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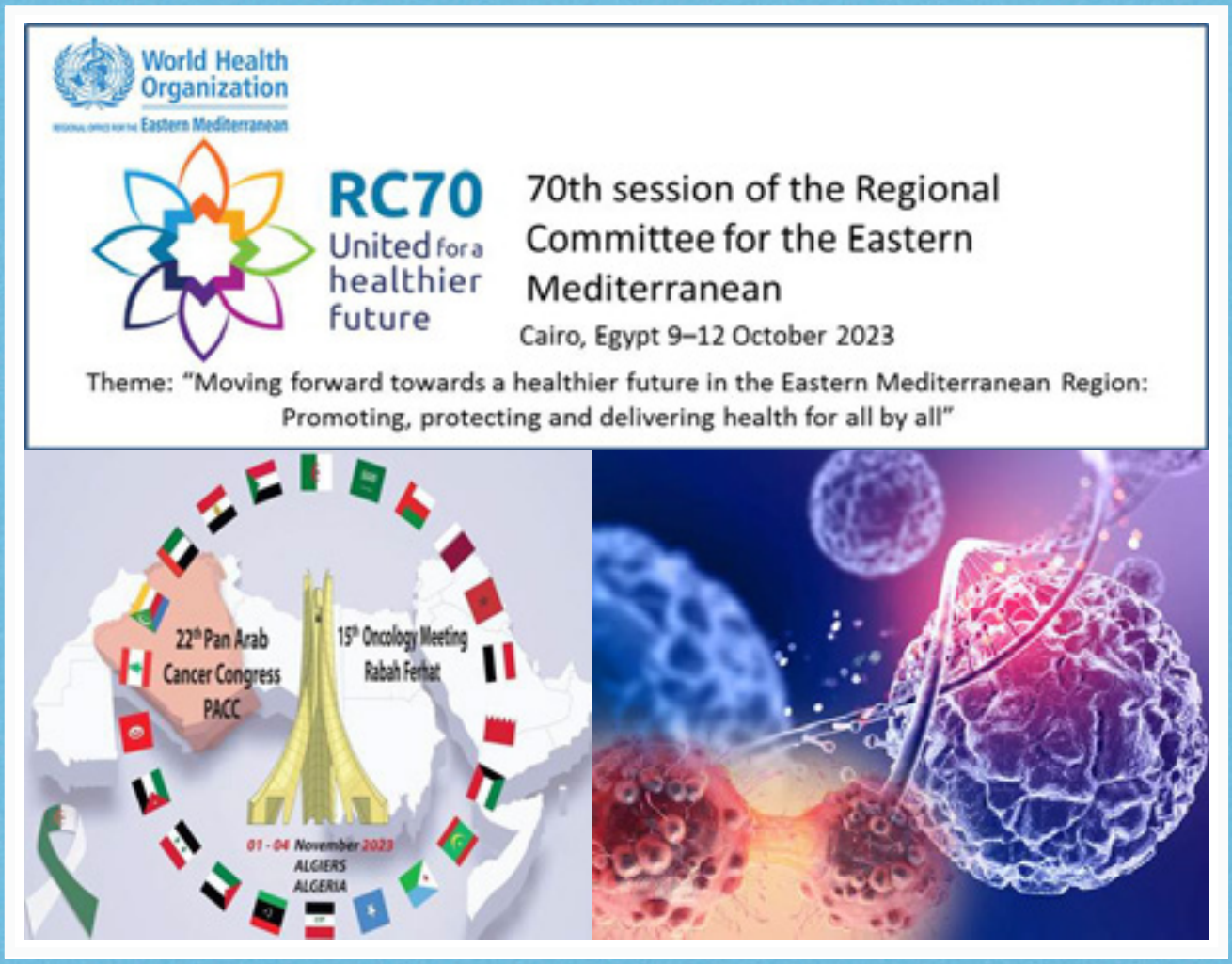

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Original Article

Comparison of Effectiveness of Moringa Oleifera Leaves Extract Gel (2%) with Retino A (0.1%) Cream for Treatment of Oral Leukoplakia: Double Blinded Randomized Control Trial

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Abstract

Aim: The study aims to evaluate and compare the efficacy of Moringa oleifera leaf extract gel (2%) & Retino A cream (0.1%) in reducing the size of lesions in oral leukoplakia.

Objectives: The present study aimed to evaluate the efficacy of two interventions, Moringa oleifera mucoadhesive gel and Retino–A cream, in reducing the size of lesions in patients with oral leukoplakia. Specifically, the objectives were: (1) to assess the efficacy of Moringa oleifera mucoadhesive gel in determining the reduction in lesion size, (2) to assess the efficacy of Retino–A cream in determining the reduction in lesion size, and (3) to compare the efficacy of Moringa oleifera mucoadhesive gel (2%) in determining the change in lesion size in oral leukoplakia patients.

Methods: Clinically diagnosed cases of oral Leukoplakia were included in this study. The sample size is 72. Thirty–six patients had lesion sizes ranging from 2– 4 cm, and 36 patients had lesion sizes ranging from 4.1 – 6 cm that were equally distributed in the case and control groups using the chit system. The case and control groups had 36 patients with an equal size range of lesions. The case and control group participants will be advised topical application of the intervention and Retino–A thrice daily using a sterile cotton bud.

Results: M. oleifera gel (2%) was found to be more effective in the reduction in the size of the lesion as compared to Retino–A in the treatment of oral leukoplakia patients.

Conclusion: This study showed that M. oleifera mucoadhesive gel (2%) is an effective and safe treatment option for oral leukoplakia patients. It demonstrated a significant reduction in lesion size compared to Retino–A cream (0.1%) after 3 months of therapy, without any reported adverse effects. However, long–term follow–up studies are needed to evaluate its long–term effectiveness. The potent antioxidant property of M. oleifera makes it a promising candidate for further studies with concentration variations and in other potentially malignant oral disorders, such as lichen planus and OSMF. The development of chemotherapeutic drugs from M. oleifera for cancer treatment should also be considered. Overall, M. oleifera appears to be a promising natural alternative to synthetic drugs for the treatment of oral leukoplakia.

Key words: Leukoplakia, Oral leukoplakia, premalignant lesion, precancer, potentially malignant disorders.

Introduction

Oral potentially malignant disorders (OPMDs) are a cluster of diseases that should be recognized at the initial stage. The most prevalent OPMDs with a higher malignant transformation rate are oral leukoplakia, oral erythroplakia, and oral submucous fibrosis.[1]

Leukoplakia is the terminology used for a precancerous non–scrapable white lesion, and World Health Organization (WHO) describes leukoplakia as “a white plaque of

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questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer.[2]

Over the past thirty years, the epidemiologic data from several countries show the prevalence of oral leukoplakia ranges between 1.1–11.7 %, with a mean value of 2.9 percent.[3] In Banoczy's study, 87% of individuals with leukoplakia were having the habit of smoking tobacco, & 77% of those who developed malignancy smoked tobacco[4.] According to Gupta et al, the association of tobacco with leukoplakia ranged from 47% to 73% among villagers living in India found in a 10–year follow–up.[5]

Tobacco is the main etiological factor for oral leukoplakia. Smoking habits in India have a varying relationship with locally established mixed tobacco habits, such as smoking, chewing, and a combination of the two (chewing betel quid and bidi smoking).[3] Tobacco products contain many carcinogenic agents; nitrosamines are the most carcinogenic agents. These carcinogenic agents were found to be abundant in the saliva of smokers as well as non–smokers and are linked with oral leukoplakia.[6]

The conservative treatment employs local and systemic chemo–preventive agents such as beta carotene, vitamin A, systemic lycopene, and topical bleomycin (a chemotherapeutic agent) with partial effectiveness.[7] Antioxidants such as Beta carotene, Retinol, Retinoids, ascorbic acid, Alpha–tocopherol, and Vitamin A are commonly used to manage oral leukoplakia, commonly used topical preparation is Retino–A cream available commercially.[8] Among the antioxidants, administering retinoic acid and beta–carotene has shown some efficacy in resolving oral leukoplakia.[9] Vitamin A/tretinoin is essential for the normal pathway of epithelial cell differentiation. The use of antioxidants, such as vitamin A, reduces free radicals, thus reducing oxidative stress and preventing cellular changes in oral leukoplakia.[10]

Moringa oleifera is a tropical shrub native to India, also known as the 'drumstick tree'.[11] Moringa oleifera leaves demonstrate potent antioxidant activity.[12] A study on moringa oleifera reported that it provides 7 times more vitamin C than oranges and 10 times more vitamin A than carrots.[11]

Moringa oleifera has abundant bio–flavonoids and antioxidants, anti–inflammatory, anti–cancer, and anti–diabetic properties.[13] M. oleifera is proven to have higher free radical scavenging activity evaluated by two methods DPPH and ABTS assays.[14] The extract obtained from leaves of M. oleifera proved to demonstrate anti–cancer properties causing apoptosis in breast and colorectal cell lines.[15] Moringa oleifera leaf extract is a potential oral anti–cancer drug contender, which was shown to induce apoptosis in human hepatocellular carcinoma cell lines.[16]

Thus, this study intended to evaluate the in–vitro and clinical efficacy of Moringa oleifera mucoadhesive gel (2%) in treating oral leukoplakia.

Methodology:

Test for flavonoids

Flavonoids were extracted from about 10 grams of M. oleifera leaves using 100 ml of 80 percent aqueous methanol at room temperature. Whatman filter paper No.42 was used to filter the entire solution. The filtrate was then transferred to a crucible and evaporated until dry over a water bath before being measured with a weighing machine. Flavonoids in the amount of 4.2 grams were obtained.[17]

Cell viability assay of M.oleifera gel (2%): 18,19

For evaluating cell viability for MO mucoadhesive gel MTT assay was employed. For this assay mouse fibroblast cell line (L929) was employed. The reaction of live cells' mitochondrial dehydrogenase enzymes with the tetrazolium rings of a soluble Dimethyl sulfoxide (DMSO) is used to dissolve crystals. The color of the solution represents the quantity of surviving cells, which is proportional to the amount of formazan. A multi–well scanning spectrophotometer is used to measure the color. 4 µl of cell suspension was seeded in a microplate and incubated for 24 hours. Later M. oleifera gel (10 µl) was added and DMSO 10% was used as a negative control and the ELISA microplate was incubated for 48 hours. A spectrophotometer was used to check the vitality of the cells 48 hours later. Each well was filled with MTT solution and incubated for 3 hours to measure cell survival. Then, after gently replacing 150 µl of MTT medium with DMSO and pipetting to remove formazan crystals that had formed, the absorbance at 540 nm was measured using an ELISA plate reader (Figure 1). The procedure was repeated 3 times. The results of the MTT assay are summarized in Table–2.

2b. Clinical study

Clinically diagnosed cases of Oral Leukoplakia were included in this study. The sample size is 72. Through a stratified randomized sampling procedure, 72 individuals with oral leukoplakia were evenly divided into case and control groups. Through a chit method, 36 patients with lesion sizes ranging from 2 to 4 cm and 36 patients with lesion sizes ranging from 4.1 to 6 cm were randomly assigned to the case and control groups. So, the case and control groups had 36 patients with equal size range lesions.

2.1 Subjects and Clinical Trial Registration:

The study included 36 patients with the equal number of size range lesions, who were divided into two groups: the case group (Moringa oleifera leaves extract group) and the control group (Retino–A 0.1% cream). The case group

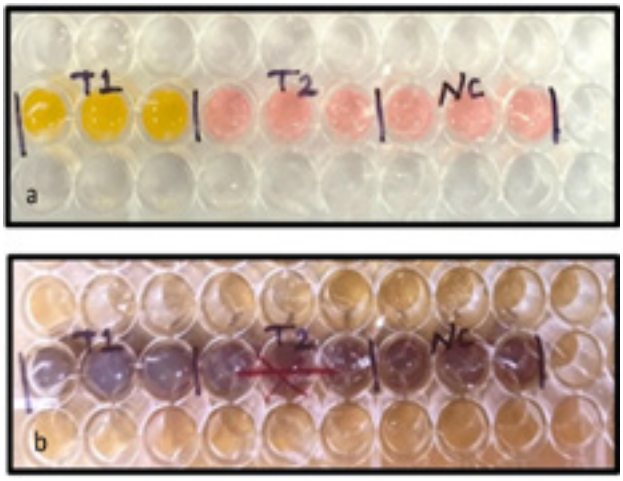

Figure 1 a–b: MTT Assay at baseline B and after 48 hours

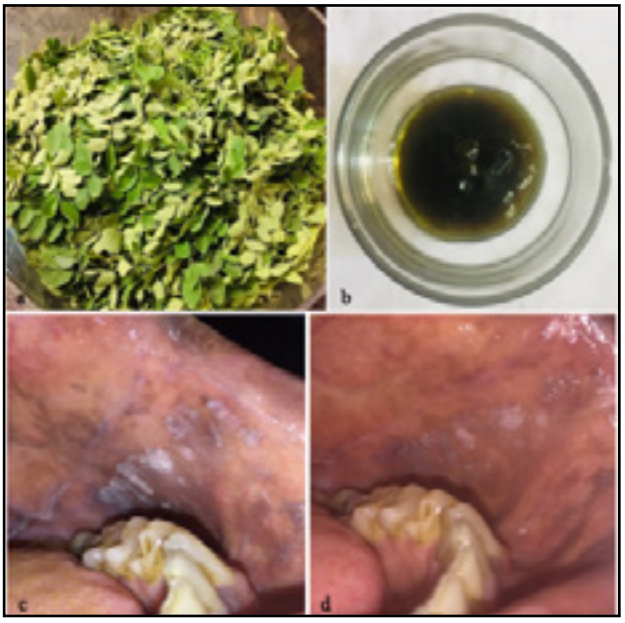

Figure 2 a–d: M. oleifera leaves (Drumstick leaves); M. oleifera mucoadhesive gel (2%); Clinical presentation at baseline in M. oleifera gel (2%) group;Clinical presentation post–treatment in M. oleifera gel (2%) group

received treatment with Moringa oleifera leaves extract mucoadhesive gel, while the control group was treated with Retino–A 0.1% cream. This randomized controlled trial (RCT) was registered in the Clinical Trial Registry India (CTRI) with registration number CTRI/2020/10/028529.Case group (Moringa oleifera leaves extract group)

Ingredients	For 100 ml of gel
1.Carbopol 940	1300mg
2.Distilled water	100
3.M. oleifera leaves extract	2000mg
4.TEA(Triethanolamine)	Qs
5.Methyl paraben	18 mg
6.Ethyl paraben	2mg

Table 1: Composition of Moringa oleifera mucoadhesive gel (2%)

Concentration	Optical Density	Mean	% Cell viability
Negative control	0.455	0.368	100
	0.339		
	0.31		
M. oleifera gel (2%)	0.5	0.377	102.4457
	0.331		
	0.421		

Table 2 : Result of MTT assay.

Group	No. of participants	Mean (mm)	Std. Deviation
M. oleifera Group	33	36.818	14.629
Retino–A Group	32	35.531	15.574

Table 3: Mean (± standard deviation) at baselines of Retino–A and M. oleifera group using unpaired t–test

Variables	Case group		Control group	
	M. oleifera (2%) group		Retino–A (0.1%) group	
	N=33		N=32	
	Baseline	Post therapy	Baseline	Post therapy
Mean (mm)	36.818	8.424	35.531	17.843
Std. deviation	14.629	5.612	15.574	8.621

Table 4: Mean (± Standard deviation) at baseline and post–therapy using paired t–test in case and control group

	Group	N	Mean (mm)	Std.Deviation	t–value	p–value
Reduction difference at baseline and post–therapy	M. oleifera group group	33	28.393	9.930	4.765	0.000012
	Retino–A group	32	17.687 Baseline	8.054		

Table 5: Mean (± Standard deviation) of reduction difference of size ((B–PT) at Baseline(B) and post–therapy(PT) in M. oleifera(2%) and Retino–A (0.1%) groups using unpaired t–test

2.2 Inclusion and Exclusion Criteria:

The inclusion criteria for this study included homogenous leukoplakia with a size range of 2–6 cm in the greatest dimension, patients above 18 years of age, and those willing to participate in the study. The exclusion criteria included non–homogenous leukoplakia, leukoplakia with a maximum dimension of more than 6 cm, leukoplakia involving the tongue and floor of the oral cavity, patients undergoing treatment for any other potentially malignant diseases except oral leukoplakia, and pregnant and lactating females.

2.3 Clinical estimation of lesion:

In the present study, the response to the treatment was assessed by quantitative unidimensional measurement using a dental vernier caliper, and measurement of the longest dimension of the lesion was done pre and post–treatment in both case and control groups.

This study was a double–blind randomized controlled trial; the 1st observer dispensed the interventions, and the size of the lesion was measured by 2nd observer who was unaware of the interventions prescribed to the patients.

The measurement was done at baseline and after 1, 2, and 3 months of the therapy. The pre and post–treatment measurements were compared, and statistical analysis was done for the final assessment.

2.4 Preparation of the mucoadhesive gel

Leaves of M. oleifera (Figure 2a) were procured from Kakati, Belagavi (altitude–752 meters above sea level, humidity–13%, temperature– 34oC). Leaves were washed with distilled water and shade dried for 4–5 days. Dried leaves were coarsely powdered in the grinder. Extraction of leaves was done by maceration procedure using a hydroalcoholic method using 70:30 ethanol: distilled water. Leaves were kept in a flask dipped in 70% aqueous ethanolic solution for 72 hours in a shaker at 75 rpm & room temperature and extracted repeatedly. Whatman's filter paper no.42 was used to filter the solution. The obtained filtrate was transferred to a crucible and dried over a water bath at 40oC. The dry content obtained was weighed on the weighing machine. The extract obtained was stored at 2–8oC temperature.

The laminar airflow was UV irradiated for half an hour before the preparation of the gel. Carbopol 940 was used as a gelling agent. 2% of the gel was prepared by adding 2gm of extract / 100 ml of distilled water.TEA was used to enhance the gelling property. Methyl and ethylparaben were used as preservatives. The obtained gel (Figure 2b) was stored in a sterile condition below 8o C of temperature. The composition of the gel is given above in Table 1.

Results

In this study, a total of 72 participants were enrolled. 36 in the control group (Retino–A) and 36 in the case group (M. oleifera), respectively. We lost the follow–up of 3 patients from the M.oleifera group and 4 patients from the Retino–A group after 1st visit. Results were based on the inter–group comparison of the change in size in the M.oliefera and Retino–A groups (Pre and post–treatment) and intragroup comparisons at baseline and post–therapy.

Statistical analysis was done using SPSS version 26 software. An unpaired t–test was used to compare the statistical difference between the data distribution of the case and control group at baseline. The resolution of the lesion in the case and control groups were compared using a paired t–test at baseline and post–therapy. The intergroup analysis of Retino–A (0.1%) and M.oleifera gel (2%) post–treatment was done using an unpaired t–test for post–treatment values, and a separate unpaired t–test was done using baseline (B) values and post–treatment (PT) values difference (B – PT) to determine which group caused the most reduction in the size of the lesion. This study had 25 males and 8 females in the M.oleifera group and 23 males and 9 females in the Retino–A group.

The maximum number of subjects in the M.oleifera group was above 55 years (39.39%), followed by 45–54 years (30.30%), and the least in the younger age group of 25–34 years (12.12%). The Retino–A group had similar distribution i.e.; maximum subjects in the age group above 55 years (53.12%), followed by the 35–44 years age group (18.75), and least in the 45–54 years age group (12.5%).

An unpaired t–test was employed to compare the baselines of both groups; there was no statistical difference found in the lesion size between the groups at baseline, and there was a normal distribution of data among both groups (table–3). A paired t–test was used for intra–group analysis to compare the baseline and post–therapy regression in the lesion size in each group (table–4).

In the Moringa oleifera group, the mean value and standard deviation at baseline were (36.818 ± 14.629), and post–treatment was (8.424 ± 5.612); the result was statistically significant at p–value ($p<0.001$), clinical presentation pre and post–treatment among M.oleifera group are shown in figures 2c and 2d. In the Retino–A group, the mean value and standard deviation at baseline were (35.531±15.574) and the post–treatment was (17.843 ± 8.621); the result was statistically significant at p–value ($p<0.001$).

To compare the efficacy of the M.oleifera group and Retino–A group post–treatment unpaired t–test was used. The mean and standard deviation of the M.oleifera and Retino–A group were (8.424± 5.612) and (17.843 ± 8.621),

respectively. M. oleifera was found more effective in the reduction of the size of the lesion.

To compare the effectiveness of both the group difference in the lesion size at baseline and post–treatment were taken from each group and analyzed using an unpaired t–test. In the M. oleifera group mean and standard deviation values were (28.393 ± 9.930) respectively, whereas, in the Retino–A group, the values were (17.687± 8.054). There was a statistical difference between both the group and the M. oleifera group was found to be more effective in the reduction of the size of oral leukoplakia at the mean difference of (10.71±1.876) at p–value ($p<0.001$) given in table–5.

Discussion:

"Oral leukoplakia is a whitish non–scrapable patch that cannot be diagnosed clinically or pathologically as any other disease and is not caused by physical or chemical causes, with the exception of tobacco habits". The malignant potential of leukoplakia was first suggested by Sugar and Benoczy in 1957.[4]

Recent literature has shown the use of various treatment modalities such as topical and systemic antioxidants such as lycopene, tretinoin, etc.[8] M. oleifera mucoadhesive gel is an ideal topical agent because of its has potent antioxidant activity, low cell toxicity, well–tolerated, cost–effective. Furthermore, it is a popular and readily available herb in India. M. oleifera gel can be used in patients with systemic diseases such as diabetes, hypertension, and liver disorders without any adverse effects as it has anti–hyperglycemic, anti–hypertensive, and hepatoprotective properties.[20] The antioxidants present, such as bioflavonoids i.e. quercetin, and Kaempherol, reduce oxidative stress by scavenging reactive oxygen species in the cell.[12] It also has anti–cancer properties, which provide added therapeutic effects in premalignant conditions such as leukoplakia.[14]

In the present randomized controlled trial, we evaluated the efficacy of M. oleifera gel (2%) for topical application among patients with oral leukoplakia and compared it with Retino–A cream (0.1%). Seventy–two patients with clinically diagnosed cases of oral leukoplakia were included in the study. Thirty–six patients were enrolled in each group. The test group received M. oleifera gel (2%), and the control group received Retino–A cream(0.1%). Although there was a loss of follow–up of 3 patients in the test group and 4 patients in the control group, a total of 33 patients received M. oleifera gel (2%) treatment and 32 patients received Retino–A cream (0.1%) treatment.

In this RCT, around 73.84% of patients were males, and 26.15% were females. According to some studies oral leukoplakia is predominant in men,[21] although other studies found no gender predilection.[22,23] Around 46.15% of the patients were above 55 years, which was consistent with the findings of Amagsa et al.[24] Retino–A cream (0.1%) i.e, tretinoin is an antioxidant most commonly used in chemo–preventive agents; it is most commonly employed for the management of oral leukoplakia.

The choice of M. oleifera as a chemo–preventive agent was based on the preclinical in–vitro studies demonstrating its potent antioxidant activity and anti–cancer properties.M. oleifera was found to be effective against and caused apoptosis of colorectal cell lines, hepatocellular carcinoma, and breast cancer cell lines.[25–27]

In previous studies, many herbal agents as a topical preparation have been employed in managing oral leukoplakia, such as curcumin, Calendula Officinalis, green tea extract, and lycopene.[28] This is the first study that demonstrates the therapeutic efficacy of M. oleifera in the form of a mucoadhesive gel in vivo. Although tretinoin is most commonly used in the management of oral leukoplakia, according to the literature M. oleifera is 10 times more potent than tretinoin as an antioxidant with no cellular toxicity.[11] Retino–A (tretinoin) cream is commonly used for the management of acne vulgaris.[28] At present there is no oral topical chemotherapeutic agent available for the management of oral leukoplakia. Oral physicians prescribe topical tretinoin cream as an alternative. M. oleifera gel is the 1st ever oral mucoadhesive gel prepared for the management of oral leukoplakia, thus it has better tolerability, better therapeutic efficacy and it is more palatable as compared to Retino–A cream.

The scope for further research is wide open to evaluating the effect of M. oleifera in non–homogenous leukoplakias and other pre–malignant diseases such as lichen planus etc, as M. oleifera is a potential candidate as a chemo–preventive agent. Also, long–term follow–up of these patients is required to evaluate the long–term therapeutic effects on oral leukoplakia and the recurrence of this pre–malignant lesion.

Potentially malignant disorders like oral leukoplakia are one of the most commonly encountered in the oral cavity. Previous epidemiological studies have proven the use of antioxidants as a potent treatment modality in a topical form. One such herbal therapeutic agent is M. oleifera, most commonly found in India and known for its potent antioxidant property reported in various in–vitro studies. Also, it has anti–cancer properties and can thus be used as

a potential chemotherapeutic agent. It is proven effective in in–vitro studies against hepatocellular carcinoma, colorectal carcinoma, and breast cancer. As the commercial form of M. oleifera in topical form is not available, it was formulated in this RCT.

Conclusion:

The present clinical trial concluded that patients who received M. oleifera mucoadhesive gel (2%) presented a significant reduction in lesion size compared to those who received Retino–A cream (0.1%). This could be attributed to the potent antioxidant property of M. oleifera, which reversed hyperkeratosis, the earliest change in oral leukoplakia. All patients responded positively to M. oleifera gel, and none of the patients in this study reported local irritation or adverse effects with M. oleifera gel. Long–term follow–up of oral leukoplakia patients is required to evaluate the long–term effectiveness of M. oleifera mucoadhesive gel in oral leukoplakia patients. M. oleifera appears to be a safe and promising antioxidant for the topical treatment of oral leukoplakia. Further studies should be conducted with concentration variations and a more extended follow–up period. An initiative should be taken to introduce the herbal gel at the commercial level. Additionally, it can be employed in studies with other potentially malignant oral disorders, such as lichen planus and OSMF. The development of chemotherapeutic drugs for the treatment of cancer from MO should also be considered.

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Funding and Conflict of Interest:

This study is self–funded. The authors declare there are no conflicts of interest, all the authors had full access to the study data.

Refrences

1. Yardimci G, Kutlubay Z, Engin B, Tuzun Y. Precancerous lesions of oral mucosa. World Journal of Clinical Cases: WJCC. 2014 Dec 12;2(12):866.
2. Warnakulasuriya S, Johnson NW, Van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. Journal of oral pathology & medicine. 2007 Nov;36(10):575–80.
3. Bánóczy J, Gintner Z, Dombi C. Tobacco use and oral leukoplakia. Journal of dental education. 2001 Apr;65(4):322–7.
4. Bánóczy J. Follow–up studies in oral leukoplakia. Journal of maxillofacial surgery. 1977 Jan 1;5:69–75.
5. Gupta PC, Mehta FS, Daftary DK, Pindborg JJ, Bhonsle RB, Jalnawalla PN, Sinor PN, Pitkar VK, Murti PR, Irani RR, Shah HT. Incidence rates of oral cancer and natural history of oral precancerous lesions in a 10–year follow–up study of Indian villagers. Community dentistry and oral epidemiology. 1980;8(6):283–333.
6. orter S, Gueiros LA, Leão JC, Fedele S. Risk factors and etiopathogenesis of potentially premalignant oral epithelial lesions. Oral surgery, oral medicine, oral pathology and oral radiology. 2018 Jun 1;125(6):603–11.
7. Parlatescu I, Gheorghe C, Coculescu E, Tovaru S. Oral leukoplakia–An update. Maedica. 2014 Mar;9(1):88.
8. Kaugars GE, Silverman Jr S, Lovas JG, Thompson JS, Brandt RB, Singh VN. Use of antioxidant supplements in the treatment of human oral leukoplakia review of the literature and current studies. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 1996 Jan 1;81(1):5–14.
9. Ribeiro AS, Salles PR, da Silva TA, Mesquita RA. A review of the nonsurgical treatment of oral leukoplakia. International journal of dentistry. 2010 Feb 23;2010.
10. Gorsky M, Epstein JB. The effect of retinoids on premalignant oral lesions: focus on topical therapy. Cancer. 2002 Sep 15;95(6):1258–64.
11. Gopalakrishnan L, Doriya K, Kumar DS. Moringa oleifera: A review on nutritive importance and its medicinal application. Food science and human wellness. 2016 Jun 1;5(2):49–56.
12. Sreelatha S, Padma PR. Antioxidant activity and total phenolic content of Moringa oleifera leaves in two stages of maturity. Plant foods for human nutrition. 2009 Dec;64(4):303–11.
13. Lin M, Zhang J, Chen X. Bioactive flavonoids in Moringa oleifera and their health–promoting properties. Journal of functional foods. 2018 Aug 1;47:469–79.
14. Charoensin S. Antioxidant and anti–cancer activities of Moringa oleifera leaves. Journal of Medicinal Plants Research. 2014 Feb 17;8(7):318–25.
15. Al–Asmari AK, Albalawi SM, Athar MT, Khan AQ, Al–Shahrani H, Islam M. Moringa oleifera as an anti–cancer agent against breast and colorectal cancer cell lines. PloS one. 2015 Aug 19;10(8):e0135814.
16. Jung IL, Lee JH, Kang SC. A potential oral anti–cancer drug candidate, Moringa oleifera leaf extract, induces the apoptosis of human hepatocellular carcinoma cells. Oncology letters. 2015 Sep 1;10(3):1597–604.
17. Patel NI, Patel PI, Patel DH, Desai SH, Meshram DH. Phytochemical analysis and antibacterial activity of Moringa oleifera. International Journal of Medicine and Pharmaceutical Sciences. 2014;4(2):27–34.
18. Grela E, Kozowska J, Grabowiecka A. Current methodology of MTT assay in bacteria – A review. Acta histochemica. 2018 May 1;120(4):303–11.
19. Vajrabhaya LO, Korsuwannawong S. Cytotoxicity evaluation of a Thai herb using tetrazolium (MTT) and sulforhodamine B (SRB) assays. Journal of Analytical Science and Technology. 2018 Dec;9(1):1–6.

20. Vergara–Jimenez M, Almatrafi MM, Fernandez ML. Bioactive components in Moringa oleifera leaves protect against chronic disease. Antioxidants. 2017 Dec;6(4):91.

21. Einhorn J, Wersäll J. Incidence of oral carcinoma in patients with leukoplakia of the oral mucosa. Cancer. 1967 Dec;20(12):2189–93.

22. Jr SS, Gorsky M, Ms FL. Oral leukoplakia and malignant transformation. A followup study of 257 patients. Cancer. 1984 Feb 1;53(3):563–8.

23. Schepman KP, Van der Meij EH, Smeele LE, Van der Waal I. Malignant transformation of oral leukoplakia: a follow–up study of a hospital–based population of 166 patients with oral leukoplakia from The Netherlands. Oral oncology. 1998 Jul 1;34(4):270–5.

24. Amagasa T, Yamashiro M, Ishikawa H. Oral leukoplakia related to malignant transformation. Oral Science International. 2006 Nov 1;3(2):45–55.

25. Khor KZ, Lim V, Moses EJ, Abdul Samad N. The in vitro and in vivo anti–cancer properties of Moringa oleifera. Evidence–Based Complementary and Alternative Medicine. 2018 Jan 1;2018:2–14.

26. Parvathy MV, Umamaheshwari A. Cytotoxic effect of Moringa oleifera leaf extracts on human multiple myeloma cell lines. Trends in Medical Research. 2007;2(1):44–50.

27. Nair S, Varalakshmi KN. Anti–cancer, cytotoxic potential of Moringa oleifera extracts on HeLa cell line. J Nat Pharm. 2011 Jul 1;2(3):138–42.

28. Salehi B, Lopez–Jornet P, Pons–Fuster López E, Calina D, Sharifi–Rad M, Ramírez–Alarcón K, Forman K, Fernández M, Martorell M, Setzer WN, Martins N. Plant–derived bioactives in oral mucosal lesions: A key emphasis to curcumin, lycopene, chamomile, aloe vera, green tea and coffee properties. Biomolecules. 2019 Mar;9(3):106.