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THE PENETRATING ABILITY OF SILYBUM MARIANUM FLAVOLIGNANS IN VITRO

CAPACITATEA PENETRANTĂ A FLAVOLIGNANILOR DIN SILYBUM MARIANUM IN VITRO

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Rezumat. În articol sunt prezintate rezultatele determinării capacității de penetrare a flavolignanilor din armurariu, utilizând modele *in vitro*. Cea mai bună capacitate de penetrare a flavolignanilor este asigurată de combinația extractului uscat de armurariu cu suma saponinelor totale de lemn dulce (89,57% masă), standardului de silimarină cu aceleași saponine (85,75% masă), extractului uscat de armurariu cu lecitină de soia (84, 37% masă). La utilizarea dodecilsulfatului de sodiu (SDS), are loc o diferențiere clară a curbelor de absorbție, fapt ce permite utilizarea SDS ca model pentru studierea absorbției flavolignanilor.
Cuvinte cheie: *Silybum marianum*, capacitate de penetrare, flavolignani.
Abstract. The article presents the results of determining the penetrating ability of milk thistle flavolignans using *in vitro* models. The best penetrating abilityof flavolignans is provided by a combination of milk thistle dry extract with the total licorice saponins (89.57% by weight), standard of sylimarinewith saponins (85.75% by weight), dry extract with soy lecithin (84, 37% by weight). With the use of sodium dodecyl sulfate (SDS), a clear differentiation of the absorption curves occurs, which may allow the use of SDS as a model for studying the absorption of flavolignans.
Keywords: Milk thistle, penetrating ability, flavolignans.

INTRODUCTION

Dosage forms based on milk thistle fruits are widely represented on the modern pharmaceutical market and are used for pharmacotherapy of liver diseases such as alcoholic and non-alcoholic cirrhosis, drug liver pathology (DIL), viral hepatitis. The antitoxic, antioxidant, anti-inflammatory and antifibrotic effects of silymarin and positive effect on the processes of cellular regeneration of hepatocytes have been well studied.

Recent studies indicate the promise of using milk thistle flavolignans in the treatment of iron overload caused by β-thalassemia major, where silymarin is used as an adjuvant to standard iron chelators [1]. Research is also underway regarding its use in the complex therapy of prostate cancer [2].

The activity of silymarin against SARS-CoV-2 infection is currently being studied. It has been proven to work on multiple targets at once in the fight against COVID-19. First, silibinin prevents the development of a cytokine storm in severe disease by directly inhibiting STAT3. Secondly, it is an inhibitor of RNA-dependent RNA polymerase, allowing effective suppression of viral replication, thereby reducing viral load [3]. Thirdly, during COVID-19 therapy, drugs can be used that negatively affect the liver, which justifies the use of silymarin as a hepatoprotector.

However, milk thistle flavolignans have low oral bioavailability (the absolute bioavailability of silibinin is about 0.95%) [4]. Therefore, this work will consider methods for increasing the bioavailability of silymarin and the nature of the penetrating ability of flavolignans through semipermeable membranes *in vitro*.

MATERIALS AND METHODS

The objects of research were a dry extract based on water-acetone extraction from crushed plant raw materials (Silybum marianum fructus manufactured by Biotest Ltd), «Legalon® 140» capsules, and a standard sample of silymarin for thin layer chromatography (TLC).

Dialysis membranes were used to study the penetrating ability; Phosphate buffered saline with the addition of ursodeoxycholic acid and pancreatin, pH = 7.4, solution volume 3.6 ml, was used to simulate the intestinal environment. For the model of systemic blood flow, a similar buffer was used that did not contain bile acids and enzymes, pH = 7.4, solution volume 18 ml.

Soy lecithin, sodium dodecyl sulfate (SDS), as well as the total triterpene saponins obtained by extraction with acetone and nitric acid (20: 1), followed by precipitation of the totalsaponins with an aqueous solution of ammonia from plant raw materials (Glycyrrhizae radices).

The incubation was carried out at 38.0 ° C for 6 hours. Sampling for spectrophotometry was carried out after 30, 60, 90, 120, 180, 270 and 360 minutes.

The content of silymarin was determined spectrophotometrically using the methodology of the State Pharmacopoeia of the Republic of Belarus. The optical density was measured at a wavelength of 289 nm. The concentration (mg / ml) of silymarin was calculated using the formula obtained on the basis of the calibration graph:

$$X, \% = \frac{A * 0,3 - 0,0017}{0,7511}$$

where A is the optical density of the test solution.

The data obtained was processed in Microsoft Office Excel 2016, the Data Analysis package.

RESULTS AND DISCUSSIONS

Figure 1 shows the dependence of the concentration

of penetrated flavolignans on the incubation time without the addition of surfactants. The charts have two minimums: at 60 and 120 minutes. At 90 minutes, a peak is observed, then, starting from 180 minutes, the concentration of penetrated silymarin increases, reaching its maximum at 270 minutes, after which it reaches a plateau.

Table 1 shows the percentage of penetration of silymarin in the test samples. It follows from it that the greatest penetrating ability is possessed byLegalon® 140 (69.83%).

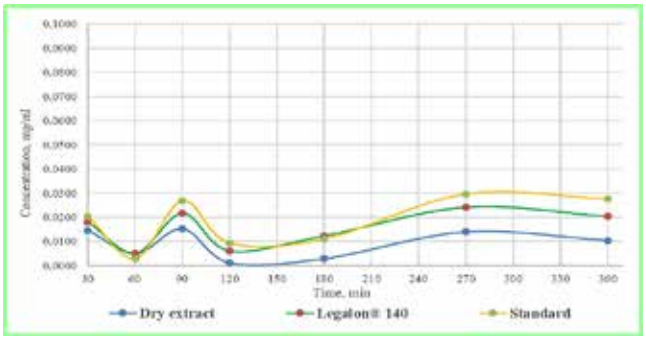

Figure 1. Graphical dependence of the concentration (mg / ml) of penetrated flavolignans from time (min)

Table 1. Mass fraction of flavolignans passed through the membrane

	Initial weight of silymarin in solution, mg	Peak mass of penetrated flavolignans, mg	Percent penetration
Dry extract	1.4523	0.2748	18.92%
Legalon® 140	0.6231	0.4351	69.83%
Standard	1.2289	0.5430	44.18%

Further, the stability of flavolignans in a buffer without the use of a membrane was investigated. Figure 2 shows the dependence of the concentration of flavolignans on the incubation time. It follows from this that the concentration of flavolignans decreases sharply at 60 minutes, then increases again at 90 minutes and gradually decreases with further incubation.

Figure 3 shows the dependence of the concentration of penetrated flavolignans on the incubation time after adding 3% (by weight) soy lecithin to the test solutions, followed by filtration. The penetration curves for the dry extract and the standard of sylimarinhave the minimum concentration values at the points corresponding to 30 minutes of incubation, then a peak is observed at 90 minutes, after which the concentration decreases again and gradually reaches a plateau. Schedule forLegalon® 140 has a slightly different character. It is observed at least at 30 minutes, however, in the time interval of 60-360 minutes, fluctuations in concentration indicators are insignificant.

Table 2 shows the percentage of silymarin penetration in the test samples after the addition of soy lecithin. It follows from it that the greatest penetrating ability is possessed bydry extract (84.37%).

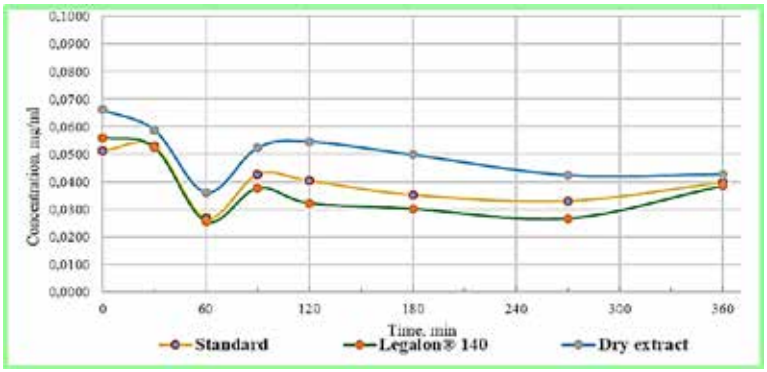

Figure 2. Dependence of the concentration (mg / ml) of flavolignans on the incubation time (min)

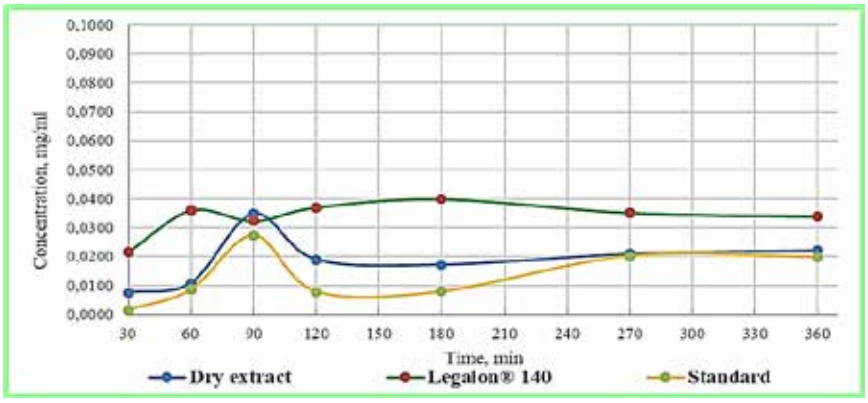

Figure 3. Graphical dependence of the concentration (mg / ml) of penetrated flavolignans on the time (min) after the addition of soy lecithin

Table 2. Mass fraction of flavolignans passed through the membrane after adding soy lecithin

	Initial weight of silymarin in solution, mg	Peak mass of penetrated flavolignans, mg	Percent penetration
Dry extract	0.7453	0.6289	84.37%
Legalon® 140	1.5602	0.7164	45.92%
Standard	0.7818	0.4905	62.74%

Figure 4 shows the dependence of the concentration of penetrated flavolignans on the incubation time after adding 3% (by weight) sodium dodecyl sulfate to the test solutions, followed by filtration. The graphs have a minimum concentration at 30 minutes, a peak is observed at 120 minutes, after which there is a slight decrease in concentration with a subsequent increase. No plateau at 360 minutes is observed.

Table 3 shows the percentage of penetration of silymarin in the test samples after the addition of sodium dodecyl sulfate. It follows from it thatdry extract has the greatest penetrating ability (15.93%).

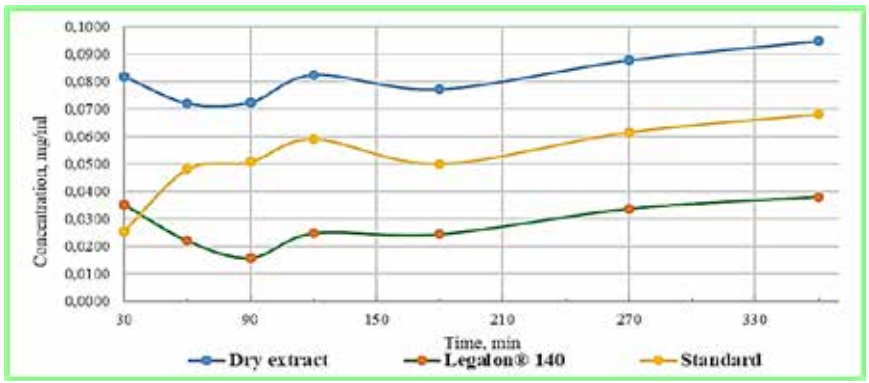

Figure 4. Graphical dependence of the concentration (mg / ml) of penetrated flavolignans versus time (min) after the addition of sodium dodecyl sulfate (SDS)

Table 3. Mass fraction of flavolignans passed through the membrane after adding sodium dodecyl sulfate (SDS)

	Initial weight of silymarin in solution, mg	Peak mass of penetrated flavolignans, mg	Percent penetration
Dry extract	1.7988	0.2865	15.93%
Legalon® 140	2.9530	0.0817	2.77%
Standard	2.3448	0.1905	8.13%

Figure 5 shows the dependence of the concentration of penetrated flavolignans on the incubation time after adding 3% (by weight) of the totallicorice saponins to the test solutions, followed by filtration. Penetration graphs forLegalon® 140and standard have minimum concentration values at 60 and 120 minutes, a peak is observed at 90 minutes. Schedule fordry extract has a maximum concentration at 30 minutes, minimum - at 60. The dependence after 120 minutes for three samples is similar. At first, an increase in concentration is observed up to 270 minutes, after which it decreases slightly.

Table 4 shows the percentage of silymarin penetration in the test samples after adding total of the licorice saponins. It follows from it thatdry extract has the greatest penetrating ability (89.57%).

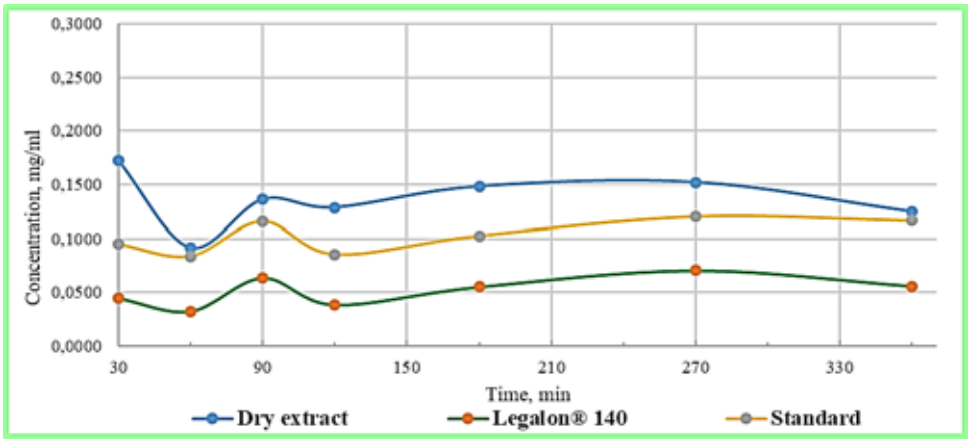

Figure 5. Graphical dependence of the concentration (mg / ml) of penetrated flavolignans on time (min) after adding the total licorice saponins

Table 4. Mass fraction of flavolignans passed through the membrane after adding the sum of licorice saponins

	Initial weight of silymarin in solution, mg	Peak mass of penetrated flavolignans, mg	Percent penetration
Dry extract	3.4687	3.1068	89.57%
Legalon® 140	1.4277	1.1879	83.20%
Standard	2.4406	2.0927	85.75%

Table 5 shows the penetration rates (in percent) of silymarin from pure samples and after the addition of various surfactants. It follows from this that the addition of soy lecithin increases the penetrating ability of flavolignans by an average of 45.20%, total licorice saponins – by 94.47% compared to the initial parameters. The addition of sodium dodecyl sulfate, on the other hand, reduces the passage of silymarin through the semipermeable membrane by 79.82% compared to the initial one.

Table 5. Percentage of penetration of flavolignans through the membrane

	Dryextract	Legalon® 140	Standard	Average
No surfactant added	18.92%	69.83%	44.18%	44.31%
With added soy lecithin	84.37%	45.92%	62.74%	64.34%
With the addition of SDS	15.93%	2.77%	8.13%	8.94%
With the addition of the total licorice saponins	89.57%	83.20%	85.75%	86.17%

CONCLUSIONS

In the course of the work, it was experimentally found that the addition of various surfactants to varying degrees affects the penetrating ability of flavolignans in vitro.

The best penetrating power is provided by a combination of dry extract with the total licorice saponins (**89.57%** by weight), standard of sylimarin with saponins (**85.75%** by weight), dry extract with soy lecithin (**84,37%** by weight).

Samples with sodium dodecyl sulfate have the lowest penetration rates. Legalon® 140, standart and dry extract have penetration rates of 2.77%, 8.13% and 15.93% by weight, respectively. The addition of SDS shifts the peaks from 90 to 120 minutes, and there is also a clear differentiation of the curves, which may allow the use of sodium dodecyl sulfate as a model for studying the absorption of silymarin.

The use of combined drugs based on milk thistle with lecithin, or milk thistle with licorice saponins has two rationales. Firstly, it will help to increase the bioavailability of silymarin in the gastrointestinal tract, which is rather low due to the poor solubility of flavolignans in water. Secondly, glycyrrhizic acid in the total licorice saponins, as well as phospholipids in lecithin, are themselves used for the prevention and treatment of liver diseases, and the combination of these substances can significantly increase the therapeutic effect of drugs based on them.

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