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MICROBIOLOGICAL QUALITY OF HEPATOPROTECTIVE HERBAL MEDICINES

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CONFLICT OF INTERESTS

Authors have declared that no competing interests exist.

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Abstract---*Phyto therapy in many cases has no side effects and toxicity, unlike synthetic medicines. Researches on hepatoprotective phytopreparations and determination of the important indicator Microbiological quality will contribute to the benefit of the phytotherapy for the treatment of hepaticdiseases.* ***The purpose*** *of the research is to analyze and determine the microbiological quality of liquid and dry extract, and coated tablets which have been developed on the basis of the noted above herbals with hepatoprotective activity****. Materials and methods.*** *Microbiological quality was determined by the method, described in SP XI, 2nd edition, page 193, change 2, dated 12.10.2005, category 3B, by the indicator "Microbiological quality".* ***Results*** *show that the liquid and dry extract as well as the coated tablets comply with the SP requirements by the indicator "Microbiological Quality". The results are included in the drafts of temporal pharmacopoeia article for the presented medicinal forms.*

Keywords---*hepatoprotective herbal medicine, phytotherapy, microbiological quality, liquid extract, dry extract, coated tablets, microorganisms.*

I. INTRODUCTION

Scientific and practical interest in the medications based on plant raw materials has rapidly increased for the recent years, despite the development of synthesis chemistry. The popularity of the phyto (or plant) preparations' use is due to their mild therapeutic effect, less toxicity and naturalness of most of them, i.e. proximity in origin.

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The great source for the using of phytopreparationsis nature itself certainly. Considerable contribution is made in researching on chemical, pharmacological and clinical therapeutic effects of the herbal medicinal plants, which now are the significant base for the production of pharmaceutical medications.

For the last years there is an increasing demand for phytopreparations, especially for those, which have an hepatoprotective effect. Any medicinal herb is a mini-biosynthetic laboratory, for chemical compounds like glycosides, alkaloids, resins, oleoresins, etc. Basing on this fact it is important to develop and produce new types of hepatoprotective phytopreparations [14].

The liver is the most important organ that plays an important role in maintaining various physiological processes in the body, especially the function of purification. It plays a central role in the detoxification and excretion of many exogenous and endogenous compounds, particularly fighting with allergens, poisons and toxins. By neutralizing, it turns them into safe and easily removable compounds. The liver also removes excess hormones, neurotransmitters (e.g. adrenaline, serotonin, etc.) from the body, as well as toxic products of metabolism - ammonia, phenol, ethanol, acetone, etc. The liver is actively involved in digestion, converting various energy sources (fats, amino acids, glycerin, etc.) into glucose. Besides, it regulates a carbohydrate metabolism, storing vitamins A, D and B12, cations of various trace elements, especially iron, cobalt and copper. In case of vitamin deficiency, the liver helps to synthesize vitamins A, B, C, D, E, K, PP and folic acid. The liver splits cholesterol, regulates lipid metabolism, synthesizes hormones and enzymes for the duodenum and small intestine, where main food splitting processes occur.

Hence, any injury to it or its dysfunction has serious implications for the health of the affected person. Every year, about 18,000 people are reported to die due to liver cirrhosis caused by hepatitis, although viral infection is one of the main causes for hepatic injury [13].

The human body identifies almost all medicines as foreign substances (i.e., xenobiotics) and subjects them to various chemical processes (such as metabolism) to make them suitable for elimination. Although almost all tissues in the body have some ability to metabolize chemicals, smooth endoplasmic reticulum in liver is the principal “metabolic clearing house” for both endogenous chemicals (e.g., cholesterol, steroid hormones, fatty acids, and proteins), and exogenous substances (e.g., drugs). But themain role of the liver is the clearance and transformation of chemicals, that makes it susceptible to medication-induced injury[15].

As it known, hepatic diseases of various types represent a major health burden and result in significant increase in mortality worldwide. Viral hepatitis, alcoholic/nonalcoholic fatty liver disease, liver fibrosis, cirrhosis, hepatocellular carcinoma, and drug-induced liver injury are of major health concerns claiming millions of lives. Pharmaceutical medicines are often associated with liver injury and hence provide limited benefits in treating liver diseases [16].

Therefore, there is a need of effective medications with a low incidence of side-effects. Phytopreparations potentially constitute such a group. In recent years many researchers have examined the effects of herbals to support liver function and treat hepatic diseases [17]. Thus, treating hepatic diseases with phytopreparations seems highly attractive in spite of the advances in conventional medicine in the last decades. Many factors contribute to phytopreparation’s appeal, including the claim that it may both treat and prevent diseases. This fact makes it to believe that these treatments are safe because they are “natural” and, therefore, harmless alternative to conventional medicine. In addition, phytopreparations are often exempt from rigorous regulations in many countries, and prescriptions are usually not required for these inexpensive products.

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Besides, it is very important to note several phytomolecules including flavonoids, alkaloids, glycosides and saponins obtained from various herbal sources have been reported as potent hepatoprotective agents. Plant tissues contain a wide variety of compounds with antioxidant activity. Phenolic compounds (flavonoids and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids and amines), carotenoids, lignans and terpenes were reported to possess antioxidative activity in suppressing the initiation or propagation of the chain reactions [18]. Flavonoids and phenolic compounds are the main antioxidative substances of phytopreparations. Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin. Over 4000 different flavonoids have been described. They may have beneficial health effects because of their antioxidant properties and their inhibitory role in various stages of tumor development[19].

The medicinal herbs with established hepatoprotective activity, discussed in the research,are Licorice (*Glycyrrhiza glabra)* root,cornsilk(Stigma maydis) and herbaHyperici (*Hypericum graveolens*). One of the common used of them isLicorice, the root of *Glycyrrizauralensis*, *Glycyrrhiza glabra* Linné or *Glycyrrhiza inflata* Batalin (Fabaceae), which has been used as traditional medicine since ancient times. In particular, Licorice was used as a medical raw material for such purposes as antidote, antitussive expectorant, relaxant, to relieve pain that occurs because of a sudden nervous breakdown of muscle or tissue, to reduce weight gain, to increase white blood cell count, and also because of its diuretic and anti-inflammatory effects. The biologically active components of Licorice are liquiritins, liquiritigenin, glycyrrhizic acids and flavones. A number of studies have been performed to analyze and characterize primary and secondary metabolites of Licorice [20].

On the basis of the above stated, it is relevant to use a ready medicinal form of hepatoprotective action for prevention and complex treatment of hepatobiliary system diseases, which contribute to normalizing liver work.

Starting with the development of new pharmaceutical preparations, at all stages from the production to a consumer, it is necessary to evaluate the probability of the risk of poor-quality medications production and to improve the systems of control and quality assurance. A special place is reservedto the control of microbiological quality of medications. Medications, which are not sterilizedin the process of manufacturing can be contaminated with microorganisms and therefore must be tested for microbiological quality.

The object of the studyisa combined liquid and dry extract, andcoated tablets, containing in its compositionLicorice (*Glycyrrhiza glabra)* root,cornsilk*(Stigma maydis*)and herbaHyperici (*Hypericum graveolens*).

The aim and tasks of the study. Several medicinal herbals have been identified which have significant hepatoprotective activity with minimal systemic adverse effects. The aim of the researchis to analyze and determine the microbiological quality of liquid and dry extract, and coated tablets which have been developed on the basis of the noted above herbals with hepatoprotective action.

II. MATERIALS AND METHODS

In microbiological conditions, pharmaceutical products can be divided into two groups: sterile and non-sterile. Non-sterile drugs must satisfy the appropriate microbiological purity criteria which are included in pharmacopoeial monographs. Pharmacopoeial studies are prepared specifically with a view to ensuring that the medicinal product is therapeutically effective and safe for the patient. The analysis comprised the results of microbiological quality tests performed before the products are marketed. The microbiological quality of medications was assessed in accordance with the indicator included in the State Pharmacopoeia (SP). But firstly it is appropriate to noteabout generally established microbiological quality parameters.

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Microbial quality parametersaccepted by WHO

The most widely accepted and used technique is that recommended by WHO for total count of microorganisms in plant materials. According to the methodology of the WHO, 10 g of sample should be suspended in 90 ml of buffer sodium chloride-peptone, adjusting the pH to 7.0. To count total aerobic bacteria, sample should be plated in duplicate, using the official technique of sowing depth on casein-soybean digest agar, and then incubated at 30-35°C for 48h. To count yeast and mold, the technique employed is the sowing depth in Sabourauddextrose plus a solution of 10% tartaric acid to obtain pH 3.0 to 3.5. The dilution is plated in duplicate and incubated at 20-25°C for 5 days. Analysis of specific pathogens, Entero- bacteriaceae and other Gram negative bacteria (E. coli, Salmonella sp., P. aeruginosa and S. aureus) consists of specific methods of cultivation and through biochemical and serological tests.

The specification of WHO for total aerobic microorganisms is not more than 107 CFU/g for the plant material for use as teas and infusions and at most 105 CFU/g for internal use. The specification of WHO for yeasts and molds are at most 104 CFU/g for the plant material for use as teas and infusions and at most 103 UFC/g for internal use. High counts of fungi are a risk because of the possibility to produce mycotoxin, such as aflatoxin, which is a carcino- gen toxin[21].

Determination of microbiological quality of hepatoprotective phytopreparationswas performed in accordance with the requirements of SP "Methods of microbiological control of medicines".

The tests were conducted in aseptic conditions, with application of methods and growth media to control all types of non-sterile medicines.

The test for microbiological quality involves quantitative determination of viable bacteria and fungi, as well as identifying certain species of microorganisms the presence of which is quite unacceptable in non-sterile medicines.

The test was carried out in aseptic conditions using the mentioned methods and nutrient media to control all kinds of non-sterile medicines, as well as raw materials used in their production.

Sampling and preparation of medications fortests.

An average sample with capacity not less than 50 g (ml) is taken, consisting of equal single samples taken from a minimum of 10 different packages, from each series of medication (regardless of its volume). For one medication analysisseparate samples of 10 g (ml) are used for each of the sections of the test described below. In total, 30 g (ml) of medicationis used for one analysis. In case if there is necessity to confirm the accuracy of the results, a retest is carried out only for the present section of the research, using a sample of 10 g (ml).

Depending on the physical properties of the dosage form, the testing sample is prepared as a solution, suspension or emulsion:

- solid medicinal forms which are difficult to dissolve, are crushed by appropriate equipment and are suspended in a phosphate buffer solution pH 7.0 or other suitable liquid nutrient medium recommended for the particular test type;
- liquid medicinal forms, as well as solid forms which dissolve rapidly and almost completely in phosphate buffer solution pH 7.0 or appropriate nutrient medium in dilution 1:10, are prepared in the form of solutions or suspensions;

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Prepared dilution samples are used to determine the total number of bacteria and fungi in 1 g (ml) of medication and to identification of the bacteria absence from the Enterobacteriaceae, Pseudomonas aeruginosa and Staphylococcus aureus families.

Quantitative determination of microorganisms

The test is carried out by anagar two-layer method in Petri dishes of diameter 90-100 mm. The medication sample of 10 g (ml) was dissolved, suspended or emulsified in a phosphate buffer solution pH 7.0, so that the final volume of the solution (suspension, emulsion) was 100 ml. Inoculations are looked through daily.

Determination of the total number of bacteria

The prepared sample solution (suspension, emulsion) is added by 1 ml to each of the two tubes with 4 ml of melted and cooled from 45 to 50 ºCgrowth medium N1. Then the content of the tube is quickly stirred and transferred to the Petri dish containing 15-20 ml of frozen growth medium N1. The upper layer of agar is distributed evenly by quick shaking of the Petri dish. After the solidification of medium, the dishes are turned over and incubated for 5 days at 30 to 35ºC. After 48 hours and finally after 5 days, the number of bacterial colonies in two dishes is counted, the average value is found, and the number of bacteria in 1 g (ml) of the sample is calculated by multiplying it by the dilution index.

For obtaining reliable results, the results of only those dishes, where the colonies have grown from 30 to 300 are taken into account. If there is no growth at dilution of sample 1:10, the results are noted as following: "1 g (ml) of medication (raw material) contains less than 10 bacteria".

Determination of the total number of fungi

The test is carried out by the agar two-layer method described above, using growth medium N2. Inoculations are incubated during 5 days at the temperature from 20 to 25 ºC. After 72 hours and finally after 5 days, the total number of colonies of yeast and mold fungi is counted in two dishes, the average value is found, and by multiplying it by the dilution index, i.e. by 10, the number of fungi in 1 g (ml) of the sample is calculated. In case if the number of fungal colonies per dish exceeds 300, further dilutions of the sample are done. All colonies of fungi are taken calculated in the dish, even if their number is less than 30.

The analysis was carried out in Petri dishes with sterile growth media: meat peptone agar - for quantitative determination of grown colonies, blood agar - for determination of hemolytic strains of bacteria, yolk-salt agar - for determination of pathogenic staphylococcus, Endo - for detection of intestinal groups of bacteria, Saburo - for identification of fungi. The testing medications in different concentrations (1: 2: 4) were transferred to the growth media, not less than in three Petri dishes. All dishes with inoculations were incubated in a thermostat at 37 °C except Saburo, at 20-25 °C. The initial results analysis was performed in 24 hours and 48 hours, the final - in 5 days. After incubation, the calculation and identification of grown colonies was carried out.

III. RESULTS

According to the results obtained in liquid and dry extracts, as well as in coated tablets, the total number of aerobic bacteria in 1 g composed 300 colony-forming units (CFU) (notdetermined at repeated microbiological analysis), the total number of yeast and mold fungi was less than 10 CFU. E.Coli, Salmonella, Ps. aeruginosa, St. Aureus and other gram-negative bacteria were not found in the process of the test.

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Table 1. Test results on different media of liquid and dry extracts for the presence of microorganisms

Identified microorganisms	Testing samples		
	Liquid extract	Dry extract	Coated tablets
MAOAM – mesophilic, aerobic and optional anaerobic microorganisms	absent	absent	absent
Bacteria of enterotoxigenic Escherichia coli	absent	absent	absent
E.coli	absent	absent	absent
B.cereus	absent	absent	absent
St.aureus	absent	absent	absent
Pathogenic flora	absent	absent	absent
Mold	absent	absent	absent
Yeast	absent	absent	absent

The analyzed liquid and dry extracts and coated tablets comply with the requirements of the index, according to which such medications should contain not more than 105 aerobic bacteria in 1 g, $10^4$of yeast mold fungi at the absence of Pseudomonas aureginosa, Staphylococcus aureus and not more than 10^3 of other intestinal bacteria. Evaluation of the quantitative and qualitative composition of the contamination flora did not reveal deviations from the requirements of SP XI.

IV. CONCLUSION

Using of phytopreparationson the base of plant extracts is widely preadin the treatment of a variety of clinical diseases, with large efforts being made to elucidate their modes of action. From year to year there is a tendency of choosing phytopreparations by the great number of patients who suffer from hepatic diseases. Future researches will cover extensive methodological improvements to separate the real therapeutic value of these medicines from unsubstantiated hopes associated with them. The active components of the herbals and phyto preparations should be isolated and tested through, and what is most important –to passtests organized in accordance with the articles of SP, for rational clinical use of the medications. Such controlled laboratory studies with herbals and especially their active ingredients in hepatic diseases are important, to discover their effect on the disease.

The proposed medicinal form of liquid extract, dry extract and coated tablets on its basis produced from medicinal plants: Licorice (*Glycyrrhiza glabra)* root,cornsilk(Stigma maydis) and herbaHyperici (*Hypericum graveolens*)complies with the requirements of SP, according to the indicator "Microbiological quality." The results are included in the drafts oftemporal pharmacopoeia article for the presentedmedicinal forms.

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