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Design of cissus–alginate microbeads revealing mucoprotection properties in anti-inflammatory therapy

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ABSTRACT

Cissus gum has been employed as polymer with sodium alginate in the formulation of diclofenac microbeads and the *in vivo* mucoprotective properties of the polymer in anti-inflammatory therapy assessed in rats with carrageenan-induced paw edema in comparison to diclofenac powder and commercial diclofenac tablet. A full 2^3 factorial experimental design has been used to investigate the influence of concentration of cissus gum (X_1); concentration of calcium acetate (X_2) and stirring speed (X_3) on properties of the microbeads. Optimized small discrete microbeads with size of 1.22 ± 0.10 mm, entrapment efficiency of 84.6% and t_{80} of 15.2 ± 3.5 h were obtained at ratio of cissus gum:alginate (1:1), low concentration of calcium acetate (5% w/v) and high stirring speed (400 rpm). *In vivo* studies showed that the ranking of percent inhibition of inflammation after 3 h was diclofenac powder > commercial tablet = cissus > alginate. Histological damage score and parietal cell density were lower while crypt depth and mucosal width were significantly higher ($p < 0.05$) in the groups administered with the diclofenac microbeads than those administered with diclofenac powder and commercial tablet, suggesting the mucoprotective property of the gum. Thus, cissus gum could be suitable as polymer in the formulation of non-steroidal anti-inflammatory drugs ensuring sustained release while reducing gastric side effects.

1. Introduction

Natural biodegradable polymers such as starches and gums present a fairly broad area of active research in controlled drug delivery [1,2]. The advantage of these natural polymers is that they are broken down into biologically acceptable molecules that are metabolized and removed from the body *via* normal metabolic pathways [3,4]. These natural polymers are hydrophilic and are more amenable to physical and chemical modifications using simple processes than the widely used synthetic polymers [5–8].

Alginate, a linear hetero-polysaccharide, extracted from different types of algae has been used for the formulation of microbeads under mild process conditions. However, some of the disadvantages attributed to alginate polymers include their susceptibility to degradation in acidic environments of the stomach and the fact that when alginate gel is formed in the presence of calcium ions, the integrity of the beads may be affected by monovalent ions or chelating agents such as phosphates, lactates and citrates, which absorb calcium ions [9]. Furthermore, they form microbeads, which have cracked and porous surfaces that can lead to relatively fast diffusion of moisture and other fluids, thus reducing the barrier properties in unfavorable environmental conditions [10]. Blending alginate with natural polymers such as gums and starches is a recent innovation that has been shown to be effective in overcoming many of these limitations by enhancing encapsulation efficiency and drug release properties of microbeads [4,11–13].

Cissus gum, obtained from the plant *Cissus pulpunea* Guill and Perr, Family Ampelidacae, is one of the numerous underutilized gums distributed in many parts of Africa, especially the savannah region. The plant has great propensity for retaining water and thus remains fresh almost throughout the year. Cissus gum is popularly referred to as food gum with a wide range of local applications in Africa where it is used as soup thickener and remedy for indigestion [14]. Cissus gum is a hydrophilic polysaccharide that swells rapidly

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in cold water and a 2% dispersion has been shown to attain a viscosity of 11.6 Pa s. It has a particle density of 1.59 g cm^3 and glass transition temperature of 264.2 °C [12]. The gum has been evaluated as a binder in pharmaceutical tablets where it was reported to produce tablets with high mechanical strength and slow drug release properties [15]. Cissus gum along with three other natural gums namely khaya, albizia and irvingia gums, has also been characterized and used, for the formulation of microbeads by ionic gelation method using zinc chloride as the chelating agent [12]. However, relatively low entrapment efficiency and short dissolution times were obtained for cissus gum, in comparison to sodium alginate and the other natural gum-alginate blends that were evaluated. Of the four natural gums evaluated, cissus gum showed more promise due to its use as remedy for indigestion suggesting probable mucoprotective properties, which could be useful in anti-inflammatory therapy.

Thus in the present study, optimized diclofenac sodium microbeads have been formulated from blends of cissus gum and sodium alginate using a total polymer concentration of 2.50% w/v, polymer to drug ratio of 4:1, and calcium acetate as cross-linking agent. The optimized microbeads were administered orally to rats with carrageenan-induced inflammation exhibited as paw edema and the anti-inflammatory effects compared with those of commercial diclofenac tablet and diclofenac powder. Histological examinations were also carried out to investigate inflammatory changes and/or presence of micro-hemorrhagic lesions in the stomach in order to evaluate the mucoprotective potential of the natural gum.

Diclofenac sodium, a potent non-steroidal anti-inflammatory drug with short biological half-life (1–2 h), exhibits adverse effects such as gastrointestinal disturbances, peptic ulceration and gastrointestinal bleeding, with long-term use [16]. Formulation of diclofenac as controlled release microbeads using a polymer with mucoprotective properties will offer a means of delivering the drug in a sustained manner with reduced side effects.

2. Materials and methods

2.1. Methods

Diclofenac sodium was obtained from Fagron GmbH & Co (Barsbüttel, Germany) while sodium alginate and calcium acetate were obtained from Carl Roth GmbH & Co (Karlsruhe, Germany). Cissus gum was obtained from the stems of *C. pulpunea* from local farmers in Tose village, South West region of Nigeria. All other reagents were of analytical grade.

2.2. Extraction of cissus gum

Cissus gum was obtained from the stem of *C. pulpunea* using established procedure [12]. Briefly, cissus stem was soaked in chloroform water for 48 h to allow the gum to diffuse out of the stem. The gum was strained through a calico cloth to remove extraneous materials and then precipitated using absolute ethanol, washed with diethyl ether and then dried in hot air oven at 40 °C for 48 h. The died gum was pulverized, passed through a 150 μm sieve mesh sieve.

2.3. Preparation of microbeads

Diclofenac sodium microbeads were prepared by ionic gelation from gel blend of cissus gum and sodium alginate to obtain gum to alginate ratios of 1:1 and 2:1, with a total polymer concentration of 2.50% w/v. Appropriate quantity of diclofenac sodium was added such that the total polymer to drug ratio was 4:1. The resulting dispersion was extruded into calcium acetate solutions (5% w/v and 10% w/v) maintained under gentle agitation (300 and 400 rpm) using a syringe with 0.90 mm needle at a dropping rate of 2 mL/min. The formed beads were allowed 30 min curing time for cross-linking and then collected by decantation. The collected beads were washed with distilled water and dried for 24 h in hot air oven at 40 °C.

2.4. Characterization of microbeads

2.4.1. Size and morphology

The particle sizes of 100 microbeads were determined by using a computerized microscope fitted with a colored video (Letz Laborlux II, Wetzlar, Germany). The morphology and surface characteristics of the microbeads were analyzed using scanning electron microscopy (Hitachi Model S-2460N Taichung, Taiwan) at an accelerating voltage of 25 kV.

2.4.2. Entrapment efficiency

Diclofenac microbeads (50 mg) were crushed in a glass mortar and suspended in 10 mL of phosphate buffer, pH 6.8. After 24 h, the solution was filtered, appropriately diluted using phosphate buffer and analyzed spectrophotometrically at 274 nm using UV/Vis spectrophotometer (LAMBDA 12 Perkin Elmer GmbH, Ueberlingen, Germany). The drug entrapment efficiency (E) was calculated using the formula:

$$E\ (\%) = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100 \quad (1)$$

2.4.3. Drug release study

The *in vitro* drug release profile of the microbeads was determined in 900 mL of phosphate buffer, pH 6.8, maintained at 37 ± 0.5 °C, using the paddle method (USP XXI) rotated at a speed of 50 rpm. The absorbance was determined at 274 nm using UV/VIS spectrophotometer (LAMBDA 12 Perkin Elmer GmbH, Ueberlingen, Germany).

Data obtained from *in vitro* dissolution studies were fitted to zero order, first order, Higuchi, Hixon–Crowell, Korsemeyer–Peppas and Hopfenberg equations, to determine the kinetics and mechanism(s) of drug release from the microbeads [17,18]. The model of best fit was identified by comparing the values of correlation coefficients.

2.5. Experiment design

Factorial experimental design, which has been used as an efficient tool to obtain an appropriate mathematical model with minimum experiments, was used for the optimization of the formulation design [19,20] and to determine the effects of various formulation factors on the characteristics of the drug formulations [21]. A full 2^3 factorial design was performed (eight batches), using three independent process parameters, namely: cissus gum concentration (X_1), concentration of calcium acetate (X_2) and stirring speed (X_3) at two levels, high (+1) and low (−1). The individual and interactive effects of X_1, X_2 and X_3 on yield of microbeads, bead size, entrapment efficiency and dissolution time (t_{80}) were determined. Potential variables such as the ratio of total polymer to drug, curing time, needle size and dropping height were kept constant in the experimental design. The results obtained were then subjected to regression analysis using the software MINITAB (version 15, Pennsylvania, U.S.A). Response surface plots were obtained from the data.