Development and Evaluation of New Choleretic Agent

Ferubko EV1,*, Rendyuk TD2, Dargaeva TD1, Nikolaev SM3

ABSTRACT

Background: The aim of the research is the determination of pharmacological activity during the development of the optimal way of a new choleretic agent obtaining. The multicomponent herbal medicinal product under the code name Hexaphyte (herbal multicomponent dry extract) is developed. It contains the following species of medicine plant raw materials: Helichrysum arenarium L., Tanacetum vulgare L., Rosa sp., Urtica dioica L., Mentha piperita L., Glycyrrhiza glabra L. in the ratio of 6:2:2:1:1. Materials and Methods: Herbal tea was extracted three times with hot water at the temperature of 75-85º C; water extracts were combined, filtered and dried. Hexaphyte was standardized by the content of phenolic compounds – sum of flavonoids in terms of luteolin standard and isosalipurposide standard. Phenolic compounds (flavonoids) are dominating substances (35-37%) in the obtained extract. The obtained multicomponent medicine under the code name Hexaphyte was administered per os in the experimental and therapeutic dose of 250 mg/kg to the laboratory animals having experimental induced damages of gallbladder and liver. Results: In Hexaphyte the content of the flavonoid sum (CFS) referred to luteolin standard at a wavelength of 350 nm was not less than 4%; CFS referred to isosalipurposide standard at a wavelength of 315 nm was not less than 15%. It was revealed in the results of the realized experiments that it has a choleretic effect superior in a number of indicators to the activity of the Allochol reference drug. The pharmacotherapeutic effect of Hexaphyte at experimental damages of gallbladder and liver is due to the presence of biologically active substances, mainly of phenolic nature. Conclusion: The obtained results of the research argue the feasibility of using the developed multicomponent medicine Hexaphyte containing biologically active substances of phenolic nature in the prevention and complex treatment of hepatobiliary system diseases.

Key words: Choleretic herbal medicine Hexaphyte, Experimental damage of liver and gallbladder, Preclinical studies, Choleretic activity.

INTRODUCTION

Cholecystitis is the common disease of the gastroduodenal zone. If the gallbladder inflammatory process is prolonged, functional and organic changes in the liver can occur.1 As known, the gallbladder and biliary tract diseases have the nature of a chronic course with seasonal exacerbations. In this regard, it is advisable to use herbal medicinal products for prevention and treatment.2,3 The range of herbal medicines with choleretic activity is limited, and it is represented by such drug preparations as Flamin, Caleflon, Hofitol, Allochol, etc.4 Therefore, the search and development of new effective multicomponent choleretic drugs are important.

Previously, we developed the choleretic herbal tea. Its composition and the ratio of the components were selected experimentally taking into account the significance of individual links in the mechanism of the liver, gall bladder, and biliary tract damages development.5,7 Flowers of dwarf everlast and tansy (fam. Asteraceae) contain flavonoids that stimulate bile formation and have antispasmodic activity in relation to the smooth muscles of the gastrointestinal and biliary tracts.5 Leaves of peppermint (fam. Lamiaceae) contain flavonoids promoting digestion by stimulating the secretion of hydrochloric acid and enzymes in the stomach.6 Roots of liquorice (fam. Fabaceae) are rich in saponins having a pronounced anti-inflammatory effect and flavonoids with a moderate choleretic effect.8 Leaves of common nettle (fam. Urticaceae) and rose hips (fam. Rosaceae) contain a complex of biologically active substances that contribute to the metabolism normalization, stimulate tissue regeneration in the gastrointestinal tract, increase the body’s resistance and stimulate the liver detoxification function.9,10 Thus, according to the literature data, there is information about the anti-inflammatory, the antispasmodic and choleretic effects of biologically active substances that are components of dwarf everlast and tansy flowers, rose hips, common nettle and of peppermint leaves, as well as liquorice roots. In regard to the above, in the scope of the task, the exploration of the choleretic activity of the mentioned herbal composition is promising and prognostic.

It is known that flavonoids, coumarins, xanthones, tannins, phenolic acids, phenolicarboxylic acids, etc. are classified as phenolic compounds. The content of biologically active substances in total extract preparations is usually calculated in terms of any dominant component. In the case of our choleretic herbal drug, the dominant components providing choleretic activity are luteolin (in tansy) and isosalipurposide (in dwarf everlast). Given this
circumstance, in the normative documents developed for such means, the inclusion of such techniques is accepted. This approach is generally accepted in the development and standardization of herbal medicines. In this regard, we use the procedure for determining phenolic compounds to standardize the Hexaphyte (choleretic agent obtained from herbal tea) by the amount of biologically active substances (flavonoids).

The aim of the research is pharmacological activity determination during the development of an optimal way of a new choleretic agent obtaining.

MATERIALS AND METHODS

Plant material

The object of the research is herbal composition under the code name Hexaphyte containing flowers of dwarf everlast (Helichrysum arenarium L.), flowers of tansy (Tanacetum vulgare L.), rose hips (Rosa sp.), leaves of common nettle (Urtica dioica L.), leaves of peppermint (Mentha piperita L.), roots of liquorice (Glycyrrhiza glabra L.). Raw materials purchased in the pharmacy network of the Russian pharmaceutical company “Krasnogorsleskredstva” were used as the objects.

Hexaphyte extract preparation

To confirm the choleretic effect based on data of chemical composition the following method of the medicine obtaining is suggested. The herbal tea containing flowers of dwarf everlast, flowers of tansy, rose hips, leaves of common nettle, leaves of peppermint, and roots of liquorice in the ratio of 6:2:2:1:1 was extracted three times with water at the temperature of 75-85°C. The combined extracts are purified by filtration and dried. The resulting extract contains polysaccharides, flavonoids, carotenoids, organic acids, vitamins, macro- and microelements, essential oils, and other natural compounds.

Flavonoid content (phenolic compounds) determination

Dwarf everlast and tansy flowers are the main medicinal raw materials that are part of Hexaphyte and determines its choleretic effect. In this regard, the procedure of quantitative determination in Hexaphyte of the sum of biologically active substances (flavonoids) in terms of the dominant components of tansy (luteolin) and immortelle (isosalipurposide) using UV-spectrophotometry was developed. The absorption of Hexaphyte solutions in 20% ethanol is subject to the Beer–Lambert–Bouguer law from the standard samples (luteolin) and isosalipurposide (GSO) at the wavelength of 315 nm and 350 nm, respectively. The content of the sum of flavonoids in terms of luteolin standard and isosalipurposide standard at a wavelength of 350 nm is not less than 4%. The content of flavonoids at a wavelength of 315 nm is not less than 15%. The dominating components of the extract obtained are phenolic compounds – flavonoids (35-37%).

Animal studies

The experiments were performed on 120 outbred mature male white rats with an initial body weight of 180-200 g, and on 40 Guinea pigs both male and female with an initial body weight of 400-500 g. The animals were obtained from the Federal State Budgetary Scientific Institution Biomedical Technology Research Center of the Federal Medical and Biological Agency of Russia; they lived in the vivarium and were provided with free access to food and water. Pharmacological studies were carried out according to “Rules for the work using experimental animals”, “Regulations accepted by the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes”, Order of the Ministry of Health of the Russian Federation No. 199n dated 04/01/2016 “On the approval of the rules of good laboratory practice”, according to the norms of good laboratory practice.
practice (GLP). The research design was approved by the Bioethical Commission of the All-Russian Research Institute of Medicinal and Aromatic Plants. This work was carried out in accordance with the Federal Law "On Medicinal Products" and the "Guidelines for Conducting Preclinical Studies of Medicines".

Hexaphyte choleretic activity at predetermined experimental and therapeutic dose of 250 mg/kg has been under study using the models of experimental cholecystitis and D-galactosamine hepatitis. The herbal choleretic agent Allochol CJSC Vifitech (Russia) registered in the Russian State Register of Medicines at a dose of 250 mg/kg was applied as the reference preparation.

Experimental cholecystitis in guinea pigs was induced with intraperitoneal injections of hexenal in a dose of 50 mg/kg. An abdominal cavity was opened in guinea pigs, and then a partial sampling of bile was performed with thin injection needles; then 0.1 ml of a 3% solution of H₂O₂ provided by (LLC “Rosbio”, Russia, Pharmacopeial monograph of the enterprise 42-4677-08) was injected into the gallbladder cavity. Acute liver damage was caused in rats by a single intraperitoneal injection of D-galactosamine hydrochloride (Sigma-Aldrich (Merck), USA, chemically pure) in a dose of 1.0 g/kg of body weight. The degree of choleretic activity of the extract was evaluated by the rate of secretion and by the total amount of bile excreted, as well as by the content in the bile of its main ingredients: bilirubin, bile acids and cholesterol. Statistical processing of the data obtained, which distribution was close to the normal one, was carried out by the parametric method by Student’s t-test using the software package Statistica 10.0 (USA). P-values ≤0.05 represented significant differences.

**RESULTS AND DISCUSSION**

The choleretic action of Hexaphyte was an understudy in the conditions of experimental cholecystitis. Hexaphyte was administered into guinea pigs intragastrically once a day for 10 days for therapeutic and prevention purposes in the experimental therapeutic dose of 250 mg/kg of body weight, starting from the 2nd day after the injection of hydrogen peroxide. Herbal preparation Allochol in a dose of 250 mg/kg of guinea pig weight was used as a reference drug. Animals of the control group received purified water in the same volume according to a similar treatment plan. The results of the studies are shown in Table 1. Under Hexaphyte action bile secretion rate in guinea pigs is accelerated at test day 3 by 25%, at day 7 – by 4%, at day 14 – by 22% compared to the control data. Reference drug-influenced bile secretion reaction in guinea pigs to a lesser extent. Thus, under experimental Hexaphyte pharmacotherapy of gallbladder damages in guinea pigs, reliably expressed the choleretic effect of the studied extract was revealed. Hexaphyte was slightly superior to Allochol in this type of pathological process according to the level of pharmacotherapeutic effect.

It is known that it is necessary to correct the main pathogenetic factors of damage to the organs of the hepatobiliary system in order to achieve full recovery, reduce the likelihood of relapse and chronicity. Hexaphyte impact on bile formation function of the liver in outbred white rats with D-galactosamine induced hepatitis has been under study as well.

Primary, the rats were arranged into the following groups: intact (10 rats); control (10 rats); experimental 1 (10 rats); experimental 2 (10 rats) ones. Animals of the first experimental group were administered with Hexaphyte into the stomach through a tube in an experimental and therapeutic dose of 250 mg/kg, once after the injection of the damaging agent. Animals of the second experimental group were administered with Allochol reference drug in the isoeffective dose of 500 mg/kg according to a similar regimen. The animals of the control group were provided with equivolue amounts of purified water according to a similar treatment plan. The results of the studies are shown in Table 2.

Based on data presented in Table 2 we found out that study of bile formation function of the liver shown a statistically significant increase in the bile secretion rate due to Hexaphyte administration, more by 63% compared with the control. Allochol reference drug had a significantly pronounced choleretic effect, increasing the rate of choleresis by 33% compared with the control. Bile acid content in bile was more by 36% and 42% compared with the control under the administration of Hexaphyte and Allochol respectively.

Under Hexaphyte treatment, the content of glycocholic, glycochenodeoxycholic, and taurocholic bile acids in bile increased

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<th>Table 1: Changes in bile secretion rate under Hexaphyte action at experimental cholecystitis in guinea pigs, (M ± m).</th>
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<td><strong>Groups of animals</strong></td>
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**Note:** hereinafter * – P-values ≤0.05 represented significant differences between data of control and experimental groups.

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<th>Table 2: Hexaphyte impact on bile formation function of liver in outbred white rats with D-galactosamine induced hepatitis, (M ± m).</th>
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<td><strong>Choleresis indices</strong></td>
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<td><strong>Bile secretion rate, mg/min per 100g of body weight</strong></td>
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<td><strong>Bile acid content in bile, mg %</strong></td>
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<td><strong>Glycochenodeoxycholic acid content in bile, mg %</strong></td>
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<td><strong>Glycochenodeoxycholic acid content in bile, mg %</strong></td>
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<td><strong>Bilirubin concentration in bile, µmol /L</strong></td>
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<td><strong>Cholesterol concentration in bile, µmol /L</strong></td>
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significantly by 133%, 41%, and 68% respectively; and there was
observed an accelerated cholesterol excretion with bile, more by 60%
compared with the control. Sherlock S. et al. and Muriel P. found that
experimental therapy of toxic liver lesions with hepatoprotective agents
is characterized by a gradual restoration of the liver structure, a decrease
in the manifestations of the mesenchymal inflammatory reaction and
cholestatic disorders. Thus, multicomponent medicine Hexaphyte
obtained has a significant choleretic effect in the D-galactosamine
induced hepatitis model that is comparable in a number of indicators
with the effect of the Allochol reference drug.

CONCLUSION
In the results of the conducted experiments, it was revealed that per
administration of multicomponent medicine under the code name
Hexaphyte an experimental and therapeutic dose of 250 mg/kg to
the laboratory animals having experimental induced damages of
gallbladder and liver has a choleretic effect superior in a number of
indicators to the activity of the Allochol reference drug.

The pharmacotherapeutic effect of Hexaphyte at experimental
damages of gallbladder and liver is due to the presence of biologically
active substances, mainly of phenolic nature. The obtained results
of the research argue the feasibility of applying the developed
multicomponent medicine Hexaphyte containing biologically active
substances of phenolic nature in the prevention and complex treatment
of hepatobiliary system diseases.

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CONFLICTS OF INTEREST
None.

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GRAPHICAL ABSTRACT

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