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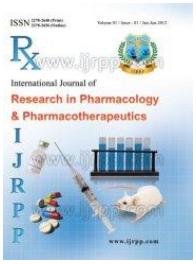

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Formulation development and evaluation of herbal lozenges for the treatment of recurrent aphthous stomatitis

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ABSTRACT

The currently available locally acting formulations to treat recurrent aphthous stomatitis (RAS) are either less efficacious or they are not comfortable for the use in the patients. Lozenges are one of the locally acting palatable oral formulations that may be used for the treatment of RAS. The aim of the present research work was to formulate locally acting efficacious herbal lozenges for the treatment of RAS which can provide a good patient compliance. As the part of preformulation study, estimation of secondary plant metabolites (Total phenolics and flavanoids) and compatibility study was carried out to formulate lozenges by moulding method. Trial batches were prepared for the optimization of bases (Isomalt, Liquid glucose and Honey) to formulate lozenges. The evaluation tests i.e. moulding time, hardness, friability, weight variation, *in vitro* drug release were carried out for the optimization of the batch. Evaluation of batches LC-LOZ 1 to LC-LOZ 9 was carried out. The diameter, thickness and hardness of lozenges were found to be 14.87±0.077mm, 7.15±0.005mm and147±0.16 N respectively. The weight uniformity was found to be 1.1±0.11 gm. The friability of lozenges was found to be less than 1% .The weight variation of all the formulations was found to be close to 1 gm which complied with the official standards. In clinical study the patients were divided into two groups and the number and size of ulcers were measured at baseline. After the treatment, follow up was taken after 24 hours, 48 hours and 72 hours. LC-LOZ was found to be more effective than Alarsin, a routinely used marketed formulation.

Keywords: RAS (Recurrent Aphthous Stomatitis), Phenolics, Flavanoids.

INTRODUCTION

Canker sores (Aphthous stomatitis) are small oval sores, red in colour, that affect the mucous membranes inside the mouth that are usually developed on the inner cheeks, gums or lips and occasionally the tongue. Recurrent aphthous stomatitis (RAS) is a disorder characterized by recurring ulcers in the oral mucosa in patients with no other signs of disease [1].

Herbal medicines are a treasure house of information, from which we may derive leads to fill many blank spots in the modern medicine traditionally; the plant is utilized in many disease conditions. The studies in this dissertation were conducted to obtain experimental evidence on the therapeutic efficacy of Liquorice and Catechu [2].

Various drugs used to prevent RAS (Recurrent Aphthous Stomatitis include choline salicylate, folic acid, benzocaine, amlexanox, local anaesthetic, locally acting steroids and antimicrobials. Beside these allopathic drug treatments, uses of herbal drugs are increasing from last decade. Herbal medicinal products are widely used around the world, increasingly so in Western nations too[1]. The sale of herbal medicines has increased considerably over the last 10 years in the industrialised countries. This growing trend to use herbal medicines to treat a wide range of problems [2]. Plant medicines are generally considered to be safer and possibly devoid of adverse effects as compared to synthetic drugs [3].

Use of liquid dosage formulations in mouth ulcers cannot provide long lasting localized effects. Further, one cannot control effectively the dose. Solid dosage forms like tablet, capsule, lozenges can overcome these disadvantages [4].While using herbal medicines, the identification of safety signals are of great importance. Liquorice is used to provide anti-inflammatory action whereas; catechu is an astringent [5]. Both, anti-inflammatory action and astringent action are required at the local level and they may produce adverse effects if they enter into the systemic circulation.

Effective locally acting solid dosage herbal formulations for the treatment of RAS are not available anywhere to the best of our knowledge. Lozenges appear to be a better formulation due to providing local effects with minimum irritation [6, 7]. Lozenges are solid preparations that are intended to dissolve in mouth or pharynx [8]. They may contain one or more medicaments in a flavoured and sweetened base. The aim of this present research work was to develop a safe and efficacious formulation of herbal lozenges using root of *Glycyrrhiza glabra* Linn and heartwood of *Acacia catechu* Wild to treat recurrent aphthous stomatitis. To achieve the goal, herbal lozenges were formulated using hydroalcoholic extract of herbal drugs and isomalt (Galen IQ) as confectionary base.

MATERIALS AND METHODS

Methodology

Preformulation studies

Estimation of active phytoconstituents in plant extraxts

Total phenolics estimated in *Glycyrrhiza glabra* by the Folin Ciocalteu's Reagent as per method of Baharam et al [9] .The percentage of total phenolics was calculated using Gallic acid method. This was done in triplicate manner.The quantification of total flavanoids in the sample was done by using aluminium chloride method as described by Baharam et al. [9, 10]. Assay content of Glycyrrhetinic acid from *Glycerrhiza glabra* was measured using colorimetric method and optical density was measured at 545 nm as per the method of Singleton VL et al [11].

One gram of dried powdered bark was extracted four times with acetone: water (70:30), by stirring at room temperature for 15 minutes. The final volume of the extract was adjusted to 100 ml with acetone: water (70:30) (=CT solution)**.**The catechin content was measured using colorimetric method at 540 nm [11].

FORMULATION DEVELOPMENT AND STANDARDIZATION

Formulation of lozenges

All the required ingredients were properly weighed as per the formulation and the process of formulation was carried out step wise in the following manner given below:

Accurately weighed quantity of Galen IQ 990 and liquid glucose were taken in beaker. This mixture was heated. Once the mixture gained enough viscosity, gradually the temperature was lowered. Once optimum temperature is achieved, the accurately weighed quantity of liquorice powder extract and black catechu powder extract was added, with stirring to ensure uniform distribution of drug in the mixture. The temperature was allowed to lower and during the cooling phase, the flavors were added with continuous stirring. Then immediately these lozenges were poured in the mould and allowed to cool and set. Then after cooling, they were removed from the moulds. The lozenges were then suitably packed [12].

Evaluation of lozenges [13]

The prepared lozenges were evaluated for hardness, weight variation, thickness and diameter, friability, mouth dissolving time and In-vitro dissolution studies.

The hardness of the lozenges was determined by manual method by using Pharmatron 8M, Dr. Scheleuniger, Germany, where the force required to break the lozenges was noted.

The weight variation was conducted by weighing 10 lozenges individually and calculating the average weight and comparing the individual lozenges weight to the average value.

The thickness and diameter of lozenges were determined using Pharmatron 8M, Dr.Scheleuniger, Germany. Took out Three lozenges from each batch and average values were calculated.

The friability of the lozenges was determined using Roche Friabilator. Weighed lozenges were placed in the friabilator and operated for 4 min at 25 rpm. The percentage friability was calculated.

In Mouth dissolving time test the time taken by the candy to dissolve completely was determined by the USP Disintegration apparatus, where hard candy lozenges were placed in each tube of the apparatus and time taken for the lozenges to dissolve completely was noted by using simulated salivary fluid (pH 6.8) 37 °C.

In-vitro dissolution testing for the amount of glycyrrhizinic acid and catechins released with different concentration of confectionary base was studied using dissolution parameters.

CLINICAL STUDY

Study design

A total 100 patients with RAS were volunteered in the study. These patients were randomly selected from outpatient department of GMERS Medical College Valsad. The study was performed in accordance with the guidelines established by the IEC (Institutional Ethics Committee) and was approved by IEC. The patient attending the OPD of the hospital were considered for study after their proper consent was obtained in signed official consent format.

A total of 100 patients, aged between 18 and 70 years, who were diagnosed as suffering from Aphthous stomatitis and who were willing to give informed consent were included in the study. They were included if they are suffering from 24 hours not more than 48 hours of aphthous stomatitis.

Patients having ulcerative oral lesion other than aphthous ulcers and herpetiform ulcers, taking any other medicine for aphthous stomatitis were excluded from the study. Patient who was pregnant, lactating and not psychologically stable were also excluded from the study.

NOAEL from preclinical study was found to be 500 mg/kg. So, the Human Equivalent Dose (HED) for clinical study was found to be 80 mg/kg. In this clinical study all the patients were divided into two groups. Out of 100 patients were taken. 50 patients were given 1gm LC-LOZ four times in a day. 50 patients were on Alarsine mouth paint thrice in a day. Comparison between LC-LOZ and alarsine mouth paint was done after 24, 48 and 72 hours.

Parameters

Clinical examination was performed to assess the number and size with calibrated periodontal prob. Size of ulcers and numbers of ulcers were estimated after 24, 48, 72 hours of drug administration.

Statistical analysis

All data were expressed as Mean ± SEM and were analyzed by one way ANOVA single factor.The results was considered to be statistically significant when $p<0.05$.

RESULTS

Preformulation studies

From the prefromulation studies we found that the *Glycyrrhiza glabra* contains 0.691 (Total phenolics % w/w) and 0.504 (Total flavonoids % w/w). *Acacia catechu* contains 0.888 (Total phenolics % w/w) and 1.339 (Total flavonoids % w/w). We also found that the Mixture of *Glycyrrhiza glabra* and *Acacia catechu* contain 1.8 (Total phenolics % w/w) and 1.98 (Total phenolics % w/w). Our formulation LC-LOZ contains 2.6 (Total phenolics % w/w) and 2.95 (Total phenolics % w/w).

Formulation studies

Evaluation of batches LC-LOZ 1 to LC-LOZ 9 showed diameter and thickness of lozenges were in range between 14.84±0.019 to 14.87±0.077mm and 6.94±0.025 to 7.15±0.005mm respectively. The hardness of lozenges was found to be in range between 124±0.64 to 147±0.16 N. The weight

uniformity was found to be between 0.876±0.51 to 1.1±0.11 gm. The friability of lozenges was found to be less than 1%. These are within the acceptance criteria according to I.P. 2007. The weight variation of all the formulations was found to be close to 1 gm which complied with the official standards (Table no.1)

In-Vitro Dissolution Study

In vitro drug release studies were carried out using USP XXIII (Beaker Method) apparatus ,with 100ml of dissolution medium maintained at 37±0.5°C for 5, 10, 15, 20 Min at 50 rpm.0.1 N HCl + pepsin is equivalent to GI fluid (Simulated salivary fluid).

LC-LOZ was subjected to physicochemical evaluation parameters such as Moulding time (min), Drug Content (% w/w) and Mouth dissolving time. The results of these studies were found to be within the limits and given in (Table no.2)

There was a time dependent decrease in number of ulcers by treated with both LC-LOZ and Alarsin mouthpaint. After 24 hrs patients treated with LC-LOZ showed significant decrease in number of ulcers (1.58±0.0760) as compared to baseline value (2.88±0.0789). After 48 hrs decrease in number of ulcers (0.78±0.0822) as compared to baseline value (2.88±0.0789) which was also found to be significantly different statistically. After 24 hrs LC-LOZ treated group showed more decrease as compared to Alarsin treated group ($p<0.05$) but it was not significant. After 48 hrs the decrease in number of ulcers in LC-LOZ treated group (0.78±0.0822) was observed significantly ($p<0.05$) greater than that from Alarsin mouth paint group (1.02±0.0925). After 72 hrs no ulcer was observed in both LC-LOZ and Alarsin treated group (Figure no.1)

There was a time dependent decrease in size of ulcers by treated with both LC-LOZ and Alarsin mouthpaint. After 24 hrs patients treated with LC-LOZ showed significant decrease in size of ulcers (2.38±0.0693) as compared to baseline value (3.14±0.0700). After 48 hrs decrease in size of ulcers (0.78±0.1188) as compared to baseline value (3.14±0.0700) which was also found to be significantly different statistically. After 24 hrs LC-LOZ treated group showed more decrease as compared to Alarsin treated group ($p<0.05$) but it was not significant. After 48 hrs the decrease in size of ulcers in LC-LOZ treated group (0.78±0.1188) was observed significantly ($p<0.05$) greater than that from Alarsin mouth paint group (1.14±0.1143). After 72 hrs no ulcer was observed in both LC-LOZ and Alarsin treated group (Figure no.2)

Table no 1: Evaluation of batches (LC-LOZ 1 – LC-LOZ 9)

Batches	Diameter (mm)(n=10)	Thickness (mm)(n=10)	Weight Uniformity (gm) (n=20)	Hardness (N)(n=5)	Friability (%)(n=5)
LC-LOZ 1	14.86±0.10	7.15±0.05	0.924±1.64	125±0.13	0.56±0.26
LC-LOZ 2	14.84±0.19	6.94±0.25	0.96±1.05	137±0.12	0.33±0.14
LC-LOZ 3	14.87±0.77	7.05±0.11	1±1.51	147±0.16	0.23±0.0.9
LC-LOZ 4	14.86±0.12	7.0±0.01	1.1±0.11	140±0.18	0.33±0.17
LC-LOZ 5	14.86±0.16	6.96±0.16	0.974±1.15	143±0.45	0.29±0.06
LC-LOZ 6	14.85±0.12	7.05±0.11	0.876±0.51	130±0.62	0.41±0.22
LC-LOZ 7	14.84±0.43	7.01±0.91	1±0.09	124±0.64	0.55±0.05
LC-LOZ 8	14.87±0.11	6.99±0.19	0.911±0.64	129±0.55	0.39±0.10
LC-LOZ 9	14.86±0.20	7.05±0.10	1± 0.56	135±0.28	0.31±0.02

Table no: 2 Evaluation of batches LC-LOZ 1 to LC-LOZ 9

Batches	Moulding time (Min)	Time required to completely dissolve (Min)(n=10)	Drug Content (% w/w) (Mean± S.D.)	
			Glycyrrhitinic acid (n=3)	Catechins (n=3)
LC-LOZ 1	5	22±0.52	98.5±0.22	98.32±0.45
LC-LOZ 2	14	16±0.67	98.16±0.66	96.26±0.64
LC-LOZ 3	4	20±0.21	96.27±0.36	96.97±0.28
LC-LOZ 4	4	20±0.18	98.01±0.41	98.61±0.13
LC-LOZ 5	16	15±0.37	97.80±0.19	97.74±0.16
LC-LOZ 6	7	17±0.14	97.64±0.23	95.48±0.12
LC-LOZ 7	7	18±0.42	95.89±0.79	98.51±0.18
LC-LOZ 8	6	15±0.33	96.05±0.16	96.86±0.62
LC-LOZ 9	5	20±0.11	98.21±0.29	97.93±0.55

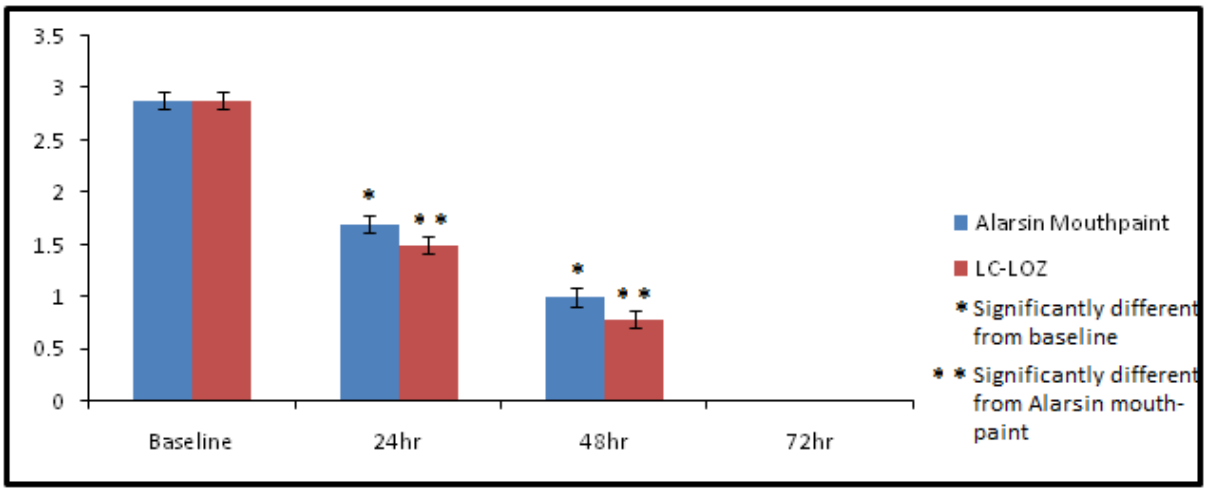

Figure 1: Comparisons of number of ulcers between LC-LOZ and Alarsin mouth paint

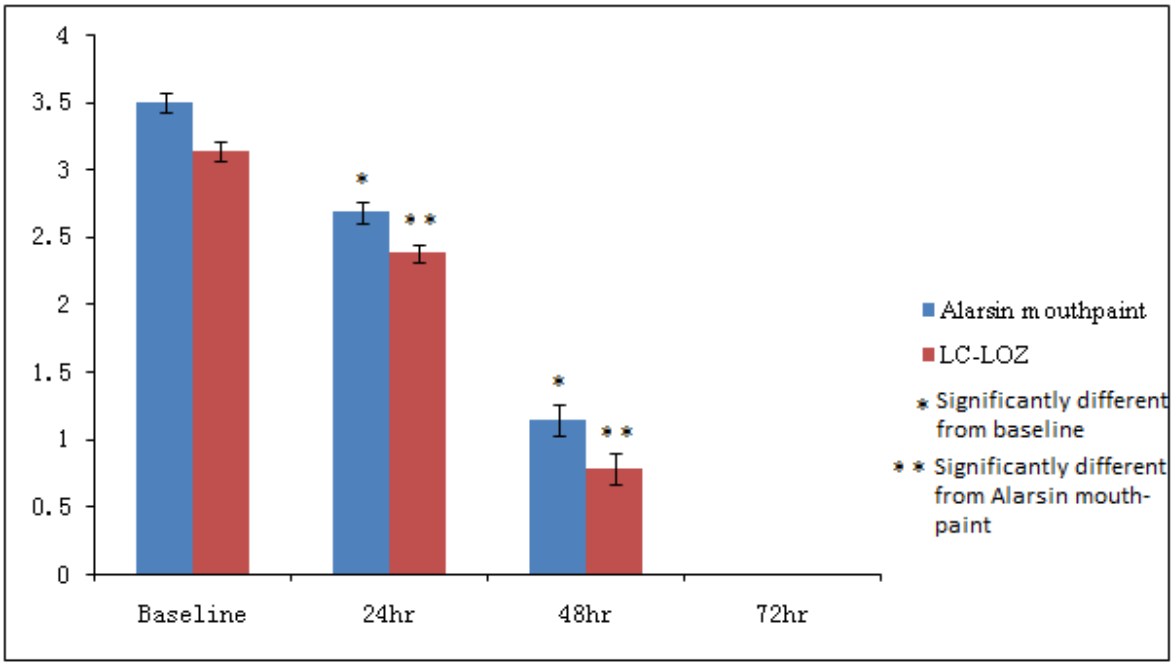

Figure 2: Comparisons of size of ulcers between LC-LOZ and Alarsin mouth paint

DISCUSSION

Recurrent aphthous stomatitis is one of the most common oral ailments. The patient of RAS presents with painful, recurring ulcers of the oral cavity. Diagnosis of RAS rests on features: a history of recurrent ulcers since childhood or adolescence and presence of typical multiple round or ovoid ulcers on examination. Although most cases of RAS are idiopathic, a careful history taking and physical examination is essential to rule out any secondary cause. A number of systemic conditions can give rise to oral ulcerations resembling RAS [14].

If the history and clinical examination are characteristic of RAS, routine laboratory testing is not necessary in most individuals. A complete blood count and measurements of levels of red-cell foliate, serum vitamin B12 and serum ferritin is suggested by few authors [15]. These investigations are useful only if there are other clinical findings suggestive of nutritional or haematological abnormalities. Immune deregulation in a genetically susceptible individual is also accepted and reasonably documented cause of RAS. Immuno-pathogenesis of RAS probably involves a cell mediated immune response. Few studies have shown an alteration in the T-cell fractions in individuals of RAS. [16]

Traditional medicine has maintained greater popularity all over developing world and the use is rapidly on the increase. Hence, the present study was undertaken to evaluate the safety and efficacy of LC-LOZ in aphthous stomatitis. Both herbal drugs are known to have a good content of tannins which may be the active constituents in relieving aphthous stomatitis.

In the present study, lozenges containing Liquorice and catechu extract were developed as convenient to the patient drug delivery form for aphthous stomatitis. During the dissolution of a solid drug form in the saliva, a solution of active substance in the mouth is delivered. The concentration of the drug in the fluids of oral cavity depends of multiple parameters [17].

The local application allows the tannins to produce astringent effect (the perceived mechanism of action) and hence promote healing and reduce pain relief. To enhance this effect and standardise the use a lozenges formulation was prepared. A total of 100 patients suffering from aphthous stomatitis participated in the study. Majority of the patients participating in the trial reported the cause of stomatitis to be constipation followed by insomnia and trauma. Other causes include spicy food, use of antibiotics etc.

All the patients were explained the reasons and therapy of ulcer and then the informed consent was obtained. The effect of the LC-LOZ and Alarsin was compared in patients of aphthous stomatitis. All the patients enrolled showed improvement after the 1st

day which was statistically significant. Both the treatment groups were found to have no ulcers by the 3rd day. This clearly indicates that both the test formulation and Alarsin were effective in improving the healing time. Statistically LC-LOZ was found to be more effective as compared to Alarsin in improving the healing time of aphthous stomatitis. Similar results were obtained for size of ulcers.

The results of clinical study indicate that constipation is one of the most common etiology for RAS. Panduranga M et al [18] in his study evaluated 50 patients with RAS and he found 25% was associated with constipation.

The pathophysiology of RAS may not differ from etiology point of view because we found improvement with Alarsin mouthpaint and LC-LOZ in all patients.

It was interesting to observe that catechu possess antioxidant activity because of tannin content. It helps to prevent cell damage. Karuna S et al evaluated that the young pods of *Acacia catechu* exhibit good amount of peroxide and DPPH free radical scavenging activity and has good potential to be exploited as source of antioxidants as well as foods [19] Liqurice also have tannin. Karami et al showed antioxidant activity of *Glycyrrhiza glabra* Linn.root extract using in-vitro model. [20]

It has the additional advantage of favourable taste- The major active component of liquorice is saponins known as glcyrrhizin, also known as glycrrhizic acid, which is an extremely sweet [20, 21]. None of the patients in any of the groups reported any adverse event. Both drugs have tannin. So both drugs produce astringent property. Hence the combination of both drugs produces synergistic effect. This raises the possibility of contribution of their ulcer healing.

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