Section A-Research paper

Formulation and evaluation of buccal film containing leaf extract of *Mangifera indica* against an oral traumatic ulcer in rats

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ABSTRACT

The present study was aimed to formulate and evaluate traumatic ulcer in the cheek mucosa of rats using methanol extract of *Mangifera indica* (MEMI) buccal filim. *M.indica* leaves were extracted with methanol and were formulated by using the water soluble ingredients (HPMC, Gelatin, Chitosan, SaCMC, PVP, PVA, and Pectin). MEMI containing F6 was found to an optimized formulation and was subjected to preliminary evaluation. A total of 24 adult male rats grouped as four, six in each Group 1- ulcer control, Group 2- Standard (Triamcinolone 0.1% ointment), Group 3- received 200mg/kg of MEMI buccal film, Group 4- received 400mg/kg of MEMl buccal film for 10 days. Ulceration of the left cheek mucosa was provoked by abrasion using scalpel blade. Body weight and their ulcers were measured with a 0.5-mm precision digital paquimeter. At the end of the study, check mucosa containing the induced ulcer was collected and given for histopathological study. Optimized formulation (F6) showed the maximum release within 6 hrs. The ulcerated area, body weight and percentage of wound contraction were significantly ($p<0.001$) normalized as that of normal rats. The proposed cheek mucosa ulcer model in rats can be considered an efficient process that offers reliable, reproducibility, and a low cost. Anti-ulcer herbal buccal film thus prepared by simple, low cost, nontoxic and eco-friendly method using the methanol leaf extract of *M. indica.* So it proved that this leaf extract can be further developed as alternative for currently used antibacterial and antiulcer agent.

Key Words: *Mangifera indica*, Buccal Film, *Invitro* dissolution study, zone of inhibition, Anti-Ulcer Activity

INTRODUCTION

Oral traumatic ulcers are relatively common and typically the result of mechanical trauma. Normal locations for traumatic ulcers include non-keratinized surfaces such the cheek mucosa, tongue's edge, lips, gingiva, and hard and soft palates. Other types of irritation, such as brushing, which produces linear erosion along the free gingival edge and is occasionally linked to areas of hyperkeratosis, can result in lesions in the cheek mucosa and gums. These ulcers are often ovoid in shape, painful, and localised to the damaged area. They may appear as a large erythematous border encircling a white-yellow necrotic centre. They may appear as a large erythematous border encircling a white-yellow necrotic centre. They typically have a diameter of 1 cm and develop towards spontaneous healing without leaving a scar over a period of 10 to 14 days. It is also well recognised that a wide range of illnesses, including auto-immune disorders, malignant neoplasias, infectious processes, nutritional deficiencies, allergic reactions, traumatisms, and iatrogenic causes, frequently present as ulcers. The progression of an ulcer's clinical profile can be divided into four stages of development. The first stage is represented by the symptomatic aspect, which is characterised by discomfort, mucosal roughness, and pruriginous sensation in the first 24 hours. Pre-ulcerative stage two includes erythema, a localised macula with discrete elevation and hard consistency, as well as a superficial circular membrane, an erythematous halo, and pain. The third stage is the ulcerative stage, which occurs between days 1 and 3 and is characterised by a yellowish and necrotic membrane. The ulcer floor develops a whitish-yellow fibrinous exudate, and the erythematous halo then persists, giving the lesion a crateriform appearance. The ulcer is at its most advanced level at this point, and the pain is reduced to a simple annoyance. The stage of repair occurs naturally[1].

Mangoes belong to genus Mangifera which consists of about 30 species. *Mangifera indica* trees are flowering plant family Anacardiaceae. According to ayurveda, varied medicinal properties are attributed to different parts of mango tree. Various parts of plant are used as a dentrifrice, antiseptic, astringent, diaphoretic, stomachic, vermifuge, tonic, laxative and diuretic and to treat diarrhea, dysentery, anaemia, asthma, bronchitis, cough, hypertension, insomnia, rheumatism, toothache, leucorrhoea, haemorrhage and piles. All parts are used to treat abscesses, broken horn, rabid dog or jackal bite, tumour, snakebite, stings, datura poisoning, heat stroke, miscarriage, anthrax, blisters, wounds in the mouth, tympanitis, colic, diarrhea, glossitis, indigestion, bacillosis, bloody dysentery, liver disorders, excessive urination, tetanus and asthma[(2)].

The wound healing potential of its bark in adult diabetic rats was investigated [(3)]. Similarly ethosomal gel applied topically possessed wound healing activity [(4)]. Aqueous leaf extract of *M. indica* has wound healing potentials and was evaluated in Wistar rats [(5)]. The antiulcer potential of the petroleum ether and ethanol extracts of leaves of *M. indica* against aspirin-induced gastric ulcer assay. They found out 13 phytoconstituent from ethanol and 8 constituents from petroleum ether extracts by HPTLC finger print analysis [(6)].

Topical application of a new paste formulation (Orabase paste containing hyaluronic acid, rosemary extract, and metronidazole) had a stronger healing effect on mouth ulcer. It resolved inflammation during specific phases of mucosal healing and significantly advanced wound healing at different stages. It also afforded a better healing response in terms of reduced wound contraction [(7)]. *In vivo* oral wound healing models and emerging regenerative therapies published during the past twenty years. Studies were evaluated by injury models, therapy interventions, and outcome measures. The success of therapeutic approaches was assessed, and research outcomes were compared based on current hallmarks of oral wound healing. Various studies were scientifically reported on the leaves, but no substantial works done for the formulations. Therefore an attempt was done to screen the anti-ulcer activity of formulated buccal film of matrix containing methanol extract of *M. indica.*

MATERIALS AND METHODS

Plant collection, identification and Authentication

The leaves of *M. indica* were collected in the month of October - November 2020 from Alappuzha district, Kerala, India and was authenticated by Dr. C. Dileep, Associate Professor, Department of Post Graduate Studies and Research in Botany, Sanatana Dharma College Alappuzha.

Preparation of extract

The collected leaves were cleaned, shade dried and coarsely powdered and stored in an air tight container. It was extracted by continuous hot Soxhlet extraction method. The powdered leaves were defatted with petroleum ether and further extracted with of methanol. The collected extract was named as methanolic extract of *M. indica* (MEMI) were concentrated on rotary evaporator and stored in refrigerator until used.

Preparation of Buccal films

Buccal films are preferably formulated using the solvent casting method, where by the water soluble ingredients are dissolved to form a clear viscous solution and the drug along with other excipients is dissolved in suitable solvent then both the solutions are mixed and stirred and finally casted in to the Petri plate and dried. Water soluble hydrocolloids used to prepare films are HPMC, HPC, SA, NaCMC and Pectin.

Ethical clearance

The research project was submitted before the Institutional animal ethical committee (IAEC) and approved the research protocol No: SJCP/IAEC/2018/2/26.

Pre-formulation study

Infra-red spectroscopy, solubility study, determination of λmax and preparation of calibration curve of extract were conducted.

Fabrication of buccal films

Different formulations (f1 – f7) were tried using various combinations of polymers like pectin, gelatin, chitosan, sodium CMC, HPMC, polyvinyl pyrrolidone, polyvinyl alcohol and propylene glycol to prepare *M. indica* buccal film.

Evaluation of characteristics of the prepared films

The following criteria's were evaluated 1. Physical appearance and surface texture, 2. Weight variation, 3. Mean thickness, 4. Swelling index, 5. Folding endurance, 6.Surface pH, 7. Moisture loss & moisture absorption studies, 8. Tensile strength, 9. Elongation at break, 10. Drug content determination, 11. *In vitro* release study, 12. Determination of order of release of drug from buccal film by graphical method and 13. Determination of bio-adhesive strength of optimized films were conducted for all the seven formulation.

Evaluation of anti-ulcer property [8] of *M. indica* buccal film

A total of 24 adult male rats (250-300g) were selected for this study. They were grouped in to four six in each:

Group1: The ulcerated rats were treated with buccal film without extract.

Group2: The ulcerated rats were treated with triamcinolone (0.1%) ointment.

Group3: The ulcerated rats were treated with 200 mg /kg of *M. indica* formulation (F6).

Group4: The ulcerated rats were treated with 400 mg/kg of *M. indica* formulation (F6).

The rats were kept in plastic cages and allowed to access to water and commercial food. The animals were anesthetized with 10% chloral hydrate (30ml/0.1g) via intraperitoneal injection, and the surgical process was initiated the eyelid reflex and a constant breathing rhythm

ceased. The animals were observed during the post-operatory period until the total recovery of reflexes.

After anaesthetic stage was reached, each animal was put on a surgical table in dorsal decubitus and immobilized with adhesive tape. The mucosa was sterilized by using a swab covered in 0.12% chlorhexidine digluconate. The ulceration was made on the left cheek mucosa by abrasion using a nº 15 scalpel blade.

To standardize the lesion area, an 8-mm-diameter demarcator was used. The operation technique was standardized for all the animals and was performed by the same examiner. The animals were observed daily for 10 days. They were weighed and their ulcers were measured with a 0.5-mm precision digital paquimeter (D= biggest diameter and d= smaller diameter) calculate the area (A= π. r. R) (Table 2). The animals were gradually sacrificed: each group was sacrificed in intervals of 24 hours through the end of the 10-day study period. After the animals were sacrificed, a section of the cheek mucosa containing the induced ulcer was collected.

The collected cheek mucosa fragments were identified and immersed in 10% formolin for 24 hours. After fixation, the specimens were macroscopically analysed. The histological characteristics of the ulcer along with their corresponding cicatrization phases were described. 0. No ulcer / remodeled connective tissue, 1. No ulcer / fibrosis + slight chronic inflammation, 2. With ulcer / fibrosis + moderate chronic inflammation, 3. With ulcer / chronic inflammation process (granulation tissue), 4. With ulcer / acute process (dilated vessels, mixed inflammatory infiltrate with neutrophilis).

Statistical analysis

All data were analyzed by using the comparison of ulcerated areas among groups was analyzed using one way ANOVA followed by the Student-Newman-Keuls test. The results were expressed as± SEM. A value of $p<0.001$ was considered a statistically significant response in all cases.

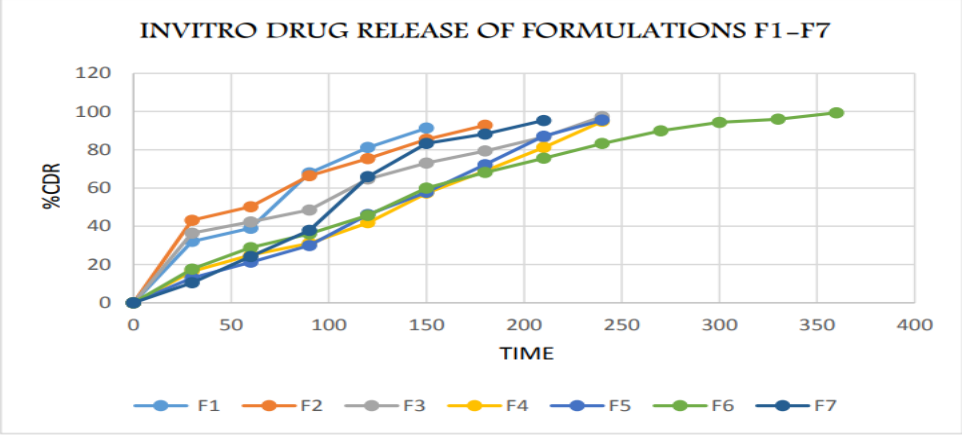
RESULTS

Different formulations (f1 – f7) were tried using various combinations of polymers and their ratios were listed in Table 1

Table 1 Formulation chart

Ingredients	Formulations						
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**
MEMI	200mg	200mg	200mg	200mg	200mg	200mg	200mg
Pectin (%w/v)	4	4					
Gelatin (%w/v)			3	3			
Chitosan (%w/v)				2	2	2	2
NaCMC(%w/v)	1.5		1.5				
HPMC(%w/v)		1.5		1.5		1.5	1.5
PVP (%w/v)					1	1	
PVA(%w/v)					1		1
PG(%v/v)	5	5	5	5	5	5	5
Peppermint flavour	1.5	1.5	1.5	1.5	1.5	1.5	1.5

The extract with F6 was found to the optimized formulation among the total seven formulations. It was finalized based on the % drug release (Figure 1).



The effect of MEMI buccal film on body weight and ulcer curative were illustrated in Table 2. The body weight was not reduced from the starting to end of the treatment schedule. It indicated that all the animals were healthy and their food intake was not affected by the injury caused by the study protocol. Similarly, the diameter of the wound was reduced drastically in a dose dependent manner. A significant ($p<0.001$) reduction in wound surface was found with high dose of extract containing buccal film.

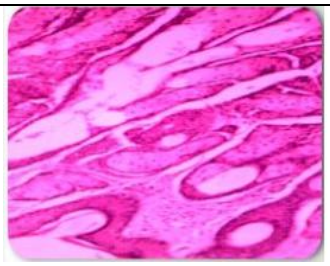
Table 2: Effect of MEMI on traumatic mouth ulcer and body weight

Parameters	Treatment groups			
	Group 1	**Group 2**	**Group 3**	**Group 4**
Body weight (initial)	265±2.3	255±3.4	260±2.7	265±1.7
Body weight (Final)	270±3.2	260±1.6	280±3.8	275±1.9
	Wound surface area (mm)			
Day 1	49.5±0.6	48.8±0.6	49.6±0.6	48.83±0.6
Day 2	49±0.6	45±0.5	38±0.6[a]	41±0.6[a]
Day 3	49±0.4	38±0.4	36±0.6[a]	34.8±0.5[a]
Day 4	48±0.6	37±0.6	33±0.5[a]	28±0.6[a]
Day 5	48±0.6	35±0.8	31±0.2[a]	22±0.6[a]
Day 6	40.6±0.4	29.8±0.1	28±0.6[a]	14±0.6[a]
Day 7	37±0.6	24±0.6	23±0.6[a]	11±0.9[a]
Day 8	32.6±0.4	21±0.6	20±0.6[a]	8.3±0.4[a]
Day 9	30.2±0.6	18±0.6	14.5±0.7[a]	4.2±0.5[a]
Day 10	29±0.6	10±0.7	8.3±0.4[a]	1.8±0.3[a]

All the data were expressed as± SEM analysed by using student NEWMAN- KEULS were a***p<0.001.

Histopathological analysis

The histological profile was composed by a lymphoplasmocytic inflammatory infiltrate, proliferation of fibroblasts and some neoformed capillaries. Images of histopathological analysis were presented in Figure 2.



Group 1
Section shows tissues lined by squamous epithelium. One are shows lobules of sebaceous gland. There are scattered inflammatory cells. Normal mucous glands are also seen. Deeper portion shows striated muscle tissue, which appear normal.

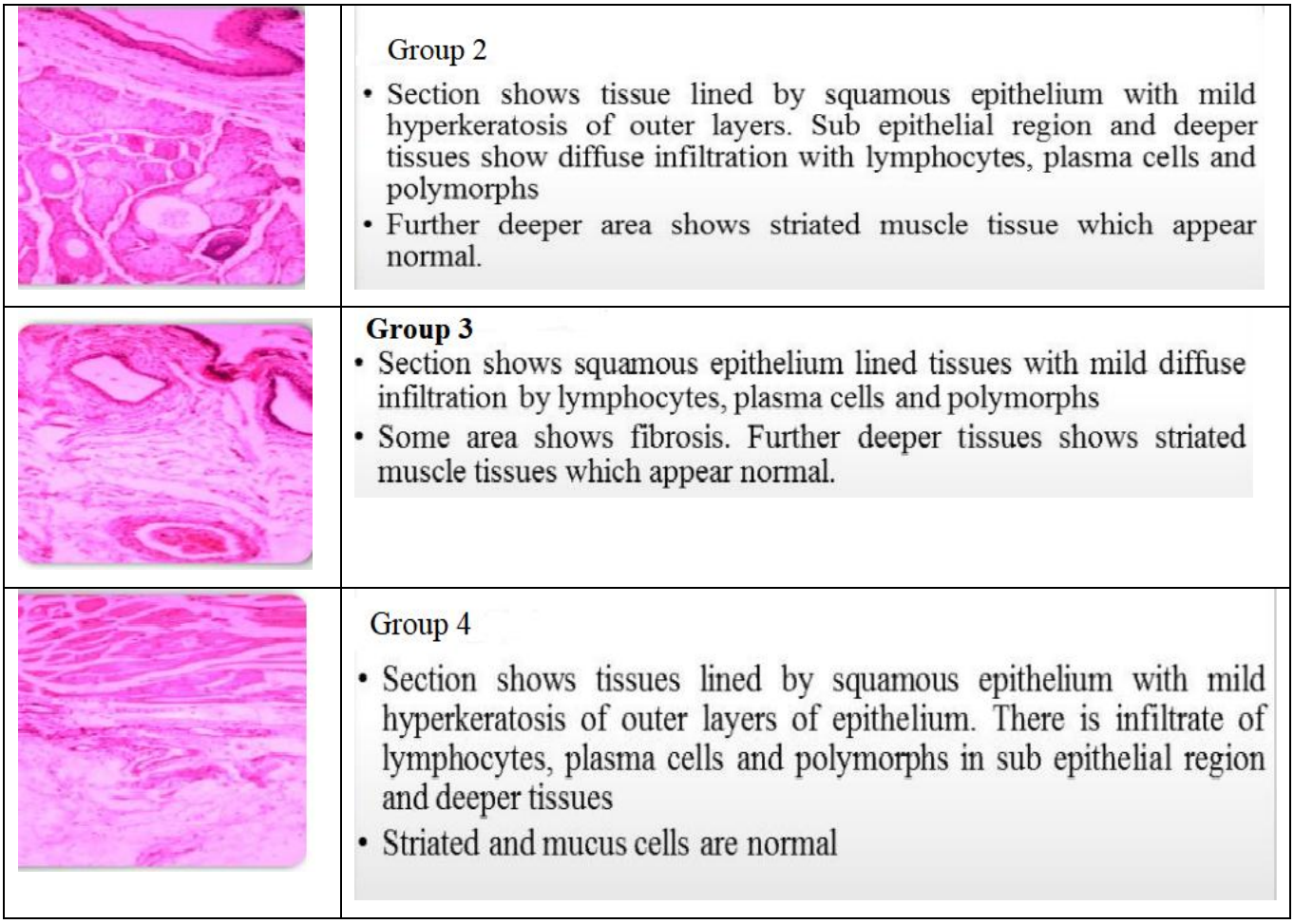


Figure 2 : Histopathaological analysis of buccal mucosa

DISCUSSION

Oral ulcer or mucositis can be very painful and significantly affect nutritional intake, mouth care, and quality of life, especially patients receiving high-dose chemotherapy prior to hematopoietic cell transplantation, oral mucositis is the single most debilitating complication of transplantation. In patients receiving chemotherapy for solid tumors or lymphoma, the rate of infection during cycles with mucositis was more than twice that during cycles without mucositis and was directly proportional to the severity of mucositis[9]. Similarly, traumatic ulcers are the most common inflammatory ulcerative conditions in the oral cavity, and the cheek mucosa is the most traumatized site. Such traumas are caused by poorly adapted prosthesis or occlusal disharmony, tooth crowns or fractured restorations and even by accidental bites while chewing or habits. Although it is the focus of intense research, there is no consensus in the literature about a definite therapeutic protocol, and many times inadequate treatments, such as the use of synthetic

substances were used for the treatment of such pathological condition[10]. Treatments, such as the use of synthetic substances, phytotherapy and herbs are used by some of the population to heal the wound [11]. Therefore, there is a need to establish experimental models able to test a natural substance in the form of pharmaceutical formulation was designed and evaluated with proper animal model.

Matrix formulations are controlled release (CR) drug delivery system which delivers the drug locally or systemically at a predetermined rate for a specified period of time. The goal of such systems is to provide desirable delivery profiles that can achieve therapeutic plasma levels by reduction in dosage schedule, reduction in side effects, better patient compliance. In this, the drug release is dependent on polymer properties, thus the application of these properties can produce well characterised and reproducible dosage forms[12]. The desired polymer containing matrix was sorted by physical, mechanical and in-vitro drug release procedures. Finally F6 was identified as an optimised formulation with two different doses of MEMI.

In India, the ethnomedicinal uses of leaves of *M. indica* indicated for diarrhoea, ulcers, diabetes, dysentery, cough, gall bladder and kidney diseases, hemorrhages, wounds, diseases in throat and hiccups, burns, and scalds. Several studies have proven the pharmacological potential of different parts of mango trees such as leaves, bark, fruit peel and flesh, roots, and flowers as anticancer, anti-inflammatory, antidiabetic, antioxidant, antibacterial, antifungal, anthelmintic, gastroprotective, hepatoprotective, immunomodulatory, antiplasmodial, and antihyperlipemic [13]. The present study focused on the healing of oral ulcer in rat model. Formulation with MEMI showed anti-ulcer property as the protective effect on oral mucosa. The core antibacterial and antioxidant properties may accelerate the complete healing of ulcer in mouth.

CONCLUSION

The cicatrization process of the mucosa in rats is similar to cicatrization in human beings. The proposed cheek mucosaulcer model in rats can be considered an efficient process that offers reliable, reproducibility, and a low cost. These results support positive evidence that MEMI can be used as an effective and safe medical tool in the treatment of oral ulcer. So it proved that this leaf extract can be further developed as alternative for currently used antibacterial and antiulcer agent.

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