Development and evaluation of novel lozenges containing marshmallow root extract

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Abstract: Lozenges (tablets intended to be dissolved slowly in the mouth) were evaluated as delivery system for polysaccharides extract from *Althaea officinalis* L. (marshmallow) root. The aim of investigation was to improve of the efficacy of convenient preparations for the treatment of irritated oropharyngeal mucosa and associated dry irritable cough. The formulations studied were prepared with water extract of roots of *Althaea officinalis* L. The polysaccharides extract was obtained by ultrasonification. Acute oral toxicity (LD 50 p.o.) of the obtained extract was estimated in mice. Four models of lozenges based on different excipients were formulated. The characteristics of the preparations: resistance to crushing, friability testing, disintegration time and drug release properties were evaluated.

Keywords: marshmallow root extract, lozenges, drug delivery systems.

INTRODUCTION

Dry cough may be very disturbing to the patient and as such relief from it is frequently sought. Use of analgesics, local anesthetics, antiseptics and NSAIDs can be effectively helpful in intense cases of acute sore throat, where self treatment is often seen. Despite oral treatment with analgesics or NSAIDs, the topical remedies like oral spray, troche or gargle when applied to the oropharyngeal mucosa can demonstrate more effective symptomatic relief and cure an acute sore throat. The usage of lozenges to comfort and heal the upper respiratory tract redness or irritation is to high extent safe, inexpensive and very accepted. Lozenges are solid, single-dose preparations designed to be sucked to obtain, usually, a local effect in the oral cavity and the throat (Sastry *et al*., 2000). This is very convenient dosage form for the slighter attacks of cough with throat irritability. The lozenges erode slowly (over 5-10 min) in the mouth and so release the drug dissolved in the saliva (Shojaei, 1998; Nagoba *et al*., 2011). The choice of filler and binder is of particular importance in the formulation of lozenges, as these excipients should contribute to rate of dissolution and to pleasant taste or feeling during tablet dissolution (Jensen *et al*., 1991; Herbert *et al*., 1991).

Aqueous extract of *Althaea officinalis* L. (*A. officinalis*) is used as an ancient remedy for symptoms of irritation of the oropharyngeal mucosa and the dry cough associated with it. The roots of *A. officinalis* contain water-soluble polysaccharides (about 5-11%), mono and di-saccharides, flavonoids, phenolic acids, coumarins, starch, tannins, phytosterols and other compounds. The efficacy of the extract is mainly due to bioadhesive effects of the polysaccharides, which form a protective film coating on the oropharyngeal mucosa, alleviating local irritation and inflammation (EMEA, 2009; ESCOP, 2003). Properties of *A. officinalis* isolated polysaccharides have been studied in several pharmacological experiments. Schmidgall *et al.,* (2000) examined the bioadhesive effects of highly purified polysaccharides from various medicinal plants on porcine buccal membrane. *A. officinalis* derived polysaccharides showed moderate level of adhesion to epithelial tissue, forming a film, which in turn allows the mucosa to heal and stay protected from the harmful effects of physical and microbial agents (Capek *et al.,* 1988). Recently, Deters *et al.,* (2010) demonstrated that aqueous extract from *A. officinalis* stimulate cellular activity and proliferation of epithelial KB cells. *In vitro* evidence demonstrated antimicrobial effect of methanol extract *of A. officinalis* root on number periodontal pathogens resident in the oral cavity (Iauk *et al.,* 2003; EMEA, 2009) and it can be assumed that the reduction of microbial invasion of saliva contributes to the healing process. In an experimental model, Wagner and Proksch (1985) established a weak immune-activating effect of the extract and polysaccharides from *A. officinalis*. In addition, Althaea-mucilage, an acidic polysaccharide isolated from *A. officinalis*, has been demonstrated to have an anti-complement activity on normal human serum (Yamada *et al.,* 1985). The positive antitussive effects of *A. officinalis* have been extensively studied in experimental models (Nosálová, 1992; Nosálová *et al.,* 1992; Nosálová *et al.,* 1993) as well as in human clinical trials. Efficacy and tolerability of marshmallow syrup has been demonstrated in a post-marketing surveillance study in 313 children and in a retrospective observational study in 599 children (Fasse *et al.,* 2005; EMEA, 2009). Efficacy of *A. officinalis* on dry cough associated with ACE inhibitors has been demonstrated in a double-blind, placebo-controlled clinical trial with 63 patients (Rouhi and Ganji, 2007).

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The aim of the present study is to develop and evaluate lozenges containing dry polysaccharide extract of marshmallow root for the treatment of inflamed oropharyngeal mucosa and associated dry irritable cough.

MATERIALS AND METHODS

Plant material

The roots of cultivated *Althaea officinalis* L. were obtained in the autumn of 2011, from Botanical garden, Sofia University "St. Kl. Ohridski", Bulgaria, where voucher specimen (PhH 4589) is deposited.

Animals

Male albino mice, strain H, aged 6-7 weeks 22-25g, kept under standard conditions in an animal house (water and food *ad libitum*, 12/h dark/light cycle) were used throughout the experiments. The ambient temperature of the room was maintained at 21±1ºC and 50% relative humidity. The animals were treated per orally. All experiments were performed in full accordance with the respective Bulgarian and European guidelines for Care and Use of Laboratory Animals and approved by the Ethical Committee of Medical University of Sofia. Testing was performed between 09.00 and 13.00 h.

Chemicals and drugs

Mannitol and sorbitol were purchased from Merck KGaA (Darmstadt, Germany). PEG 6000, powder and sucrose were obtained from Sigma-Aldrich GmbH (Germany). Xanthan gum was obtained from S.D Fine Chemicals (Mumbai). Magnesium stearate was purchased from (Herwe Chemisch-technische Erzeugnisse, Germany) and colloidal silica dioxide (Aerosil 200) from Degusa (Germany). Ethanol 96% was obtained from Merck (Germany). Purified water was obtained by distillation. All chemicals and reagents used were of analytical grade.

Acute toxicity (LD50)

Acute toxicity was estimated by the Up-and-Down Procedure according to the OECD Test Guideline 425 (1998). Mice were per orally (p.o.) treated with the polysaccharides extract previously dissolved in 40°C distilled water in doses of 2000, 3000, 4000 and 5000 mg/kg b.w. Animals were observed daily for clinical signs or mortality over a period of two weeks following the treatment.

Extraction of polysaccharides from the roots of Althaea officinalis L.

An ultrasonic apparatus Sonica 4300 (Italy) was used for extraction of polysaccharides. The extraction was carried out at ultrasonic power 300 W, frequency of 45 kHz, temperature 25°C for 120 min. 200 g roots of *A. officinalis* were dry milled and sieved trough 5 mm sieve. The sieved roots were socked in 1L purified water and shaken for 15 minutes. The mixture was extracted ultrasonically at above presented conditions. The obtained extract was filtered trough the cellulose membrane HVCO (3.5 kDA Roth, Karluhe, Germany) and the filtrate was precipitated with 96% ethanol. Ethanol 70% (v/v) was added to the precipitate and centrifuged. After vacuum drying the sediment was dissolved in purified water and freeze-dried.

Assay of polysaccharides

A modified spectrophotometric method was used (Pawar and D'Mello, 2011) to assay of polysaccharides in dry extract as a galactose at 490 ± 2 nm. The standard curve was drowning at concentration range of galactose from 10 to 80 μg/ml. The equation of fitted curve is y = 0.0036 + 0.0046.x, at correlation coefficient (R^2 = 0,9994). Sample preparation: 0.2000 g of freeze-dried extract was heated with 40 ml purified water in reflux condenser for 1 hour and filtrated. The obtained filtrate was precipitated with 96% ethanol and centrifuged. The sediment obtained was dissolved in 100.0 ml hot water (solution A). 10.0 ml from the solution A were diluted to 100.0 ml with water (solution B). 1.0 ml of solution B was mixed with 1 ml of 5% phenol solution and 5 ml sulfuric acid. The obtained mixture was equilibrated 30 min at room temperature. The absorbance was measured spectrophotometrically at 490 nm and the quantity of polysaccharides as galactose was calculated.

Preparation of model lozenges

Model lozenges contain 20 mg dry extract and excipients. Models were prepared by direct compression using a single position tablet press (model EK 0, Korsch, Berlin, Germany) supplied with the 13 mm biconvex punches at 8 kN pressure force. Average weight of lozenges was about 850 mg.

Characteristics of model formulation

Resistance to crushing of tablet: was performed by the method of progressive loading according to the requirements of Eur. Ph. 7.0, apparatus Erweka type TBH 30, Germany.

Friability testing: was performed according to the requirements Eur. Ph. 7.0, apparatus Erweka TAR 20, Germany.

Disintegration time: was performed by the method described in Eur. Ph. 7.0 in buffer solution with pH 7.2, apparatus Erweka ZT 3.

Drug release studies: Drug dissolution was observed in dissolution test apparatus (model Erweka DT 600, Hensenstmm, Germany), USP paddle method at 50 rpm, in 250 ml phosphate buffer with pH 7.2 and 37 ± 0.5°C. The samples of 1.0/ml were withdrawn at 5, 10, 15, and 30 minutes and were filtered trough 0.45 μm cellulose filter. The quantity of polysaccharides as galactose was

analyzed by analytical procedure. 1 ml of filtrated sample was mixed with 1 ml of 5% phenol solution and 5 ml sulfuric acid. The obtained mixture was equilibrated 30 min at room temperature. The absorbance was measured spectrophotometrically at 490 nm versus blank solution. Blank solution is consisting from all ingredients of lozenges in the same concentrations and at the same conditions of the dissolution experiment without presence of extract.

RESULTS

Acute toxicity study (LD50)

The extract is characterized by a very low degree of toxicity. The acute toxicity LD50 of *A. officinalis* extract in albino mice was found to be above 5000 mg/kg according to the method of OECD 425 (1998).

Extraction and assay of polysaccharides from the roots of Althaea officinalis L.

The polysaccharides extract from *A. officinalis* was obtained by ultrasonification procedure described above. The yield of extraction was about 10%. The quantity of polysaccharides of extract developed was evaluated to 95.9%.

Preparation of model lozenges

By the method of direct compression four model lozenges compositions based on different excipients were prepared. The compositions of model formulations are given in table 1. The formulation A1 and A3 are identical but prepared by different technological methods. The formulation A1 was prepared with ordinary mixing of components, while model A3 was obtained by previously mixing of the extract with PEG 6000. The lozenges were obtained by 8 kN pressure force.

Table 1: Composition of different model of lozenges

Ingredients (mg)	Formulation code			
	A1	A2	A3	A4
A. officinalis extract	20	20	20	20
Sorbitol	280	-	280	250
Mannitol	-	280	-	-
PEG 6000, powder	71	71	71	71
Sucrose, crystalline	225	225	225	200
Sucrose, powder	225	225	225	200
Xanthan gum	-	-	-	80
Magnesium stearate	4.5	4.5	4.5	4.5
Colloidal silica dioxide	4.5	4.5	4.5	4.5
Orange flavor	22	22	22	22

Characteristics of model formulations

The obtained models are characterized with good technological parameters, which are presented in table 2.

Table 2: Characteristics of model lozenges

Characteristics	Formulation code			
	A1	A2	A3	A4
Mass of tablets (mg)	852± 1.2	852± 1.8	852± 0.9	852± 2.1
Mechanical strength (N)	100± 5.4	95± 3.9	97± 4.7	110± 5.3
Friability (%)	0.5	0.5	0.6	1
Disintegration time (min)	7-8	8-9	6-7	>30

The obtained model lozenges were with necessary uniformity of mass, mechanical strength about 100 N and low friability-no more than 1%. Disintegration time was in the range of 6-9 minutes for models A1-A3. As expected, model A4 had slower disintegration time, over 30 minutes, because of presence of xanthan gum in the formulation.

Drug release profile of model lozenges is presented in fig. 1. The results from the investigations show that the use of different excipients leads to significant differences in the kinetics of release from model formulations.

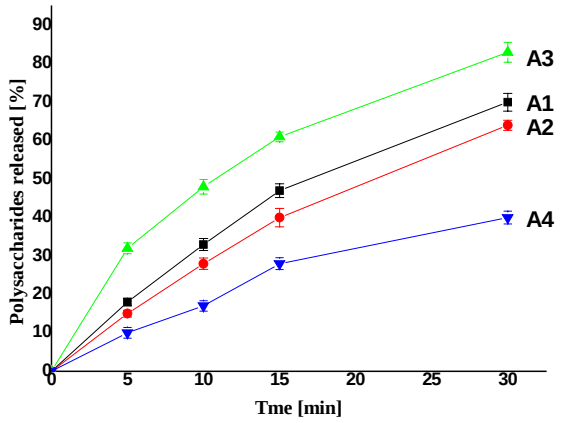

Fig. 1: Dissolution profile of polysaccharides from model lozenges

Models based on sorbitol and mannitol (A1 and A2) show relatively closed kinetic of drug release. The little difference between them can be explained by the lower water solubility of mannitol than sorbitol. The rate of release of the drug from model A3 is higher than model A1. This is due to different technology of preparation (see above). The preliminary mixing of extract with PEG ensures its better hydrophilization and then higher dissolution rate from the lozenges. The rate of drug release from model A4 is the lowest. This is logically, by reason of inclusion of xanthan gum in the formulation.

According to the results and considering the route of administration, it seems that the model lozenges A3 provides optimal variant of excipients with release of polysaccharide extract over 80% at 30 min.

DISCUSSION

A great number of plant extracts are frequently used for upper respiratory tract disorders, including sore throat. Sore throat is considered a minor ailment but may be very disturbing to the patient and as such relief from it is frequently sought (Fasse *et al.,* 2005). In addition to analgesic effect of NSAIDs, the topical remedies like lozenges provide effective symptomatic relief of pain and inflammation in acute sore throat. The usage of lozenges to comfort and heal the throat redness or irritation is to high extent safe, inexpensive and very accepted. Marshmallow extract has been used medicinally since ancient times. In the present study, polysaccharides extract from marshmallow root, has been studied for acute toxicity in mice. It was found that the extract is practically nontoxic, as p.o. LD50 was above 5 g/kg body weight. The efficacy of the extract is mainly due to bioadhesive effects of the polysaccharides, which form a protective film coating on the oropharyngeal mucosa, alleviating local irritation and inflammation (EMEA, 2009; ESCOP, 2003).

In the present study, lozenges containing marshmallow root extract were developed as convenient to the patient drug delivery form for soothing sore throat symptoms. 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 (Richards and Xinq, 1993). The physical and chemical characteristics of the drug form and the physiology of the oral cavity both influence the amount of drug in the mouth (Perushotham *et al.*, 2011). Four models of lozenges based on different excipients were formulated by the method of direct compression. All of the obtained models were characterized with good technological parameters. The results from the drug release studies show that the use of different ingredients leads to the considerable differences in the release profiles from model formulations. An addition of xanthan gum in model lozenges A4 increased disintegration time (over 30 min) and retain *in vitro* drug release rate (40% for 30 min) of the lozenges since xanthan gum could apparently enhance the viscosity of the lozenge matrix. According to our results, the model lozenges A3 possess optimal technological characteristics in the object of disintegration and *in vitro* drug release (over 80% of polysaccharide extract was released at 30 min).

In conclusion, results of the present study would contribute to the development of drug form (lozenges) for local delivery of marshmallow root extract for the treatment of irritated oropharyngeal mucosa and associated dry cough.

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