermann's test	No appearance of blue colour	Negative
Flavonoids	Sulphuric acid test	Deep yellow solution observed	Positive
	Lead acetate tests	Yellow precipitate observed	Positive
Proteins	Biuret test	Appearance of Violet colour observed	Positive
Fats and oil (fixed oil)	Solubility test in Chloroform	Soluble	Positive
Volatile oil	Solubility test in 90 % Alcohol	Soluble	Positive

Positive- Present; Negative- Absent

Table 8: ORGANOLEPTIC EVALUATION

Formulation	Colour	Odour	Texture
Nigella sativa (75 % w/v) cream	Creamy white	Pleasant aromatic odour of black seed oil	Smooth
Clobetasol propionate (0.05 % w/w) gel	White	Pleasant	Smooth

Both the formulations showed stability in their colour, odour, and texture for the evaluation period of 3 months as noted by the organoleptic examiner. Also, there were no signs of liquefaction or phase separation.

Table 9: pH OF THE FORMULATIONS

Formulation	pH				
	Day 0	**Day 15**	**1 month**	**2 months**	**3 months**
Nigella sativa (75 % w/v) cream	7.34	7.26	7.21	7.18	7.02
Clobetasol propionate (0.05 % w/w) gel	6.89	6.85	6.92	6.96	6.88

Table 10: VISCOSITY OF THE FORMULATIONS

Formulation	Viscosity (cP)				
	Day 0	**Day 15**	**1 month**	**2 months**	**3 months**
Nigella sativa (75 % w/v) cream	510 ± 25	521 ± 13	528 ± 18	535 ± 31	524 ± 28
Clobetasol propionate (0.05 % w/w) gel	704 ± 21	708 ± 26	738 ± 31	714 ± 28	728 ± 28

All the values are expressed as mean ±SD in cP

Table 11: MUCOADHESIVE PROPERTY TESTING

Formulation	Weight that showed complete detachment of the tissue side pan (g)	Mean tensile strength (g)
Nigella sativa (75 % w/v) cream	40	41.7
	45	
	40	
Clobetasol propionate (0.05 % w/w) gel	35	36.7
	40	
	35	

All values are expressed in g- grams.

Table 12: CYTOTOXICITY TESTING OF *Nigella sativa* (75 % w/v) CREAM AND CLOBETASOL PROPIONATE (0.05 % w/w) GEL BY MTT ASSAY ON L929 MOUSE FIBROBLAST CELLS

Compound	Optical density (OD)	Mean optical density (OD)	% Viability
Control	0.462	0.446	100
	0.447		
	0.430		
Clobetasol propionate 0.05 % gel	0.452	0.412	92.23
	0.287		
	0.496		
Nigella sativa 75 % cream (conc.1)	0.531	0.407	91.34
	0.491		
	0.201		
Nigella sativa 75 % cream (conc. 2)	0.331	0.416	93.20
	0.423		
	0.494		
Nigella sativa 75 % cream (conc.3)	0.450	0.425	95.13
	0.416		
	0.409		

Both the formulations i.e., *Nigella sativa* (75 % w/w) cream and Clobetasol propionate (0.05 % w/w) gel were not cytotoxic and depicted a good tolerability on L929 mouse fibroblast cells.

CLINICAL STUDY:

A total of 60 clinically diagnosed OLP patients (40 moderate cases and 20 severe cases) were included in this study to get randomly allocated to Group I (*Nigella sativa* 75 % w/v cream) and Group II (Clobetasol propionate 0.05 % gel) using concealed chit method. Five patients of Group I (3 moderate and 2 severe) and three patients of Group II (2 moderate and 1 severe) failed to report for the 15^{th} day follow-up due to travelling issues. Additionally, in Group II, *Candida* infection (Figure 25) was observed in 3 patients (1 moderate and 2 severe) during the 30^{th} day follow-up and hence the intervention was stopped to initiate antifungal therapy. Therefore, a total of 25 patients (17 moderate and 8 severe) in Group I and 24 patients (16 moderate and 8 severe) in Group II were remaining after exclusion of the patients for the aforementioned reasons and the data pertaining to those same patients were included in the statistical analysis. However, the baseline and 15^{th} day follow-up data of the patients who had *Candida* infection during the 30^{th} day follow-up in Group II were considered. There were no side-effects neither any signs of toxicity reported during the treatment or follow-up in Group I.

STATISTICAL ANALYSIS:

The statistical analysis was carried out utilizing-

- Sample size (n) was 25 in Group I and 27 in Group II (excluding *Candida* infection cases (3) n = 24), α=5 %, β error= 5 %, power= 85 %.
- **Shapiro-Wilk test** for checking the distribution of data (normality) only for size of the lesion (mm) data (ratio data) which showed a normal distribution.

Hence, parametric tests were done for size of the lesion (mm) and non-parametric tests were done for burning sensation- NRS scores (ordinal data).

Burning sensation (NRS score):

- **Mann-Whitney U test** for intergroup comparison of burning sensation (NRS score) between Group I and II for moderate and severe OLP cases.
- **Wilcoxon signed rank test** for intragroup comparison of burning sensation (NRS score) at different time points in Group I and Group II for moderate and severe OLP cases.
- **Friedman's test** for comparison of burning sensation (NRS score) at baseline to that at 15^{th} day, 30^{th} day, and 45^{th} day in Group I and Group II for moderate and severe OLP cases.
- **Dunn's Post-Hoc test** for pairwise comparison of burning sensation (NRS score) at baseline, 15^{th} day, 30^{th} day, and 45^{th} day in Group I and Group II for moderate and severe OLP cases.

Size of the lesion (mm):

- **Unpaired *t*-test** for intergroup comparison of size of the lesion (mm) between Group I and II for moderate and severe OLP cases.
- **Paired *t*-test** for intragroup comparison of size of the lesion (mm) at different time points in Group I and Group II for moderate and severe OLP cases.
- **One-way repeated measures ANOVA test** for comparison of size of the lesion (mm) at baseline to that at 15^{th} day, 30^{th} day, and 45^{th} day in Group I and Group II for moderate and severe OLP cases.

- **Bonferroni's Post-Hoc test** for pairwise comparison of size of the lesion (mm) at baseline, 15th day, 30th day, and 45th day in Group I and Group II for moderate and severe OLP cases.

Table 13: Distribution of patients by age groups in Group I and Group II

Age group	Group I- N (%)		Group II- N (%)		Total- N (%)
	Moderate	Severe	Moderate	Severe	
≥ 30 years	4 (23.5)	1 (12.5)	2 (11.8)	1 (10.0)	8 (15.4)
31-40 years	3 (17.6)	2 (25.0)	2 (11.8)	3 (30.0)	10 (19.2)
41-50 years	6 (35.3)	1 (12.5)	6 (35.2)	0 (0.0)	13 (25.0)
≥ 51 years	4 (23.5)	4 (50.0)	7 (41.2)	6 (60.0)	21 (40.4)
Total	17 (100)	8 (100)	17 (100)	10 (100)	52 (100)
N	25		27		

The majority of subjects in Group I were in the age group of ≥ 51 years (8- 32 %) followed by 41-50 years (7- 28 %) and least in the 31-40 years (5- 20 %) and ≤ 30 years (5- 20%).

The majority of subjects in Group II were in the age group of ≥ 51 years (13- 48.3 %) followed by 41-50 years (6- 22.2 %) and least in the 31-40 years (5- 18.5 %) and ≤ 30 years (3- 11 %).

Graph 1: Distribution of patients by age groups in Group I and Group I

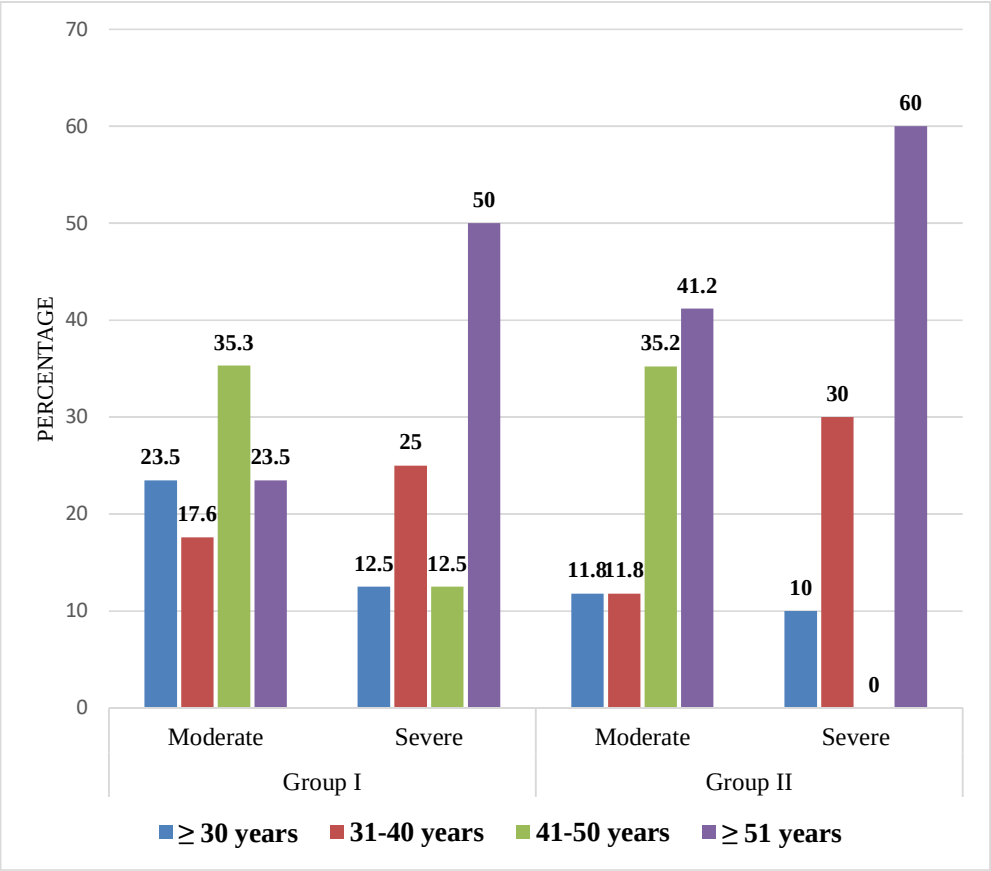


Table 14: Mean age of the patients in Group I and Group II

Group	N	Mean Age ± SD (years)
Group I	25	44.5 ± 13.5
Group II	27	48.7 ± 13.3

The mean age of OLP occurrence in Group I was 44.5 ± 13.5 years and in Group II was 48.7 ± 13.3 years. The graphical representation of the same is given in Graph 2.

Graph 2: The mean age of OLP occurrence in Group I and Group II

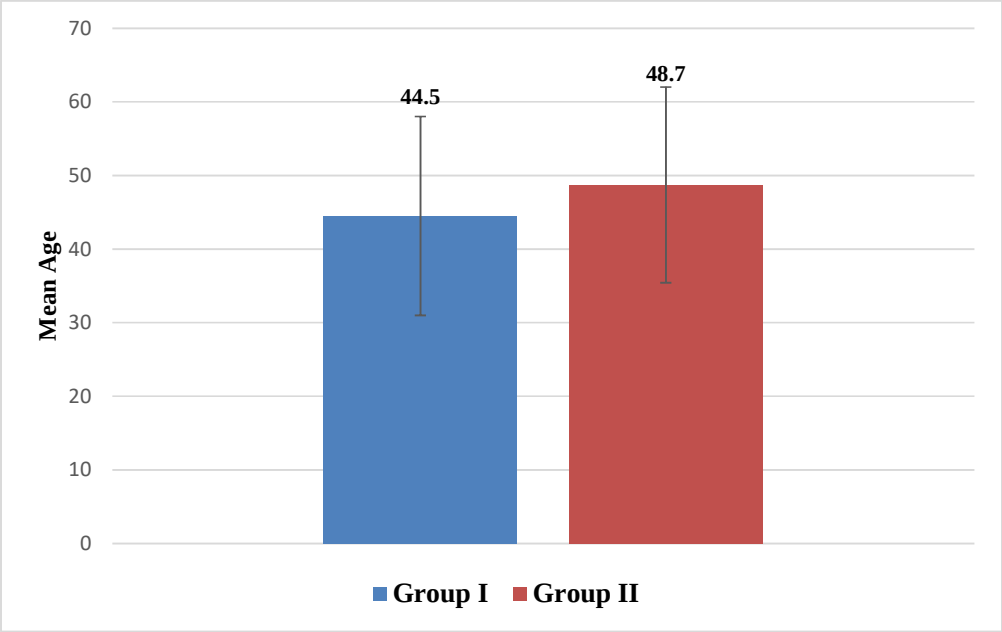


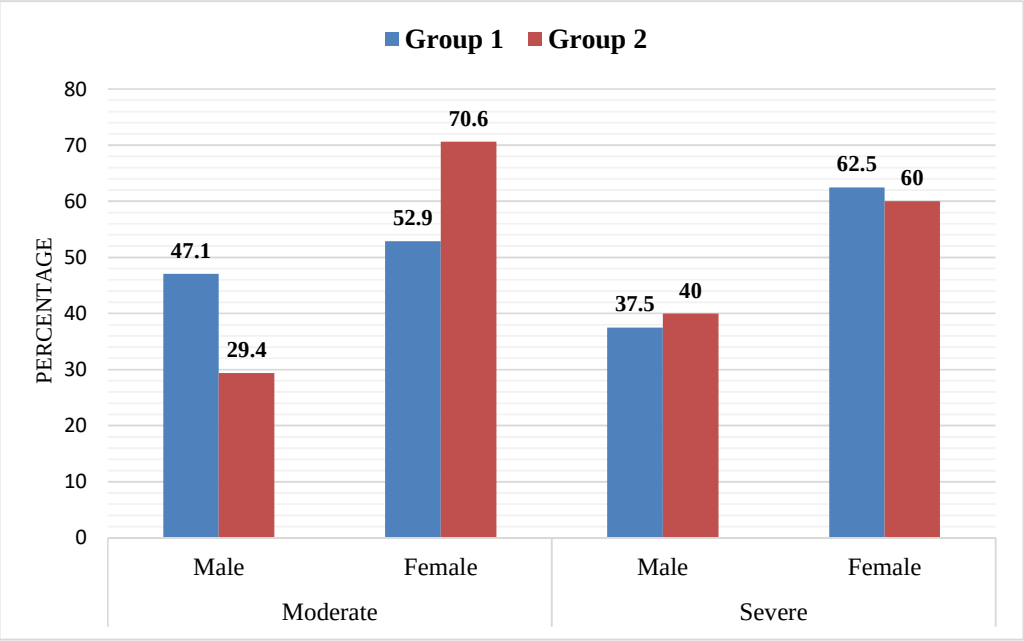
Table 15: Distribution of gender of patients in Group I and Group II

Gender	Group I- N (%)		Group II- N (%)		Total- N (%)
	Moderate	Severe	Moderate	Severe	
Males	8 (47.1)	3 (37.5)	5 (29.4)	4 (40.0)	20 (38.5)
Females	9 (52.9)	5 (62.5)	12 (70.6)	6 (60.0)	32 (61.5)
Total	17 (100)	8 (100)	17 (100)	10 (100)	52 (100)
N	25 (11 males, 14 females)		27 (9 males, 18 females)		

The subjects included both males and females as participants in Group I and Group II.

The graphical representation of the distribution of gender of patients in Group I and Group II is given in Graph 3.

Graph 3: Distribution of gender of patients in Group I and Group II



ANALYSIS RELATED TO BURNING SENSATION (NRS SCORE)

Table 16: Intergroup comparison of burning sensation (NRS score) at different time points between Group I and Group II for Moderate OLP cases

Timepoint	Group	N	Mean ± SD	Mean rank	Sum of ranks	Mann-Whitney U-value	Z-value	p-value
Baseline	Group 1	17	4.1 ± 2.2	14.4	244.5	91.5	-1.845	0.065
	Group 2	17	5.7 ± 2.4	20.6	350.5			
15th day	Group 1	17	2.2 ± 2.0	14.4	244.5	91.5	-1.844	0.065
	Group 2	17	3.7 ± 2.4	20.6	350.5			
30th day	Group 1	17	0.9 ± 1.3	14.2	240.5	87.5	-1.849	0.064
	Group 2	16	1.8 ± 1.7	20.0	320.5			
45th day	Group 1	17	0.5 ± 0.8	15.0	255.5	102.5	-1.613	0.107
	Group 2	16	0.2 ± 0.5	19.1	305.5			

**p-value ≤ 0.05- Statistically significant; Test Applied: Mann-Whitney U test; 95 % CI. The values of burning sensation (NRS scores) are presented as mean ± Standard deviation (SD).*

At the baseline, the mean NRS score (burning sensation) for Moderate OLP cases was 4.1 ± 2.2 mm and 5.7 ± 2.4 mm in Group I and II, respectively.

At 15th day, the mean NRS score (burning sensation) for Moderate OLP cases was 2.2 ± 2.0 mm and 3.7 ± 2.4 mm in Group I and II, respectively.

At 30th day the mean NRS score (burning sensation) for Moderate OLP cases was 0.9 ± 1.3 mm and 1.8 ± 14.5 mm in Group I and II, respectively.

At 45th day, the mean NRS score (burning sensation) for Moderate OLP cases was 0.5 ± 0.8 mm and 0.2 ± 0.5 mm in Group I and II, respectively.

When intergroup comparison of mean NRS scores was done between Group I and Group II for Moderate OLP cases, there was no statistically significant difference ($p > 0.05$) in the NRS scores (burning sensation) at all the different time points i.e., at baseline, at 15th day, at 30th day, and 45th day.

In Group I, mean NRS score at the baseline is 4.1 ± 2.2 in Moderate OLP cases.

- Mean NRS score at the baseline was 4.1 ± 2.2 and on 15th day was 2.2 ± 2.0. The mean difference between baseline and 15th day is 1.9. The % of change at 15th day is 46.3 %.
- Mean NRS score at the baseline was 4.1 ± 2.2 and on 30th day was 0.9 ± 1.3. The mean difference between baseline and 30th day is 3.2. The % of change at 30th day is 78.05 %.
- Mean NRS score at the baseline was 4.1 ± 2.2 and on 45th day was 0.5 ± 0.8. The mean difference between baseline and 60th day is 3.6. The % of change at 45th day is 87.8 %.

In Group II, mean NRS score at the baseline is 5.7 ± 2.4 in Moderate OLP cases.

- Mean NRS score at the baseline was 5.7 ± 2.4 and on 15th day was 3.7 ± 2.4. The mean difference between baseline and 15th day is 2. The % of change in 15th day is 35.1 %.
- Mean NRS score at the baseline was 5.7 ± 2.4 and on 30th day was 1.8 ± 1.7. The mean difference between baseline and 30th day is 3.9. The % of change in 30th day is 68.4 %
- Mean NRS score at the baseline was 5.7 ± 2.4 and on 45th day was 0.2 ± 0.5. The mean difference between baseline and 45th day is 5.5. The % of change in 45th day is 96.5 %.

The graphical representation is given in Graph 4.

Table 17: Intergroup comparison of burning sensation (NRS score) at different time points between Group I and Group II for Severe OLP cases

Timepoint	Group	N	Mean ± SD	Mean rank	Sum of ranks	Mann-Whitney U-value	Z-value	p-value
Baseline	Group 1	8	6.3 ± 2.7	11.9	95.5	20.5	-1.770	0.077
	Group 2	10	4.6 ± 1.4	7.6	75.5			
15^{th} day	Group 1	8	4.3 ± 2.5	11.4	91.5	24.5	-1.398	0.162
	Group 2	10	2.7 ± 1.2	8	79.5			
30^{th} day	Group 1	8	2.5 ± 1.8	11.6	93	7	-2.715	0.007*
	Group 2	8	0.50 ± 0.8	5.3	43			
45^{th} day	Group 1	8	0.9 ± 1.5	9.5	76	24	-1.105	0.269
	Group 2	8	0.3 ± 0.7	7.5	60			

**p-value ≤ 0.05- Statistically significant; Test Applied: Mann-Whitney U test; 95 % CI. The values of burning sensation (NRS scores) are presented as mean ± Standard deviation (SD).*

At the baseline, the mean NRS score (burning sensation) for Severe OLP cases was 6.3 ± 2.7 mm and 4.6 ± 1.4 mm in Group I and II, respectively.

At 15^{th} day, the mean NRS score (burning sensation) for Severe OLP cases was 4.3 ± 2.5 mm and 2.7 ± 1.2 mm in Group I and II, respectively.

At 30^{th} day the mean NRS score (burning sensation) for Severe OLP cases was 2.5 ± 1.8 mm and 0.50 ± 0.8 mm in Group I and II, respectively.

At 45^{th} day, the mean NRS score (burning sensation) for Severe OLP cases was 0.9 ± 1.5 mm and 0.3 ± 0.7 mm in Group I and II, respectively.

When intergroup comparison of mean NRS scores was done between Group I and Group II for Severe OLP cases, there was no statistically significant difference ($p > 0.05$) in the NRS scores (burning sensation) at baseline, 15th day, and 45th day except at 30th day where there was a statistically significant difference ($p \leq 0.05$).

In Group I, mean NRS score at the baseline is 6.3 ± 2.7 in Severe OLP cases.

- Mean NRS score at the baseline was 6.3 ± 2.7 and on 15th day was 4.3 ± 2.5. The mean difference between baseline and 15th day is 2. The % of change at 15th day is 31.7 %.
- Mean NRS score at the baseline was 6.3 ± 2.7 and on 30th day was 2.5 ± 1.8. The mean difference between baseline and 30th day is 3.8. The % of change at 30th day is 60.3 %.
- Mean NRS score at the baseline was 6.3 ± 2.7 and on 45th day was 0.9 ± 1.5. The mean difference between baseline and 60th day is 5.4. The % of change at 45th day is 85.7 %.

In Group II, mean NRS score at the baseline is 4.6 ± 1.4 in Severe OLP cases.

- Mean NRS score at the baseline was 4.6 ± 1.4 and on 15th day was 2.7 ± 1.2. The mean difference between baseline and 15th day is 1.9. The % of change in 15th day is 41.3 %.
- Mean NRS score at the baseline was 4.6 ± 1.4 and on 30th day was 0.5 ± 0.8. The mean difference between baseline and 30th day is 4.1. The % of change in 30th day is 89.1 %
- Mean NRS score at the baseline was 4.6 ± 1.4 and on 45th day was 0.3 ± 0.7. The mean difference between baseline and 45th day is 4.3. The % of change in 45th day is 93.48 %.

The graphical representation is given in Graph 4.

Graph 4: Comparison of Group I and Group II with mean NRS scores at different time points

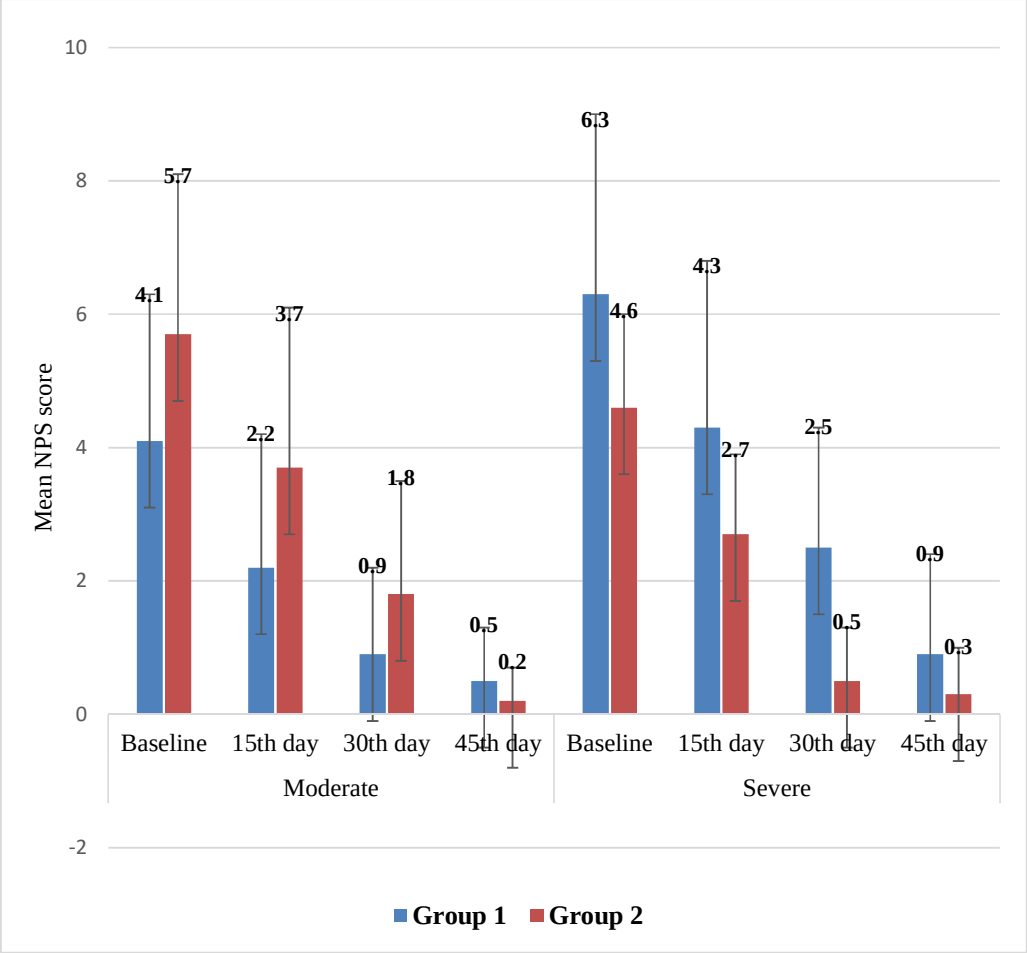


Table 18: Intragroup comparison of burning sensation (NRS score) at different time points in Group I and Group II for Moderate OLP cases

Group	Time points	N	Z-value	p-value
Group I	Baseline	17	-3.589	<0.001**
	15th day	17		
	Baseline	17	-3.538	<0.001**
	30th day	17		
	Baseline	17	-3.527	<0.001**
	45th day	17		
Group II	Baseline	17	-3.559	<0.001**
	15th day	17		
	Baseline	17	-3.532	<0.001**
	30th day	16		
	Baseline	17	-3.526	<0.001**
	45th day	16		

**p-value ≤ 0.05- Statistically significant, p-value ≤ 0.001-Highly statistically significant; Test Applied: Wilcoxon signed rank test; 95 % CI.*

When intragroup comparison of NRS scores (burning sensation) in Group I and in Group II for Moderate OLP cases was done, there was highly statistically significant reduction ($p \leq 0.001$) between the values at baseline and at different time points i.e., at baseline, at 15th day, at 30th day, and 45th day.

The graphical representation is given in graph 5.

Table 19: Intragroup comparison of burning sensation (NRS score) at different time points in Group I and Group II for Severe OLP cases

Group	Time points	N	Z-value	p-value
Group I	Baseline	8	-2.848	0.004*
	15^{th} day	8		
	Baseline	8	-2.825	0.005*
	30^{th} day	8		
	Baseline	8	-2.814	0.005*
	45^{th} day	8		
Group II	Baseline	10	-2.850	0.004*
	15^{th} day	10		
	Baseline	10	-2.539	0.011*
	30^{th} day	8		
	Baseline	10	-2.555	0.011*
	45^{th} day	8		

**p-value ≤ 0.05- Statistically significant; Test Applied: Wilcoxon signed rank test; 95 % CI.*

When intragroup comparison of NRS scores (burning sensation) in Group I and in Group II for Severe OLP cases was done, there was a statistically significant reduction ($p \leq 0.05$) between the values at baseline and at different time points i.e., at baseline, at 15^{th} day, at 30^{th} day, and 45^{th} day.

The graphical representation is given in graph 5.

Graph 5: Comparison of different time points with mean NRS scores in Group I and Group II

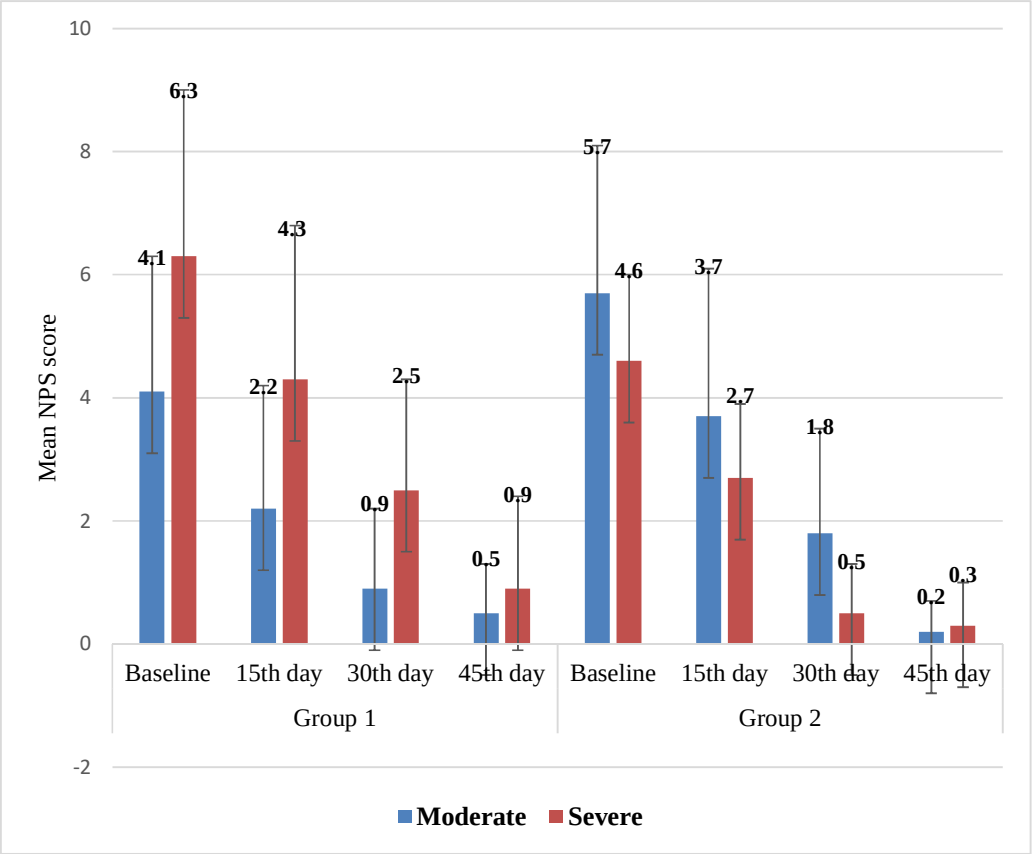


Table 20: Comparison of burning sensation (NRS score) at baseline to that at 15th day, 30th day, and 45th day in Group I and Group II for Moderate OLP cases

Group	Mean Rank				Chi-square value	*df*	p-value
	At baseline	At 15th day	At 30th day	At 45th day			
Group I	4.0	2.7	1.9	1.4	46.115	3	<0.001**
Group II	4.0	3.0	1.8	1.2	45.560	3	<0.001**

**p-value ≤ 0.05- Statistically significant, p-value ≤ 0.001- Highly statistically significant; Test Applied: Friedman's test; 95 % CI.*

When comparison of NRS scores (burning sensation) in Group I and Group II for Moderate OLP cases was done between the different time points i.e., at baseline, at 15th day, at 30th day, and 45th day, there was a highly statistically significant difference ($p \leq 0.001$) between the values.

Table 21: Pairwise comparison of burning sensation (NRS score) at baseline, 15th day, 30th day, and 45th day in Group I for Moderate OLP cases

Group I- Moderate OLP		N	Z-value	Std. Error	p-value
Baseline	15th day	17	1.265	0.443	0.026*
	30th day	17	2.118	0.443	<0.001**
	45th day	17	2.618	0.443	<0.001**
At 15th day	Baseline	17	1.265	0.443	0.026*
	30th day	17	0.853	0.443	0.324
	45th day	17	1.353	0.443	0.013*
At 30th day	Baseline	17	2.118	0.443	<0.001**
	15th day	17	0.853	0.443	0.324
	45th day	17	0.500	0.443	1.000
At 45th day	Baseline	17	2.618	0.443	<0.001**
	15th day	17	1.353	0.443	0.013*
	30th day	17	0.500	0.443	1.000

**p-value ≤ 0.05- Statistically significant, p-value ≤ 0.001- Highly statistically significant; Test Applied: Dunn's Post-Hoc test; 95 % CI.*

When pairwise comparisons of the NRS scores (burning sensation) at baseline, 15th day, 30th day, and 45th day in Group I for Moderate OLP cases were done, there was a highly statistically significant difference ($p \leq 0.001$) between Baseline Vs 30th day and Baseline Vs 45th day; a statistically significant difference ($p \leq 0.05$) between Baseline Vs 15th day and 15th Vs 45th day; whereas no statistically significant difference ($p > 0.05$) between 15th day Vs 30th day and 30th day Vs 45th day.

Table 22: Pairwise comparison of burning sensation (NRS score) at baseline, 15th day, 30th day, and 45th day in Group II for Moderate OLP cases

Group II- Moderate OLP		N	Z-value	Std. Error	p-value
Baseline	15th day	17	1.000	0.456	0.171
	30th day	16	2.125	0.456	<0.001**
	45th day	16	2.750	0.456	<0.001**
At 15th day	Baseline	17	1.000	0.456	0.171
	30th day	16	1.125	0.456	0.082
	45th day	16	1.750	0.456	0.001**
At 30th day	Baseline	17	2.125	0.456	<0.001**
	15th day	17	1.125	0.456	0.082
	45th day	16	0.625	0.456	1.000
At 45th day	Baseline	17	2.750	0.456	<0.001**
	15th day	17	1.750	0.456	0.001**
	30th day	16	0.625	0.456	1.000

**p-value ≤ 0.05- Statistically significant, p-value ≤ 0.001- Highly statistically significant; Test Applied: Dunn's Post-Hoc test; 95 % CI.*

When pairwise comparisons of the NRS scores (burning sensation) at baseline, 15th day, 30th day, and 45th day in Group II for Moderate OLP cases were done, there was a highly statistically significant difference ($p \leq 0.001$) between Baseline Vs 30th day, Baseline Vs 45th day, and 15th day Vs 45th day; whereas no statistically significant difference ($p > 0.05$) between Baseline Vs 15th day, 15th Vs 30th day, and 30th Vs 45th day.

Table 23: Comparison of burning sensation (NRS score) at baseline to that at 15th day, 30th day, and 45th day in Group I and Group II for Severe OLP cases

Group	Mean Rank				Chi-square value	*df*	p-value
	At baseline	At 15th day	At 30th day	At 45th day			
Group I	4.0	2.9	2.1	1.0	23.734	3	<0.001**
Group II	4.0	3.0	1.7	1.4	22.973	3	<0.001**

**p-value ≤ 0.05- Statistically significant, p-value ≤ 0.001- Highly statistically significant; Test Applied: Friedman's test; 95 % CI.*

When comparison of NRS scores (burning sensation) in Group I and Group II for Severe OLP cases was done between the different time points i.e., at baseline, at 15th day, at 30th day, and 45th day, there was a highly statistically significant difference ($p \leq 0.001$) between the values.

Table 24: Pairwise comparison of burning sensation (NRS score) at baseline, 15th day, 30th day, and 45th day in Group I for Severe OLP cases

Group I- Severe OLP		N	Z-value	Std. Error	p-value
Baseline	15th day	8	1.063	0.645	0.599
	30th day	8	1.938	0.645	0.016*
	45th day	8	3.000	0.645	<0.001**
At 15th day	Baseline	8	1.063	0.645	0.599
	30th day	8	0.875	0.645	1.000
	45th day	8	1.938	0.645	0.016*
At 30th day	Baseline	8	1.938	0.645	0.016*
	15th day	8	0.875	0.645	1.000
	45th day	8	1.063	0.645	0.599
At 45th day	Baseline	8	3.000	0.645	<0.001**
	15th day	8	1.938	0.645	0.016*
	30th day	8	1.063	0.645	0.599

**p-value ≤ 0.05- Statistically significant, p-value ≤ 0.001- Highly statistically significant; Test Applied: Dunn's Post-Hoc test; 95 % CI.*

When pairwise comparisons of the NRS scores (burning sensation) at baseline, 15th day, 30th day, and 45th day in Group I for Severe OLP cases were done, there was a highly statistically significant difference ($p \leq 0.001$) between Baseline Vs 45th day; a statistically significant difference ($p \leq 0.05$) between Baseline Vs 30th day and 15th Vs 45th day; whereas no statistically significant difference ($p > 0.05$) between Baseline Vs 15th day, 15th day Vs 30th day and 30th day Vs 45th day.

Table 25: Pairwise comparison of burning sensation (NRS score) at baseline, 15th day, 30th day, and 45th day in Group II for Severe OLP cases

Group II- Severe OLP		N	Z-value	Std. Error	p-value
Baseline	15th day	10	1.063	0.645	0.599
	30th day	8	2.313	0.645	0.002*
	45th day	8	2.625	0.645	<0.001**
At 15th day	Baseline	10	1.063	0.645	0.599
	30th day	8	1.250	0.645	0.317
	45th day	8	1.563	0.645	0.093
At 30th day	Baseline	10	2.313	0.645	0.002*
	15th day	10	1.250	0.645	0.317
	45th day	8	0.313	0.645	1.000
At 45th day	Baseline	10	2.625	0.645	<0.001**
	15th day	10	1.563	0.645	0.093
	30th day	8	0.313	0.645	1.000

**p-value ≤ 0.05- Statistically significant, p-value ≤ 0.001- Highly statistically significant; Test Applied: Dunn's Post-Hoc test; 95 % CI.*

When pairwise comparisons of the NRS scores (burning sensation) at baseline, 15th day, 30th day, and 45th day in Group II for Severe OLP cases were done, there was a highly statistically significant difference ($p \leq 0.001$) between Baseline Vs 45th day; a statistically significant difference ($p \leq 0.05$) between Baseline Vs 30th day and 15th Vs 45th day; whereas no statistically significant difference ($p > 0.05$) between Baseline Vs 15th day, 15th day Vs 30th day, 15th day Vs 45th day, and 30th day Vs 45th day.

ANALYSIS RELATED TO SIZE OF THE LESION

Table 26: Intergroup comparison of size of the lesion (mm) at different time points between Group I and Group II for Moderate OLP cases

Group		N	Mean	SD	*t*-value	*df*	p-value
Baseline	Group 1	17	126.5	62.7	-0.439	32	0.664
	Group 2	17	136.5	70.7			
15th day	Group 1	17	65.8	30.9	-0.202	32	0.842
	Group 2	17	68.1	35.3			
30th day	Group 1	17	28.0	13.8	0.127	31	0.900
	Group 2	16	27.4	14.5			
45th day	Group 1	17	8.9	6.7	-0.402	31	0.691
	Group 2	16	9.8	6.6			

**p-value ≤ 0.05- Statistically significant; Test Applied: Unpaired-t test; 95 % CI; The values of size of the lesion (mm) are presented as Mean and Standard deviation (SD).*

At the baseline, the mean size of lesion for Moderate OLP cases was 126.5 ± 62.7 mm and 136.5 ± 70.7 mm in Group I and II, respectively.

At 15th day, the mean size of lesion for Moderate OLP cases was 65.8 ± 30.9 mm and 68.1 ± 35.3 mm in Group I and II, respectively.

At 30th day, the mean size of lesion for Moderate OLP cases was 28.0 ± 13.8 mm and 27.4 ± 14.5 mm in Group I and II, respectively.

At 45^{th} day, the mean size of lesion for Moderate OLP cases was 8.9 ± 6.7 mm and 9.8 ± 6.6 mm in Group I and II, respectively.

When intergroup comparison of mean size of the lesion (mm) was done between Group I and Group II for Moderate OLP cases, there was no statistically significant difference ($p > 0.05$) in the size of the lesion (mm) at all the different time points i.e., at baseline, at 15^{th} day, at 30^{th} day, and 45^{th} day.

The graphical representation is given in Graph 6.

Table 27: Intergroup comparison of size of the lesion (mm) at different time points between Group I and Group II for Severe OLP cases

Group		N	Mean	SD	*t*-value	*df*	p-value
Baseline	Group 1	8	163.1	72.5	0.112	16	0.912
	Group 2	10	159.3	71.2			
15^{th} day	Group 1	8	97	35.6	0.900	16	0.381
	Group 2	10	79.5	44.7			
30^{th} day	Group 1	8	47	14.5	0.823	14	0.424
	Group 2	8	39.2	22.3			
45^{th} day	Group 1	8	15.2	6.7	1.227	14	0.240
	Group 2	8	10.9	7.1			

**p-value ≤ 0.05- Statistically significant; Test Applied: Unpaired-t test; 95 % CI; The values of size of the lesion (mm) are presented as Mean and Standard deviation (SD).*

At the baseline, the mean size of lesion for Severe OLP cases was 163.1 ± 72.5 mm and 159.3 ± 71.2 mm in Group I and II, respectively.

At 15^{th} day, the mean size of lesion for Severe OLP cases was 97 ± 35.6 mm and 79.5 ± 44.7 mm in Group I and II, respectively.

At 30^{th} day, the mean size of lesion for Severe OLP cases was 47 ± 14.5 mm and 39.2 ± 22.3 mm in Group I and II, respectively.

At 45^{th} day, the mean size of lesion for Severe OLP cases was 15.2 ± 6.7 mm and 10.9 ± 7.1 mm in Group I and II, respectively.

When intergroup comparison of mean size of the lesion (mm) was done between Group I and Group II for Severe OLP cases, there was no statistically significant difference ($p > 0.05$) in the size of the lesion (mm) at all the different time points i.e., at baseline, at 15th day, at 30th day, and 45th day.

The graphical representation is given in Graph 6.

Graph 6: Comparison of Group I and Group II with mean size of the lesion at different time points

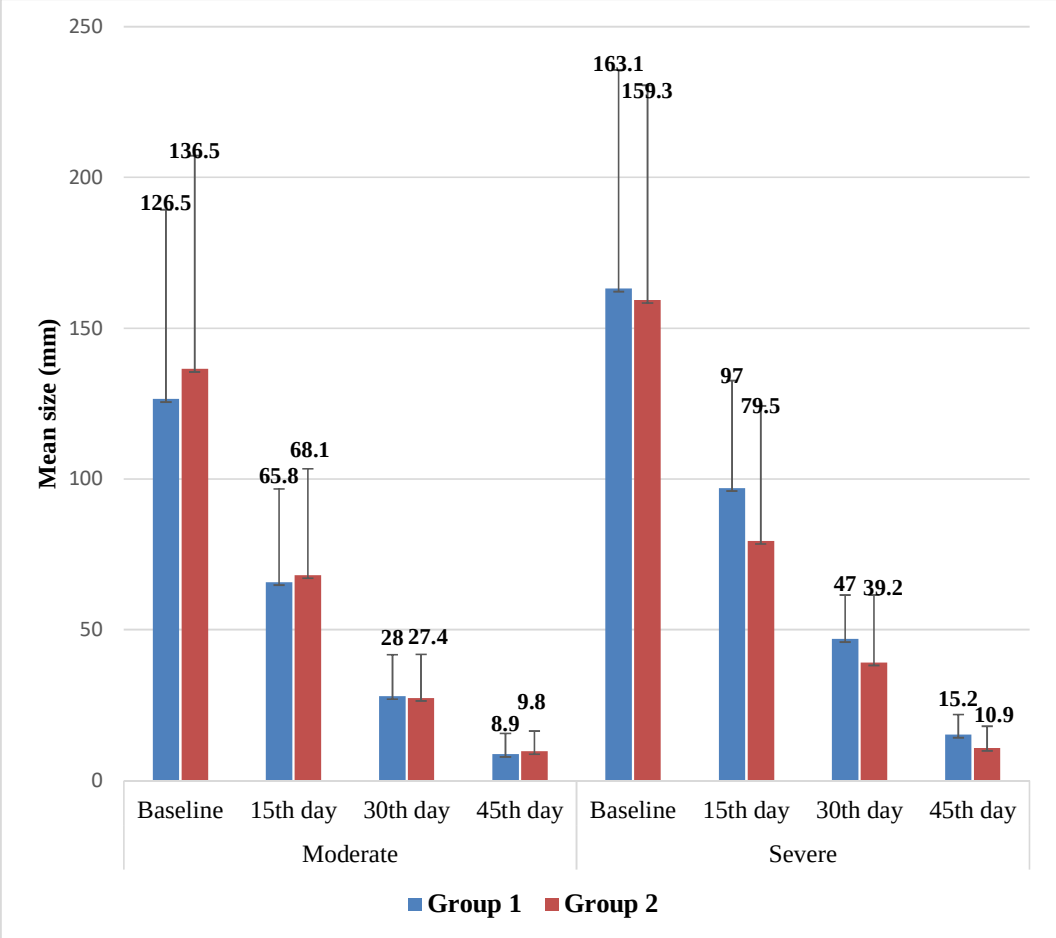


Table 28: Intragroup comparison of size of the lesion (mm) at different time points in Group I and Group II for Moderate OLP cases

Group	Time points	n	Mean	SD	*t*-value	*df*	p-value
Group I	Baseline	17	126.5	62.7	7.139	16	<0.001**
	15^{th} day	17	65.8	30.9			
	Baseline	17	126.5	62.6	7.893	16	<0.001**
	30^{th} day	17	28	13.8			
	Baseline	17	126.5	62.7	8.258	16	<0.001**
	45^{th} day	17	8.9	6.7			
Group II	Baseline	17	132.9	71.4	7.362	15	<0.001**
	15^{th} day	17	68.1	35.3			
	Baseline	17	132.9	71.4	7.284	15	<0.001**
	30^{th} day	16	27.4	14.5			
	Baseline	17	132.9	71.4	7.237	15	<0.001**
	45^{th} day	16	9.8	6.6			

**p-value ≤ 0.05- Statistically significant, **p-value ≤ 0.001- Highly statistically significant; Test Applied: Paired-t test; 95 % CI; The values of size of the lesion (mm) are presented as Mean and Standard deviation (SD).*

When intragroup comparison of mean size of the lesion (mm) in Group I and in Group II for Moderate OLP cases was done, there was highly statistically significant reduction ($p \leq 0.001$) between the values at baseline and at different time points i.e., at baseline, at 15^{th} day, at 30^{th} day, and 45^{th} day.

In Group I, mean size of the lesion at the baseline is 126.5 ± 62.7 mm in Moderate OLP cases.

- Mean size of the lesion at the baseline was 126.5 ± 62.7 mm and on 15th day was 65.8 ± 30.9 mm. The mean difference between baseline and 15th day is 60.7 mm. The % of change at 15th day is 48 %.
- Mean size of the lesion at the baseline was 126.5 ± 62.7 mm and on 30th day was 28 ± 13.8 mm. The mean difference between baseline and 30th day is 98.5 mm. The % of change at 30th day is 77.8 %.
- Mean size of the lesion at the baseline was 126.5 ± 62.7 mm and was 45th day was 8.9 ± 6.7 mm. The mean difference between baseline and 60th day is 117.6 mm. The % of change at 45th day is 92.9 %.

In Group II, mean size of the lesion at the baseline is 132.9 ± 71.4 mm in Moderate OLP cases.

- Mean size of the lesion at the baseline was 132.9 ± 71.4 mm and on 15th day was 68.1 ± 35.3. The mean difference between baseline and 15th day is 64.8 mm. The % of change in 15th day is 49 %.
- Mean size of the lesion at the baseline was 132.9 ± 71.4 mm and on 30th day was 27.4 ± 14.5. The mean difference between baseline and 30th day is 105.5 mm. The % of change in 30th day is 79.3 %
- Mean size of the lesion at the baseline was 132.9 ± 71.4 mm and on 45th day was 9.8 ± 6.6. The mean difference between baseline and 45th day is 123.1 mm. The % of change in 45th day is 92.6 %.

The graphical representation is given in Graph 7.

Table 29: Intragroup comparison of size of the lesion (mm) at different time points in Group I and Group II for Severe OLP cases

Group	Time points	n	Mean	SD	*t*-value		p-value
Group I	Baseline	8	163.1	72.5	4.360	7	0.003*
	15^{th} day	8	97	35.6			
	Baseline	8	163.1	72.5	5.190	7	0.001**
	30^{th} day	8	47	14.5			
	Baseline	8	163.1	72.5	6.262	7	<0.001**
	45^{th} day	8	15.1	6.7			
Group II	Baseline	10	158.1	79.7	6.389	9	<0.001**
	15^{th} day	10	79.5	44.7			
	Baseline	10	158.1	79.7	5.500	7	0.001**
	30^{th} day	8	39.3	22.3			
	Baseline	10	158.1	79.7	5.481	7	0.001**
	45^{th} day	8	10.9	7.1			

**p-value ≤ 0.05- Statistically significant, **p-value ≤ 0.001- Highly statistically significant; Test Applied: Paired-t test; 95 % CI; The values of size of the lesion (mm) are presented as Mean and Standard deviation (SD).*

When intragroup comparison of mean size of the lesion (mm) in Group I and in Group II for Severe OLP cases was done, there was a highly statistically significant reduction ($p \leq 0.001$) between the values at baseline and at different time points i.e., at baseline, at 15^{th} day, at 30^{th} day, and 45^{th} day except for the values between baseline and 15^{th} day in Group I, where there was only a statistically significant reduction ($p \leq 0.05$).

In Group I, mean size of the lesion at the baseline was 163.1 ± 72.5 mm in Severe OLP cases.

- Mean size of the lesion at the baseline was 163.1 ± 72.5 mm and on 15th day was 97 ± 35.6 mm. The mean difference between baseline and 15th day is 66.1 mm. The % of change at 15th day is 40.52 %.
- Mean size of the lesion at the baseline was 163.1 ± 72.5 mm and on 30th day was 47 ± 14.5 mm. The mean difference between baseline and 30th day is 116.1 mm. The % of change at 30th day is 71.2 %.
- Mean size of the lesion at the baseline was 163.1 ± 72.5 mm and on 45th day was 15.1 ± 6.7 mm. The mean difference between baseline and 60th day is 148 mm. The % of change at 45th day is 90.7 %.

In Group II, mean size of the lesion at the baseline was 158.1 ± 79.7 mm in Severe OLP cases.

- Mean size of the lesion at the baseline was 158.1 ± 79.7 mm and on 15th day was 79.5 ± 44.7. The mean difference between baseline and 15th day is 78.4 mm. The % of change in 15th day is 49.6 %.
- Mean size of the lesion at the baseline was 158.1 ± 79.7 mm and on 30th day was 39.3 ± 22.3. The mean difference between baseline and 30th day is 118.8 mm. The % of change in 30th day is 75.1 %
- Mean size of the lesion at the baseline was 158.1 ± 79.7 mm and on 45th day was 10.9 ± 7.1. The mean difference between baseline and 45th day is 147.2 mm. The % of change in 45th day is 93.1 %.

The graphical representation is given in Graph 7.

Graph 7: Comparison of different time points with mean size of lesion in Group I and Group II

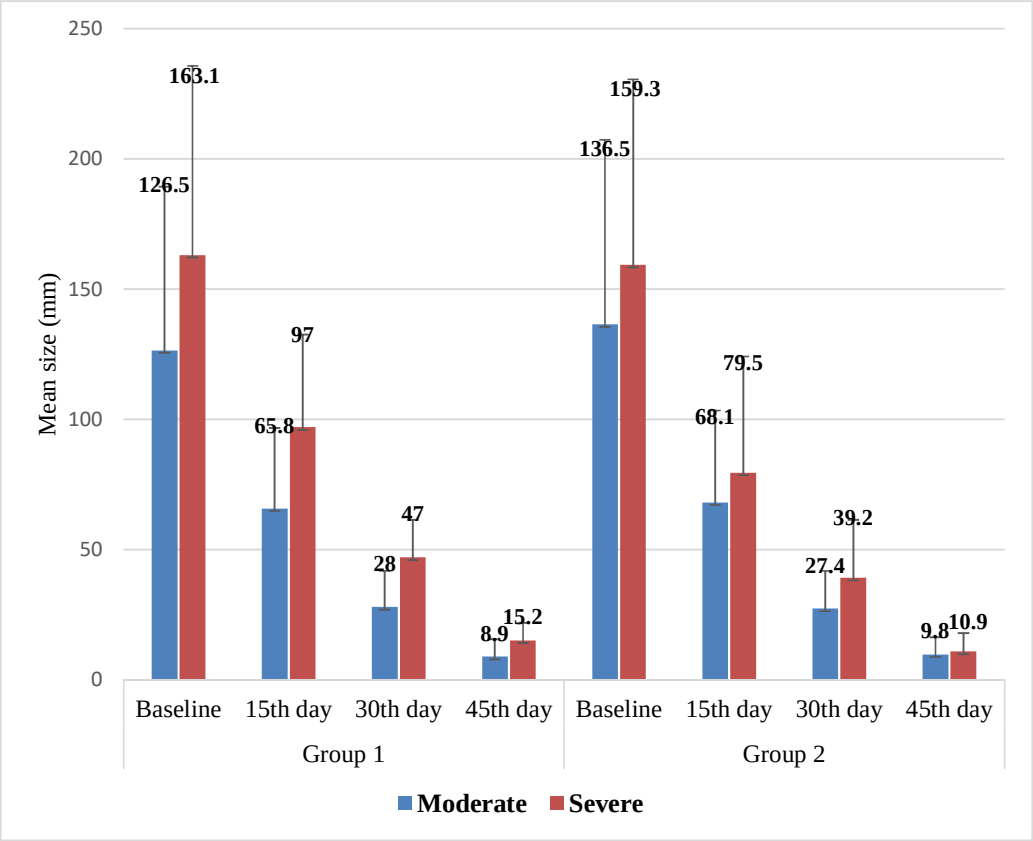


Table 30: Comparison of size of the lesion (mm) at baseline to that at 15th day, 30th day, and 45th day in Group I and Group II for Moderate OLP cases

Group	Mean size of the lesion (mm) ± SD (95 % CI)				F-value	*df*	p-value
	At baseline	At 15th day	At 30th day	At 45th day			
Group I	126.5 ± 62.7	65.8 ± 30.9	28 ± 13.8	18.9 ± 13.8	22.581	14	<0.001**
Group II	136.5 ± 70.67	68.1 ± 35.3	27. 4 ± 14.5	10.2 ± 6.4	15.374	13	<0.001**

**p-value ≤ 0.05- Statistically significant, **p-value ≤ 0.001- Highly statistically significant; Test Applied: One-way repeated measures ANOVA test; 95 % CI. The values of size of the lesion (mm) are presented as Mean ± Standard deviation (SD).*

When comparison of mean size of the lesion (mm) in Group I and Group II for Moderate OLP cases were done between the different time points i.e., at baseline, at 15th day, at 30th day, and 45th day, there was a highly statistically significant difference ($p \leq 0.001$) between the values.

Table 31: Pairwise comparison of size of the lesion (mm) at baseline, 15th day, 30th day, and 45th day in Group I for Moderate OLP cases

Group I- Moderate OLP		n	Mean Difference	Std. Error	p-value
Baseline	15th day	17	60.6	8.5	<0.001**
	30th day	17	98.5	12.5	<0.001**
	45th day	17	117.6	14.2	<0.001**
At 15th day	Baseline	17	-60.6	8.5	<0.001**
	30th day	17	37.8	5.0	<0.001**
	45th day	17	56.9	6.7	<0.001**
At 30th day	Baseline	17	-98.5	12.5	<0.001**
	15th day	17	-37.8	5.0	<0.001**
	45th day	17	19.1	2.4	<0.001**
At 45th day	Baseline	17	-117.6	14.2	<0.001**
	15th day	17	-56.9	6.7	<0.001**
	30th day	17	-19.1	2.4	<0.001**

**p-value ≤ 0.05- Statistically significant, **p-value ≤ 0.001- Highly statistically significant; Test Applied: Bonferroni's Post-Hoc test; 95 % CI.*

When pairwise comparisons of the mean size of the lesion (mm) at baseline, 15th day, 30th day, and 45th day in Group I for Moderate OLP cases were done, there was a highly statistically significant difference ($p \leq 0.001$) in all the comparisons.

Table 32: Pairwise comparison of size of the lesion (mm) at baseline, 15th day, 30th day, and 45th day in Group II for Moderate OLP cases

Group II- Moderate OLP		n	Mean Difference	Std. Error	p-value
Baseline	15th day	17	65.3	9.3	<0.001**
	30th day	16	105.6	14.5	<0.001**
	45th day	16	123.1	17.0	<0.001**
At 15th day	Baseline	17	-65.3	9.3	<0.001**
	30th day	16	40.2	5.8	<0.001**
	45th day	16	57.8	8.2	<0.001**
At 30th day	Baseline	17	-105.6	14.5	<0.001**
	15th day	17	-40.2	5.8	<0.001**
	45th day	16	17.6	2.9	<0.001**
At 45th day	Baseline	17	-123.1	17.0	<0.001**
	15th day	17	-57.8	8.2	<0.001**
	30th day	16	-17.6	2.9	<0.001**

**p-value ≤ 0.05- Statistically significant, **p-value ≤ 0.001- Highly statistically significant; Test Applied: Bonferroni's Post-Hoc test; 95 % CI.*

When pairwise comparisons of the mean size of the lesion (mm) at baseline, 15th day, 30th day, and 45th day in Group II for Moderate OLP cases were done, there was a highly statistically significant difference ($p \leq 0.001$) in all the comparisons.

Table 33: Comparison of size of the lesion (mm) at baseline to that at 15th day, 30th day, and 45th day in Group I and Group II for Severe OLP cases

Group	Mean size of the lesion (mm) ± SD (95 % CI)				F-value	*df*	p-value
	At baseline	At 15th day	At 30th day	At 45th day			
Group I	163.1±72.5	97 ± 35.6	47 ± 14.5	15.1 ± 6.7	25.975	5	0.002*
Group II	159 ± 71.1	79.5 ± 44.7	39.3 ± 22.3	10.9 ± 7.1	7.414	5	0.027*

**p-value ≤ 0.05- Statistically significant; Test Applied: One-way repeated measures ANOVA test; 95 % CI. The values of size of the lesion (mm) are presented as Mean ± Standard deviation (SD).*

When comparison of mean size of the lesion (mm) in Group I and Group II for Severe OLP cases was done between the different time points i.e., at baseline, at 15th day, at 30th day, and 45th day, there was a statistically significant difference ($p \leq 0.05$) between the values.

Table 34: Pairwise comparison of size of the lesion (mm) at baseline, 15th day, 30th day, and 45th day in Group I for Severe OLP cases

Group I- Severe OLP		n	Mean Difference	Std. Error	p-value
Baseline	15th day	8	66.1	15.2	0.020*
	30th day	8	116.1	22.3	0.008*
	45th day	8	148	22.6	0.003*
At 15th day	Baseline	8	-66.1	15.1	0.020*
	30th day	8	50	8.3	0.003*
	45th day	8	81.9	10.5	0.001**
At 30th day	Baseline	8	-116.1	22.3	0.008*
	15th day	8	-50	8.3	0.003*
	45th day	8	31.9	3.3	<0.001**
At 45th day	Baseline	8	-148	23.6	0.003*
	15th day	8	81.9	10.5	0.001**
	30th day	8	-31.9	3.3	<0.001**

**p-value ≤ 0.05- Statistically significant, **p-value ≤ 0.001- Highly statistically significant; Test Applied: Bonferroni's Post-Hoc test; 95 % CI.*

When pairwise comparisons of the mean size of the lesion (mm) at baseline, 15th day, 30th day, and 45th day in Group I for Severe OLP cases were done, there was a statistically significant difference ($p \leq 0.05$) in all the comparisons except for those between 15th day Vs 45th day and 30th day Vs 45th day, where there was a highly statistically significant difference ($p \leq 0.001$).

Table 35: Pairwise comparison of size of the lesion (mm) at baseline, 15th day, 30th day, and 45th day in Group II for Severe OLP cases

Group II- Severe OLP		n	Mean Difference	Std. Error	p-value
Baseline	15th day	10	79.5	15.8	0.009*
	30th day	8	118.9	21.6	0.005*
	45th day	8	147.2	26.9	0.006*
At 15th day	Baseline	10	-79.5	15.8	0.009*
	30th day	8	39.4	11.9	0.077
	45th day	8	67.8	16.9	0.030*
At 30th day	Baseline	10	-118.9	21.7	0.005*
	15th day	10	-39.4	11.9	0.077
	45th day	8	28.4	6.9	0.028*
At 45th day	Baseline	10	-147.3	26.9	0.006*
	15th day	10	-67.8	16.9	0.030*
	30th day	8	-28.4	6.9	0.028*

**p-value ≤ 0.05- Statistically significant; Test Applied: Bonferroni's Post-Hoc test; 95 % CI.*

When pairwise comparisons of the mean size of the lesion (mm) at baseline, 15th day, 30th day, and 45th day in Group II for Severe OLP cases were done, there was a statistically significant difference ($p \leq 0.05$) in all the comparisons except for those between 15th day Vs 30th day, where there was no statistically significant difference ($p > 0.05$).

DISCUSSION

Lichen Planus is a common autoimmune chronic inflammatory mucocutaneous disease that involves the skin and oral mucosa [1]. The global pooled prevalence is 1.01 % [132] and is commonly seen in 30-60 years of age with slightly higher female predilection [3]. It is associated with pain and burning sensation in the oral cavity, thereby severely debilitating the patients' quality of life.

Although, the exact etiology of OLP is unknown, it is believed to be a T-cell mediated autoimmune disease in which the auto-cytotoxic CD8+ T cells trigger apoptosis of the oral basal epithelium [4,5].

Various treatment modalities such as topical and systemic drug therapy including triamcinolone propionate, clobetasol propionate, tacrolimus, pimecrolimus, cyclosporine, etc.; ultraviolet therapy (PUVA) therapy; laser therapy; and other natural alternatives are used to treat OLP. However, these modalities are known to cause various side-effects [69].

Clobetasol ointment (0.05%) is the most commonly used corticosteroid for treating OLP. The rationale behind its usage is its anti-inflammatory and immunomodulatory property [69]. Although Clobetasol propionate is the gold standard drug prescribed, it has adverse effects like adrenal suppression on long term systemic use and causes atrophy of epithelium and candidiasis on topical use. Clobetasol propionate (0.05%) mouthwash can cause adverse effects like hirsutism and moon face between 4^{th} week and 6^{th} week, from the initiation of therapy [63]. Other minor adverse effects have been associated with fluticasone propionate spray such as bad taste and smell, dry mouth, nausea, sore throat, swollen mouth and candidiasis [69,134].

In the recent years, there is a rise in the prevalence of recalcitrant cases of OLP as reported in the study by **Thongprasom K and Dhanuthai K (2008)** who evaluated the efficacy of topical tacrolimus ointment in comparison to the triamcinolone acetonide (0.1 %). Although, topical tacrolimus (0.1 %) ointment showed a better therapeutic response initially, relapses occurred within 3 to 9 weeks of cessation of the therapy and the common side effect from both the drugs was temporary burning sensation or stinging sensation at the site of the topical drug use [135].

Owing to the aforementioned adverse effects of the corticosteroids and to overcome the recurrence, the current study was planned to use the herbal medication of *Nigella sativa* cream for the treatment of Oral Lichen Planus in an aim to develop an alternative herbal modality in view of its minimal or no side effects.

Nigella sativa is an annual flowering plant with prophetic significance that is known for centuries for its medicinal values along with potent anti-inflammatory, analgesic, antioxidant, and immunomodulatory properties. In the past literature, *Nigella sativa* extracts are known to be effective to prevent and treat various oral conditions like oral mucositis, oral ulcers, gingivitis, periodontitis, and dental caries, etc. [89]. Thymoquinone, a major therapeutic ingredient responsible for the medicinal properties is found in the volatile oil in major concentration.

The current study included sixty clinically diagnosed cases of OLP to receive the *Nigella sativa* (75 % w/v) cream and Clobetasol propionate (0.05 % w/w) gel equally. Three (11.1 %) patients of Group II (corticosteroid) in this study had *Candida* infection during the 30th day follow-up, however, no other side-effects were elicited. This was in accordance to **Thongprasom K and Dhanuthai K (2008)** who

found candidiasis as the only common side effect of 0.3 % and 0.5 % triamcinolone acetonide mouth wash in 4 out of 35 OLP cases in comparison to cyclosporine [135]. After excluding the drop-outs and opportunistic *Candida* infection cases, a total of 49 patients with 25 patients in *Nigella sativa* group and 24 patients in Clobetasol propionate group, who completed the 45 days of topical therapy.

In the current study, the mean age of the patients was 44.5 ± 13.5 years in Group I and 48.7 ± 13.3 years in Group II which is consistent with the study done by **Chitturi RT *et al* (2015)** that included 58 OLP patients of Indian origin with a mean age of 45.7 years [136]. In both the groups, the majority of the subjects were in the age group ≥ 51 years followed by 41-50 years and least in the 31-40 years and ≤ 30 years that is in line with the epidemiological evaluation of OLP by **Axell and Rundquist (1987)** where the highest prevalence was found between 55-74 years of age [137]. This could be due to higher level of stress, decreased healing potential, and increased consumption of multiple medications in older age due to medically compromised status contributing to lichenoid reactions. However, the recent trend has reported the age of occurrence of this disease to be low as 28 years in concordance to the 15.4 % of the patients in the age group of ≤ 30 years in this study. This can be attributed to the increased levels of stress at a younger age in the recent years that may be related to their jobs, lifestyle changes, and economic burden, as stress is one of the etiological factors of OLP.

The present study included OLP patients of either sex with 38.5 % males and 61.5 % females thereby clearly indicating a female predilection. This finding was in conjunction to the studies done by **Silvermann S *et al* (1985)** [138]**, Omal PM *et al* (2012)** [139]**, Kumar T *et al* (2014)** [140]**, Varghese Soma Susen *et al* (2016)** [141]**, and**

AE Thomas *et al* (2017) [142]**,** where there were 68 %, 63 %, 64 %, 65 %, and 74.6 % of females, respectively. This could be attributed to the hormonal influences in the females, especially estrogen that has a protective effect on the oral mucosa.

Reticular type of OLP cases were the highest in number (57.7 %) followed by erosive (28.8 %), atrophic (7.7 %), bullous (3.8 %) types, with plaque type being the least (1.9 %) in the present study. This finding is consistent with that of **Bagewadi A & Bhoweer AK (2011)** [142] and **Chitturi RT *et al* (2015)** [136] who also reported reticular type being the highest (70.4 % and 59 %, respectively). However, **Mansourian A *et al* (2011)** [144] reported 51.1 % of erosive type out of 45 OLP cases and **Reddy *et al* (2012)** [145] reported erosive (45 %) & atrophic cases (35 %) out of the 40 OLP cases, in majority.

OLP patients report burning sensation on eating spicy or occasionally normal food as a common complaint and the exact mechanism of the same is not clear. However, the intolerability to spices could be due to the epithelial atrophy and increased permeability. In the current study, the mean NRS scores (burning sensation) of moderate cases in Group I and II at the baseline were 4.1 ± 2.2 and 5.7 ± 2.4, and of severe cases were 6.3 ± 2.7 and 4.6 ± 1.4, respectively. This was in accordance to **AE Thomas *et al* (2017)** [142] where it was 5.68 ± 0.78, 5.26 ± 1.098, and 5.32 ± 1.108 in Group I (Triamcinolone acetonide 0.1 %), Group II (Cure next oral gel- 3 times a day), and Group III (Cure next oral gel- 6 times a day) at baseline, respectively.

In Group I, there was 87.8 % (in moderate cases) and 85.7 % (in severe cases) of reduction in the mean NRS scores on 45^{th} day when compared to the 96.5 % (moderate cases) and 93.48 % (severe cases) of reduction in the Group II. Hence,

there was a statistically significant reduction in the burning sensation in both the groups in the current study with the effect being higher in Group II. This is in line with the findings of **AE Thomas *et al* (2017)** [142] who reported 77.39 %, 54.4 % and 64.99 % reduction in NRS scores after the therapy with triamcinolone acetonide 0.1 % buccal paste, curcumin oral gel- 3 times/ day, and curcumin oral gel- 6 times per day for three months.

In this study, the mean size of the lesion of moderate cases in Group I and II at baseline was 126.5 ± 62.7 mm and 136.5 ± 70.7 mm, and of severe cases was 163.1 ± 72.5 mm and 159.3 ± 71.2 mm, respectively. Owing to the fact that the digital photographic measurements are cumbersome, inaccurate (due to inherent magnification), and time consuming, longest unidirectional length measurement according to RECIST criteria and simple photograph were the methods of documentation, for size of the lesion, used in this study [131].

In Group I, there was 92.9 % (in moderate cases) and 90.7 % (in severe cases) of reduction in the size of the lesion on 45th day when compared to the 92.6 % (moderate cases) and 93.1 % (severe cases) of reduction in the Group II. Hence, there was a statistically significant reduction ($p < 0.001$) in the size of the lesion in both the groups of the current study with the effect being slightly higher in Group II. This was in accordance to the findings of **Mansourian A *et al* (2011)** [144] who compared the efficacy of aloe vera mouthwash with triamcinolone acetonide 0.1 % on 46 OLP patients and found that there was a significant reduction ($p < 0.001$) in the size of the lesion after a treatment period of 4 weeks with a slightly higher effect in triamcinolone acetonide group.

A cross-over clinical study conducted by **Akram Kooshki (2016)** on 40 elderly patients with knee osteoarthritis reported that the relief in the pain intensity was 0.53 times higher in *Nigella sativa* oil than the oral acetaminophen, thereby recommending *Nigella sativa* oil topical application therapy as the safe supplement for elder patients with knee osteoarthritis [107].

Agah S *et al* (2013) reported that the *Nigella sativa* oil showed a significant decrease in abdominal pain, incomplete defecation, bloating, fecal urgency, and mucus discharge in irritable bowel syndrome patients [104]. A randomized placebo-controlled trial by **Hasan Fallah Huseini (2016)** showed that the topical *Nigella sativa* oil (30 % w/w) therapy significantly reduced the VAS scores (82 %) after 1- and 2-month interval as compared to topical diclofenac and placebo in women with cyclic mastalgia [106]. The potent anti-inflammatory and analgesic properties of *Nigella sativa* oil could be the basis contributing to these results.

Nigella sativa cream is a very safe and promising medication for the treatment of Oral Lichen Planus as perceived from the findings of this study. There was a significant reduction of burning sensation as well as size of the lesion within 15 to 30 days of topical application of the *Nigella sativa* cream. Additionally, it has minimal or no side effects, despite the need for longer therapeutic intervention in OLP.

LIMITATIONS

1. *Nigella sativa* (75 % w/v) cream is an herbal preparation, and hence can be best preserved in an air-tight amber colored glass container placed in a refrigerator at a temperature of 4 °C.
2. Although, the preparations did not show any signs of degradation for a period of 3 months, the exact shelf life of the preparations was not determined.
3. Despite the mucoadhesive property evaluation of the preparations already done in this study on sheep buccal mucosa, their mucosal substantivity and salivary factors influencing its stability quotient in the oral cavity of the patients were not assessed.
4. This study involved a limited sample size of 49 subjects by the end of the study and had a short follow-up period of 45 days.

FUTURE PROSPECTS OF THE STUDY

1. Study with a larger sample size must be undertaken with variations in dose, different combinations, and longer follow-up period.
2. A long-term stability study evaluating the various physical properties and other parameters of the preparations should be conducted.
3. An initiative should be taken to introduce this drug at commercial level, with clinical trials to establish its safety and efficacy.
4. Mucosal substantivity of the preparations and the salivary factors influencing its stability quotient in topical form should be assessed inside the patients' oral cavity.

SUMMARY AND CONCLUSION

OLP is a common chronic inflammatory, oral potentially malignant disorder, associated with cell mediated immunological dysfunction [1,2] that affects the mucosal tissues and severely debilitates the quality of life of the patients. It is relatively a common disorder, affecting 1 to 2 % of the general population [3], especially between 30-60 years of age having a female predilection [4]. Despite the several factors that are predisposed to this condition, the exact etiology is still unknown.

The current treatment modalities available for treating OLP are associated with adverse effects like atrophy, ulceration, itching, stinging, feeling of warmth, etc [15]. This advocates for a need of alternative treatment modality with low cost and minimal or no side-effects for treating OLP patients. *Nigella sativa* oil, which is known to have various tested therapeutic properties, can be an effective alternative treatment in the treatment of OLP based on the current evidences [24, 25, 26, 27, 28, 29, 35, 36].

The present double blinded randomized control trial was conducted to evaluate and compare the clinical efficacy of topical *Nigella sativa* (Black seed) (75 % w/v) cream and Clobetasol propionate (0.05 %) gel in the management of Oral Lichen Planus.

As the commercial form of the *Nigella sativa* oil topical formulation is not available, it was formulated in the cream form in the Department of Pharmaceutics, KAHER's KLE College of Pharmacy, Belagavi for the present study.

A total of 60 clinically diagnosed OLP patients were included in this study and were categorized into moderate and severe OLP cases based on the severity of burning sensation given by **Malhotra *et al* (2008)** [132] by the first investigator (Stratification of patients). Subsequently, Clinical estimation of the burning sensation and size of the lesion were done by Numeric Pain Rating (NRS) scale and a standard Vernier caliper, respectively. The patients were then randomized to Group I- *Nigella sativa* (75 % w/v) cream and Group II- Clobetasol propionate (0.05 %) gel by concealed chit method by the second investigator after performing the oral patch test. Here, both the first investigator and the patient were blinded off the intervention. By the end of the study, 25 patients (17 moderate and 8 severe) in Group I and 24 patients (16 moderate and 8 severe) in Group II were remaining after exclusion of the patients who failed to report for the follow-up and those who had *Candida* infection in Group II. The patients were dispensed with 30 g of cream/ gel in sterile, concealed plastic containers and advised to apply the respective medicines twice daily after breakfast and dinner. The follow-up measurements were made at 15^{th} day, at 30^{th} day, and at 45^{th} day from the start of the topical cream/ gel therapy by the single researcher i.e., the first investigator.

The results depicted that there was a statistically significant reduction ($p \leq 0.05$) in the burning sensation and size of the lesion in both the groups on intragroup comparisons at different timepoints. Also, there was no statistically significant difference ($p > 0.05$) between the clinical efficacy of both the interventions in intergroup comparisons.

In Group I, there was 87.8 % (moderate cases) and 85.7 % (severe cases) of reduction in the mean NRS scores on 45th day when compared to the 96.5 % (moderate cases) and 93.48 % (severe cases) of reduction in the Group II. Similarly, there was 92.9 % (moderate cases) and 90.7 % (severe cases) of reduction in the size of the lesion on 45th day in Group I when compared to the 92.6 % (moderate cases) and 93.1 % (severe cases) of reduction in the Group II.

The study concluded with an outcome that the patients receiving topical *Nigella sativa* (75 % w/v) cream showed significant reduction in size of the lesion and the burning sensation in OLP patients. All the patients responded to *Nigella sativa* (75 % w/v) cream and no side effects and neither any signs of toxicity were reported during the treatment or follow-up. This could be because of the potent anti-inflammatory, immuno-modulatory and wound healing properties of *Nigella sativa* (75 % w/v) cream which alleviated the signs and symptoms in OLP patients. Thus, *Nigella sativa* (75 % w/v) cream appears to be a safe, promising, and cost-effective medication for treatment of Oral Lichen Planus.

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ANNEXURE – I

ETHICAL CLEARANCE CERTIFICATE

Research and Ethics Committee
KLE V K INSTITUTE OF DENTAL SCIENCES
KLE University
Accredited 'A' Grade by NAAC Placed in Category 'A' by MHRD (GoI)
Nehru Nagar, Belagavi - 590 010, Karnataka State
☎: 0831-2470362
FAX: 0831-2470640
Web: http://www.kledental-bgm.edu.in
E-mail:principal@kledental-bgm.edu.in

Sl. No. : 1333

CERTIFICATE

This is to Certify that the synopsis titled

COMPARATIVE EVALUATION OF THE EFFICACY OF NIGELLA SATIVA [~~15~~ 35% (W/V)] CREAM AND CLOBETASOL PROPIONATE [0.05% w/w] GEL FOR THE MANAGEMENT OF ORAL LICHEN PLANUS: A DOUBLE BLINDED RANDOMIZED CONTROL TRIAL *Submitted by*

Dr. LOKESH KUMAR. S *P. G. Student / Staff, Guided by* DR. ZAMEERA NAIK *from Department of* ORAL MEDICINE & RADIOLOGY. *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 09/11/2019

Member Secretary
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

MEMBER SECRETARY
Research & Ethical Committee
KLEVK Institute of Dental Sciences
BELAGAVI.

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman
Research and Ethical Committee
KLE VK Institute of Dental Sciences
Belgaum

ANNEXURE – II

CLINICAL TRIAL REGISTRATION

CLINICAL TRIALS REGISTRY - INDIA
ICMR - National Institute of Medical Statistics

PDF of Trial
CTRI Website URL - http://ctri.nic.in

Clinical Trial Details (PDF Generation Date :- Thu, 23 Jul 2020 09:17:51 GMT)

CTRI Number	CTRI/2020/07/026745 [Registered on: 23/07/2020] - **Trial Registered Prospectively**
Last Modified On	16/07/2020
Post Graduate Thesis	Yes
Type of Trial	Interventional
Type of Study	Drug Ayurveda Unani
Study Design	Randomized, Parallel Group, Active Controlled Trial
Public Title of Study	Black seed cream for reduction of patients burning sensation and white patches in mouth
Scientific Title of Study	"Comparative evaluation of the efficacy of Nigella sativa (75%) (w/v) cream and Clobetasol propionate (0.05%) ointment for the management of Oral Lichen Planus- a double blinded randomized control trial"

Secondary IDs if Any

Secondary ID	Identifier
NIL	NIL

Details of Principal Investigator or overall Trial Coordinator (multi-center study)

Details of Principal Investigator	
Name	Dr Lokesh Kumar S
Designation	Post graduate student
Affiliation	KLE VISHWANATH KATTI INSTITUTE OF DENTAL SCIENCES, KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH
Address	DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY KLE VISHWANATH KATTI INSTITUTE OF DENTAL SCIENCES KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH JNMC CAMPUS NEHRU NAGAR Belgaum KARNATAKA 590010 India
Phone	8148888961
Fax	
Email	lokemar95@gmail.com

Details Contact Person (Scientific Query)

Details Contact Person (Scientific Query)	
Name	Dr Zameera Naik
Designation	Professor and post graduate guide
Affiliation	KLE VISHWANATH KATTI INSTITUTE OF DENTAL SCIENCES, KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH
Address	DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY KLE VISHWANTH KATTI INSTITUTE OF DENTAL SCIENCES KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH JNMC CAMPUS NEHRU NAGAR Belgaum KARNATAKA 590010 India
Phone	9448854710
Fax	
Email	drznaik@gmail.com

Details Contact Person (Public Query)

Details Contact Person (Public Query)	
Name	Dr Lokesh Kumar S
Designation	Post graduate student

	Affiliation	KLE VISHWANATH KATTI INSTITUTE OF DENTAL SCIENCES, KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH
	Address	DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY KLE VISHWANTH KATTI INSTITUTE OF DENTAL SCIENCES KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH JNMC CAMPUS NEHRU NAGAR Belgaum KARNATAKA 590010 India
	Phone	8148888961
	Fax	
	Email	lokemar95@gmail.com

Source of Monetary or Material Support

Source of Monetary or Material Support
> KLE VISHWANTH KATTI INSTITUTE OF DENTAL SCIENCES DEPT OF ORAL MEDICINE AND RADIOLOGY KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH JNMC CAMPUS NEHRU NAGAR BELEGAVI KARNATAKA 590010

Primary Sponsor

Primary Sponsor Details	
Name	DR LOKESH KUMAR S
Address	ROOM NO 24 SANGAM BOYS HOSTEL JNMC CAMPUS NEHRU NAGAR BELAGAVI KARNATAKA 590010
Type of Sponsor	Other [POST GRADUATE STUDENT SELF FUNDED]

Details of Secondary Sponsor

Name	Address
NIL	NIL

Countries of Recruitment

List of Countries
India

Sites of Study

Name of Principal Investigator	Name of Site	Site Address	Phone/Fax/Email
DR LOKESH KUMAR S	KLE VISHWANATH KATTI INSTITUTE OF DENTAL SCIENCES	DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY KLE VISHWANTH KATTI INSTITUTE OF DENTAL SCIENCES KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH JNMC CAMPUS NEHRU NAGAR Belgaum KARNATAKA	8148888961 lokemar95@gmail.com

Details of Ethics Committee

Name of Committee	Approval Status	Date of Approval	Is Independent Ethics Committee?
RESEARCH AND ETHICS COMMITTEE	Approved	09/11/2019	No

Regulatory Clearance Status from DCGI

Status	Date
Not Applicable	No Date Specified

Health Condition / Problems Studied

Health Type	Condition
Patients	Other lichen planus

Intervention / Comparator Agent

Type	Name	Details
Intervention	Nigella sativa(Black seed) 75% (w/v) cream	Topical application of the cream on the oral mucosa twice daily

			after breakfast and dinner for 60 days will be advised. Sufficient quantity of the cream will be used based on the size of the lesion(approximately 1g per application) COMPOSITION OF CREAM Nigella sativa oil- 75ml span 60 (emulsifying agent)-20g tween 80 (emulsifying agent)-6.25g glycerine (humectant)-2.5g distilled water(9s)- 125g (Nigella sativa oil without any preservatives is purchased as Greenish Baraka black seed oil from Greenish (India) Trades Pvt Ltd, Chennai-4 with the certifications like HACCP, GMP and ISO 22000. CREAM PREPARATION PROCEDURE Step I: Weighed quantity of span 60 will be melted in china dish at 55ºC in water which will be mixed to twan 80 with glass rod. Step II: Molten mass of surfactants will be mixed with calculated quantity of Nigella sativa oil at room temperature using high speed propeller (2500 rpm) for 10 minutes. Step III: To the homogenized mixture of oil and surfactants, calculated quantity of glycerine and water will be added slowly with high speed stirring (2500 rpm) for 30 minutes. The cream will be stored in an air tight container at room temperature.
	Comparator Agent	CLOBETASOL PROPIONATE 0.05% OINTMENT	Commercially available product will be used. Topical route of application on the oral mucosa twice daily after breakfast and dinner for 60 days will be advised. Sufficient quantity of the ointment will be used based on the size of the lesion (approximately 1g). If there is complete regression in the lesion size and symptom(burning sensation) in less than 60 days, the dosage and frequency will be tapered and then will be advised to stop as it is a steroid drug.

Inclusion Criteria

Inclusion Criteria	
Age From	18.00 Year(s)
Age To	70.00 Year(s)
Gender	Both
Details	1. Patients clinically diagnosed with Oral Lichen planus (according to

	Andreason criteria) with fine white radiating striae bilaterally along with burning sensation. 2. Patients above 18 years. 3. Patients who are willing to participate in this study.

Exclusion Criteria

Exclusion Criteria	
Details	1. Patients with any other potentially malignant disorders other than Oral Lichen Planus and under treatment for the same. 2. Patients who are allergic to Clobetasol propionate and Nigella sativa (Black seed) topical preparation. 3. Patients with any other systemic disease. 4. Pregnant and lactating females. 5. Patients who have already undergone treatment for Oral Lichen planus (within 3 months)

Method of Generating Random Sequence	Stratified randomization
Method of Concealment	Sequentially numbered, sealed, opaque envelopes
Blinding/Masking	Participant and Investigator Blinded

Primary Outcome

Outcome	Timepoints
Reduction in the intenstity of burning sensation and reduction in the size of the lesion.	15 days, 30 days, 45 days, 60 days

Secondary Outcome

Outcome	Timepoints
Whether Nigella sativa 75% (w/v) cream can or cannot be used as an alternative treatment modality for the management of Oral Lichen Planus and whether it has comparable effects of Clobetasol propionate will be known	15 days, 30 days, 45 days, 60 days

Target Sample Size	**Total Sample Size=**72 **Sample Size from India=**72 **Final Enrollment numbers achieved (Total)=**Applicable only for Completed/Terminated trials **Final Enrollment numbers achieved (India)=**Applicable only for Completed/Terminated trials
Phase of Trial	Phase 2/ Phase 3
Date of First Enrollment (India)	17/09/2020
Date of First Enrollment (Global)	No Date Specified
Estimated Duration of Trial	**Years=**2 **Months=**6 **Days=**0
Recruitment Status of Trial (Global)	Not Applicable
Recruitment Status of Trial (India)	Not Yet Recruiting
Publication Details	NIL
Brief Summary	**NULL HYPOTHESIS:** There will be no change in the intensity of burning sensation and size of the lesion on application of

Nigella sativa (Black seed) (75%w/v) cream in comparison to Clobetasol propionate ointment (0.05%) in Oral Lichen Planus patients.

ALTERNATIVE HYPOTHESIS:

There will be change in the intensity of burning sensation and size of the lesion on application of *Nigella sativa* (Black seed) (75%w/v) cream in comparison to Clobetasol propionate ointment (0.05%) in Oral Lichen Planus patients.

Clinical evaluation of the efficacy of *Nigella sativa* (Black seed) (75%w/v) cream and Clobetasol propionate (0.05%) ointment:

Clinically diagnosed cases of OLP (according to Andreason) with bilateral white striations along with burning sensation will be included in this study. The clinical evaluation of burning sensation will be done using Visual analogue scale (VAS) and the size of the lesion will be measured by a standard Vernier caliper (according to RECIST criteria) by the first investigator. The second investigator will be allocating the patients asper computer generated sequence, generated by the statistician (stratified method of allocation will be followed for categorizing OLP cases into moderate and severe, using scoring of Malhotra et al,2008) as Group I and Group II (36 participants under each group). The application of the cream/ointment will be demonstrated to the patient by the second investigator and checked for adverse reaction after a time period of half an hour. The patients will then be advised for topical application of *Nigella sativa* (Black seed) cream(75%w/v) and Clobetasol propionate 0.05% ointment respectively with sterile cotton buds twice a day after breakfast and dinner.. Every patient will be given a treatment card where he/she will be asked to enter the daily application of cream/ointment. Both the patient and the first investigator will be blinded of the type of intervention. Patients will be recalled after 15, 30, 45 & 60 days for reevaluation of burning sensation with Visual analogue scale and size of the lesion (RECIST criteria) by the first investigator. The application of cream/ ointment will be advised until the healing of lesion.

RECIST CRITERIA:

The RECIST system is a simplified tumor measurement by using only the longest uni-dimensional measurement of a lesion, and for multiple lesions, using the sum of the longest diameters.

ANNEXURE – III

KLE V.K. INSTITUTE OF DENTAL SCIENCES, BELAGAVI

DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY

PATIENT CONSENT FORM

"Comparative evaluation of the efficacy of *Nigella sativa* (75%) (w/v) cream and Clobetasol propionate (0.05%) (w/w) gel for the management of Oral Lichen Planus- A Double Blinded Randomized Control Trial"

I,_____aged_____years old have been informed about my involvement in the study.

1) I agree to give my personal details like name, age, sex, address, previous dental history, medical history and the details required for the study to the best of my knowledge

2) I will cooperate with the dentist for my intraoral and/or extraoral examinations.

3) I permit the dentist to utilize the information given by me and results obtained from this study for presentation and publication purpose.

4) I understand that sometimes adverse reactions may occur on application of the cream/ gel.

5) I have been informed about the follow up after 15, 30, & 45 days and I agree to visit for the same.

6) I will not claim any returns for my cooperation in the study, even if it is being sponsored by any agency. I am participating with my own will and wish.

In my full consciousness and presence of mind, after understanding all the procedure in my vernacular language, I am willing and giving my consent to participate in this study.

Signature of the patient:…… Signature of the dentist:…….

Date:…… Name of the witness:……..

Place:…… Signature of the witness:…….

ANNEXURE – IV

ಕೆ ಲ್ ಇ ವಿಶ್ವನಾಥ್ ಕತ್ತಿ ದಂತ ಮಹಾವಿದ್ಯಾಲಯ, ಬೆಳಗಾವಿ
ಮೌಖಿಕ ಔಷಧಿ ಮಾತು ವಿಕಾರಣಶಾಸ್ತಿರ ಇಲಾಖೆ
ಒಪ್ಪಿಗೆ ಪತರ

ತುಲನಾತಮಕ ಮೌಲಾಮಾಪನ ನ ಣಿಗೆಲಲ ಸ್ತಟಿವ ಎಣ್ಣೆ (75%) ಮುಲಾಮು ಮಾತು ಕಲಒಬೆಟಸೂಲ್ ಪ್ರರಪ್ಪಯೋನೇಟ್ (೦.೦5%) ಮುಲಾಮು ಗಾಗಿ ಮೌಖಿಕ ಲಿಚೆನ್ ಪ್ಲಲನಸ್- ಡಬಲ್ ಬಿಲಿಂಡೆಡ್ ರ್ಯಿಂಡಮೈಜ್ಡ್ ಕಂಟ್ರೋಲ್ ಟರಯಲ್

ನಾನು _____ ವಯಸು _____ ವರುಷ ಆಧ್ಯಾನಧ್ಯಲ್ಲಿ ನನನ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಯ ಬಗ್ಗೊ ನನಗ್ಗ ತಿಳಿಸಲಾಗಿಧೆ.

೧) ನನನ ಹೆಸರು, ವಯಸು, ಲ್ಲಿಂಗ, ವಿಳಾಸ, ಹಲ್ಲಿನ ಇತಿಹಾಸ, ವೈದ್ಾಕೀಯ ಇತಿಹಾಸ ಮತ್ತು ಅಧ್ಾಯನಕೆ ೆ ಅಗತ್ಾವಾಧ್ ವಿವರಗಳನುನ ನೀಡಲು ಒಪ್ಪುತ್ುೀನೆ.

೨) ನಾನು ಎಲಾಿ ತ್ರಹದ್ ತ್ಾಪಸನೆಗಳೆಗ್ಗ ಒಳಗಾಗಲು ಸಹಕಾರಿಸುತ್ುನೆ.

೩) ನಾನು ನೀಡಿದ್ ಮಾಹಿತಿ ಮತ್ತು ಫಲ್ಲತಂಶಗಳನು ಪ್ರಸುುತಿ ಮತ್ತು ಪ್ರಕಬನೆ ಉದ್ದೀಶಕಾೆಗಿ ಬಳಸಿಕೊಳ್ಳುಲು ದಂತ್ ವೈದ್ಾರಿಗ್ಗ ನಾನು ಅನುಮತಿ ನೀಡುತ್ುನೆ .

೪) ಮುಲಾಮು ಉಪ್ಯೀಗಿಸುವಾಗ ಕೆಲವೊಮೆ್ಮ ಪ್ರತಿಕೊೀಲ ಪ್ರತಿಕರಯೆಗಳ್ಳ ಸಂಭವಿಸಬಹುದು ವೆಂದು ನಾನು ತಿಳಿದಿದ್ದಿನೆ

೫) ೧೫, ೩೦, ಮಾತ್ತ ೪೫ ದಿನಗಳ್ಳ ನಂತ್ರ ನಡೆಯುವ ಮರುಪ್ರಿಶೀಲನೆಗ್ಗ ನನಗ್ಗ ತಿಳಿಸಲಾಗಿದ್ ಮತ್ತು ಇಧ್ಾಗಿ ನಾನು ಭೇಟಿ ನೀಡಲು ಒಪ್ಪುತ್ುೀನೆ.

೬) ಅಧ್ಾಯನದ್ಲ್ಲಿ ನನನ ಸಹಕಾರಕಾೆಗಿ ನಾನು ಯಾವುದೇ ತ್ರಹದ್ ಏಜೆನಿಯಿಂದ್ ಪ್ರಯೀಗಿನಾಗಲು ಆಧ್ಯಯವನುನ ಪ್ಡೆಯುವುದಿಲಿ ಹಾಗು ನಾನು ಸ್ವಾಇಚೆಯಿಂದ್ ಅಧ್ಾಯನದ್ಲ್ಲಿ ನಾನು ಭಾಗವಹಿಸುತಿದ್ದಿನೆ.

ನನನ ಪೂರ್ಣಪ್ರಜೆ ೆ ಮತ್ತು ಮನಸಿನ ಉಪ್ಸಥತಿಯಲ್ಲಿ ಎಲಾಿ ಕಾಯಣವಿಧ್ಯನಗ್ಗಳನುನ ಸಥಳಿೀಯ ಭಾಷೆಯಲ್ಲಿ ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದಿನೆ ಮತ್ತು ಈ ಅಧ್ಾಯನದ್ಲ್ಲಿ ಭಾಗವಹಿಸಲು ಸಂಪೂರ್ಣ ಒಪ್ಪುಗ್ಗಯನುನ ನೀಡುತಿದ್ದಿನೆ.

ರೀಗಿಯ ಸಹಿ:_____ ದಂತ್ ವೈದ್ಾರ ಸಹಿ:_____

ದಿನಾಂಕ:_____ ಸ್ವಕಿದಾರರ ಹೆಸರು:_____

ಸಥಳ:_____ ಸ್ವಕಿದಾರರ ಸಹಿ:_____

ANNEXURE – V

के.एल.ई विश्वनाथ कट्टी दंत विज्ञान संस्था, बेलगावी

ओरल मेडिसिन आणि रेडिओलॉजी विभाग

संमती पत्र

"तुलनात्मक मूल्यांकन करिता नैजेला सटायवा मलम आणि क्लाबेटसोल प्रोपिऑनेट मलम ची कार्यक्षमता लाईकन प्लेनस च्या उपचारासाठी- एक डबल ब्लायनदेड अभ्यास"

मी,_____वय _____वर्ष माझ्या सहभागाची माहिती मला देण्यात आली आहे।

१) मी माझे वैयक्तिक तपशील जसे की नाव, वय, लिंग, पत्ता, मागील दंत इतिहास, वैद्यकीय इतिहास आणि अभ्यासासाठी आवश्यक असलेल्या तपशीलांना माझ्या सर्वोत्तम माहिती देण्यास सहमत आहे.

२) मी माझ्या इंट्राओरियल आणि / किंवा बाह्य परीक्षांसाठी दंतचिकित्सकांना सहकार्य करीन.

३) मी दंतचिकित्सकांकडून माझ्याद्वारे दिलेली माहिती आणि या अभ्यासामधून प्राप्त झालेल्या सादरीकरणासाठी आणि प्रकाशनाच्या उद्देशाने वापरण्याची परवानगी देतो.

४) मला समजले आहे की कधीकधी मलम वापरल्यास प्रतिकूल प्रतिक्रिया येऊ शकतात.

५) मला १५, ३०, ४५ आणि ६० दिवसानंतर पाठपुराव्याविषयी माहिती देण्यात आली आहे आणि मी त्यास भेट देण्यास सहमत आहे.

६)कोणत्याही अभ्यासानुसार प्रायोजित केले गेले असले तरीही अभ्यासाच्या माझ्या सहकार्यासाठी मी परताव्याचा दावा करणार नाही. मी माझ्या स्वत: च्या इच्छेने सहभाग घेत आहे.

माझ्या पूर्ण चेतने आणि मनाच्या उपस्थितीत, माझ्या स्थानिक भाषेतील सर्व प्रक्रिया समजल्यानंतर, मी या अभ्यासात भाग घेण्यास तयार आहे आणि मी संमती देतो.

रूग्णाची सही: .....

तारीख: .....

ठिकाण: .....

दंतवैद्याची सही: .....

साक्षीदाराचे नाव: .....

साक्षीदाराची सही: .....

ANNEXURE – VI

KLE V.K INSTITUTE OF DENTAL SCIENCES, BELAGAVI

DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY

"Comparative evaluation of the efficacy of *Nigella sativa* (75%) (w/v) cream and Clobetasol propionate (0.05%) (w/w) gel for the management of Oral Lichen Planus- A Double Blinded Randomized Control Trial"

CASE HISTORY PROFORMA

Name of the

patient:

Address:

Chief Complaint:

History of presenting illness:

Past medical and dental history:

Personal History:

Sleep: **Positive/**

Negative

Stress:

Positive/

Negative

General Physical Examination:

Extraoral Examination:

Intraoral Examination:

Hard tissue Examination:

Soft tissue Examination:

Examination of the specific lesion: (Oral

Lichen Planus)Erythema and related mucosal

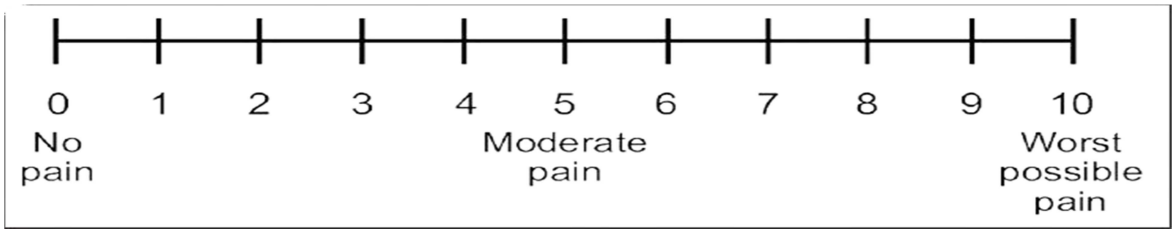
changes: Yes/ No

Side: Right Side/ Left Side/ Both

Site: Buccal mucosa/ Labial mucosa/ Floor of mouth/ Upper buccal sulcus/Lower buccal sulcus/ Upper labial sulcus/ Lower labial sulcus/ tongue

Extension:

Numerical Pain Rating Scale:



Scoring of burning sensation:

Malhotra et al, 2008 scoring

Buccal mucosa - Right- <50%/ > 50%

Left - <50%/ > 50%

Tongue- Back surface- <50%/ > 50%

Front surface- <50%/ > 50%

Lips- Upper- involved/uninvolvedLower- involved/uninvolved

Gingiva- involved/uninvolvedPalate- involved/uninvolved Total score-

Severity-Moderate/Severe

<u>Size (RECIST criteria):</u>

Provisional Diagnosis:

Investigations:

Final Diagnosis

Treatment Plan:

Follow-up:

Signs and Symptoms	Baseline	After 15 days	After 30 days	After 45 days
Burning sensation (NPS score)				
Size of the lesion (mm)				
Adverse effect if any				

ANNEXURE – VII

KLE V.K INSTITUTE OF DENTAL SCIENCES, BELAGAVI

DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY

PATIENT INFORMATION SHEET

"Comparative evaluation of the efficacy of Nigella sativa (75%) (w/v) cream and Clobetasol propionate (0.05%) (w/w) gel for the management of Oral Lichen Planus- A Double Blinded Randomized Control Trial"

Dear Patient,

You are invited to take part in a research study to examine and intervene your oral condition- Oral Lichen Planus. I would like to interview you to ask you about the symptoms of the condition. This research is a part of an MDS., main dissertation at KLE Academy of Higher Education and Research.

Before you decide whether to take part in the study it is important that you understand what the research is for and what you will be asked to do. Please take time to read the following information and discuss it with others if you wish. It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep. You will be also asked to sign a consent form. You can change your mind at any time and withdraw from the study without giving any reason. The standard of care you receive will not change whether or not you decide to participate in this study. You are welcome to phone me (@8148888961) if you would like any further information.

The purpose of this research study is to evaluate and compare the efficacy of Nigella sativa (75% w/v) cream and Clobetasol propionate (0.05% w/w) gel for the management of Oral Lichen Planus.

You have been chosen because you have been diagnosed with Oral Lichen Planus along with burning sensation. The study will involve 48 participants who will be examined and advised for topical application of Nigella sativa (75% w/v) cream or Clobetasol propionate (0.05% w/w) gel twice a day (after food) for varied time period (until the lesion heals) after thorough recording of case history and getting informed

consent. All participants will be given demonstration of application of cream/ gel and will also be checked for any allergic or adverse reactions after a period of half an hour. Nigella sativa oil is a natural remedy known for its various beneficial properties with very minimal or no side effects. The dosage to be administered is well below the LD50 value of thymoquinone to be causing any adverse reactions. Multiple photographs will be recorded during the study to compare the size of the lesion. You will be asked to report for a review and follow-up visit after 15, 30, and 45 days.

The information gained from this research will be used to publish in scientific platforms/ journals without revealing your identity to make recommendations for the best practice and the results of the study may also lead onto further studies into the management of Oral Lichen Planus.

Dr. Lokesh Kumar. S, PG Student (MDS.,)
Dept. of Oral Medicine and Radiology,
KLEVKIDS, Belagavi

ANNEXURE – VIII

GANTT CHART

Activities	August 2019 to March 2022																				
	2019	2020				2021												2022		
	Aug -Dec	Jan-M ar	Apr- Jun	Jul- Sep	Oct- Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Ethical clearance& CTRI Reg																				
Study plan & preparation, Literaturereview & data collectiontool preparation																				
Cream &gel formulation																				
Phytochemical screening/ Mucoadhesive property evaluation																				
Cytotoxicity evaluation																				
Pilot Study																				
Data collection																				
Data entry																				
Data Analysis																				
Write up & Submissi on																				
Publication																				

ANNEXURE – IX

MASTER CHART- 1

Burning sensation in Group I

NPS scores									
Patient No.	**Age**	**Gender**	**Grading**	**Severity**	**Type**	**Baseline**	**15th Day**	**30th Day**	**45th Day**
Group I- *Nigella sativa* (Black seed) (75 % w/v) cream									
1	50	M	Grade II	Moderate	Reticular	2	1	0	0
2	30	M	Grade I	Moderate	Reticular	2	1	1	0
3	43	F	Grade II	Moderate	Reticular	5	3	1	0
4	45	M	Grade II	Moderate	Reticular	3	1	0	0
5	45	M	Grade II	Moderate	Reticular	3	2	0	0
6	55	F	Grade I	Moderate	Reticular	5	4	2	1
7	23	M	Grade I	Moderate	Plaque	3	0	0	0
8	45	M	Grade I	Moderate	Reticular	8	4	0	0
9	44	M	Grade II	Moderate	Reticular	2	0	0	0
10	37	F	Grade II	Moderate	Reticular	4	2	1	0
11	29	F	Grade I	Moderate	Reticular	6	4	2	0
12	62	F	Grade II	Moderate	Atrophic	1	0	0	0
13	27	F	Grade II	Moderate	Reticular	5	2	1	0
14	28	M	Grade II	Moderate	Reticular	8	7	5	2
15	70	F	Grade I	Moderate	Reticular	7	5	2	0
16	61	F	Grade II	Moderate	Reticular	4	1	0	0
17	35	F	Grade II	Moderate	Reticular	2	0	0	0
18	52	F	Grade I	Severe	Erosive	10	8	6	4
19	58	F	Grade III	Severe	Erosive	8	5	2	1
20	67	M	Grade I	Severe	Bullous	6	5	4	2
21	34	F	Grade II	Severe	Erosive	7	4	2	0
22	46	M	Grade II	Severe	Erosive	3	2	1	0
23	29	M	Grade II	Severe	Erosive	2	1	1	0
24	38	F	Grade II	Severe	Erosive	6	2	1	0
25	59	F	Grade III	Severe	Atrophic	8	7	3	0

ANNEXURE – X

MASTER CHART- 2

Burning sensation in Group II

NPS scores									
Patient No.	**Age**	**Gender**	**Grading**	**Severity**	**Type**	**Baseline**	**15th Day**	**30th Day**	**45th Day**
Group II- Clobetasol propionate (0.05 % w/w) gel									
1	67	F	Grade I	Moderate	Reticular	7	3	2	1
2	42	M	Grade I	Moderate	Reticular	6	0	0	0
3	58	F	Grade I	Moderate	Atrophic	5	3	2	1
4	23	M	Grade I	Moderate	Reticular	6	3	2	0
5	56	F	Grade II	Moderate	Reticular	5	4	2	0
6	63	F	Grade I	Moderate	Reticular	3	2	1	1
7	48	M	Grade II	Moderate	Reticular	5	3	0	0
8	40	F	Grade II	Moderate	Reticular	10	8	6	3
9	42	F	Grade II	Moderate	Reticular	8	7	3	1
10	55	F	Grade II	Moderate	Reticular	3	1	0	0
11	54	M	Grade I	Moderate	Atrophic	2	2	0	0
12	29	M	Grade II	Moderate	Reticular	5	4	2	0
13	34	F	Grade I	Moderate	Reticular	8	5	2	1
14	50	F	Grade II	Moderate	Reticular	8	6	2	0
15	48	F	Grade II	Moderate	Reticular	2	1	0	0
16	52	F	Grade II	Moderate	Reticular	9	8	5	0
17	43	F	Grade II	Moderate	Reticular	4	2		
18	55	F	Grade II	Severe	Erosive	5	3	1	0
19	66	M	Grade II	Severe	Erosive	5	3	0	0
20	65	M	Grade I	Severe	Erosive	5	4	2	2
21	55	F	Grade II	Severe	Erosive	5	4	0	0
22	32	F	Grade II	Severe	Erosive	2	1	0	0
23	37	F	Grade II	Severe	Erosive	4	2	0	0
24	28	F	Grade I	Severe	Erosive	3	1	1	0
25	37	F	Grade II	Severe	Erosive	5	3	0	0
26	66	M	Grade III	Severe	Bullous	5	2		
27	70	M	Grade I	Severe	Erosive	7	4		

ANNEXURE – XI

MASTER CHART- 3

Size of the lesion in Group I

Size of the lesion (mm)									
Patient No.	**Age**	**Gender**	**Grading**	**Severity**	**Type**	**Baseline**	**15th Day**	**30th Day**	**45th Day**
Group I- *Nigella sativa* (Black seed) (75 % w/v) cream									
1	50	M	Grade II	Moderate	Reticular	126	82	34	9
2	30	M	Grade I	Moderate	Reticular	115	77	23	2
3	43	F	Grade II	Moderate	Reticular	189	96	37	16
4	45	M	Grade II	Moderate	Reticular	271	116	43	14
5	45	M	Grade II	Moderate	Reticular	73	40	13	0
6	55	F	Grade I	Moderate	Reticular	48	24	8	1
7	23	M	Grade I	Moderate	Plaque	94	48	16	0
8	45	M	Grade I	Moderate	Reticular	54	32	15	9
9	44	M	Grade II	Moderate	Reticular	179	98	32	13
10	37	F	Grade II	Moderate	Reticular	147	84	29	14
11	29	F	Grade I	Moderate	Reticular	77	43	28	11
12	62	F	Grade II	Moderate	Atrophic	193	111	49	10
13	27	F	Grade II	Moderate	Reticular	117	53	28	19
14	28	M	Grade II	Moderate	Reticular	121	51	23	4
15	70	F	Grade I	Moderate	Reticular	36	21	9	3
16	61	F	Grade II	Moderate	Reticular	119	47	31	5
17	35	F	Grade II	Moderate	Reticular	191	96	58	21
18	52	F	Grade I	Severe	Erosive	77	62	33	9
19	58	F	Grade III	Severe	Erosive	86	70	41	12
20	67	M	Grade I	Severe	Bullous	136	99	60	16
21	34	F	Grade II	Severe	Erosive	244	156	71	23
22	46	M	Grade II	Severe	Erosive	189	101	42	11
23	29	M	Grade II	Severe	Erosive	103	49	28	7
24	38	F	Grade II	Severe	Erosive	207	113	44	17
25	59	F	Grade III	Severe	Atrophic	263	126	57	26

ANNEXURE – XII
MASTER CHART- 4
Size of the lesion in Group II

Size of the lesion (mm)									
Patient No.	**Age**	**Gender**	**Grading**	**Severity**	**Type**	**Baseline**	**15th Day**	**30th Day**	**45th Day**
Group II- Clobetasol propionate (0.05 % w/w) gel									
1	67	F	Grade I	Moderate	Reticular	63	30	16	2
2	42	M	Grade I	Moderate	Reticular	209	107	42	9
3	58	F	Grade I	Moderate	Atrophic	91	36	14	11
4	23	M	Grade I	Moderate	Reticular	136	83	38	14
5	56	F	Grade II	Moderate	Reticular	111	58	14	0
6	63	F	Grade I	Moderate	Reticular	25	13	6	3
7	48	M	Grade II	Moderate	Reticular	148	87	27	17
8	40	F	Grade II	Moderate	Reticular	47	21	9	0
9	42	F	Grade II	Moderate	Reticular	174	89	34	8
10	55	F	Grade II	Moderate	Reticular	85	37	26	15
11	54	M	Grade I	Moderate	Atrophic	126	61	27	6
12	29	M	Grade II	Moderate	Reticular	190	104	45	13
13	34	F	Grade I	Moderate	Reticular	59	28	11	7
14	50	F	Grade II	Moderate	Reticular	156	98	34	22
15	48	F	Grade II	Moderate	Reticular	284	128	53	12
16	52	F	Grade II	Moderate	Reticular	223	102	42	18
17	43	F	Grade II	Moderate	Reticular	194	76		
18	55	F	Grade II	Severe	Erosive	258	179	62	16
19	66	M	Grade II	Severe	Erosive	287	115	66	12
20	65	M	Grade I	Severe	Erosive	126	83	53	4
21	55	F	Grade II	Severe	Erosive	104	26	18	14
22	32	F	Grade II	Severe	Erosive	84	39	19	5
23	37	F	Grade II	Severe	Erosive	149	65	27	9
24	28	F	Grade I	Severe	Erosive	71	39	12	3
25	37	F	Grade II	Severe	Erosive	186	83	57	24
26	66	M	Grade III	Severe	Bullous	141	68		
27	70	M	Grade I	Severe	Erosive	187	98		