Correction of Toxic Liver Damage with a Multicomponent Herbal Extract in an Animal Experiment

Ferubko EV¹, Nikolaev SM², Dargaeva TD³, Rendyuk TD⁴,*

ABSTRACT

Background: Digestive diseases constitute a significant part in the overall structure of human diseases. Herbal chologogues are indicated for a treatment of chronic liver, gall bladder, and bile ducts diseases. The aim of the work is to determine the choleretic effect of a new multicomponent plant extract. Materials and Methods: Multicomponent plant extract was obtained from the following types of plant materials: 300 g of immortelle flowers (Helichrysum arenarium L.), 100 g of tansy flowers (Tanacetum vulgare L.), 100 g of rose fruits (Rosa sp.), 100 g of leaves of common nettle (Urtica dioica L.), 50 g of mint leaves (Mentha piperita L.), 50 g of licorice roots (Glycyrrhiza glabra L.). The extract was standardized by the total flavonoid content. It was calculated and expressed in terms of luteolin and isosalipurposide standards (total flavonoids content: not less than 4% and 15% respectively). The animal experiments being done in 80 nonlinear male rats with initial body weight 180-200 g. In order to study a choleretic effect of multicomponent herbal extract, naive rats recieved the single experimental dose of 250 mg/kg. Pharmacotherapeutic activity was studied in white rats with CCl₄-induced hepatitis. Results: Studies indicate a pronounced choleretic effect of the studied plant extract, that is comparable with the effect of “Allochol” in intact rats experiments. The course administration of a per os (peroral) multicomponent plant extract in a dose of 250 mg/kg to white non-linear rats with tetrachloromethane liver damage has a choleretic effect: it increases the rate of bile secretion, stimulates the synthesis and secretion of cholates with bile, and also the excretion of cholesterol and bilirubin. Conclusion: The obtained research results argue the feasibility of using a multicomponent plant extract containing biologically active substances of phenolic nature in the prevention and comprehensive treatment of liver diseases.

Key words: Multicomponent extract, Choleretic effect, Experimental hepatitis.

INTRODUCTION

Digestive diseases constitute a significant part in the overall structure of human diseases. This diseases category is characterized by a relapsing course, functional disorders in the case of organic nature of disease.¹ ² Herbal chologogues are indicated for a treatment of chronic liver, gall bladder, and bile ducts diseases.³ A range of herbal drugs with choleretic activity is not wide and includes species, tablets containing purified extracts of “Flamin” (Helichrysum arenarium floridis flavonoids), “Caleflonum” (Calendulae officinalis floridis extract) and “Chophytol” (Cynarae scoumuli foliae extract), Chophytol oral solution, “Allochol” tablets et al.⁴ In view of this, the expansion of the list of herbal drugs with choleretic activity is a promising way. Within this framework, it is reasonable to create new effective plant-based medicines.⁵ ⁶ As is well known, phytopreparations have a mild, moderate and natural (physiological) effect on the body, have a gradually, but steadily developing therapeutic effect, unlike synthetic drugs. Herbal remedies have a small number of contraindications or practically do not have them. When taking herbal remedies, side effects, cases of intolerance are observed relatively rarely. From this perspective, the purpose of our study was determining the choleretic activity of multicomponent plant extract.

MATERIALS AND METHODS

Based on the literary analysis and data collected from a preliminary phytochemical study of plant material, the components of extract were substantiated including the contribution of every ingredient.⁶ ¹¹ The object of study was dry extract from plant material: 300 g of immortelle flowers (Helichrysum arenarium L.); 100 g of tansy flowers (Tanacetum vulgare L.); 100 g of rose fruits (Rosa sp. – Rosa majalis Herrn., Rosa acicularis Undl., Rosa canina L. and others); 100 g of leaves of common nettle (Urtica dioica L.); 50 g of mint leaves (Mentha piperita L.); 50 g of licorice roots (Glycyrrhiza glabra L.). The components mixture was extracted with hot water (75-85° C). The resulting extract contained polysaccharides, flavonoids, carotenoids, organic acids, vitamins, macro- and microelements, essential oils, and other natural compounds. The total flavonoid content was calculated and expressed in terms of luteolin and isosalipurposide standards (total flavonoids content: not less than 4% and 15% respectively). This biologically active substances are known for potential choleretic activity of the extract.
The study was carried out in accordance with the Russian Federation’s Federal Law “On Circulation of Medicines,” “Guidelines for Preclinical Trials of Medicinal Products”. The experiments being done in 80 nonlinear male rats with initial body weight 180-200 g. Animals were received from Federal State-Funded Institution of Science “Scientific Centre of Biomedical Technologies” of Federal Medical and Biological Agency of Russia and kept in animal facility with free access to food and water. Pharmacological research was carried out in compliance with the Order of the Ministry of Health of the Russian Federation No. 199n of April 01, 2016 “On Approval of Rules of Good Laboratory Practice” and in accordance with GLP.12 The studies were approved by local Bioethics Committee (Protocol No. 7 of October 01, 2018).

In order to study a choleretic effect of multicomponent herbal extract, naive rats received the single experimental dose of 250 mg/kg. “Allochol” (a herbal cholagogue) registered in Russian State Register of Medicinal Remedies was chosen as a standard medication at dose of 250 mg/kg.4

Bile samples were taken from anesthetized animal (thiopental sodium, 45 mg/kg) through polyethylene cannula inserted into the common bile duct. The samples were collected every hour during 4 hours. The cholagogue activity of the extract was measured by the speed of secretion and the quantity of the total excreted bile, and also by the levels of bilirubin, bile acids, and cholesterol.

Pharmacotherapeutic activity was studied in white rats with CCl₄-induced hepatitis. The water extract was given intragastrically at a dose of 250 mg/kg during the course produce choleretic effect in white rats with initial body weight 180-200 g. Animals were received from Federal State-Funded Institution of Science “Scientific Centre of Biomedical Technologies” of Federal Medical and Biological Agency of Russia and kept in animal facility with free access to food and water. Pharmacological research was carried out in compliance with the Order of the Ministry of Health of the Russian Federation No. 199n of April 01, 2016 “On Approval of Rules of Good Laboratory Practice” and in accordance with GLP.12 The studies were approved by local Bioethics Committee (Protocol No. 7 of October 01, 2018).

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RESULTS AND DISCUSSION

Pharmacological study of choleretic activity of the resulting extract compared to “Allochol” was carried out in intact white rats. The results are shown in the Tables 1-5.

Table 1: The effect of multicomponent herbal extract on the rate of bile secretion in intact rats.

| No | Drug name      | Dose, mg/kg | 1 h. | 2 h. | 3 h. | 4 h. | 5 h. | 6 h. |
|----|----------------|-------------|------|------|------|------|------|------|
| 1  | Distilled water (control) | -           | 2.6 ± 0.2 | 2.8 ± 0.1 | 2.5 ± 0.1 | 2.7 ± 0.2 | 2.2 ± 0.2 | 1.8 ± 0.2 |
| 2  | Herbal extract | 250         | 2.6 ± 0.3* | 5.0 ± 0.4* | 5.0 ± 0.3* | 4.2 ± 0.2* | 3.2 ± 0.2* | 2.5 ± 0.1* |
| 3  | “Allochol”     | 250         | 2.7 ± 0.2* | 4.1 ± 0.3* | 4.3 ± 0.2* | 3.8 ± 0.3* | 2.9 ± 0.4* | 2.2 ± 0.2* |

Note: Here and elsewhere below asterisk * denote significant differences at P<0.05.

Table 2: Effect of a complex extract on the amount of bile excreted during hours 2-5 of the experiment in intact rats.

| No | Drug name      | Dose, mg/kg | 2 h. | 3 h. | 4 h. | 5 h. |
|----|----------------|-------------|------|------|------|------|
| 1  | Distilled water (control) | -           | 168  | 150  | 162  | 132  |
| 2  | Herbal extract | 250         | 300  | 300  | 252  | 192  |
| 3  | “Allochol”     | 250         | 246  | 258  | 228  | 174  |

| No | Drug name      | Dose, mg/kg | Amount of bile excreted, mg/100 g | Total amount of bile excreted during 4 hours, mg/100 g |
|----|----------------|-------------|----------------------------------|-----------------------------------------------|
| 1  | Distilled water (control) | -           | 612                              | 1044                                           |
| 2  | Herbal extract | 250         | 143                              | 286                                            |
| 3  | “Allochol”     | 250         | 906                              | 1746                                           |

Table 3: Effect of a multicomponent herbal extract and “Allochol” on the bile acids levels in intact rats.

| No | Drug name      | Dose, mg/kg | Amount of bile acids within 1 hour, mg/100 g | Total amount of bile acids during 4 hours, mg/100 g |
|----|----------------|-------------|-----------------------------------------------|-----------------------------------------------|
| 1  | Distilled water (control) | -           | 1,51                                           | 5,05                                          |
| 2  | Herbal extract | 250         | 3,09                                           | 9,53                                          |
| 3  | “Allochol”     | 250         | 2,58                                           | 8,29                                          |
### Table 4: Effect of a multicomponent herbal extract and “Allochol” on the bile cholesterol levels in intact rats.

| №  | Drug name                  | Dose, mg/kg | Amount of cholesterol within 1 hour, mg/100,0 g | Total amount of cholesterol during 4 hours, mg/100,0 g |
|----|----------------------------|------------|-----------------------------------------------|------------------------------------------------------|
|    |                            |            | 2 h.   | 3 h.   | 4 h.   | 5 h.   |                                                    |
| 1. | Distilled water (control)  | -          | 0.020  | 0.016  | 0.019  | 0.011  | 0.066                                             |
| 2. | Herbal extract             | 250        | 0.036  | 0.042  | 0.045  | 0.023  | 0.146                                             |
| 3. | “Allochol”                 | 250        | 0.044  | 0.036  | 0.027  | 0.015  | 0.122                                             |

### Table 5: Effect of a multicomponent herbal extract and “Allochol” on the bile bilirubin levels in intact rats.

| №  | Drug name                  | Dose, mg/kg | Amount of bilirubin within 1 hour, mg/100,0 g | Total amount of bilirubin during 4 hours, mg/100,0 g |
|----|----------------------------|------------|-----------------------------------------------|------------------------------------------------------|
|    |                            |            | 2 h.   | 3 h.   | 4 h.   | 5 h.   |                                                    |
| 1. | Distilled water (control)  | -          | 0.018  | 0.015  | 0.015  | 0.013  | 0.061                                             |
| 2. | Herbal extract             | 250        | 0.033  | 0.030  | 0.030  | 0.023  | 0.116                                             |
| 3. | “Allochol”                 | 250        | 0.027  | 0.025  | 0.026  | 0.017  | 0.095                                             |

### Table 6: Bile excretion rate dynamics under effect of a multicomponent extract in white rats with experimental hepatitis induced by CCl₄.

| Experimental conditions          | Bile excretion rate during 4 hours, mg/min per 100 g of body weight |
|----------------------------------|---------------------------------------------------------------|
|                                  | 1 hour | 2 hours | 3 hours | 4 hours |
| Intact rats                      | 5.4 ± 0.3 | 5.2 ± 0.2 | 5.2 ± 0.4 | 5.2 ± 0.4 |
| Control rats (CCl₄ + H₂O)        | 3.7 ± 0.3 | 3.2 ± 0.3 | 2.9 ± 0.2 | 2.6 ± 0.2 |
| Control rats (CCl₄ + an extract) | 5.3 ± 0.2** | 5.0 ± 0.3** | 4.1 ± 0.4* | 3.8 ± 0.3** |
| Control rats (CCl₄ + “Allochol”) | 5.2 ± 0.3* | 4.8 ± 0.2* | 4.0 ± 0.3 | 4.3 ± 0.2* |
| Control rats (CCl₄ + H₂O)        | 4.4 ± 0.4 | 4.4 ± 0.3 | 4.3 ± 0.3 | 4.0 ± 0.4 |
| Control rats (CCl₄ + an extract) | 6.0 ± 0.5* | 5.8 ± 0.4* | 6.2 ± 0.3** | 6.2 ± 0.2** |
| Control rats (CCl₄ + “Allochol”) | 5.8 ± 0.4 | 5.6 ± 0.3* | 5.8 ± 0.4 | 5.8 ± 0.3 |
| Control rats (CCl₄ + H₂O)        | 4.3 ± 0.1 | 4.0 ± 0.2 | 4.5 ± 0.2 | 3.9 ± 0.2 |
| Control rats (CCl₄ + an extract) | 6.6 ± 0.3** | 6.0 ± 0.5** | 5.8 ± 0.4* | 5.6 ± 0.5** |
| Control rats (CCl₄ + “Allochol”) | 6.2 ± 0.2* | 5.7 ± 0.4 | 5.5 ± 0.3* | 5.3 ± 0.3* |
| Control rats (CCl₄ + H₂O)        | 4.7 ± 0.4 | 4.4 ± 0.4 | 4.4 ± 0.4 | 3.3 ± 0.3 |
| Control rats (CCl₄ + an extract) | 4.8 ± 0.2 | 5.5 ± 0.1* | 5.6 ± 0.3** | 5.0 ± 0.1** |
| Control rats (CCl₄ + “Allochol”) | 4.5 ± 0.2 | 5.2 ± 0.2* | 5.3 ± 0.3* | 4.7 ± 0.2* |

Note: Here and elsewhere below asterisk * denote that the differences between control and experimental groups are significant at $P < 0.05$; asterisks ** denote that the differences between control and experimental groups are significant at $P < 0.01$. 

Pharmacognosy Journal, Vol 12, Issue 1, Jan-Feb, 2020
and preventive care of hepatobiliary system diseases. Biologically active substances of phenolic nature for combined therapy is reasonable to use the multicomponent herbal extract containing excretion of cholesterol and bilirubin. The given results prove that it stimulates synthesis and excretion of the cholates, and also induces induced liver damage. The extract increases the rate of bile excretion, altogether, these data demonstrate a significant choleretic effect of a herbal extract, which is comparable to effect of “Allochol” in experiment in intact rats. Per os administration of the extract at dose of 250 mg/kg during the course produce choleretic effect in white rats with CCl₄-induced liver damage. The extract increases the rate of bile excretion, stimulates synthesis and excretion of the cholates, and also induces excretion of cholesterol and bilirubin. The given results prove that it is reasonable to use the multicomponent herbal extract containing biologically active substances of phenolic nature for combined therapy and preventive care of hepatobiliary system diseases.

**ACKNOWLEDGMENT**

This paper was financially supported by “Russian Academic Excellence Project 5-100”.

**CONFLICTS OF INTEREST**

None.

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GRAPHICAL ABSTRACT

ABOUT AUTHORS

- **Ferubko Ekaterina Vladimirovna**: Candidate of Medical Sciences, Head of the Department of Experimental and Clinical Pharmacology, All-Russian Scientific Research Institute of Medicinal and Aromatic Plants (Russia, Moscow). Research interests: study of targeted pharmacological activity and interpretation of the mechanism of action of promising herbal medicines.

- **Nikolaev Sergey Matveevich**: Doctor of Medical Sciences, professor, chief researcher at the Laboratory of Experimental Pharmacology of Institute of General and Experimental Biology of the Siberian Branch of the RAS (Russia, Ulan-Ude). Research interests: study of targeted pharmacological activity and interpretation of the mechanism of action of promising herbal medicines.

- **Dargaeva Tamara Darizhapovna**: Doctor of Pharmaceutical Sciences, professor, chief researcher at the Department of Phytochemistry and Standardization, All-Russian Research Institute of Medicinal and Aromatic Plants (Russia, Moscow). Research interests: search for promising sources of biologically active substances and the creation on their basis of highly effective herbal medicines.

- **Rendyuk Tamara Danilovna**: Candidate of Pharmaceutical Sciences, Associate Professor, Department of Pharmaceutical Natural Sciences, Institute of Pharmacy, Sechenov University (Russia, Moscow). Research interests: search for promising sources of biologically active substances and the creation on their basis of highly effective herbal medicines.

Cite this article: Ferubko EV, Nikolaev SM, Dargaeva TD, Rendyuk TD. Correction of Toxic Liver Damage with a Multicomponent Herbal Extract in an Animal Experiment. Pharmacog J. 2020;12(1):168-72.