PharmacologyOnLine

Archives • 2021 • vol.3 • 1502-1511

COMPARATIVE STUDY OF THE EFFECT OF PHARMACEUTICAL COMPOSITIONS OF THICK EXTRACT OF COMMON TANSY FLOWERS (TANACETUM VULGARE L.) AND ESSENTIAL OILS AT THE BILIARY FUNCTION OF THE LIVER AND THE COMPOSITION OF BILE

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Abstract

The aim of the study is to compare the effect of the created pharmaceutical compositions (PC), consisting of a thick extract of common tansy flowers (Tanacetum vulgare L.) (TECTF) and essential oils of lavender, mint, cloves, on the biliary function of the liver of healthy animals and, according to the results of the study, the choice of the optimal PC.

Studied PC: TECTF at a dose of 100 mg/kg in combination with lavender essential oil (LEO) at 5% of the TECTF dose, i.e. at a dose of 5 mg/kg (TECTF+LEO); TECTF at a dose of 100 mg/kg in combination with mint essential oil at 5% of the TECTF dose, i.e. at a dose of 5 mg/kg (TECTF+MEO); TECTF 100 mg/kg in combination with clove essential oil at 5% of the TECTF dose, i.e. 5 mg/kg (TECTF+CEO). The comparison drug was a complex phytopreparation that contains themint essential oil, "Cholelesan" produced by the corporation "Arterium" (Ukraine) as an analogue of the research objects in terms of indications for use and therapeutic effect. The study was carried out at intact outbred male white rats. During the experiment, the animals were kept in standard vivarium conditions with a natural day-night light regime and free access to water and food. Everything was carried out in accordance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" and the methodological recommendations of the State Medical Center of the Ministry of Health of Ukraine "Preclinical Studies of Medicines".

A study was carried out of the influence of the investigated substances at the volume and rate of secreted bile, the content of bile acids and cholesterol in it, as well as on the cholato-cholesterol coefficient. The results of the study were assessed by using a package of standard statistics programs; for all types of statistical analysis, differences were considered significant for $p < 0,05$.

It has been established that PC TECTF+LEO has a pronounced efficacy in combination with a high level of safety and can serve as a basis for the creation of a drug with pronounced choleretic and cholekinetic activity, capable of preventing the appearance of cholesterol stones in the gallbladder.

Keywords: *choleretic activity, common tansy flowers, essential oil, diseases of the hepatobiliary system.*

Introduction

Currently, diseases of the hepatobiliary tract are widespread among people of working age, which determines the high economic significance of this medical and social problem [1, 20, 21, 26, 27]. Factors contributing to the onset and development of liver damage are viral infections, the intake of medicinal substances, the ingestion of toxic agents and concomitant chronic diseases of a systemic nature [19, 22, 23, 25]. Despite the successes in the chemical synthesis of new drugs, the study of medicinal plants of the domestic flora is relevant [28, 29, 30]. In particular, common tansy (Tanacetum vulgare L.) has long been known for its choleretic, cholekinetic and antispasmodic properties [2]. Essential oils of plants – complex multicomponent mixtures of chemicals, are also of interest from the point of view of pharmacotherapy of liver diseases [3, 31, 32, 33]. Essential oils of mint, lavender and clove exhibit pronounced antispasmodic, choleretic, anti-inflammatory and antioxidant effects [4, 34, 35].

The current trend of the pharmaceutical market is an increase in the number of combined drugs, which have a number of advantages over monocomponent: polymodal effect and low probability of patient's adverse reactions.

In the context of this direction, new pharmaceutical compositions, including dense extract of common tansy flowers (Tanacetum vulgare L.) (TECTF) and essential oils of lavender, mint, clove, were developed at the National University of Pharmacy [5].

The aim of this study was to comparatively investigate the effect of the created pharmaceutical compositions, consisting of TECTF and essential oils of lavender, mint, cloves, at the biliary function of the liver of healthy animals and, according to the results of the study, at the choice of the optimal pharmaceutical composition.

Methods

The studied TECTF was obtained at the Department of Botany, NUPh under the guidance of prof. T. Gontova. Tansy flowers were crushed to a particle size of 2-3 mm and extracted three times with 70% water-ethanol solution. The ratio of the mass of raw materials to the total volume of the extractant is 1:5. The resulting extracts were combined and left to stand for 24 hours at a temperature of 2-3°C, after which they were filtered and evaporated on a rotary vacuum evaporator until a thick mass with a moisture content of no more than 25%.

The resulting TECTF was studied by thin-layer chromatography, which made it possible to determine 3 dominant phenolic compounds: luteolin, luteolin-7-glycoside, and chlorogenic acid. The quantitative content of phenolic substances in TECTF was determined by spectrophotometry. The data obtained made it possible to standardize TECTF in terms of the total content of flavonoids – 3,69 % (in terms of luteolin) and the amount of hydroxycinnamic acids – 16,88 % (in terms of chlorogenic acid) [6, 7].

The study of the effect of standardized pure TECTF and a pharmaceutical composition at the biliary function of the liver of healthy animals was carried out on the basis of the Educational and Scientific Institute of Applied Pharmacy of the National Pharmaceutical University, certified by the State Expert Center of the Ministry of Health of Ukraine. Preliminary safety tests of pure TECTF were carried out and its toxicological characteristics were studied. It was determined that the intragastric administration of TECTF to sexually mature rats of both sexes at a dose of 5000 mg/kg does not lead to the death of animals and does not have a negative effect on the state of organs and systems of animals in general. This allows the extract to be classified as practically non-toxic. Also, in the course of preliminary screening studies at healthy intact rats, a conditionally effective dose of the pure extract was selected – 100 mg/kg animal weight [8].

Based on the results of the analysis of the reference literature data – State Register of Medicines of Ukraine and the Compendium of Medicines Directory, phytopreparations containing essential oils presented on the domestic pharmaceutical market were identified [9]. The subsequent analysis of the ratio of the components of their composition determined the choice of the essential oil content in the composition in a volume of 5% of the conditionally effective dose of the pure extract [10].

The objects of this study were pharmaceutical compositions (PC): TECTF at a dose of 100 mg/kg in

combination with lavender essential oil at 5% of the TECTF dose, i.e. at a dose of 5 mg/kg (TECTF+LEO); TECTF at a dose of 100 mg/kg in combination with mint essential oil at 5% of the TECTF dose, i.e. at a dose of 5 mg/kg (TECTF+MEO); TECTF 100 mg/kg in combination with clove essential oil at 5% of the TECTF dose, i.e. 5 mg/kg (TECTF +CEO). The comparison drug was a complex phytopreparation containing mint essential oil, "Cholelesan" produced by the "Arterium" corporation (Ukraine) as an analogue of the objects of study in terms of indications for use and therapeutic effect. The reference drug "Cholelesan" contains the following components: extract of dense fruits of wild carrots and calendula flowers ((7,75-13,4):1) – 60 mg; dry extract of sandy everlasting flowers (40: 1) – 50 mg; curcumin C3 ((64-66): 1) – 20 mg; turmerone oil – 5 mg; peppermint essential oil – 7.5 mg.

The animals received the objects of study in the form of a freshly prepared aqueous suspension 1 time per day, 1 hour before feeding, considering the maximum volume of liquid allowed for administration into the rat's stomach. A suspension of pharmaceutical compositions was prepared immediately before administration to animals: volatile essential oils were applied to the Neusilin carrier substance, then the carrier-fixed essential oils were added to TECTF and 1-2 drops of Tween-80 emulsifier were added to form a suspension. The contents of the capsules of the comparison drug "Cholelesan" were also dissolved in water with the addition of 1-2 drops of Tween-80.

The subjects of the study and the reference drug were administered to the animals once a day one hour before feeding for 14 days. The animals were divided into groups: 1 – intact control (animals received distilled water); 2 – animals receiving TECTF at a dose of 100 mg/kg; 3 – animals receiving PC TECTF+LEO; 4 – animals receiving PC TECTF+MEO; 5 - animals receiving PC TECTF+CEO; 6 – animals that received the reference drug "Cholelesan" ("Arterium", Ukraine) at a dose of 35 mg/kg, as an analogue of pharmacological activity. The dose of the reference drug was calculated based on the recommendations of the instructions for the coefficients of species sensitivity of Yu. A. Rybolovlev [11].

The study was carried out at intact outbred male white rats. During the experiment, the animals were kept in standard vivarium conditions with a natural day-night light regime and free access to water and food. All were carried out in accordance with the «European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes» [12] and methodological recommendations of the State Medical Center of the Ministry of Health of Ukraine «Preclinical studies of drugs» [13]. The distribution of animals into groups in the experiment was carried out according to the principle of randomization.

The studied pharmaceutical compositions and the reference drug were administered to animals intragastrically by use of a metal atraumatic probe in the form of a freshly prepared aqueous suspension 1 time per day 1 hour before feeding. Suspension of pharmaceutical compositions was prepared immediately prior to inoculation of animals. Intact control animals were injected with a volume of water equivalent to the weight of the animal, taking into account the maximum allowable volume of liquid for injection into the rat's stomach. The study agents were administered throughout the 14-day study period and on the day of the experiment one hour before the start of bile collection.

The day before the collection of bile, the animals were deprived of food, leaving free access to water. On the 14th day of the experiment, the objects of study were administrated 1 hour before the start of bile collection, the animals of the intact control group received an equivalent amount of distilled water. The animals were anesthetized by intraperitoneal injection of sodium thiopental at a dose of 50 mg/kg, fixed on the operating table, and the abdominal cavity was opened with a 2 cm long incision in the epigastric region. In anesthetized animals, the location of the entry of the bile duct into the duodenum was found and a polyethylene cannula tube was fixed in the duct, through which all the secreted bile entered the test tube. The indicator of the volume of bile secreted in 60 minutes was recorded hourly after the administration of the studied compositions and the reference drug by taking bile from a test tube with a graduated syringe. The collection of bile was carried out for 3 hours and the rate of bile secretion was

calculated (mg/min/100 g of body weight of the animal).

In the secreted bile, the content of bile acids and cholesterol was determined, and the cholato-cholesterol coefficient was calculated. To determine the content of bile acids and cholesterol in bile, a method is used based on the ability of a cooled solution of iron (III) chloride to form colored complexes with bile acids and cholesterol, which have absorption maxima at different wavelengths. The study is carried out as follows: bile is diluted 20 times with 96 ° ethanol, centrifuged for 5 minutes at a speed of 1500 rpm, 3.5 ml of a 0.1% solution of iron (III) chloride in glacial acetic acid are added to 0.1 ml of the supernatant, which is preliminarily mixed in the cold with concentrated sulfuric acid in a ratio of 1:1. At the 20th minute from the beginning of the reaction, the optical density of the reaction product with cholesterol is determined at a wavelength of 480 nm. At the 30th minute, the mixture is placed in a thermostat at 37℃ for 20 minutes and determine the optical density of bile acids at a wavelength of 385 nm. A 0.1% solution of iron (III) chloride is used as a comparison solution. The bile acid (BA) content (mg /%) is determined by the formula: Cba = 114 * D385 * P, where Cba is the content of bile acids; D385 – the value of the optical density of the solution at 385 nm; P – dilution of bile. Cholesterol content (mg /%) is determined by the formula: Cch = 50*(D480-0.04D385)*R, where Cch is the cholesterol content; D480 – the value of the optical density of the solution at 480 nm; D385 – the value of the optical density of the solution at 385 nm; P – dilution of bile [14].

The results of the study were assessed by use of a package of standard statistics programs; for all types of statistical analysis, the differences were considered significant at $p < 0,05$ [15, 16].

Results and discussion

This pharmacological search consists in the experimental identification of the maximum choleretic efficacy of one of the studied PC and pursues the goal of its choice. As you know, true choleretics stimulate bile formation, increasing the production of bile acids, and increase the difference in osmotic pressure between blood and bile. Thus, true choleretics prevent the formation of cholesterol stones in the gallbladder.

The stimulating effect on the excretory function of the liver of the studied pharmaceutical compositions and the reference drug was assessed by the increase in the volume of bile secreted during the experiment and by the rate of its excretion. The content of bile acids and cholesterol in the secreted bile was also determined and the cholato-cholesterol coefficient was calculated, which is a characteristic of the lithogenicity of bile. The obtained numerical data of the experimental results are presented in table 1 and 2.

TECTF as a monocomponent substance and in all studied compositions significantly stimulates bile secretion, increasing the volume of bile secreted by experimental animals and the rate of its expiration in comparison with intact ones by 1.4-1.8 times (Table 1). It should be noted that the herbal preparation of the complex composition "Cholelesan" presented on the pharmaceutical market of Ukraine, an analogue of the objects of research in terms of therapeutic effect, which has been successfully used in clinical practice since 2017, is reliably (Newman-Kels criterion, $p < 0.05$) inferior to pure TECTF and PC for exhibited choleretic action.

Based on the physiology of the digestive process, the most important is the stimulation of bile secretion immediately after a meal, thus the maximum manifestation of the choleretic effect of the drug in the first hour after taking it is preferable. The maximum choleretic effect at the 1st hour of observation was shown by PC TECTF+LEO in terms of the absolute value of the volume of excreted bile, which was 0.58 ml, which is 5% more than the indicator of pure TECTF, 7% more than the indicator of the TECTF+MEO composition and 15% - TECTF+CEO.

It was also found that the choleretic effect of both pure TECTF and PC decreases gradually over time after administration, while the choleretic activity of the reference drug tends to rapidly fade away after 1 hour of observation. From table it can be seen that the cholekinetic effect of the reference drug «Cholelesan» decreases by 52% at the 2nd hour of observation and by 59% by the end of the experiment. The choleretic activity of pure TECTF decreases by 27% and 51% at the 2nd and 3rd hours of

observation, respectively, the activity of the compositions decreases, respectively, as follows: PC TECTF+LEO by 27% and 46%, PC TECTF+MEO by 9% and 29%, PC TECTF+CEO by 13% and 62%.

Analyzing this trend in the long term, we can conclude that long-term use of the compositions of TECTF+MEO and TECTF+CEO may contribute to the development of biliary dysfunction, hypotension of the sphincter of Oddi, followed by reflux of the contents of the duodenum into the biliary tract [17]. A gradual and more significant decrease in choleretic activity after 2 and 3 hours after ingestion, inherent in pure TECTF and PC of the TECTF+LEO composition, contributes to the maximum physiological course of the digestion process.

Also noteworthy is the effect of TECTF as a monocomponent substance and investigated pharmaceuticals. compositions on the composition of bile and its colloidal properties. A decrease in the cholato-cholesterol coefficient and an increase in lithogenic indices (Table 2) indicate colloidal destabilization of bile and an increased tendency to stone formation. An important therapeutic characteristic of a potential choleretic drug is the ability to increase the content of bile acids in bile, increase the cholato-cholesterol ratio and decrease the lithogenicity of bile [18].

Data analysis table. 2 shows that the most pronounced effect at the content of bile acids in bile is exerted by PC TECTF+LEO. According to this indicator, this object is significantly ($p < 0.05$, Newman-Kels test) superior to TECTF, other compositions and the reference drug. The content of bile acids in the TECTF+LEO group is 968.29 mg/%, which is 24% higher than that of the group of animals of intact control, by 12% in the group that received TECTF, by 11% in the group that received PC TECTF+MEO, by 17 % in the group that received PC TECTF+CEO and by 9% in the group that received the herbal preparation, an analogue of the pharmacological action «Cholelesan ».

It should also be noted that under the conditions of using the objects of study at healthy young animals, there was no significant effect on the cholesterol content in bile. The indices of the pure TECTF and PC TECTF+LEO groups were reduced versus the indices of the intact control group by 4.7% and 2.7%, respectively. It follows from this that the objects under study show a tendency to manifest a hypocholesterolemic effect, but at the same time the normal ratio of bile components in healthy animals does not change in the absence of pathology.

It is necessary to highlight the influence of the research objects on the cholato-cholesterol coefficient, reflecting the colloidal stability of bile. The value of the indicator in the group receiving PC TECTF+LEO is statistically significant ($p < 0.05$, Newman-Kels test) exceeds the indicators of all other groups. The value of the cholato-cholesterol coefficient for the TECTF+LEO group is 34.69, which is by 26% higher than the group of animals of the intact control, by 9% in the group of animals receiving pure TECTF, by 13% in the group of animals receiving PC TECTF+MEO, by 18 % in the groups of animals that received PC TECTF+CEO and by 13% in the groups of animals that received a reference drug.

The result of the study is the selection of an optimal PC composition that meets the requirements for a drug – a true choleretic: PC TECTF+LEO has a beneficial effect on the colloidal stability of bile, reducing its lithogenicity by increasing the cholato-cholesterol ratio by increasing the content of bile acids in bile. This PC contributes to the maximum saturation of bile with cholesterol during the interdigestive period, i.e. a gradual decrease in choleretic activity 2 and 3 hours after ingestion, which probably contributes to the maximum physiological prevention of the appearance of biliary sludge.

Evaluating the results obtained in this study, it should be noted that PC TECTF+LEO has a pronounced efficacy in combination with a high level of safety and can serve as a basis for creating a drug with pronounced choleretic and cholekinetic activity, capable of preventing the appearance of cholesterol stones in the gallbladder.

Conclusions

1. It was found that PC TECTF+LEO has the most pronounced stimulating effect at the excretory function of the liver, which is determined by a significant increase in both the volume of excreted bile and the rate of its outflow.

2. It was found that PC TECTF+LEO reliably surpasses the multicomponent herbal preparation for comparison drug «Cholelesan» in terms of the severity of the choleretic and cholekinetic effects.

3. It was found that PC TECTF+LEO significantly increases the cholato-cholesterol ratio, thereby reducing the lithogenicity of bile.

4. The data obtained are an experimental justification for the further study of PC TECTF+LEO as a promising choleretic agent in order to create a drug based on it.

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Table 1. Choleretic and cholekinetic activity of study objects

Groups	The volume of excreted bile, ml			Bile secretion rate, mg/min/100 g		
	1 h	2 h	3 h	1 h	2 h	3 h
IC (n=6)	0,35± 0,02	0,26± 0,03	0,14± 0,02	2,72± 0,20	1,99± 0,31	1,09± 0,17
TECTF, 100 mg/kg (n=6)	0,55± 0,03*	0,40± 0,03*	0,27± 0,03*	4,19± 0,35*	3,05± 0,33*	2,07± 0,21*
TECTF+LEO, 100+5 mg/kg (n=6)	0,58± 0,04*	0,42± 0,06*	0,31± 0,03*	3,93± 0,35*	2,86± 0,46*	2,14± 0,22*
TECTF+MEO, 100+5 mg/kg (n=6)	0,54± 0,07*	0,49± 0,03 */**/***	0,38± 0,07 */***	3,70± 0,51	3,36± 0,21*	2,63± 0,50 */**/***
TECTF+CEO, 100+5 mg/kg (n=6)	0,49± 0,03 */***	0,43± 0,03*	0,39± 0,02 */***	3,47± 0,27	3,02± 0,21*	1,30± 0,04 ***
Cholelesan, 35 mg/kg (n=6)	0,44± 0,05 */**/***	0,21± 0,07	0,18± 0,06	3,17± 0,27 */**/***	1,52± 0,57	1,31± 0,45***

Notes:

IC – group of animals of intact control;
TECTF – a group of animals that received a dense extract of common tansy flowers;
TECTF+LEO – a group of animals that received TECTF in combination with lavender essential oil;
TECTF+MEO – a group of animals that received TECTF in combination with peppermint essential oil;
TECTF+CEO – a group of animals that received TECTF in combination with clove essential oil;
Cholelesan – a group of animals that received the reference drug «Cholelesan»;
* – significantly relative to the indices of the intact control group (Newman-Kels test), p<0,05;
** – significantly relative to the indicators of the TECTF group (Newman-Kels test), p<0,05;
*** – reliably relative to the indicators of the TECTF + LEO group (Newman-Kels test), p<0,05;
n – the number of animals in the group.

Table 2. Influence of research objects on the content of bile acids, cholesterol and lithogenicity of bile

Groups	Bile acid content, mg/%	Cholesterol content, mg/%	Cholato-cholesterol ratio
IC, (n=6)	736,62±10,74	28,59±0,63	25,77±0,66***
TECTF, 100 mg/kg, (n=6)	853,60±13,15 */***	27,26±0,96	31,35±1,20*/***
TECTF+LEO, 100+5 mg/kg, (n=6)	968,29±10,64 */**	27,83±1,38	34,69±1,50*/**
TECTF+MEO, 100+5 mg/kg, (n=6)	860,25±10,15 */***	28,44±0,38	30,25±0,65*/***
TECTF+CEO, 100+5 mg/kg, (n=6)	801,35±11,87 */**/***	28,27±0,76	28,35±0,62*/**/***
Cholelesan, 35 mg/kg, (n=6)	880,86±8,27 */**/***	29,33±1,60*	30,11±1,56*/***

Notes:

IC – group of animals of intact control;

TECTF – a group of animals that received a dense extract of common tansy flowers;

TECTF+LEO – a group of animals that received TECTF in combination with lavender essential oil;

TECTF+MEO – a group of animals that received TECTF in combination with peppermint essential oil;

TECTF+CEO – a group of animals that received TECTF in combination with clove essential oil;

Cholelesan – a group of animals that received the reference drug «Cholelesan»;

* – significantly relative to the indices of the intact control group (Newman-Kels test), p<0,05;

** – significantly relative to the indicators of the TECTF group (Newman-Kels test), p<0,05;

*** – reliably relative to the indicators of the TECTF + LEO group (Newman-Kels test), p<0,05;

n – the number of animals in the group.