



Fabrication and Evaluation of Herbal Buccal Patch Containing Leaf Extract of *Anacardium occidentale*

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ABSTRACT

The goal of the present research was to develop and characterize traumatic ulcers in the rat cheek mucosa using a buccal methanol extract of *Anacardium occidentale* (MEAO). *A. occidentale* leaves were prepared implementing the water-soluble components (HPMC, Gelatin, Chitosan, NaCMC, PVP, PVA, and Pectin) after being extracted with methanol. MEAO that contains F6 was discovered to be an optimal formulation and has undergone through a preliminary assessment. 24 adult male rats were divided into four groups of six each, with group 1 receiving a control treatment for ulcers, group 2 receiving standard treatment (Amoxicillin), group 3 receiving 200 mg/kg of MEAO buccal film, and group 4 receiving 400 mg/kg of MEAO buccal film for 10 days. Scalpel blade abrasion caused mucosal ulceration on the left cheek. A 0.5-mm preciseness digital paquimeter was used to evaluate body weight and the severity of their ulcers. Check mucosa containing the induced ulcer was taken and imposed for histological analysis at the final stage of the study. Maximum release was seen within 6 hours of the optimized formulation (F6). Body weight, the dimension of the ulcer, and the proportion of wound contraction were all considerably ($p < 0.001$) standardized to those of rats under healthy conditions. The suggested rat cheek mucosa ulcer model can be viewed as an effective procedure that provides consistency, repeatability, and affordability. *Anacardium occidentale* leaf extract in methanol was used to create an anti-ulcer herbal buccal film in an easy, affordable, safe, and environmentally responsible manner. So it was established that this leaf extract may be expanded upon as an equivalent for the antibacterial and antiulcer medications already in use.

Key Words: *Anacardium occidentale*, Buccal Film, *In vitro* dissolution study, Zone of inhibition, Anti-Ulcer Activity

Introduction

The buccal route is a desirable method of administration for systemic drug delivery because it misses hepatic first pass metabolism, gives excellent bioavailability, and provides direct access to the circulation throughout the body through the internal jugular vein (Jacob S et al., 2021). Buccalbioadhesive films offer considerable benefits over conventional dose forms for the treatment of numerous diseases due to their ability to release topical medications in the mouth cavity at a gradual and controlled rate. It is essential to have a complete comprehend of the physiological environment in which buccally delivered dosage forms exist as well as the different variables that may have an impact on physiological characteristics before developing such approaches (Rohani Shirvan A et al., 2021). The bulk of *in vitro* dissolving studies of buccal dosage forms do not take into account the numerous features of biological fluids like saliva because they are not fully understood.

Anacardium occidentale, a versatile tropical tree that grows to a height of around 10-15 meters (m), belongs to the Anacardiaceae family. They naturally grow on rather dry soil, but when cultivated, they do well in tropical rain forests. Numerous resources and goods can be made by cashew trees (Salehi B et al., 2019). In Nigeria, medicinal uses for the leaf, bark, and apple are being investigated (Borges J. 2021). Extracts from the leaves, stems, and bark are widely used to treat diarrhea, dysentery, and colonic pain. Additionally, it is said to have anti-inflammatory, anti-diabetic, and anti-ulcerogenic characteristics. The presence of several phytochemical components, including phenolic, triterpenoids, carbohydrates, xanthoprotein, and flavonoids, was discovered in the extracts made from methanol of cashew nuts (Gaitan-Jimenez S. Y et al., 2022).

A mouth ulcer healed more quickly after being topically applied with a new paste formulation (Orabase paste with hyaluronic acid, rosemary extract, and metronidazole). At specific locations in the mucosal healing process, it reduced inflammation and greatly sped up various stages of wound healing (Silva et al., 2023). In terms of a superior healing response, it also allowed for less wound contraction. Novel restorative therapeutics and *in vivo* oral wound healing models published throughout the past 20 years (Rao S et al., 2011). Injury models, therapeutic strategies, and outcome metrics were used to assess studies. Based on existing indicators of oral wound healing, the efficacy of therapeutic strategies was evaluated, and study results were compared. Numerous scientific studies on the leaves have been published, but no significant formulation research has been done. Therefore, a test was conducted to evaluate the anti-ulcer properties of a matrix-based buccal film containing methanol extract of *Anacardium occidentale*.

Materials and methods

Plant collection, Identification and Authentication

The leaves of *Anacardium occidentale* were collected in the month of October - November 2022 from Alappuzh adistrict, Kerala, India and were authenticated by Dr. Sreeja Krishnan, Assistant Professor, Department of Post Graduate Studies and Research in Botany, Sree Narayana College, Cherthala.

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 (Karami Z et al., 2015). The collected extract was named as methanolic extract of *A. occidentale* (MEAO) 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, Poly vinyl Pyrrolidone, Poly vinyl Alcohol, Chitosan , Gelatin, NaCMC, PropyleneGlycol and Pectin (Semalty A et al., 2010).

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 λ_{max} and preparation of calibration curve of extract were conducted (Senta-Loys Z et al., 2017).

Fabrication of buccal films

Different formulations (F1 – F7) were tried using various combinations of polymers like pectin, gelatin, chitosan, sodium CMC, HPMC, polyvinyl pyrrolidone, Propylene Glycol and polyvinyl alcohol to prepare *Anacardium occidentale* buccal film (Table 1).

Ingredients	Formulations						
	F1	F2	F3	F4	F5	F6	F7
MEAO	200 mg	200 mg	200 mg	200 mg	200 mg	200 mg	200 mg
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

Table 1. Formulation chart

Evaluation of characteristics of the prepared films

1. Physical appearance and surface texture, 2. Weight variation, 3. Mean thickness, 4. Swelling index (Preis M et al., 2014). 5. Folding endurance, 6.Surface pH, 7.Moisture loss & moisture absorption studies, 8.Tensile strength, 9.Elongation at break, 10.Drug content determination (Zaman M et al., 2018). 11. *In vitro* release study, 12.Determination of order of release of drug from buccal film by graphical method and 13.Determination of bioadhesive strength of optimized films were conducted for all the seven formulation (Kottke D et al., 2020).

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).

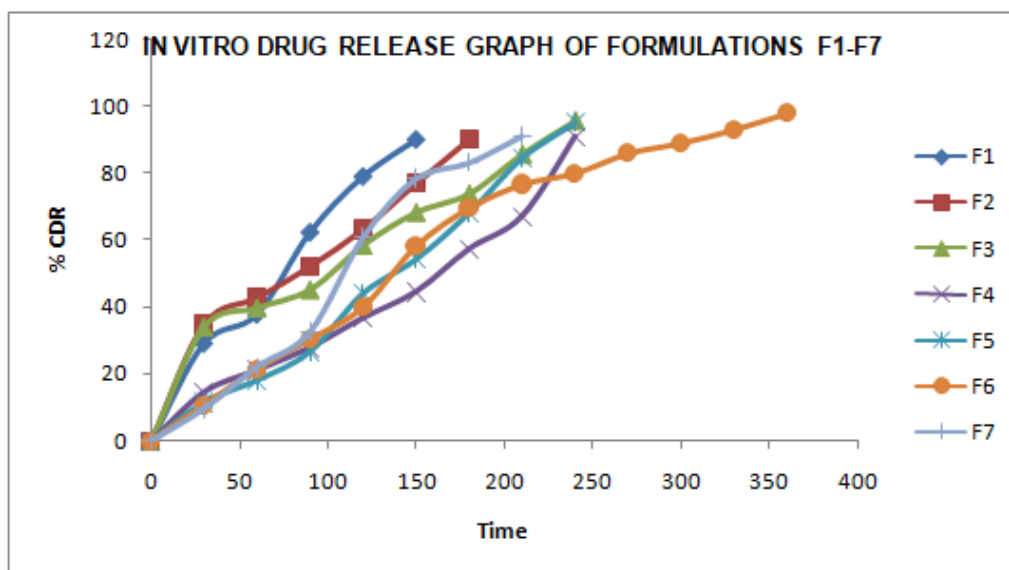


Figure 1:- Comparison of *In vitro* drug release graph of formulations F1-F7

Evaluation of anti-ulcer property of *Anacardium occidentale* 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 Amoxicillin.

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

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

The rats had been kept in plastic cages with access to water and bought from a store food. Intraperitoneal injection of 10% chloral hydrate (30ml/0.1g) was used to anesthetize the animals. When the surgical procedure began, the eyelid reflex and a regular breathing pattern had stopped. From the time after surgery until their reflexes had fully recovered, the animals were watched.

Each animal was placed on a surgical table in dorsal decubitus after reaching an esthetic stage, and was then taped immobile. A swab dipped in 0.12% chlorhexidine digluconate was used to sanitize the mucosa. An n° 15 scalpel blade was used to abrasion the mucosa of the left cheek to create the ulcers.

Using a demarcator with an 8-mm diameter, the lesion area was uniformed. The same examiner performed the operation on all the animals using the same standardized procedure. For ten days, the animals were watched every day. In order to compute the area ($A = \pi \cdot r \cdot R$), their weights and ulcers were measured using a 0.5-mm accuracy digital paquimeter ($D =$ highest diameter and $d =$ smallest diameter) (Table 2). Each group of animals was gradually slaughtered at intervals of 24 hours until the ending of the 10-day study period. A piece of the cheek mucosa containing the created lesion was removed after the animals were slaughtered.

After being identified, the recovered cheek mucosa fragments were placed in 10% formol for 24 hours. The specimens were macroscopically analyzed after informol fixation. The histological features of the ulcer and the stages of cicatrization that correlate to them were characterized.

0. No ulcer or connective tissue remodeling. 1. No ulcer / fibrosis + modest chronic inflammation; 2. With ulcer / fibrosis + moderate chronic inflammation; 3. With ulcer / chronic inflammation process (granulation tissue); 4. With ulcer / acute process (dilated arteries, mixed inflammatory infiltrate with neutrophils).

Results

Days	Treatment groups (Area)			
	Group 1	Group 2	Group 3	Group 4
Body weight (initial)	264±2.4	254±3.6	259±2.8	264±1.6
Day 1	48.5±0.7	47.8±0.8	48.6±0.8	47.86±0.4
Day 2	48±0.5	44±0.6	37±0.5 ^a	40±0.8 ^a
Day 3	48±0.4	37±0.5	35±0.6 ^a	33.7±0.6 ^a
Day 4	47±0.5	36±0.7	32±0.4 ^a	27±0.8 ^a

Day 5	47±0.3	34±0.7	30±0.4 ^a	21±0.8 ^a
Day 6	39.6±0.3	28.7±0.2	27±0.5 ^a	13±0.7 ^a
Day 7	36±0.7	23±0.5	22±0.5 ^a	10±0.8 ^a
Day 8	31.6±0.5	20±0.6	19±0.6 ^a	7.4±0.6 ^a
Day 9	29.2±0.7	17±0.7	13.6±0.6 ^a	3.2±0.8 ^a
Day 10	28±0.4	9±0.8	7.4±0.3 ^a	1.2±0.2 ^a
Body weight (Final)	269±3.3	259±1.8	279±3.6	274±1.8

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

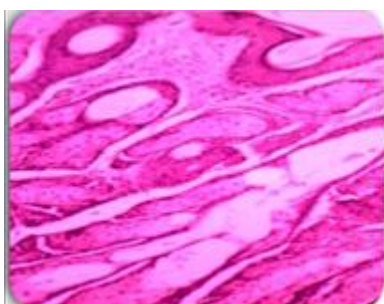
Table 2: Effect of MEAO on traumatic mouth ulcer

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 below.

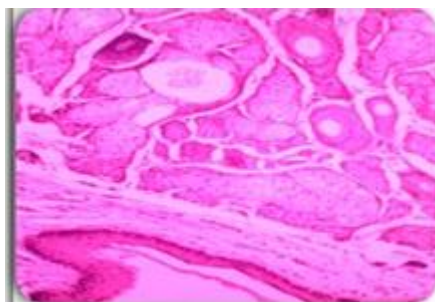
Histopathological analysis of buccal mucosa

Group 1



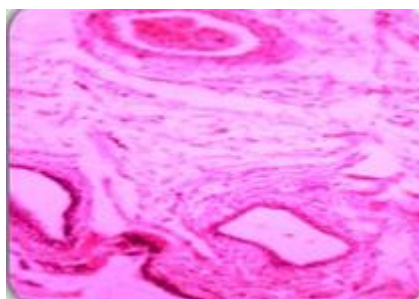
This section demonstrates tissues with squamous epithelium linings. One of them shows the lobules of the sebaceous gland. Inflammatory cells are present everywhere. Additionally, normal mucous glands are visible. The deeper area displays what appears to be typical striated muscle tissue.

Group 2



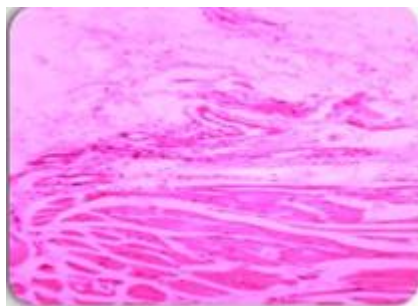
This section shows segment with mild outer-layer hyperkeratosis and squamous epithelium-lined tissue. Lymphocytes, plasma cells, and polymorphs are diffusely infiltrating deeper tissues and the sub epithelial region. A deeper region displays what seems to be normal striated muscle tissue.

Group 3



Portion with diffuse lymphocyte, plasma cell, and polymorph infiltration in tissues bordered by squamous epithelium. Some of the tissue has fibrosis. In deeper tissues, striated muscle tissues look normal.

Group 4



This section shows tissues that are bordered by squamous epithelium and have only mild hyperkeratosis on the outer layers of the epithelium. Lymphocytes, plasma cells, and polymorphs are present in the subepithelial region and deeper tissues. Striations and mucus cells are typical.

Statistical analysis

All the data were by one way ANOVA and the results were expressed as \pm SEM analysed by using student NEWMAN- KEULS method (n= 6).

Discussion

Primarily in patients receiving high-dose chemotherapy prior to hematopoietic cell transplantation, oral mucositis is the single most incapacitating consequence of transplantation. It can be extremely painful and have a substantial negative impact on nutritional intake, oral hygiene, and quality of life. The rate of infection during cycles with mucositis was more than twice as high as during cycles without mucositis in patients undergoing chemotherapy for solid tumors or lymphoma, and it was inversely correlated with the degree of mucositis. A certain percentage of the population make use of treatments such the usage of synthesized substances, phytotherapy, and herbs to heal the wound. As a result, it is important to develop experimental models that can test a natural ingredient that has been formulated into pharmaceutical and evaluated using compatible animal models.

Controlled release (CR) drug delivery systems distribute the drug locally or systemically at a predetermined rate for a predetermined amount of time using matrix formulations. The purpose of these systems is to offer desirable delivery profiles that can attain therapeutic plasma levels through a reduction in dosing schedule, a decrease in side effects, and improved patient compliance. The application of these qualities can result in well-characterized and repeatable dosage forms because the drug release in this situation is based on polymer properties. By using physical, mechanical, and *in-vitro* drug release techniques, the desired polymer-containing matrix was separated. The optimized formulation F6 with two separate MEAO dosages was finally identified.

The ethnomedical uses of *Anacardium occidentale* leaves in India included the treatment of hemorrhages, wounds, disorders of the throat and hiccups, burns, and scalds, as well as diarrhea, ulcers, diabetes, dysentery, cough, gall bladder and kidney ailments. Different cashew tree parts, including the leaves, bark, fruit peel and flesh, roots, and flowers, have been shown to have pharmacological potential as anticancer, anti-inflammatory, antidiabetic, antioxidant, antibacterial, antifungal, anthelmintic, gastroprotective, hepatoprotective, immunomodulatory, antiplasmodial, and antihyperlipidemic agents. The current study concentrated on the rat model's ability to cure mouth ulcers. A formulation containing MEAO demonstrated anti-ulcer properties as the oral mucosa's protective effect. The primary antibacterial and antioxidant characteristics can promote the full recovery of a mouth ulcer.

Conclusion

Similar to human cicatrization, the mucosal cicatrization process takes place in rats. The suggested rat cheek mucosa ulcer model can be viewed as an effective procedure that is reliable, reproducible, and affordable. These outcomes provide compelling proof that MEAO can be utilized as a safe and successful medical technique to treat mouth ulcers. Thus, it was demonstrated that this leaf extract might be further developed as a substitute for the antibacterial and antiulcer agents that are now in use.

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