Research Article

EFFECTIVE MUCOADHESIVE BUCCAL PATCHES: WOUND HEALING ACTIVITY OF CURCUMIN & CENTELLA ASIATICA EXTRACT COMPARED TO rhEGF

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ABSTRACT

Curcumin, Asiaticoside & madecassoside are the active constituent extracts from Curcuma longa and *Centella asiatica* respectively were known for the remedy as healing agent. The invention is to produce muco-adhesive buccal patches, which heals the lesion/ injury with replacing patches. Extraction of *Curcumin* was done with soxhlet apparatus and extraction of *Asiaticoside & madecassoside* was performed with Subcritical water extraction. Muco-adhesive buccal patches were made with the aid of polyvinylpyrrolidone (PVP K-30) with the dimension of 1.5×1.5 cm and same also for rhEGF. The comparative study has been carried out between the plant extracts and rhEGF powder as mucoadhesive buccal patches in 5 human volunteers .The average healing time of plant extracts and rhEGF are 36.6 ± 0.46 hrs & 52.16 ± 2.82 hrs, and SEM of the same is 0.232 & 1.262 respectively. The t-test, degree of freedom (df) was calculated as 12.0807 and 8 respectively. Thus, the two-tailed p-value was calculated as 0.0001 which is consider to be extremely statistically significant, also standard error of difference was 1.283. The difference in time interval of healing lesion is not so much and we can accept the plant extract , combination of *curcumin* and *Centella Asiatica* as a mucoadhesive buccal patches with satisfactory results.

Keywords: Muco-adhesive buccal patches , rhEGF, *Curcumin*, *Asiaticoside & madecassoside*

INTRODUCTION

Curcuma longa rhizome extracts were evaluated for antibacterial activity against pathogenic strains of Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*) bacteria. Essential oil was found to be most active and its activity was compared to standard antibiotics gentamycin, ampicillin, doxycycline and erythromycin in these strains. Only the clinical isolate of *S. aureus* showed more sensitivity towards essential oil fraction than the standard strain. The use of essential oil from turmeric as a potential antiseptic in prevention and treatment of antibacterial infections has been suggested [1].

The wound healing process involves extensive oxidative stress to the system, which generally inhibits tissue remodeling. In the present study, an improvement in the quality of wound healing was attempted by slow delivery of antioxidants like curcumin from collagen, which also acts as a supportive matrix for the regenerative tissue. Curcumin incorporated collagen matrix (CICM) treated groups were compared with control and collagen treated rats. Biochemical parameters and histological analysis revealed that increased wound reduction, enhanced cell proliferation and efficient free radical scavenging in CICM group [2]. Curcumin is an oil-soluble pigment, practically insoluble in water at acidic and neutral pH, and soluble in alkali. Preparations of water-soluble curcumin by incorporation into various surfactant micellar systems (e.g. sodium dodecyl sulfate, cetylpyridinium bromide, gelatine, polysaccharides, polyethylenglycol, cyclodextrins) have been reported (Humphrey, 1980, Tonnesen, 2002) [3].

The report describes the healing of cutaneous wounds in experimental animals grafted with collagen-glycosaminoglycan (GAG) matrix/Silastic (Dow Corning Corp., Midland, MI) bilayers; assesses the feasibility of using collagen-GAG matrix as a vehicle for delivering culture-selected, autogenous fibroblasts to cutaneous wound sites; and evaluates the use of collagen-GAG/Silastic bilayers as mucosal substitutes [4]. A method for evaluating mucoadhesive properties of polymer films that employs a model mucosa and wetting of tissues imitating physiological conditions is proposed. The results show evidence for good mucoadhesive properties of natural biocompatible polymers such as alginate and chitosan [5] .

Bioadhesion can be defined as a phenomenon of interfacial molecular attractive forces amongst the surfaces of the biological substrate and the natural or synthetic polymers, which allows the polymer to adhere to the biological surface for an extended period of time [6-9].

Based on the theories of the adhesion, it can be summarized that the mucoadhesive property of a polymer can be tailored by changing the parameters which has the capacity to alter the interaction among the polymer and the mucosal layer. In this section, attempts will be made to analyze some of the parameters which can tailor the mucoadhesive property of a given polymer.

Polymers usually diffuse into the mucosal layer and thereafter adhere to the layer by forming intermolecular entanglements. With the increase in the molecular weight (MW) of the polymer chain there is an increase in the mucoadhesiveness of a polymer. In general, polymers having MW ≥ 100, 000 have been found to have adequate mucoadhesive property for biomedical applications. A typical example is polyethylene glycol (PEG). PEG of 20,000 MW shows negligible mucoadhesive property while PEG of 200,000 MW exhibits improved mucoadhesiveness and the PEG of 400,000 MW has got excellent mucoadhesiveness.

Similarly, polyoxyethylene of 7,000,000 MW has exhibited excellent mucoadhesive property and could be tried for the development of buccal delivery systems [10]. The evaluation of the potential bioadhesive behaviour of chitosan and thiolated chitosan (chitosan-TBA)-coated poly(isobutyl cyanoacrylates) (PIBCA) nanoparticles. Nanoparticles were obtained by radical emulsion polymerisation with chitosan of different molecular weight and with different proportions of chitosan/chitosan-TBA. Mucoadhesion was *ex vivo* evaluated under static conditions by applying nanoparticle suspensions on rat intestinal mucosal surfaces and evaluating the amount of nanoparticles remaining attached to the mucosa after incubation.

The analysis of the results obtained demonstrated that the presence of either chitosan or thiolated chitosan on the PIBCA nanoparticle surface clearly enhanced the mucoadhesion behaviour thanks to non-covalent interactions (ionic interaction and hydrogen bonds) with mucus chains. Both, the molecular weight of chitosan and the proportion of chitosan-TBA in the formulation influenced the nanoparticle hydrodynamic diameter and hence their transport through the mucus layer. Improved interpenetration ability with the mucus chain during the attachment process was suggested for the chitosan of high molecular weight, enhancing the bioadhesiveness of the system [11]. Epidermal Growth Factor, Human Recombinant (rhEGF) is a 6.2kDa protein that is mitogenic for a variety of

mammalian cell types [12-16]. EGF stimulates the proliferation and differentiation of epithelial cells from skin, the cornea, lung and tracheal tissue and the gastrointestinal tract. EGF also promotes growth and migration of keratinocytes and enhances the proliferation of fibroblasts and embryonic cells. Thus, EGF plays an important role in wound healing and organogenesis.

MATERIALS AND METHOD

Extraction of natural drug

Extraction of Curcumin

The 250 gm rhizome of *Curcuma longa* was dried and powdered. Then it was allowed to stuff into the soxhlet apparatus and extraction was initiated with solvent 90% alcohol of about 250 ml, for about 72 hrs at 45- 50 º C. The extract was decolorized with activated charcoal and then concentrate. Which was dried in room temperature overnight, yielding 6 gm of *curcumin.*

Subcritical water Extraction of *Centella asiatica*

The whole plant were allowed to dry in 40-45 º C temperature and made powder. Deionized water were filled into a glass reservoir and was first purged for 2 h to remove dissolved O2. Teflon filter which is placed at the entrance of the pump purified deionized water, thus, solid particles were not allowed to enter the pump. A water feed pump (Reciprocating pump, Jasco Co., PU-2080, flow rate= 0.1-9.9 mL/min, maximum pressure= 50 MPa) was used to deliver water through system with various flow rates. The powdered drug was induced into the column in the assembly. Deionized water was heated before entering the extraction column (height=12.5 cm, inner diameter (ID) = 0.9 cm, and outer diameter (OD) = 1.3 cm) using a coil preheater that was placed in an oven at 250 º C. The system pressure was controlled by a back pressure regulator (TESCOM Co., pressure= 400 bars). And finally the extracted materials get collected into container Shown in (**figure 1).** The Extraction yield 7 mg/gm asiaticoside and 11 mg/gm madecassoside respectively.
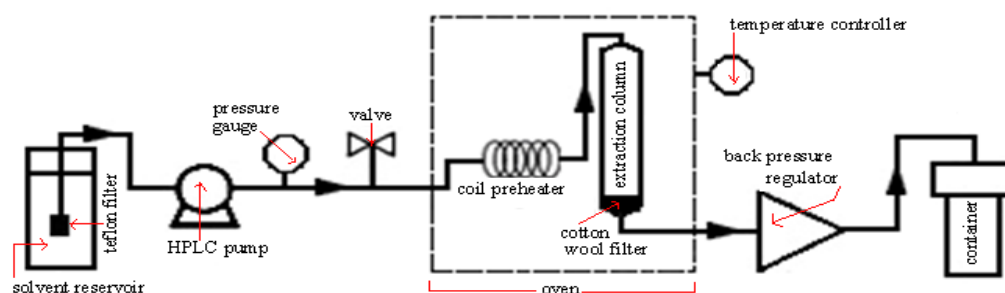

Fig. 1: Subcritical water extraction of asiaticoside and madecassoside from *Centella asiatica*

Preparation of mucoadhesives buccal patches (MABP)

MABP of Curcumin and *Centella asiatica* extracts

One gram of chitosan was dissolved in 100 mL 1.5% (*V/V*) acetic acid under occasional stirring for 48 h. The resulting viscous chitosan solution was filtered through nylon gauze to remove debris and suspended particles. To improve patch performance and release characteristics, a water-soluble hydrophilic additive, polyvinylpyrrolidone (PVP K-30), was added. The drug (Curcumin 10 mg with *Asiaticoside* cum *madecassoside* 10:10 mg) and PVP K-30 were added into the chitosan solution under constant stirring. Propylene glycol (5%, *V/V*) was added into the solution as plasticizer under constant stirring. This viscous solution was left overnight at room temperature to ensure a clear, bubble-free solution. The solution was poured into a glass Petri dish (6 cm diameter) , (figure 2) and allowed to dry at ambient temperature till a flexible film was formed. Dried films were carefully removed, checked for any imperfections or air bubbles and cut into patches of 16 mm in diameter, containing 9 mg drug per patch. The patches were packed in aluminum foil and stored in an airtight glass container to maintain the integrity and elasticity of the patches.

MABP of epidermal growth factor, human, recombinant (rhEGF)

The dried powdered was gifted from "Biotech Equity Research Group" , bank Street Fort, Mumbai and PVP K-30 were added into the chitosan solution under constant stirring. Propylene glycol (5%, *V/V*) was added into the solution as plasticizer under constant stirring. This viscous solution was left overnight at room temperature to ensure a clear, bubble-free solution. The solution was poured into a glass Petri dish (6 cm diameter) and allowed to dry at ambient temperature till a flexible film was formed.
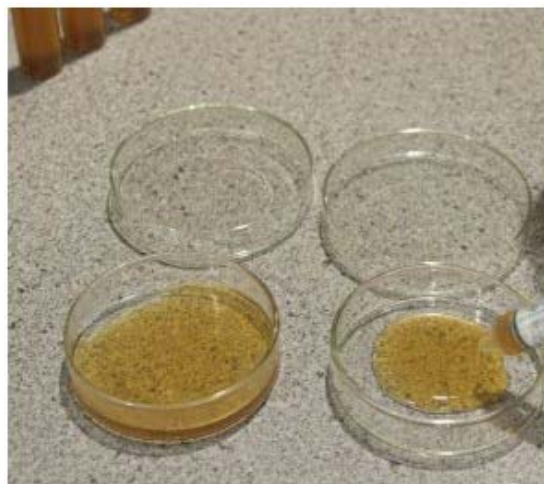

Fig. 2: MABP preparation viscous collection of Curcumin and Centella Asiatica Extracts

Comparative study of rhEGF with the extract formulation of MABP

Out of 10 patients of "Jay arogya hospital", Gwalior, M.P., India, 5 patients were examined for the healing of buccal mucosa lesion through MABP made of extracts and rest 5 patients with rhEGF (MABP) . Thus the mucoadhesives patches of extracts healing shown in (figure 3) and mucoadhesive patches of rhEGF are represented in (Table 1).

Table 1: Healing times according to lesion sizes of both the plant extract and rhEGF of MABPs

No. of Patients	Healing time with plant extract as MABP	Lesion size	Healing time with rhEGF as MABP	Lesion size
1	37 hrs	0.45 mm	49 hrs	0.32 mm
2	36.4 hrs	0.32 mm	56.1 hrs	0.49 mm
3	35.9 hrs	0.3 mm	52 hrs	0.41 mm
4	37.2 hrs	0.5 mm	53.6 hrs	0.39 mm
5	36.8 hrs	0.39 mm	50.1 hrs	0.32 mm
Average	36.6 ± 0.46 hrs		52.16 ± 2.82 hrs	
SEM	0.232		1.262	
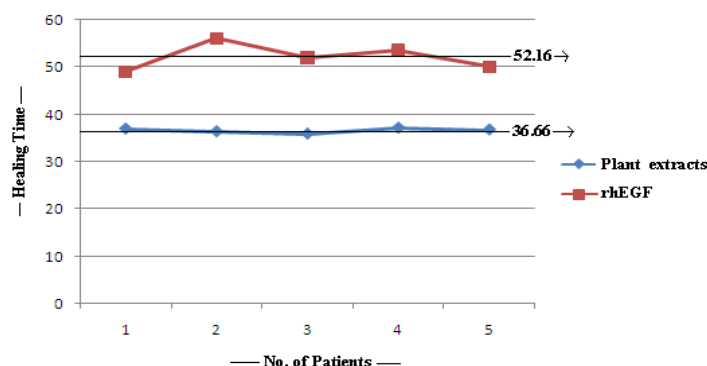
Fig. 3: Graphical representation of healing time vs No. of patients for MABP of plant extract and rhEGF.

The statistical evaluation has been carried out for both the category of formulation (Plant extract of MABP & rhEGF of MABP).

The mucoadhesive buccal patch was design in (Figure 4), The size of this patch is 1.5×1.5 cm which normally covers entire circumference of lesion which basically occurs within this size ranges.

Fig. 4: Mucoadhesive buccal patch with 1.5×1.5 cm dimension.

RESULTS AND DISCUSSION

Out of 10 patients selected for the entire study of healing activity, 5 volunteers are selected for the plant extract study and 5 volunteers for the rhEGF study. It has been noticed that the range of lesions are from 0.3 to 0.5 mm only. The healing time of MABP of plant extract for 5 patients are 37, 36.4, 35.9, 37.2, 36.8 hrs and the healing time of MABP of rhEGF of 5 patients are 49, 56.1, 52, 53.6, 50.1 hrs and thus the average cum standard deviation healing time acquired was 36.6 ± 0.46 and 52.16 ± 2.82 hrs respectively of MABP. So, thus the SEM estimated was 0.232 of plant extract and 1.262 of rhEGF of MABP. Shown in table 1. The t-test, degree of freedom (df) was calculated as 12.0807 and 8 respectively. Thus, the two-tailed p-value was calculated as 0.0001 which is consider to be extremely statistically significant, also standard error of difference was 1.283. On its application of MABPs to the lesions or scar injury of buccal mucosa the healing takes place on consecutive replacement of patches after 6 hrs time interval. The result were represented by (figure 5).
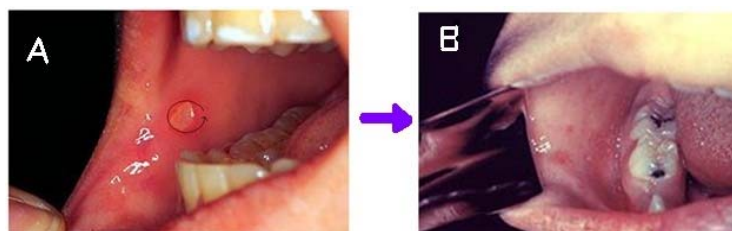
Fig. 5: Its represents the healing of patient from A to B image after application of MABP

As the lesion in buccal mucosa occurs due to the accidental bits of teeth or mucosa eruption due to hot food matters/pungent chemical reaction of food matters. No, such remedial measures of local application were there till now. But, the patches of these plant extracts show remarkable results on replacement in every 6 hrs of interval.

CONCLUSION

On the basis of experiments, results and discussion the P- value shows the statistical valuation of 0.0001 which is extremely statistically significant. Thus, its stands the difference in time interval of healing lesion is not so much and we can accept the plant

extract , combination of curcumin and Centella Asiatica as a mucoadhesive buccal patches with satisfactory results.

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