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Research Article

HEPATOPROTECTIVE ACTIVITY OF SILYMARIN FLOATING DRUG DELIVERY SYSTEM AGAINST ANTI TUBERCULOSIS DRUG

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ABSTRACT

A Gastroretentive floating controlled drug delivery system containing Silymarin was prepared in the form of tablets and evaluated for its processing parameters, in vitro release in 0.1 N HCl. Eight formulations were prepared by using rate controlling polymers such as HPMC K4M and Eudragit RS100, alkalizing agent sodium bicarbonate and solubilizing agent poly vinyl Pyrrolidone (PVP K30). Floating tablets were prepared by direct compression method. The preformulation studies and tablet evaluation tests were performed and results were within the limits. Tablets remained buoyant over 20 hours in the release medium and the amount of sodium bicarbonate found to be significant for not only to remaining buoyant without causing disintegration of the tablet. The different ratios of polymers 15% and 20% showed the significant difference in the drug release with increasing in the concentration of solubilizing agent PVP K30. All the formulations exhibited diffusion dominant drug release. Stability studies for all formulations were conducted for a period of 60 days at 4º±2ºC, 27º±2ºC and 45º±2ºC

respectively and the formulations showed no significant changes in physical appearance, drug content and in-vitro drug release even after 60 days. The control release of the drug from the dosage form shows the hepatoprotective activity against Isoniazid (INH) + Rifampcin (RIF) induced hepatotoxicity in rats.

Keywords: Floating drug delivery system, controlled drug release, low-density polymers, alkalizing agent, Silymarin, Isoniazid (INH) and Rifampcin (RIF).

Introduction

Tuberculosis is one of the most common infectious diseases. In India, pulmonary tuberculosis is one of the major causes for adult deaths. Isoniazid (INH) and Rifampcin (RIF), the first line drugs used for tuberculosis chemotherapy are associated with hepatotoxicity[1]. Oxidative stress as one of the mechanism for INH + RIF induced hepatic injury. INH and RIF are metabolized in the liver by acetylation and hydrolysis. The metabolites of INH and RIF are hepatotoxins and produces liver damage.

Majority of normally formed free radicals (free oxygen) are removed by the action of reduced glutathione. Glutathione is an enzyme present in the liver. Glutathione is reduced as a result of administration of INH and RIF. By reduction in glutathione, Lipid Peroxidation (LPO) process (i.e., oxidation of lipids) initiated. As the result of LPO, hepatic tissues are injured.

Silybum marianum seed extract has been called Silymarin and consisting of Silybin, Silychristin, Silydianin and Isosilybin. The flavonoid Silybin constitutes 60% to 70% of Silymarin used as an effective treatment of for liver disease[2].

In view of this, the present study was aimed at evaluating the Hepatoprotective activity of Silymarin formulations against INH and RIF induced Hepatotoxicity in albino rats.

MATERIAL AND METHODS

Materials

Silymarin, Hydroxy propyl methyl cellulose (HPMC K4M) and Eudragit RS100 were obtained as gift sample from Micro Labs, Hosur. Polyvinyl pyrrolidone (PVP K30) was obtained from Granules India Ltd., Hyderabad. Other reagents and solvents were of analytical grade.

Method

Preparation of Silymarin floating tablets[3]

The drug, polymers (at different ratios), PVP K30, sodium lauryl sulfate (SLS), sodium bicarbonate ($NaHCO_3$) and lactose were blended thoroughly in a motor and pestle and then passed through sieve no. 100. The powder blend was mixed with talc (2%) and tablets were prepared by direct compression method using a single punch-tableting machine (Minipress-I) with hardness 6 kg/cm^2. Eight formulations were prepared and coded them from F1 to F8. The details of composition of each formulation are shown in **Table-1**.

Table1: Formulations of Silymarin floating tablets

S.No.	INGREDIENTS	FORMULATIONS OF SILYMARIN FLOATING TABLETS*							
		F1	F2	F3	F4	F5	F6	F7	F8
01.	SILYMARIN	280	280	280	280	280	280	280	280
02.	HPMC K4M	75	100	100	--	--	--	50	50
03.	EUDRAGIT RS 100	--	--	--	75	100	100	50	50
04.	$NaHCO_3$	70	70	70	70	70	70	70	70
05.	PVP K30	20	20	40	20	20	40	20	40
06.	SLS	3	3	3	3	3	3	3	3
07.	TALC	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S
08.	LACTOSE	45	20	--	45	20	--	20	--

* Weight of one tablet is 500mg.

F1 - HPMC K4M (15%w/w) and PVP K30 (4%w/w); F2 - HPMC K4M (20%w/w) and PVP K30 (4%w/w); F3 - HPMC K4M (20%w/w) and PVP K30 (8%w/w); F4 – Eudragit RS100 (15%w/w) and PVP K30 (4%w/w); F5 – Eudragit RS100 (20%w/w) and PVP K30 (4%w/w); F6 – Eudragit RS100 (20%w/w) and PVP K30 (8%w/w); F7 – HPMC K4M (10%w/w), Eudragit RS100 (10%w/w) and PVP K30 (4%w/w); F8 – HPMC K4M (10%w/w), Eudragit RS100 (10%w/w) and PVP K30 (8%w/w)

EVALUATION OF GRANULES (OR) POWDER BLEND

Preformulation studies such as angle of repose, bulk density, tapped density, compressibility index and Hausner's ratio were determined for their micromeritic properties.

EVALUATION OF FLOATING TABLETS

To design tablets and tablets production quality, the formulated tablets were evaluated for hardness test[4] (using Monsanto Hardness Tester), friability test[5] (using Roche Friabilator) and weight variation test[6].

Buoyancy / Floating test[7]

The time interval between introduction of tablet into the dissolution medium and its floatation to the top of the dissolution medium was termed as buoyancy lag time (BLT). The duration upto which the tablet floats on the dissolution medium was taken as duration of buoyancy (DB). Both BLT and DB were determined using USP 24 type II dissolution apparatus in 900ml of 0.1N HCl at 37°±1°C.

Drug content

Five tablets from each formulation were taken and grinded into fine powder. From this equivalent to 100mg of Silymarin powder was weighed and dissolved in sufficient quantity of methanol and diluted with 0.1 N HCl. The samples were analysed spectrophotometrically at 286 nm[8].

IR Spectral Analysis

A drug-polymers interaction was studied by using FTIR[8] (Shimadzu, Japan, Model-8400s). IR Spectral Analysis of pure Silymarin, HPMC K4M, Eudragit RS100 were carried out. The peaks and the patterns produced by the pure drug were compared with peaks and patterns of pure drug with combination of polymers.

Dissolution studies[3]

In vitro drug release of all the formulations were carried out using USP–type II dissolution apparatus (paddle type). The dissolution medium, 900 ml 0.1N HCl solution, was placed into the dissolution flask maintaining the temperature of 37 $\pm$ 0.5^{o}C and rpm of 50. One Silymarin tablet was placed in the dissolution apparatus. The apparatus was allowed to run for 7 hours. Samples measuring 10 ml were withdrawn every 30mts intervals upto 7 hours. The fresh dissolution medium (37^{o}C) was replaced every time with the same quantity of dissolution medium to maintain the same volume of dissolution medium. Collected samples were diluted upto 100ml with 0.1N HCl and analyzed at 286nm using 0.1N HCl as blank. The cumulative percentage drug release was calculated.

Drug release kinetics (Curve Fitting Analysis) [9,10,11]

To analyze the mechanism of the drug release rate kinetics of the dosage form, the data obtained were fitted into zero order, first order, Higuchi's model and Korsmeyer's equation release models. The model with the highest correlation coefficient was considered to be the best model. The value of 'n' in the Korsmeyer's model indicates the release mechanism.

Stability studies [12]

It was carried out to evaluate the stability of the drug. All the formulations were stored at 4°±2°C, 27°±2°C (RH 65%$\pm$5%) and 45°$\pm$2°C (RH 75%$\pm$5%) temperatures for 60 days. Two tablets were taken from all the stored samples at the intervals of 15th, 30th, 45th and 60th days. The drug content

analysis and in vitro release studies were carried out to determine the percentage of Silymarin released using U.V. spectrophotometric at 286nm. Stability studies are used to find out whether any chemical degradation of Silymarin formulations takes place or not.

Pharmacodynamic studies

Healthy Wister albino rats of either sex weighing about 150-200gm are selected and used in the present study. The animals were acclimatized for 2 weeks prior to experiment. The animals were free to access standard pellet food and water.

Isoniazid (INH) and Rifampcin (RIF) were used to produce hepatotoxicity in rats. INH and RIF were dissolved in sterile distilled water and administered at the dose of 12.5mg/kg body weight to 24 animals for14 days.

These above animals were divided into 4 groups [Group IIA, Group IIB, Group IIC and Group IID] and treated orally as follows:

- **Group I:** 6 rats were treated with 1ml of 1%w/v Carboxy Methyl Cellulose (CMC) for 14 days (normal control) at the dose of 12.5mg/kg body weight.
- **Group II:** 24 rats were treated with INH + RIF for 14 days (10mg/kg). On 15^{th} day these 24 animals were divided into 4 groups (i.e., IIA, IIB, IIC and IID).
- **Group IIA:** Among 24 animals treated with INH + RIF, 6animals were selected and served as hepatotoxic control and was not treated with any drug.
- **Group IIB:** 6 rats were treated with Silymarin tablets without polymer suspended in 1%w/v CMC at the dose of 12.5mg/kg body weight.
- **Group IIC:** 6 rats were treated with drug + HPMC K4M (F3) suspended in 1%w/v CMC at the dose of 12.5mg/kg body weight.

- **Group IID:** 6 rats were treated with drug + Eudragit RS 100 (F6) suspended in 1%w/v CMC at the dose of 12.5mg/kg body weight.

Biochemical analysis

Several plasma proteins, including bilirubin, are synthesized in the liver, and the liver also regulates plasma lipids and lipoproteins. Therefore, the levels of total protein and total bilirubin can be used as indicators of liver function. Serum ALT, AST, ALP and GGTP activities are considered as good markers of hepatic injury and hepatocellular integrity. The activities of these enzymes before starting the treatment (as baseline values) and after the treatment of rats with the drugs are measured.

Blood samples obtained from tail vein was centrifuged for 10min at 3000rpm to separate the serum and serum samples were stored in a deep freezer until they could be analyzed. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamyl-3-carboxy-4-nitroanilide (GGTP) activities and the levels of serum albumin, serum total protein, and serum total bilirubin were determined by Hitachi-917 auto-analyzer.

On the 15^{th} day, Group IIB, IIC and IID animals were treated with Silymarin tablet without polymer, Silymarin with HPMC K4M and Silymarin with Eudragit RS 100 respectively. After 7 hours of these drug treatments the blood samples were collected, centrifuged and serum samples were analyzed to determine the above parameters. The results are presented in **Table-2**.

Table 2: Effect of various formulations of Silymarin in different biochemical parameters in INH + RIF induced hepatotoxic rats.

Groups		Total Bilirubin (U/L)	Total Protein (U/L)	Alkaline Phosphatase (ALP) (U/L)	Aspartate Transaminase (AST) (U/L)	Alanine Transaminase (ALT) (U/L)	Gamma glutamyl-3-carboxy-4-nitroanilide (GGTP) (U/L)
Group I		0.62 ± 0.06	9.52 ± 0.04	121.6 ± 3.01	124.40 ± 3.64	35.46 ± 1.14	94.8 ± 1.88
Group II	**IIA**	2.01 ± 0.12	4.28 ± 0.08	340.0 ± 6.64	424.41± 9.62	152.80 ± 3.40	180.8 ± 6.20
	IIB	1.21 ± 0.04*	5.71 ± 0.10*	204.61 ± 1.16*	254.64 ± 1.16*	91.68 ± 1.62*	108.48 ±1.28*
	IIC	1.31 ± 0.11*	6.18 ± 0.02*	221.84 ± 1.21*	275.86 ± 1.06*	99.32 ± 1.87*	117.52 ± 1.90*
	IID	1.27 ± 0.04*	5.99 ± 0.10*	214.20 ± 1.06*	267.37 ± 1.15*	96.26 ± 1.40*	113.90 ± 1.28*

Each value represents the average value of 6 readings.

* Significant relative to hepatotoxic control $p < 0.01$.

RESULTS AND DISCUSSION

The Silymarin floating tablets using two polymers and in combination of polymers with different ratios was prepared by direct compression method. Before compression of the powder, preformulation studies such as Bulk density, Tapped density, Angle of repose, Compressibility index and Hausner's ratio were determined for all formulations. All the parameters are within the acceptable limits for powder bled to show good flow properties while formulating tablets.

The tablets were prepared and evaluated for the hardness, friability, weight variation, drug content and buoyancy determination. The buoyancy lag time of all formulations was ranged from 96 to

174 sec. and duration of buoyancy was more than 20h. These results exhibited satisfactory floatable ability because of their low density and internal voids.

IR spectrum of pure Silymarin and Silymarin with combination of polymers showed no significant interactions between the drug and polymers and they are compatible with each other

The in-vitro dissolution studies of all the formulations are performed. The percentage drug release at the end of 7 h from F1, F2, F3, F4, F5, F6, F7 and F8 were found to be 40.26%, 36.16%, 45.21%, 39.67%, 34.15%, 43.95%, 35.29% and 44.43% respectively. As the ratio of the polymers (HPMC K4M and Eudragit RS100) increased from 15% to 20%, the percentage drug release was decreased that is in formulation F2 (36.16%) and F5 (34.15%) when compared to the formulations F1 (40.26%) and F4 (39.67%). The formulations F3, F6 and F8 (i.e., 45.12%, 43.95% and 44.43%) showed slightly higher percentage drug release when compared to the formulations F2, F5 and F7 (i.e., 36.16%, 34.15% and 35.29%) due to solubilization property of PVP K30 and increased concentration of PVP K30 from 4% to 8%. However, when the drug release of F1 and F2 (using HPMC K4M) is compared with the F4 and F5 (using Eudragit RS100) showed less drug release due to the less water permeability of Eudragit RS100 when compared with HPMC K4M [13].

The results of four kinetic models namely zero order equation, first order equation, Higuchi's equation and Korsmeyer's equation. All formulations follow the first order release rate (R^2: 0.8814 to 0.9423). Higuchi's equation proved that (R^2= 0.9544 to 0.9829) the diffusion is the dominant mechanism in all the formulations[10]. In Korsmeyer's equation if n=0.45 or n<0.45, it is Fickian diffusion[11]. Kosmeyer's plot proved that the n values are <0.45 for all the formulations. Therefore all formulations follow Fickian release mechanism[11].

Stability studies were performed for all the formulations. All the formulations were stored at 4°±2°C, 27°±2°C (RH 60±5%) and 45°±2°C (RH 75±5%) for 60 days. After an interval of 15th, 30th,

45th and 60th days the samples were withdrawn and tablet evaluation tests were conducted. There was no colour change and there were no deviations in all tests. There was no deviations in the percentage of drug release also. It showed that all formulations remain stable for 60 days.

In present study, administration of INH + RIF treated rats showed an increase in the levels of total bilirubin, serum marker enzymes such as AST, ALP, ALT, GGTP and reduction in total protein in the hepatotoxic control rats (Group-IIA) at the end of 7 hours when compared to the control group of animals without any drug (Group-I). It indicates that liver has been injured.

Animals treated with Silymarin tablets without polymer and Silymarin floating tablet formulations (F3 and F6) showed significant decrease in serum hepatic enzymes and increase in total protein as compared to the hepatotoxic control.

The silymarin tablets without polymer showed significant decrease in the serum marker enzyme levels (AST = 254.64, ALP = 204.4, ALT = 91.68, GGTP = 108.48) and total bilirubin (1.21) with significant increase in total protein (5.71) level when comparing hepatotoxic group animals (Group IIA). This may be due to immediate release of Silymarin from the tablets prepared without polymers. But in Silymarin floating tablets prepared with polymers HPMC K4M and Eudragit RS 100 showed the following levels:

Drug + HPMC K4M (F3): AST = 275.85, ALP = 221.84, ALT = 99.32, GGTP = 117.52, total bilirubin = 1.31 and total protein = 6.18.

Drug + Eudragit RS 100 (F6): AST = 267.85, ALP = 214.50, ALT = 96.26, GGTP = 113.90, total bilirubin = 1.27 and total protein = 5.99.

These results showed that there was slight increase in the serum marker enzymes, total bilirubin and slight decrease in the total protein when compared to Silymarin tablet without polymers. This may be due to the controlled release of Silymarin from the floating tablets prepared by the polymers. After 7 hours

it showed that levels of serum marker enzymes (AST, ALP, ALT and GGTP) and total bilirubin was decreased with increase in total protein. If the treatment is continued for 14 to 15 days serum marker enzymes, total bilirubin and total protein may reach the normal values.

From this study, Silymarin floating tablet formulations showed the significant protective action against hepatotoxic effect produced by Isoniazid (INH) and Rifampicin (RIF).

Conclusion

The present study was carried out to develop the floating drug delivery system using HPMC K4M and Eudragit RS100 polymers as carriers. The results of experimental studies of Silymarin floating tablets proved that the powder blend showed good flow properties, tablet evaluation tests are within the acceptable limits, IR spectral analysis proved that there was no drug-polymer interaction, percentage drug release was controlled and the formulations were stable after storing at different temperatures for 60 days. Thus, results of the current study clearly indicates, a promising potential of the Silymarin floating system as an alternative to the conventional dosage form. The Pharmacodynamic activity of Silymarin showed the protective action against hepatotoxicity in animals.

Further, clinical investigation of Silymarin floating tablets in human volunteers may improve the patient compliance. Such an attempt will be useful to release Silymarin floating drug delivery system in the market in the near future.

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