

BIOLOGICAL RESEARCH

UDC 612.6:615.322:615.331

DOI: 10.15587/2519-8025.2023.275942

INVESTIGATION OF THE INFLUENCE OF DRY EXTRACTS OF BUPLEURUM AUREUM AND SALSOLA COLLINA L. ON THE ANTIMICROBIAL EFFECT OF CO-TRIMOXAZOLE**Olga Naboka, Alla Kotvitska, Nataliia Filimonova, Alla Glushchenko, Olga Filiptsova, Alina Volkova**

Scientific data on the pharmacodynamics of dry extracts of *Bupleurum aureum* and *Salsola collina* L. based on the results of studying the antimicrobial effect and the similar effect of co-trimoxazole when they are used together have been supplemented. The investigated phytoextracts do not show antimicrobial properties, but they do not change the antimicrobial effect of co-trimoxazole when they are used in combination.

The aim of the study was to experimentally investigate the antimicrobial effect of extracts of *Bupleurum aureum* and *Salsola collina* L. and establish the possible antagonistic effect of these extracts on the antimicrobial drug co-trimoxazole when used together.

Materials and methods. The research was conducted in May 2016. Screening of the antimicrobial effect of extracts of *Bupleurum aureum* and *Salsola collina* L. and establishing of the possible antagonistic effect of these extracts on the antimicrobial drug co-trimoxazole when they are used together was carried out in the laboratory of the Department of Microbiology of the National Pharmaceutical University, which has a certificate of attestation 045/14 dated 28.10.2014. For determination of antimicrobial activity, the agar diffusion method ("well" method), which is based on the ability of medicinal substances to penetrate the agar layer, was used. A set of reference strains of microorganisms was used: *S. aureus* ATCC 6538, *E. coli* ATCC 8739, *P. aeruginosa* ATCC 9027, *B. subtilis* ATCC 6633, *C. albicans* ATCC 10231. Petri dishes were filled with two layers of solid nutrient medium. The lower layer - 10 ml of melted "cold" AGV agar (medium No. 3), the upper layer - nutrient medium for the corresponding test strain. After cooling the lower layer of agar, three thin-walled steel cylinders (inner diameter – 6.0 ± 0.1 mm, height - 10.0 ± 0.1 mm) were placed on it at an equal distance from each other and from the edge of the cup. The top layer was poured around the cylinders – 13.5 ml of agar, melted and cooled to 45-48°C, mixed with the seed dose of the test microorganism (1.5 ml of microbial suspension, the concentration corresponding to the type of microorganism). After cooling the upper layer of agar, the cylinders were removed with sterile tweezers and 0.25-0.3 ml of the studied drug was added to the resulting wells. The results were recorded after 24 h by measuring the zone of growth inhibition, including the diameter of the wells. Measurements were made with an accuracy of 1 mm, while focusing on the complete absence of visible growth.

The obtained data were analyzed using the methods of variational statistics. The significance level is $p < 0.05$. The studied plant extracts of *Bupleurum aureum* (aqueous and alcoholic) and *Salsola collina* L. (aqueous and alcoholic) were used in doses of 0.005 mg/ml and 0.01 mg/ml, which corresponded to doses of 5 mg/kg and 10 mg/kg. Experimental data were also processed by parametric (Newman-Keuls) and non-parametric (Mann-Whitney) methods of variational statistics, using the Statistica 6.0 statistical software package; differences were considered statistically significant at $p < 0.05$.

Results. At the final stage, the determination of the antimicrobial effect of water and alcohol extracts of *Bupleurum aureum* and *Salsola collina* L. at doses of 1 mg/ml and 5 mg/ml was carried out, and the effect of BAS of these extracts on the antimicrobial effect of co-trimoxazole when used together was determined. In the course of the study, it has been established, that the addition of the above-mentioned extracts to the co-trimoxazole formulation does not affect its initial antimicrobial properties.

Conclusion. Today, drug-induced liver injury remains one of the most important problems of hepatology and pediatrics, pharmacological science pays a lot of attention to the search for new effective and harmless drugs with a hepatoprotective effect, and the improvement of existing drugs is primarily aimed at increasing their specificity and reducing side effects related to the pharmacological properties of the drug. Currently, there is increasing interest in medicinal plants as a source of various biologically active substances (BAS), which provide a wide spectrum of pharmacological action of the agent, which allows to immediately affect various links of the pathogenesis of liver diseases. The analysis of scientific sources made it possible to establish that medicinal products of plant origin, thanks to BAS, possess polymodality of effects and reveal a versatile complex effect on the course of pathological processes in the body. Most drugs are characterized by good tolerability, absence of

withdrawal syndrome and toxicity to parenchymal organs. Medicinal plants are used not only as monopreparations, but also in combination with synthetic drugs and as raw materials for obtaining BAS

Keywords: *pediatrics, co-trimoxazole, phytoextracts, hepatoprotectors, antimicrobial action, combined drugs*

How to cite:

Naboka, O., Kotvitska, A., Filimonova, N., Glushchenko, A., Filiptsova, O., Volkova, A. (2023). Investigation of the influence of dry extracts of bupleurum aureum and Salsola collina L. on the antimicrobial effect of co-trimoxazole. ScienceRise: Biological Science, 1 (34), 00-00. doi: <http://doi.org/10.15587/2519-8025.2023.275942>

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1. Introduction

In recent years, the problem of drug-induced liver injury (DILI) has become more and more relevant, because they can be caused by about 1,000 drugs, more than 200 of which are potentially hepatotoxic [1, 2]. There is also data that when patients take six or more types of drugs at the same time, the probability of developing structural and functional changes in the liver reaches up to 80 % [3]. At the same time, the destructive effect is caused not so much by the drugs themselves, but by their reactive metabolites, which are formed in the liver with the participation of the biotransformation system of xenobiotics [4].

According to world statistics, 50 % of cases of acute liver injury are caused by drugs. Among them are analgesics/NSAIDs (paracetamol, diclofenac sodium, indomethacin, nimesulide), antibacterials (tetracycline, co-trimoxazole), antihypertensives (enalapril, methyldopa) and many others [5–7].

Co-trimoxazole as a combined antibacterial agent according to the ATS classification belongs to the group of antimicrobial agents for systemic use of the subgroup J01EE01 Sulfamethoxazole and trimethoprim. Analytical research of the pharmaceutical market of co-trimoxazole drugs, carried out with the help of content analysis of official sources of information (the State Register of Medicinal Products of Ukraine), data from the directory of drugs (Compendium) and the online aggregator of pharmacies, drugs, products for hygiene, health and beauty (Tabletki.ua), shows that 10 medical preparations from 8 manufacturers from three countries are currently registered in Ukraine [8–10]. The share of preparations of Ukrainian manufacturers is the largest among the registered drugs of co-trimoxazole. Fig. 1 shows the shares of co-trimoxazole drug-producing countries and pharmaceutical companies from each of them.

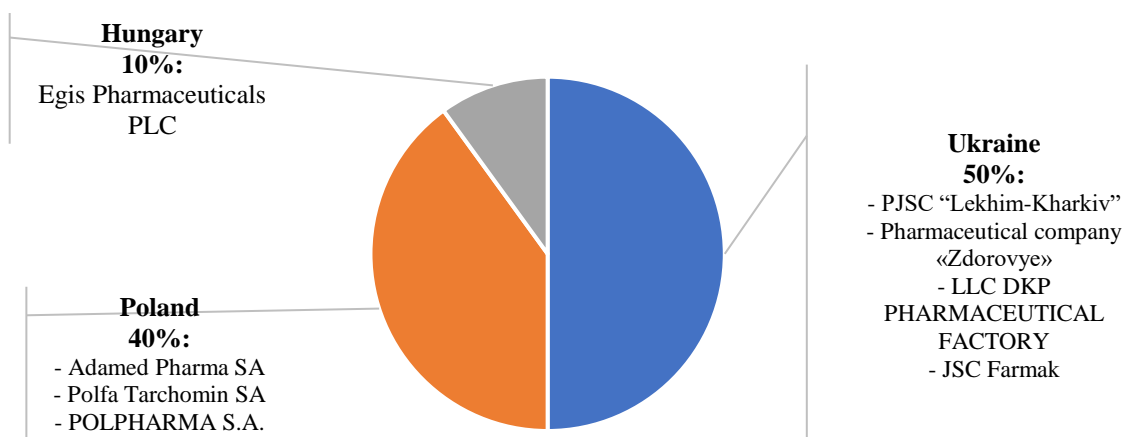


Fig. 1. Distribution of co-trimoxazole producing countries according to the State Register of Drugs of Ukraine

The structural analysis of the registered assortment of co-trimoxazole drugs showed the dominance of solid dosage forms – tablets (60 % of the assortment), presented in two dosages. Oral suspension (30 % of the assortment) and concentrate for preparation of solution for infusions (10 % of the assortment) are also presented

among the registered dosage forms (Fig. 2). Attention is drawn to the fact that only the last two dosage forms can be used in the treatment of children under 6 years of age – the concentrate for preparing a solution for infusions is recommended for babies from 6 weeks, the oral suspension – from 2 months.

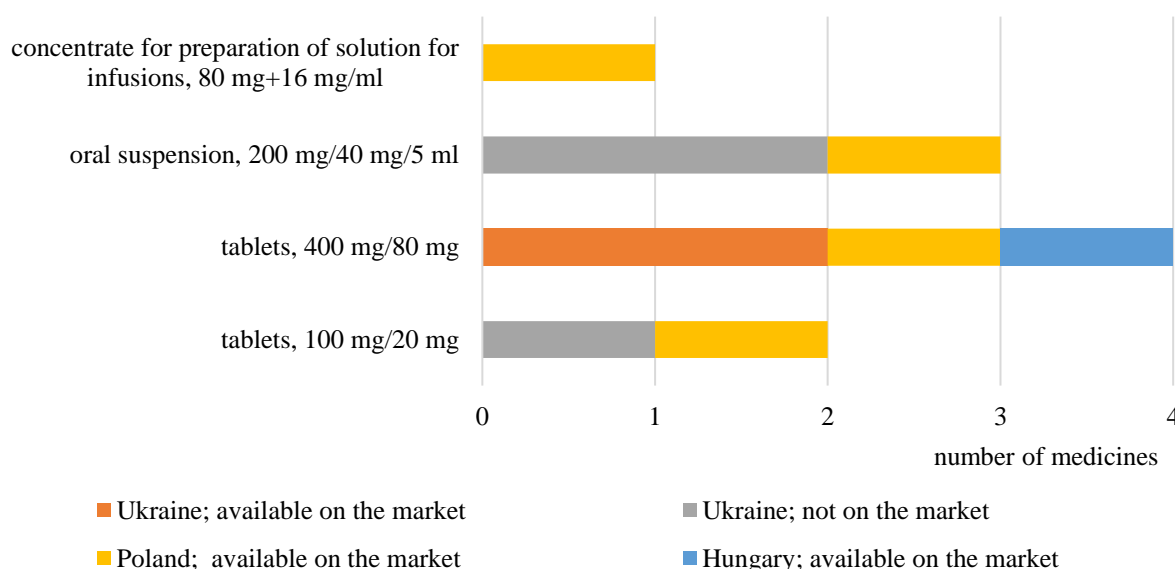


Fig. 2. Distribution of co-trimoxazole drugs by dosage forms, dosage, producing countries

It should be noted, that according to the results of the analysis of co-trimoxazole drugs available on the market, saturation of the assortment is noted at 70 %, since there are no Ukrainian-made drugs in the form of tablets 100 mg/20 mg and oral suspension 200 mg/40 mg/5ml among the offers of the drug wholesale and retail chain. Accordingly, this fact indicates the limited physical and economic availability of co-trimoxazole drugs for the population due to the dominance of foreign products available in pharmacies and emphasizes the urgency of the development of new drugs by Ukrainian manufacturers.

In Ukraine, the domestic drug co-trimoxazole under the trade name "B-tol", produced by LLC "DKP" "Pharmaceutical Factory", is widely used as an antimicrobial preparation in medical and pediatric practice, which is prescribed to adults and children from 6 months strictly according to indications, which is due to the presence of adverse reactions, in particular from the hepatobiliary and excretory systems [8].

Taking into account that DILI remains one of the most important problems of medicine and pediatrics, the improvement of existing drugs is primarily aimed at increasing their specificity and harmlessness by preventing the manifestation of side effects, associated with the mechanism of their pharmacological action, by creating combinations with potential herbal hepatoprotectors. A wide spectrum of biological action, availability in terms of price and significant harmlessness indicate the expediency and perspective of introducing herbal hepatoprotectors into the composition of children's dosage forms of drugs that can cause DILI [11]. As clinical practice has shown, children belong to the "vulnerable" group of patients, since special dosage forms have not been developed for most drugs, and the practice of off-label prescribing (not according to the instructions) is widespread in children [12]. In children, as well as in the elderly, the diagnosis of unwanted adverse reactions is difficult [13, 14]. At the same time, drug complications in children are more severe than in adults. According to literature sources, DILI most often develops in children under three years of age and in adults over 40 years of age [15].

The increase in the arsenal of domestically produced children's drugs, which is observed in Ukraine, is undoubtedly a very important fact, and one of the promising directions of modern medicine is the combination of compounds of synthetic and natural origin. This approach makes it possible due to the synergism of active substances to ensure the necessary pharmacological effect and reduce the effective dose of active substances [11]. Therefore, to eliminate the indicated undesirable effects of co-trimoxazole, we planned to introduce a phyto-component that exhibits a hepatoprotective effect, and thus, the creation of a new combined pediatric dosage form is planned.

In the light of the above, promising objects for study have become medicinal plants – *Bupleurum aureum*, *Salsola collina* L., *Fumaria Schleicheri*, artichoke (*Cynara scolymus* L.), which contain phenolic compounds, flavonoids [16], saikosaponins [17, 18], tannins, phytosterols, amino acids, micro- and macroelements, and have long been used in folk medicine for the treatment of liver diseases, exhibit antioxidant, choleric, anti-inflammatory, detoxifying, wound-healing effects [19, 20]. This became the basis for the creation and study of 16 original dry extracts from these plants, obtained using different extraction technologies (aqueous, 30 %, 50 % and 70 % alcohol extracts) in order to find an effective and harmless hepatoprotector. All of the above theoretically substantiates the relevance of the search for new potential hepatoprotectors among plant extracts from *Bupleurum aureum*, *Salsola collina* L., *Fumaria Schleicheri* and artichoke, and the experimental confirmation of the feasibility of their combined use with the antimicrobial drug co-trimoxazole to increase its safety.

According to previous studies on the model of tetrachloromethane hepatitis in rats, among 16 plant extracts, 5 were selected, namely: *Salsola collina* L. aqueous extract (SCAqE); of the *Salsola collina* L. alcoholic extract (SCAIE 30 %); *Salsola collina* L. alcoholic extract (SCAIE 50 %); *Bupleurum aureum* aqueous extract (BAAqE) and *Bupleurum aureum* alcoholic extract (BAAIE 50 %), which showed the most pronounced an-

tioxidant effect at a conditionally effective dose of 5 mg/kg. They became the subject of our further experimental studies. When studying acute toxicity on white sexually mature mice and white sexually immature rats, it has been established, that all plant extracts, selected at the screening stage, when administered intragastrically belong to toxicity class V – practically harmless substances ($LD_{50} > 5000$ mg/kg) [21].

At the next stage of the work, the pharmacological properties (membranoprotective, anti-inflammatory, choleric) of BAAqE, BAAIE 50 %, SCAqE, SCAIE 30 %, SCAIE 50 % and therapeutic effectiveness (hepatoprotective effect) of BAAIE 50 % were determined. SCAqE and SCAIE 50 % on the model of spontaneous hemolysis of erythrocytes in rats showed a pronounced membrane-protective effect at the level of 45 % and 37 % ($p < 0.05$), respectively, and in terms of expressiveness, quercetin at a dose of 50 mg/kg was statistically significantly superior by 33 % and 25 % ($p < 0.05$), and SCAqE – also vitamin E at a dose of 50 mg/kg by 20 % ($p < 0.05$) [22]. Note that against the background of acute tetrachloromethane hepatitis in rats, the membrane-protective effect of SCAqE and SCAIE 50 % was more pronounced than with spontaneous hemolysis of erythrocytes and exceeded that of quercetin by 21 % and 16 % ($p < 0.05$), respectively, which can be explained by a greater lability of erythrocyte membranes, the physical and chemical parameters of which are disturbed during pathology. The membrane-stabilizing effect of BAAIE 50 % (54.5 %, $p < 0.05$) was confirmed on the model of disruption of the osmotic environment of erythrocytes, which was at the level of vitamin E (47.7 %, $p < 0.05$) [21].

In view of the fact that an excess of lipid peroxides disrupts the physical and chemical structure of cell membranes, inhibits their enzyme systems, inactivates cytoplasmic enzymes, reduces the activity of thiol enzymes, which leads to the development of exudative and alterative processes in tissues, the anti-inflammatory activity of extracts was investigated in models of acute exudative inflammation, caused by carrageenan and zymosan and models of chronic aseptic skin inflammation in rats. Experimental data show that BAAIE 50 % and SCAIE 50 % show a moderate (29 % and 32 %, $p < 0.05$) anti-exudative effect. All extracts were inferior to diclofenac sodium at a dose of 8 mg/kg and quercetin at a dose of 50 mg/kg. At the same time, on the model of aseptic inflammation of the skin, which makes it possible to assess the effect of drugs on other inflammatory processes, the highest rate of wound healing in rats was established for BAAIE 50 % just at the second day of observations, which was 85 % ($p < 0.05$), which indicates its distinct wound-healing and anti-alterative effect. In general, the studied extracts can be arranged in the following order of decreasing antialteration activity: BAAIE 50 % (93 %) > SCAIE 50 % (78 %) > BAAqE (74 %) > ESCAqE (72 %) > SCAIE 30 % (68 %) > quercetin (65 %) [23].

It has been established, that the combined use of BAAIE 50 % or SCAIE 50 % with the antimicrobial drug co-trimoxazole in a dose of 0.7 mg/kg prevents the manifestations of cytolytic and cholestatic syndromes, normalizes transaminase activity of the blood, reduces the content of bilirubin, and the activity of alkaline phosphatase in the blood of sexually immature rats, which indi-

cates a weakening of the inflammatory process in the liver and activation of organ regeneration. The studied extracts reduced the peroxidation of lipids, increased the content of reduced glutathione and normalized the activity of catalase in experimental animals. The experiment showed that co-trimoxazole in a dose of 0.7 mg/kg with a two-week course of administration has a hepatotoxic effect. Sexually immature rats developed moderately pronounced drug-reactive subacute hepatitis with an increased level of hepatocyte apoptosis. BAAIE 50 % and SCAIE 50 % corrected the hepatotoxic effect of co-trimoxazole at a dose of 0.7 mg/kg, reduced the expression of hepatocyte apoptosis. BAAIE 50 % had the advantage. The hepatoprotective action of both extracts had practically no dose-dependent effect. In terms of hepatoprotective effect, the extract of *Salsola collina* L. was not inferior to quercetin at a dose of 50 mg/kg and inferior to silibor at a dose of 100 mg/kg. BAAIE 50 % was superior to quercetin and not inferior to silibor. During a two-week course of administration of co-trimoxazole at a dose of 0.7 mg/kg, an increased presence of apoptotic cells was observed in the kidneys of sexually immature rats. According to the apoptosis-protective effect in the kidneys, BAAIE 50 % had the advantage, it was not inferior to quercetin at a dose of 50 mg/kg and silibor at a dose of 100 mg/kg. SCAIE 50 % was inferior to comparison drugs in terms of this effect [24].

The experiment proved that co-trimoxazole in a dose of 0.7 mg/kg does not show a noticeable pancreatotoxic effect. At the same time, an increased presence of apoptotic cells was observed in the pancreas. SCAIE 50 % and BAAIE 50 % contributed to the reduction of the presence of apoptotic cells in the pancreatic islets and acinous parenchyma of the pancreas of rats, prevailed over the similar effect of quercetin at a dose of 50 mg/kg and were almost at the level (especially the extract of *Bupleurum aureum*) of silibor in a dose of 100 mg/kg [25].

The experiment showed that in the organs of immunogenesis (thymus and spleen) of rats after administration of co-trimoxazole in a dose of 0.7 mg/kg, standard signs of immune response appear. The investigated extracts of *Salsola collina* L. and *Bupleurum aureum* in doses of 5 mg/kg and 10 mg/kg softened the expression of the standard signs of the immune response to antigenic stimulation with co-trimoxazole and in terms of such "immunomodulatory effect" were on a par with the comparison drugs: quercetin in a dose of 50 mg/kg and silibor in a dose of 100 mg/kg [25].

Thus, the long-term administration of co-trimoxazole (at a dose of 0.7 mg/kg) to sexually immature rats, the equivalent of which is the therapeutic maximum daily dose for a child, caused significant changes in the structural and functional state of the liver compared to the control group, with the development of cytotoxicity, cholestasis, disorders of POL/AOS, intoxication of the body of sexually immature rats. The combined use of extracts of *Salsola collina* L. and *Bupleurum aureum* (aqueous and 50 % alcohol extracts) with co-trimoxazole reduced its organotoxicity, reliable positive changes in biochemical markers of drug-induced liver lesions were obtained. The reduction of fatty dystrophy, coagulation necrosis of hepatocytes, and reduction of the number of necrosis foci in the kidneys, pan-

creatic islets, and acinous parenchyma of the pancreas, thymus, and spleen of sexually immature rats, damaged by co-trimoxazole, was confirmed by morphological methods of using the above-mentioned extracts [24, 25].

At the final stage, it was expedient to determine the antimicrobial effect of aqueous and alcoholic extracts of *Salsola collina* L. and *Bupleurum aureum* in doses of 1 mg/ml and 5 mg/ml and to establish the action of biologically active substances of these extracts on the antimicrobial effect of co-trimoxazole when used together. This became the goal of this study.

2. Materials and methods

The research was conducted in May 2016. Screening of the antimicrobial effect of extracts of *Bupleurum aureum* and *Salsola collina* L. and establishing of the possible antagonistic effect of these extracts on the antimicrobial drug co-trimoxazole when they are used together was carried out in the laboratory of the Department of Microbiology of the National Pharmaceutical University, which has a certificate of attestation 045/14 dated 28.10.2014. For determination of antimicrobial activity, the agar diffusion method ("well" method), which is based on the ability of medicinal substances to penetrate the agar layer, was used [26]. A set of reference strains of microorganisms was used: *S. aureus* ATCC 6538, *E. coli* ATCC 8739, *P. aeruginosa* ATCC 9027, *B. subtilis* ATCC 6633, *C. albicans* ATCC 10231. Petri dishes were filled with two layers of solid nutrient medium. The lower layer – 10 ml of melted "cold" AGV agar (medium No. 3), the upper layer – nutrient medium for the corresponding test strain. After cooling the lower layer of agar, three thin-walled steel cylinders (inner diameter – 6.0 ± 0.1 mm, height – 10.0 ± 0.1 mm) were placed on

it at an equal distance from each other and from the edge of the cup. The top layer was poured around the cylinders – 13.5 ml of agar, melted and cooled to 45–48 °C, mixed with the seed dose of the test microorganism (1.5 ml of microbial suspension, the concentration corresponding to the type of microorganism). After cooling the upper layer of agar, the cylinders were removed with sterile tweezers and 0.25–0.3 ml of the studied drug was added to the resulting wells. The results were recorded after 24 h by measuring the zone of growth inhibition, including the diameter of the wells. Measurements were made with an accuracy of 1 mm, while focusing on the complete absence of visible growth.

The obtained data were analyzed using the methods of variational statistics. The significance level is $p < 0.05$. The studied plant extracts of *Bupleurum Aureum* (aqueous and alcoholic) and *Salsola Collina* L. (aqueous and alcoholic) were used in doses of 0.005 mg/ml and 0.01 mg/ml, which corresponded to doses of 5 mg/kg and 10 mg/kg. Experimental data were also processed by parametric (Newman-Keuls) and non-parametric (Mann-Whitney) methods of variational statistics, using the Statistica 6.0 statistical software package; differences were considered statistically significant at $p < 0.05$ [27].

3. Research results

The method of diffusion in agar ("wells" method) was used to determine the antimicrobial activity. The investigated plant extracts of *Bupleurum Aureum* (aqueous and alcoholic) and *Salsola Collina* L. (aqueous and alcoholic) were used in concentrations of 0.005 mg/ml and 0.01 mg/ml, which corresponded to doses of 5 mg/kg and 10 mg/kg. The results of the research are shown in Table 1.

Table 1

Antimicrobial activity of extracts of *Bupleurum Aureum* and *Salsola Collina* L., obtained by aqueous and alcohol (50 %) extraction

| Sample name | Growth inhibition zone, mm | | | | |
|-------------------------|----------------------------|----------------|--------------------|----------------------|--------------------|
| | <i>S. aureus</i> | <i>E. coli</i> | <i>B. subtilis</i> | <i>P. aeruginosa</i> | <i>C. albicans</i> |
| Co-trimoxazole | 49 | 40 | 56 | 55 | 14 |
| BAAqE, 0.005mg/ml | – | – | – | – | – |
| SCAqE, 0.005mg/ml | – | – | – | – | – |
| BAAqE, 0.01 mg/ml | – | – | – | – | – |
| SCAqE, 0.01 mg/ml | – | – | – | – | – |
| BAAIE 50 %, 0.005mg/ml | – | – | – | – | – |
| SCAIE 50 %, 0.005 mg/ml | – | – | – | – | – |
| BAAIE 50 %, 0.01 mg/ml | – | – | – | – | – |
| SCAIE 50 %, 0.01 mg/ml | – | – | – | – | – |

Note: "–" presence of the growth of microorganisms

The conducted series of studies established that the studied plant extracts did not show antimicrobial activity in concentrations corresponding to the experimental 5 mg/ml and 10 mg/ml.

The following series of studies was conducted to establish the possible antagonistic effect of the studied

extracts on the drug co-trimoxazole. Plant extracts of *Bupleurum Aureum* (aqueous and alcoholic) and *Salsola Collina* L. (aqueous and alcoholic) were used in concentrations of 1 mg/ml and 5 mg/ml. The results of the research are shown in Table 2.

Table 2

The results of the study of the action of extracts of *Bupleurum Aureum* and *Salsola Collina L.*, obtained by aqueous and alcohol (50 %) extraction, on the antimicrobial effect of co-trimoxazole

| Sample name | Growth inhibition zone, mm | | | | |
|---------------------------------------|----------------------------|----------------|--------------------|----------------------|--------------------|
| | <i>S. aureus</i> | <i>E. coli</i> | <i>B. subtilis</i> | <i>P. aeruginosa</i> | <i>C. albicans</i> |
| Co-trimoxazole | 49.2±1.2 | 40.8±1.5 | 56.5±1.5 | 56.0±0.9 | 15.2±0.7 |
| BAAqE, 1 mg/ml | – | – | – | – | – |
| SCAqE, 1 mg/ml | – | – | – | – | – |
| BAAqE, 5 mg/ml | – | – | – | – | – |
| SCAqE, 5 mg/ml | – | – | – | – | – |
| BAAIE 50 %, 1 mg/ml | – | – | – | – | – |
| SCAIE 50 %, mg/ml | – | – | – | – | – |
| BAAIE 50 %, 5 mg/ml | – | – | – | – | – |
| SCAIE 50 %, 5 mg/ml | – | – | – | – | – |
| BAAqE (1 mg/ml) + co-trimoxazole | 41.3±2.4 | 47.6±2.7 | 49.5±1.5 | 49.6±3.1 | 15.5±0.5 |
| SCAqE (1 mg/ml) + co-trimoxazole | 39.8±1.3 | 47.5±3.08 | 48.6±2.5 | 48.3±2.6 | 15.5±1.04 |
| BAAqE (5 mg/ml) + co-trimoxazole | 41.0±2.09 | 40.0±1.4 | 40.0±1.4 | 49.6±1.4 | 15.5±1.04 |
| SCAqE (5 mg/ml) + co-trimoxazole | 39.3±1.2 | 40.0±1.4 | 39.3±1.7 | 49.2±0.7 | 15.5±0.5 |
| BAAIE 50 % (1 mg/ml) + co-trimoxazole | 42.2±2.3 | 40.3±1.4 | 42.5±2.3 | 50.8±1.47 | 15.6±1.03 |
| SCAIE 50 % (1 mg/ml) + co-trimoxazole | 41.2±2.6 | 47.3±3.4 | 48.0±2.1 | 48.0±3.2 | 15.8±0.7 |
| BAAIE 50 % (5 mg/ml) + co-trimoxazole | 44.6±5.3 | 46.6±4.5 | 47.2±3.9 | 47.8±2.6 | 15.6±0.8 |
| SCAIE 50 % (5 mg/ml) + co-trimoxazole | 36.8±1.7 | 44.6±4.8 | 45.0±4.3 | 50.0±1.4 | 15.6±0.5 |

Note: "–" presence of the growth of microorganisms

According to the results of the conducted studies, it has been concluded, that no pronounced antagonism was detected between co-trimoxazole and the tested samples in doses of 1 mg/ml and 5 mg/ml.

Thus, according to the results of the conducted research, it has established been, that the addition of extracts of *Bupleurum Aureum* and *Salsola Collina L.* to the co-trimoxazole formulation does not significantly affect the initial antimicrobial properties of the latter.

4. Discussion of research results

Today, in Ukraine, liver injury, caused by drugs that are necessary for adequate antibacterial therapy, regardless of their side effects, remain one of the unsolved problems of medicine and pediatrics [28, 29]. Therefore, increasing the safety of patient treatment and preventing the manifestation of undesirable effects related to the mechanism of pharmacological action of drugs affecting the liver can be achieved by their combined use with herbal hepatoprotectors [11, 30]. The study provides a theoretical and experimental solution to the scientific problem of pharmacology, which consists in the search for new potential hepatoprotectors among plant extracts from *Bupleurum Aureum* and *Salsola Collina L.* for the pharmacocorrection of toxic manifestations of the antimicrobial drug co-trimoxazole [31].

Comparing the effectiveness of BAAIE 50 % on various models of hepatitis in rats, it can be stated, that the mechanism of its hepatoprotective action is related to the ability of the biologically active substances, included in its composition, to normalize redox processes in the liver. According to [20], saikosaponins-a and saikosaponins-d

(triterpene glycosides) were isolated from different species of *Bupleurum* (*Bupleurum Chinense* and *Bupleurum scorzoneraefolium*) and their pharmacological activity was investigated. It has been established, that saikosaponins stimulate autophagy in the cell (getting rid of old and damaged parts of the cell with the help of lysosomes), which plays a key role in protecting against infection, against the accumulation of toxic proteins and maintaining normal homeostasis. Considering the above, BAAIE 50 % increases the elimination of toxic products (medium molecules, TBA-R) from the body of animals due to the activation of autophagy by saikosaponins, which are part of it [21].

According to the results of the conducted studies, it has been concluded, that no pronounced antagonism between co-trimoxazole and the studied samples of BAAIE 50 % and SCAIE 50 % in doses of 1 mg/ml and 5 mg/ml was detected. It has been established, that the addition of extracts of *Bupleurum Aureum* and *Salsola Collina L.* to the co-trimoxazole formulation does not significantly affect its original antimicrobial properties.

Thus, on the basis of the results of our own research, when searching for new original plant hepatoprotectors for the purpose of pharmacocorrection of DILI in adults and children, in studies, conducted using adult (sexually mature) and child (sexually immature 1 month old) rats, the expediency of the combined use of the antimicrobial drug co-trimoxazole with extracts of *Bupleurum Aureum* and *Salsola Collina L.* which showed a pronounced hepatoprotective effect, was experimentally substantiated. The proposed approach will allow to optimize and to ensure proper antibacterial therapy in medical and pediatric practice when using drugs with proven hepatotoxicity.

Limitations of the study. The research was conducted using vegetative cells of reference cultures.

The prospect of further research is the study of the specific pharmacological effect of the combined dosage form of co-trimoxazole suspension and dry extract of *Bupleurum Aureum* 50 %.

5. Conclusions

1. Aqueous and alcoholic extracts of *Bupleurum Aureum* and *Salsola Collina L.* at concentrations of 0.005 mg/ml and 0.01 mg/ml, which correspond to experimental doses of 5 mg/kg and 10 mg/kg, do not show antimicrobial activity.

2. An increase in the concentration of the investigated phytoextracts by 100–200 times (dose 1 mg/ml) and 500–1000 times (dose 5 mg/ml) was not accompanied by the detection of antimicrobial activity.

3. The combined use of the antimicrobial drug co-trimoxazol with samples of aqueous and alcoholic ex-

tracts of *Bupleurum Aureum* and *Salsola Collina L.* was not accompanied by pronounced antagonism.

4. The combined use of co-trimoxazole with aqueous and 50 % alcoholic extracts of *Bupleurum Aureum* and *Salsola Collina L.* at a concentration of 1 mg/ml should be defined as the most promising among the samples.

Conflict of interests

The authors declare that they have no conflict of interest regarding this research, including financial, personal, authorship or any other kind of conflict that could influence the research and its results, presented in this article.

Funding

The study was conducted without any financial support.

Data Availability

The data will be provided upon a reasonable request.

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Received date 07.02.2023

Accepted date 21.03.2023

Published date 31.03.2023

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