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Formulation and Evaluation of Licozinat Matrix Tablet

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Abstract

Objective: Monoammonium glycyrrhizinate of Glycyrrhiza root has been used as an expectorant, detoxificator, anti-allergic, and antioxidant. Japanese researchers have been determined it has anti-viral activity in case of hepatitis A, B, C, D. We have isolated monoammonium glycyrrhizinate from Glycyrrhiza root, grown in Mongolia through a method presented in a previous study. The objective of the study was to develop prolonged release matrix tablet with hepatoprotective effect and to evaluate their pharmacotechnical qualities and the *in vivo* performance. Licozinat matrix tablets contained monoammonium glycyrrhizinate 140 mg; glycine 50 mg; LD-methionin 50 mg in each tablet.

Methods: In the present study the matrix tablet were prepared using HPMC K4000, lactose, glucose, microcrystalline cellulose, PVP-K30, talc and magnesium stearate in different ratios. The matrix tablet were prepared by wet granulation method and evaluated for weight variation, hardness, friability, *in-vitro* release, and *in vivo* study.

Results: Appropriate excipients were chosen for the matrix tablets: lactose as a diluent, 5% of PVP-K30 as a binder, HPMC as a matrix former, 3% of talc and 1% of magnesium stearate as a glidiant or lubricant. We have prepared the matrix tablets by wet granulation method and compressed the tablet mixture by a 2.5 kPa pressure. Formulation 5 (F5) was determined to be the most appropriate tablet design and it released the drug in a prolonged way during the *in vitro* testing. The hepatoprotective effect of matrix tablet in comparison to Glycyron tablet, were studied on CCl_4 induced hepatotoxicity in rats. The effect of Licozinat matrix tablet were compared with the Glycyron tablet that were administered to CCl_4 treated rats. On administration Licozinat matrix tablet decreased the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin and alkaline phosphatise (ALP).

Conclusion: We have developed and evaluated prolonged/controlled release matrix tablets with hepatoprotective effect. The Licozinat matrix tablets satisfied the quality criteria. On administration Licozinat matrix tablet decreased the level of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin and alkaline phosphatise (ALP).

Keywords: *Glycyrrhiza uralensis*; Monoammonium glycyrrhizinate; Matrix tablet; Hepatoprotective effect

Introduction

In global market of the pharmaceutical industry, tablets are the most popular method of oral drug administration. Furthermore, the rate of prolonged/controlled release matrix tablet has grown gradually in recent years [1-3]. Controlled release oral delivery systems are designed to: achieve therapeutically effective concentrations of drug in systemic circulation over an extended period of time; decrease the frequency of taking medicine; increase convenience to patients [1,2,4]. In Mongolia, in the field pharmaceutical technology the development of prolonged/controlled release matrix tablet has not been introduced yet.

In Mongolia, the morbidity rate of gastro intestinal diseases has ranked in the 2nd place of which liver diseases were the primary reason [5]. Monoammonium glycyrrhizinate from licorice root have been used as an expectorant, detoxificator, anti-allergic, and antioxidant. Japanese scientists have determined that it has anti-virus activity in patients with liver inflammation caused by hepatitis viruses A, B, C and D [6]. Therefore, we aimed to develop highly effective matrix tablet with hepatoprotective effect, containing monoammonium glycyrrhizinate extracted from licorice root grown in Mongolia.

Materials and Methods

The technological study of the developed matrix tablet Licozinat was conducted in the Laboratory of Technology and Chemistry, School of Pharmacy, Mongolian National University of Medical Sciences.

Hydroxypropyl methylcellulose (HPMC) K-4000 was bought from Shandong Liaocheng Hua Pharmaceutical Co., Ltd, China, glycine from Hubei provincial Bafeng Pharmaceuticals and Chemicals share Co., Ltd, DL- methionine from Zhangjiagang Huachang Pharmaceutical Co., Ltd, China. Polyvinylpyrrolidone K-30, glucose, lactose, microcrystalline cellulose (MCC), talc and magnesium stearate (Thianjin Well-Real Chemical Technology Co., Ltd., China).

All chemicals were of analytical reagent grade. Glycyron tablets (Lot No: 17060 produced in Minophagen Pharmaceutical Co., Ltd., Japan) were used for standard treatment group during *in vivo* study. Glycyron tablets contain monoammonium glycyrrhizinate 35 mg, glycine 25 mg and DL-methionine 25 mg in each tablet.

Formulation of matrix tablets

Tablets were prepared by wet granulation method. Each prepared tablet contained three active pharmaceutical ingredients (APIs) such as: monoammonium glycyrrhizinate 140 mg; glycine 50 mg; LD-methionin 50 mg. In order to develop appropriate tablets various excipients such as matrix former, diluents, binder, lubricant and glidiant were added. APIs and matrix former, diluent and binder were mixed properly and were granulated with the 5% solution of PVP K-30 as a binder solution. The wet mass was granulated by wet granulator (Shanghai Aligned Machinery Manufacture and Trade Co., Ltd.) through the sieve with 2 mm diameter holes and generated wet granules were dried at room temperature.

Dry granules were lubricated with talc and magnesium stearate [5]. The matrix tablets were prepared by the compression of the tablet mixture using rotary tablet machine ZP-19 (Shankhai Zhong Lian, China). In order to choose the appropriate excipients there were prepared tablets with various ratios of matrix former and different diluents (Table 1).

Ingredients		Formulation code				
		F1	F2	F3	F4	F5
Active ingredients	Monoammonium glycyrrhizinate (mg)	140	140	140	140	140
	Glycine (mg)	50	50	50	50	50
	LD- methionine (mg)	50	50	50	50	50
Matrix former	HPMC K4000 (mg)	30	45	60	85	100
	Lactose (mg)	230	--	--	--	160
Diluents	Glucose (mg)	--	215	--	175	--
	Microcrystalline cellulose (mg)	--	--	200	--	--
Binder	5% solution of PVP	qs	qs	qs	qs	qs
Lubricant and glidiant	Talc and magnesium stearate (mg)	qs	qs	qs	qs	qs
Total weight tablet (mg)		500	500	500	500	500

Table 1: Composition of Licozinat matrix tablet (All ingredients in mg/tablet).

Evaluation of matrix tablet: The quality of the prepared tablets was evaluated according to Mongolian National Pharmacopoeia's [7] methods by criteria's such as appearance, average weight, weight variation, hardness, friability, microbiological contamination and *in-vitro* dissolution study.

Determination of monoammonium glycyrrhizinate: Monoammonium glycyrrhizinate was determined from the studied tablets by a spectrophotometric HPLC method.

Methodology of high performance liquid chromatography

Chromatographic system and system suitability: Column C18 as the stationary phase and a mixture of methanol: glacial acetic acid: 0.2 mol/L ammonium acetate's solution (67:1:33) as the mobile phase. As detector a spectrophotometer set at a wavelength of 250 nm. Calculated with the reference to the peak of glycyrrhizinic acid. Flow rate: 1 ml/min, retention time is 10.3 min [8].

Reference solution: Weigh accurately 10 mg of glycyrrhizinic acid CRS in 50 ml volumetric flask, add 45 ml of the mobile phase, ultrasonicate to dissolve, cool, add the mobile phase to volume and shake well (containing 0.2 mg/ml of monoammonium glycyrrhizinate, equal to 0.1959 mg of glycyrrhizinic acid per ml).

Test solution: 20 matrix tablets were powdered in mortar. Weight accurately 35,7 mg of powdered tablets in 50 ml volumetric flask, add 45 ml of the mobile phase, ultrasonicate to dissolve, cool, add the mobile phase to volume and shake well and filter.

Procedure: Inject accurately 10 µl of each of the reference solution and the test solution into the column, respectively; calculate the content [8].

***In-vitro* dissolution study:** USP type II (Rotating basket) dissolution test apparatus(Freund Jasco DT-610) was used at 100 rpm at a temperature of 37 ± 0.5°C and 900 ml of dissolution media (Phosphate Buffer (pH=6.8)) and matrix tablet was placed in the basket [9]. 10 ml samples were taken at 0.75, 1, 2, 4, 6, 8, 10, 12, 16, 24 hours [10]. The sample (10 ml) was transferred to 25 mL volumetric flask and added mixture of Phosphate Buffer (pH-6.8): Ethanol (70:30) to volume. The sample solution was then filtered through Whatman filter No.41. The absorbance was measured by UV spectrophotometer at 254 nm.

Reference solution: The standard solution was prepared by dissolving 25 mg of glycyrrhizinic acid in mixture of Phosphate Buffer (pH=6.8): Ethanol (70:30) in 25 ml of volumetric flask and added to volume and filtered through Whatman filter No.41. Measure accurately 1 ml of filtrate to a 25 ml volumetric flask, add to volume and shake well. The absorbance was measured by UV spectrophotometer at 254 nm [11].

***In-vivo* study:** A total of 90 rats (200-250 gr) were used for this study. The animals were kept in well ventilated room in the animal house of College of Mongolian Medicine and Pharmacy, Inner Mongolia University for the Nationalities. They were given standard food pellets and allowed drinking water. Ethical clearance for the use of animals was obtained from the Committee constituted for the purpose.

A total of 90 rats were taken and divided into nine groups. Each group having ten rats (n=10). Healthy group and Control group received normal diet and drinking water. Standard treatment group received Glycyron tablet (1 mg/kg) 2 times daily for 10 days. Experimental 6 groups received Licozinat matrix tablet by various doses (1 mg/кg × 1 times daily; 2 mg/кg × 1 times daily; 3 mg/кg × 1 times daily; 1 mg/кg × 2 times daily; 2 mg/kg × 2 times daily; 3 mg/кg × 2 times daily) for 10 days. After 24 hours of the last treatment, all rats were induced liver inflammation with CCl_4. After 24 hours of the treatment with CCl_4, anesthetized with chloral hydrate and blood was collected by cardiac puncture and serum was separated for estimations of serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), serum alkaline phosphatase (ALP) and serum bilirubin. Liver was taken for histopathological examination [12].

Results and Discussion

The quality of tablet was evaluated according to Mongolian National Pharmacopoeia methods by criteria's such as appearance, average weight, weight variation, hardness, friability, microbiological

contamination and *in-vitro* dissolution study. The result is shown in Table 2.

Acceptance criteria		F1	F2	F3	F4	F5
Appearance	**Whitish, flat, beveled edged**	**Complies**	**Complies**	**Complies**	**Complies**	**Complies**
Average weight (500 mg)	0.475 - 0.525	0.503	0.508	0.505	0.501	0.502
Weight variation	± 5%	+1.9, -1.5	0.3	+1.8, -1.4	+1.4, -1.2	+0.9, -0.7
Friability	Not less than 97%	98.24	98.72	98.06	98.4	99.33
		± 0.03	± 0.02	± 0.03	± 0.02	± 0.01
Hardness	4.58-12.23 kg/cm2	5.54 ± 0.1	4.9 ± 0.12	5.16 ± 0.1	5.38 ± 0.12	5.3 ± 0.1
Monoammonium glycyrrhizinate, g	0.066-0.073	0.069	0.069	0.68	0.068	0.069
		± 0.003	± 0.001	± 0.003	± 0.001	± 0.002
Total aerobic bacteria, CFU/ml	Not more than 105	Not more than 105	Not more than 105	Not more than 105	Not more than 105	Not more than 105
Mould, CFU/ml	Not more than 102	Not reported	Not reported	Not reported	Not reported	Not reported
E. coli and Coliform/1 g	--	Not reported	Not reported	Not reported	Not reported	Not reported
Note: p<0.05 or the accuracy of the statistics 95% and formulations were evaluated and satisfied the above quality criteria by Mongolian National Pharmacopoeia methods.						

Table 2: Quality of matrix tablets.

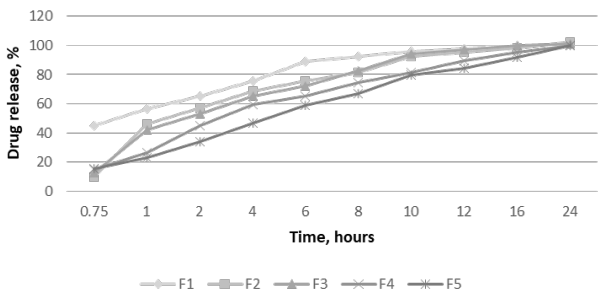

Figure 1: Drug release from the experimental formulations (n=5).

From the result, F5 design of matrix tablets released drug at 0.75, 1, 2, 4, 6, 8, 10, 12, 16, 24 hours by 15.28% ± 0.11, 22.69% ± 0.12, 34.14% ± 0.25, 46.48% ± 0.17, 58.6% ± 0.24, 66.98% ± 0.22, 79.95% ± 0.08, 84.42% ± 0.21, 92.12% ± 0.11, 99.86% ± 0.52 was determined to be the appropriate design (Figure 1).

***In-vitro* study:** For evaluating the *in vitro* drug release, USP type II (Rotating basket) dissolution test apparatus (Freund Jasco DT-610) was used at 100 rpm at a temperature of 37 ± 0.5°C and 900 ml of dissolution media. A single tablet was placed in the basket and filled 900 ml Phosphate Buffer (pH=6.8). The samples were taken at 0.75, 1, 2, 4, 6, 8, 10, 12, 16, 24 hours. The quantity of monoammonium glycyrrhizinate was measured by UV spectrophotometer at 254 nm. For matrix tablets, it needs to release 10-30% of drug for 60 minutes, 40-60% of drug after 240 minutes. But F1, F2, F3 designs of matrix tablets were release 40-60% of drug in first 60 minutes. Therefore, after 240 minutes F1, F2, F3 designs of matrix tablets release 65-76% of drug.

***In vivo* study:** The hepatoprotective effect of the chosen matrix tablets (F5) in comparison to Glycyron tablets, were studied on CCl_4 induced hepatotoxicity in rats. The licozinat matrix tablets (various doses) in parallel with the Glycyron tablet were administered before the CCl_4 induced hepatotoxicity in rats (Table 3).

Groups	Serum ALT (U/L)	Serum AST (U/L)	Serum ALP (U/L)	Serum bilirubin
Healthy	34.05 ± 6.43	85.36 ± 12.15	219.5 ± 49.94	1.13 ± 0.35
Control	473.25 ± 66.82	713.27 ± 63.21	311 ± 46.78	2.64 ± 0.2
Standard treatment/Glycyron/1 mg/кg × 2 times daily	201.6 ± 34	276.56 ± 71.11	281.12 ± 33.18	1.65 ± 0.14

Licozinat matrix tab 1 mg/кg × 1 times daily	264.48 ± 58.16	458.13 ± 76.41	281.86 ± 24.09	1.78 ± 0.36
Licozinat matrix tab 2 mg/кg × 1 times daily	197.48 ± 57.08	253.06 ± 96.18	307.2 ± 50.94	1.45 ± 0.19
Licozinat matrix tab 3 mg/кg × 1 times daily	357 ± 43.3	345.52 ± 102.35	252.67 ± 39.84	2.1 ± 0.59
Licozinat matrix tab 1 mg/кg × 2 times daily	154.3 ± 38.58	380.32 ± 71.37	261 ± 33.84	2 ± 0.22
Licozinat matrix tab 2 mg/кg × 2 times daily	330.5 ± 52.47	349.38 ± 95.43	313.6 ± 49.99	2.28 ± 0.58
Licozinat matrix tab 3 mg/кg × 2 times daily	254.8 ± 60.37	377.82 ± 100.74	281.4 ± 55.65	2.14 ± 0.25

Table 3: Defendence of Licozinat matrix tablet on serum bilirubin, ALT, AST and ALP level, $p<0.05$ or the accuracy of the statistics 95%.

From the show results, the administration of Licozinat matrix tablets 2 mg/kg doses 1 times daily decreased the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum alkaline phosphatase and the total bilirubin 1-3 times, in compared with levels of control group animals. It was approximate to standard treatment group (glycyron 1 mg/kg doses 2 times daily). Licozinat matrix tablets 2 mg/kg doses 1 times daily were determined appropriate dose and administration.

Conclusion

Controlled release "Licozinat" matrix tablets were prepared by wet granulation method. Formulation (F5) containing 20% HPMC K4000 releases in the desired manner and was determined to be the appropriate design. On administration of Licozinat matrix tablet 2 mg/kg doses 1 times daily in rats the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin and alkaline phosphatise (ALP) have decreased. The prepared Licozinat matrix tablets have hepatoprotective effect. Licozinat matrix tablets have determined some advantages such as achieved therapeutically effective concentrations of drug in systemic circulation over an extended period of time, decreased the frequency of taking medicine (one time daily), increased convenience to patients.

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