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Research Article

Formulation and *invivo* evaluation of mucoadhesive microspheres embedded *clerodendrum phlomidis* (cp) extract for prolonged antidiabetic activity

K. Jesindha Beyatricks*, S. Kavimani[2], Habeela Jainab[3], ShivaKumar[3], Bindhu[3]

**Research scholar, PRIST University, Thanjavur, Tamilnadu*

[1]Professor, Mother Therasa Post Graduate Institute of Health Sciences, Pondicherry

[2]Hillside College of Pharmacy & Research Centre, Bangalore

Corresponding Author: K. Jesindha Beyatricks

Email: jolyjesi@gmail.com

ABSTRACT

In this study an attempt was made to prepare mucoadhesive microcapsules of Clerodendrum *phlomidis* extract using alginate polymers for prolonged release. Encapsulation of extract into sodium alginate polymer was done by ionic-gelation technique. In vivo testing of the mucoadhesive microcapsules in diabetic albino rats demonstrated significant antidiabetic effect of extract. The hypoglycemic effect obtained by mucoadhesive microcapsules was for more than 16 h whereas plain CP extract produced an antidiabetic effect for only 4 h suggesting that mucoadhesive microcapsules are a valuable system for the long term delivery of CP extract. In-vivo data obtained over a 120-h period indicate that CP extract loaded alginate microspheres from batch F7 showed the better glycemic control than control and a commercial brand of the drug.

Keyword: Mucoadhesive, Ionic gelation technique, Sodium Alginate, Microspheres

INTRODUCTION

Diabetes mellitus is a most common metabolic disorder of human beings. It is global in distribution affecting 2 to 6 percent population of the World [1]. Clerodendrum phlomidis is well known drug in ayurveda and siddha medicine for treatment of diabetics. *Clerodendrum phlomoidis* L. (Family: Verbenaceae) is commonly known as *Thazhu thaazhai* in Tamil and *Arni* in Hindi [2].

β-Sitosterol is widely distributed in the plant kingdom and found in Clerodendrum phlomidis [3]. The biochemical effects of cholesterol differ from those of phytosterols. The major phytosterol sources in the human diet are vegetable oils, cereals, fruits, and vegetables [4]. Authors have reported that β-sitosterol inhibits the growth of HT-29 human colon cancer cells and induces apoptosis of human prostate cancer cells. β-Sitosterol also has the potential to function as an anti-cancer agent for controlling colon carcinogenesis [5]. Moreover,

β-sitosterol possessed potential anti-diabetic and anti-oxidant activities in streptozotocin-induced hyperglycemia models reported [6]. In addition to their cholesterol-lowering effect, β-sitosterol have anti-inflammatory, antipyretic, antineoplastic, immune-modulating and blood sugar-controlling effects.

In humans, <10% of the total dietary β-sitosterol consumed is absorbed in the intestine. In rats, ~4% of β-sitosterol is absorbed. Human dietary intake ranges from 40 to 400 mg/d. In Western diets, phytosterol intake is low, ~80 mg/d.

Due to poor solubility and bioavailability of phytosterols, the serum cholesterol-lowering effect of phytosterols is not consistent, and high dosages (up to 25 to 50 g/d) are required for efficacy [14].

Plasma concentrations of β-sitosterol ranged from 0.30 to 1.02 mg/100 ml plasma. Plasma levels were raised slightly when intakes were increased greatly, and on fixed intakes they were constant from week to week. The percentage of esterified β-sitosterol in the plasma was the same as for cholesterol. However, the rate of esterification of b-sitosterol was slower than that for cholesterol. β-sitosterol were much shorter than for cholesterol and excreted in bile as free sterol; this excretion was more rapid than that of cholesterol [7].

Novel drug delivery system has shown improved pharmacokinetic and pharmacological properties of phytoconstituents parameters which can be advantageously be used in the treatment of the diabetes mellitus [8].

Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems [9]. Microparticles are defined as spherical polymeric particles. These microparticles are constitutes an important part of these drug delivery systems, by virtue of their small size and efficient carrier characteristics [10]. However, the success of these novel microparticles is limited due to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes. It can be achieved by coupling bioadhesion characteristics to microparticles and developing novel delivery systems referred to as "bioadhesive microparticles". Bioadhesive microparticles include microspheres and microcapsules (having a core of the drug) of 1–1000 μm in diameter and consisting either entirely of a bioadhesive polymer or having an outer coating of it, respectively. Bioadhesive microparticles have advantages such as efficient absorption and enhanced bioavailability of drugs owing to their high surface to volume ratio, a much more intimate contact with the mucus layer, and specific targeting of drugs to the absorption site [11].

Preparation of Mucoadhesive Microcapsules containing CP extract

Cross-linking technique [12]

Mucoadhesive microcapsules containing CP extract were prepared employing sodium alginate in combination with three mucoadhesive polymers—sodium CMC, carbopol 934Pand HPMC as coat materials. Orifice-ionic gelation method was employed to prepare the microcapsules. Sodium alginate was dissolved in 50 ml of purified water to form a homogenous polymer solution. The active substance CP extract was added to the polymer solution (in a ratio of CP extract: polymer solution 1:1) and sonicated to form a viscous dispersion. The aqueous phase was taken in a syringe (No. 20) and extruded dropwise in 100 ml of the external oily phase (liquid paraffin) containing 0.2% Span®80 and stirring was carried out using propeller stirrer (Remi, India) at 1000 rpm. After 15 min, 2.0 ml of calcium chloride was added drop-by-drop to the emulsion and stirring was continued. The emulsion was stirred for 1 h and then centrifuged at 2000 rpm for 15 min. The microspheres were separated by filtration and washed with petroleum ether followed by water to remove the paraffin and excess cross linking agent. The dried microspheres were then stored at 25°C.

Sodium alginate microspheres were dispersed in 5 ml of 1.5 wt% mucoadhesive polymers—sodium CMC, carbopol 934P and HPMC in buffer solution (pH 6.8) for 1 h under stirring of 300 rpm as the second-step solidification. Finally, the mucoadhesive polymers coated sodium alginate microspheres and then dried at 40ºC for 6 Hrs.

Table No. 1 Formulations for CP extract loaded alginate microspheres

S.No.	Ingredients (% w/w)	F1	F2	F3	F4	F5	F6	F7	F8
1	CP extract	5	5	5	5	5	5	5	5
2	Alginate	0.75	1.5	1.5	1.5	1.5	1.5	1.5	1.5
3	Calcium chloride	2	2	2	2	2	2	2	2
	Cross linking time	30 min	30 min	15 min	30 min	45 min	30 min	30 min	30 min
4	Sodium CMC	-	-	-	-	-	1.5	-	-
5	Carbopol 934P	-	-	-	-	-	-	1.5	-
6	HPMC	-	-	-	-	-	-	-	1.5

In Vivo study [13]

The animals used for *in vivo* experiments were adult Wistar male albino rats (230-250 g) from Central Animal House of C.L.Baid Metha college of pharmacy, Chennai, India (IAEC/XLVI/02/CLB/MCP/2015). The animals were kept under standard laboratory conditions, temperature at 25±1°C and relative humidity (55 ± 5%). The animals were housed in polypropylene cages, four per cage, with free access to standard laboratory diet (Lipton feed, Mumbai, India) and water ad libitum. Guidelines of Institutional Animal Ethics Committee were followed for *in vivo* experiments.

Induction of diabetes and experimental groups

Diabetes was induced in rats by intraperitoneal injection of 50 mg/kg streptozotocin (STZ; Sigma, St Louis, MO, USA) dissolved in 0.1 mol /L sodium citrate buffer, pH 4.5. The induction of diabetes was evaluated 72 h later and rats with blood glucose levels >250 mg / dL were used in subsequent experiments. The nine experimental groups (n = 9 in each) used in the present study were as follows.

1. Group I, a vehicle-treated diabetics control group.
2. Group II, diabetic rats treated with CP extracts 1200 mg/kg per day.
3. Group III, diabetic rats treated with β-sitosterol 10 mg/kg per day (BS was dissolved in 0.5 mL olive oil).
4. Group IV, diabetic rats administered mucoadhesive microcapsules of CP extract at a dose equivalent to BS 10 mg/kg per day.
5. Group V, diabetic rats treated with glibenclamide 0.3 mg/kg per day.

Blood samples were withdrawn by the retro orbital puncture at predetermined time at 1 hour intervals up to 24 h, and were analyzed for blood glucose by glucose oxidase and peroxidise (GOD/POD) method using commercial glucose kit.

Statistical analysis

Statistical analyses were accomplished using GraphPad Prism statistical package. Student's *t*-test was used to determine the statistically significant differences between the results. Results with P values <0.05 were considered statistically significant.

Relative pharmacological availability (PA%) was calculated by using following equation. The same equation was used in calculation of the relative bioavailability (F%); however, the AUC values were substituted instead of the AAC values.

$$PA\% = \left(\frac{AAC_{Mic.}}{AAC_{Sus.}}\right) \times \left(\frac{Dose_{Sus.}}{Dose_{Mic.}}\right) \times 100$$

Where, PA is the pharmacological availability of the CP extract. $AAC_{oral\ suspension}$ and $AAC_{oral\ microcapsules}$ are area above the curve of reduction in blood glucose levels for oral and s.c. administration respectively. The average standard deviations of blood glucose levels measured in the six experimental rats are plotted versus time and the trapezoid rule is used to calculate the AAC.

RESULTS & DISCUSSION

Formulation of cp extract loaded microspheres

The formulations were compared for their release, microencapsulation efficacy, mucoadhesive property by comparing the concentration of sodium alginate and mucoadhesive polymers. The percentage of extract entrapment increased with increase in polymer concentration. This was attributed to physical interaction and/or entanglement of the greater amount of extract inside the intricate cross-linked calcium alginate gel network.

One of the ways of changing drug release from the microspheres is to change the crosslinking density of the matrix by employing various time of exposure to crosslinking agent. The effect of the exposure time to calcium chloride on the release rate of β-sitosterol has been investigated by varying the time of exposure to calcium chloride as 15 - 45 min. The results were given in **Fig.1**, which clearly indicated that increasing exposure time to $CaCl_2$ decreased the cumulative release of β-sitosterol. The β-sitosterol release was found more slowly with the percentage increase of cross-linker concentrations ($CaCl_2$) in cross-linking solutions, which can be attributed by high degree of cross-linking by higher $CaCl_2$ concentration might slower the drug release from highly cross-linked microspheres. The higher concentration of cross-linker used for the preparation of ionically gelled alginate microspheres might produce a rigid polymeric structure due to contraction of microvoids, which could facilitate poor entry of dissolution medium into the calcium ion induced ionically gelled extract-loaded alginate polymer and slow the drug release.

Invivo study

In vivo testing of the mucoadhesive microcapsules of CP extract in diabetic albino rats demonstrated significant prolonged antidiabetic effect compared to plain CP extract. The percentage reduction of blood glucose level (pharmacological response) after β-sitosterol at 1 h time period was found to be (44±4%) and the effect was maintained only for 6 h. These results are in good agreement with the findings of an earlier report.

The hypoglycemic effect obtained by F7 mucoadhesive microcapsules was for more than 16 h whereas plain CP extract produced an antidiabetic effect for only 4 h suggesting that mucoadhesive microcapsules are a valuable system for the long term delivery of CP extract. Prolonged release formulation of CP extract is significantly more effective than the immediate release formulation in reducing blood glucose levels and side effects.

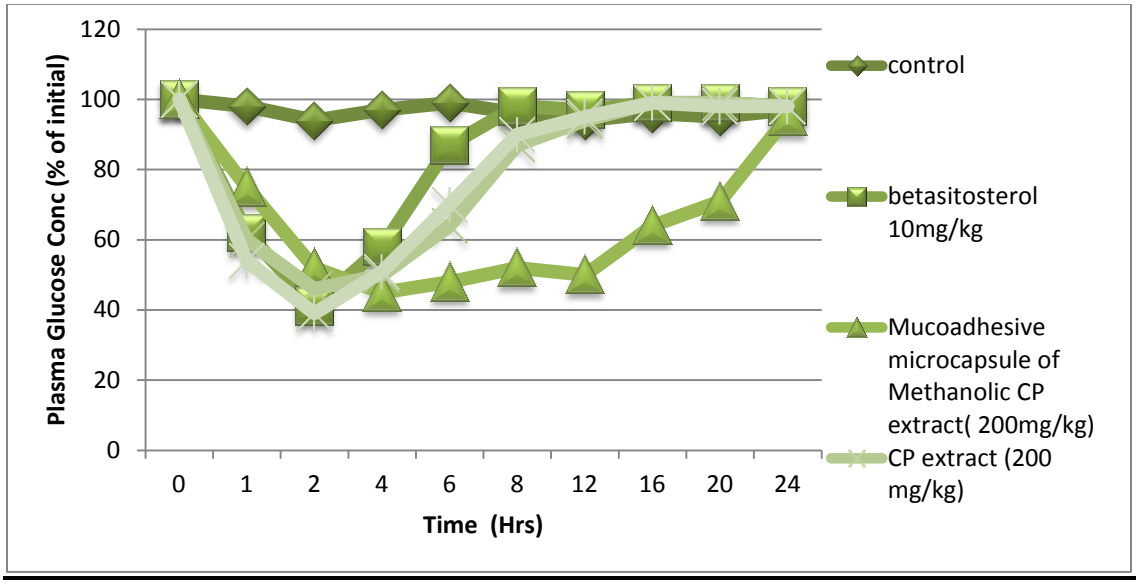

Fig.No.1 Hypoglycemic effect of control, Beta sitosterol, loaded microspheres, CP extract & standard

Table No. 2 Main pharmacokinetic parameters for plasma glucose levels after administration of different formulations to rats

Parameters	Plain CP extract	CP extract mucoadhesive micrsocapsules
CP extract dose (mg)	200	200
Minimum glucose level in % of the initial level	62.13±3.44	75.04±2.76
Time point of minimum glucose level (h)	2	6
$AAC_{0\rightarrow24}$	368±7.4	925±5.2*
Relative pharmacological efficacy (PA%)	-	2.51±4.1*

Results are expressed as (mean±SD, *n* = 6). * P<0.05

Pharmacokinetic parameters of glucose levels after dosing are shown in Table 2. The mucoadhesive microcapsules of CP extract produced minimum glucose level 55.21±3.16 % at 6 h and the reduction of glucose levels was maintained over a prolonged period of time. This result may be attributed to the improved permeation in gatrointestinal tract using microspheres carrier system, which keeps the extract for longer time in absorption site. Orally administered plain CP extract solution at the same dose showed slight reduction in blood glucose level due to poor permeability in GIT. The relative pharmacological efficacy for mucoadhesive microcapsules of CP extract (2.51±4.1%) was almost three-fold higher than the efficacy of the plain CP extract.

CONCLUSION

The microcapsules containing CP extract consisting of mucoadhesive polymer alginate could be prepared by an ionic gelation process. The microcapsules exhibited good mucoadhesive properties in an in vitro test. The in vivo study demonstrated significant blood glucose reducing activity of mucoadhesive microcapsules of CP extract. Developed mucoadhesive microcapsules are suitable for prolonged effect after oral administration of CP extract.

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