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# The Research Of The Hypericum Extracts' Pharmacological Activity

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The results of a comparative study of the acute toxicity of extracts of plants of the genus Hypericum are shown. It is found that extracts are non-toxic and exhibit anti-inflammatory and antimicrobial effect.

The aim of this Study was to investigate the pharmacological activity of extracts from medicinal herbs.

*Keyword:* Acute Toxicity, Hypericum Species, Extracts, Pre-Clinical Study, Anti-Inflammatory And Antimicrobial Effect.

**INTRODUCTION:** The centuries-long history of scientific medical use of medicinal plants is closely linked with the development of civilization. All plants contain biologically active compounds that exhibit complex effects on living organisms and have a wide range of therapeutic activity.

Flora of Ukraine is characterized by a variety of plants' species. Special attention deserve plants with long experience of use in medicine, which include species of the genus Hypericum.

The **aim of our study** was to investigate the pharmacological activity of extracts from medicinal herbs.

## MATERIALS AND METHODST

The objects of study were aqueous and aqueous-alcoholic extracts of Hypericum perforatum and Hypericum maculatum (Table 1).

***Table 1*** **: Objects of research**

| Number c/o | Medicinal plant | Raw | Type extractant | Symbol |
|---|---|---|---|---|
| 1 | Hypericum (H.) perforatum | grass | 40% ethanol | HP-4 |
| | | | Purified water | HP-0 |
| 2 | H. maculatum | grass | 40% ethanol | HM-4 |
| | | | Purified water | HM-0 |

Types of hypericum are widespread in Ukraine, they are used in scientific and traditional medicine and exhibit anti-inflammatory, antimicrobial effect and effect of healing wounds [1 - 4].

At the department of Pharmacy of Ivano-Frankivsk National Medical University, we obtained extracts from the studied raw materials. During the developing of parameters of extracts we took into account the degree of comminution of raw, the nature of the solvent, extraction time, ratio of raw-extractant, frequency of extraction, and extraction temperature limits. In establishing the optimal parameters of obtaining the extracts we were determining the quantitative content of active and extractive substances.
Analysis of the chemical composition of the obtained extracts showed that they all contain significant amount of phenolic compounds and polysaccharides [5].

Information about the use of the studied plants in folk medicine and results of their study phytochemical allowed to identify the main areas of research, namely the study of acute toxicity, anti-inflammatory and antimicrobial activity.
The study of biological activity from extracts was made with the advisory assistance of professor of biochemistry of Ivano-Frankivsk National Medical University Klimenko A.A. and professor of clinical pharmacy at the exchange rate of pharmacology and clinical pharmacology Hudyvok Y.S. Experimental work was performed in a volume of simple pharmacological screening [6, 7].

All factual material was worked out by the method of variation statistics calculation the arithmetic mean and its statistical error, the reliability of comparable values was evaluated by Student's test, the level of probability was accepted $p \leq 0,05$ [8].

Experimental studies were carried out on white mice and rats, which were standardized by physiological and biochemical indices and were in a vivarium in compliance with sanitation standards on a standard diet.

The *acute toxicity* of extracts' study was performed by the method of B.M. Shtabskyy and R.M. Gzhegockyy [9].

Acute toxicity study was carried out on outbred white male mice, weighing 20 - 24 g, who were on a regular diet. Animals were injected intragastric of aqueous preparations.

Observation of the animals was performed within 14 days. Systematic registration of indicators of each animal was performed at least once a day.

The study of *anti-inflammatory activity* of Hypericum perforatum and Hypericum maculatum extracts was performed by the method of F.P. Trynus and co-authors (1975) on rat paw edema model caused by subplantarnum introduction of flologeninus agent [10]. With this aim under the aponeurosis of sole hind paw injected 0.1 ml of 2 % aqueous solution of formalin. Experimental studies were carried out on white male rats weighing 0.20 - 0.22 kg, divided into 5 groups.

2 hours before and immediately after entering flologeninus agent animals of 1 - 4 groups orally administered water-alcohol and water extracts of Hypericum perforatum and Hypericum maculatum at doses 100 mg per 100 g body weight.

The fifth group includes the control animals, which were injected 0.1 ml of 2 % aqueous solution of formalin. Measurement of paw volume was performed before the experiment, in 1 hour, 3 hours, and at the time of greatest development of edema in 5 hours.
Effect of extracts was assessed by the ability to inhibit edema quotes rats in 1 hour, 3 hours and 5 hours.

Effect of extracts was assessed by the ability to inhibit edema quotes rats.

Anti-inflammatory efficacy was calculated using the formula:

$$\% \text{ Inhibition of inflammation} = (V_c - V_o) / V_c \cdot 100,$$

where:
$V_c$ - average volume increase of swollen feet in control;
$V_o$ - the average increase in the volume of swollen feet in treated animals.

The investigation of *antimicrobial activity* of Hypericum perforatum and Hypericum maculatum extracts was carried out using the method of active substances diffusion in agar using paper discs. The concentration of active substance on disks for extracts was 5 mg [11].
As a universal nutrient environment 5 % blood agar and daily broth cultures from 1 % sugar broth in suspension with density of 1 billion microbes were used.

1 ml of the bacterial suspension was deposited on the surface of 5 % blood agar and evenly rubbed in it. Crops were incubated at 37 $^0$C for 24 - 72 hours depending on the cultural characteristics of the studied culture. Evaluation of antimicrobial activity was performed by measuring ruler of delay zone the growth of microorganisms (in mm) around the investigational drug. As studied cultures Pseudomonas aeruginosa, Esherichia coli, Proteus vulgaris, Staphylococcus aureus, Staphylococcus epidermidis were used.

For comparison of bacteriostatic activity of studied drugs paper disks with antibiotics: ampicillin - 30 mg / disc, troleandomitsin - 30 mg / disc were used.

## RESULTS AND DISCUSSION

While studying of *acute toxicity* was found that a substance in doses 8000 - 9000 mg / kg when injected into the stomach don't cause the death of animals within 14 days. Symptoms of intoxication were not found. After drug administration for the entire period of observation in animals injected with solutions extracts normal reflexes were kept, abnormalities in behavior, clinical symptoms of intoxication and death of animals was not observed.

The results are shown in Table 2.

***Table 2:*** **The results of acute toxicity of extracts investigation**

| Number c/o | The name of the substance | $DL_{50}$, mg / kg |
|---|---|---|
| 1 | *Aqueous alcoholic extract of Hypericum maculatum* | 8000 |
| 2 | Aqueous extract of Hypericum maculatum | 8000 |
| 3 | Aqueous alcoholic extract of Hypericum perforatum | 8500 |
| 4 | Aqueous extract of Hypericum perforatum | 9000 |

As a result of the research after intragastric administration of Hypericum perforatum and Hypericum maculatum extracts at doses of 8000 - 9000 mg / kg the animal deaths were not observed (Table 2), they were neat, had satisfactory appetite, respond normally to sound and light stimuli, processes of urination and defecation were normal, respiratory failure and the court is not observed.

So, as a result of the study it is revealed that intragastric administration of extracts does not result in death of the animals that indicates a lack of toxicity and characterizes them as practically non-toxic (V class toxicity, $DL_{50}$> 5000 mg / kg) according to the classification of substances for toxicity [6].

The studies show that all studied extracts exhibit *anti-inflammatory* activity. Antiexudative activity of Hypericum perforatum and Hypericum maculatum extracts on a model of rat paw formalin inflammation is presented in Table 3.

***Table 3:*** **Antiexudative activity of Hypericum perforatum and Hypericum punctatum**

| № of group | The object of the study | *Increase the volume of quotes, %* | | |
|---|---|---|---|---|
| | | in1 hour | In 3 hours | in 5 hours |
| 1 | *HM-4* | 115 ± 2,90 | 115 ± 2,90 | 115 ± 2,90 |
| 2 | HM-0 | 120 ± 2,09 | 122 ± 1,61 | 120 ± 2,25 |
| 3 | HP-4 | 113 ± 1,93 | 116 ± 1,77 | 116 ± 2,09 |
| 4 | HP-0 | 121 ± 2,90 | 120 ± 2,58 | 120 ± 1,45 |
| 5 | - | 127 ± 3,86 | 135 ± 3,86 | 137 ± 2,74 |
| № of group | Name of drug | Rate of inflammatory reaction's inhibition, % | | |
| | | in 1 hour | in 3 hours | in 5 hours |
| 1 | *HM-4* | 44,4 | 57,1 | 59,5 |
| 2 | HM-0 | 25,9 | 37,1 | 45,9 |
| 3 | HP-4 | 51,9 | 54,3 | 56,8 |
| 4 | HP-0 | 22,2 | 42,9 | 45,9 |

The results of the studies (Table 3) show that among all investigated extracts the highest anti-inflammatory activity shows water-alcohol extract of Hypericum maculatum that inhibits the inflammatory response by 59,5 %. The results suggest promising drug development from this raw material with anti-inflammatory action.

The results of *antimicrobial studies* indicate that the water-alcohol extract retard growth of rod and koko microflora, which are often agents of nosocomial infections and pathogens of secondary infection. The most sensitive to the action of extracts are strains of Staphylococcus and the most stable are strains of Pseudomonas aeruginosa.

The results of these studies are presented in Table 4.

The studies show that the best bacteriostatic effect exhibits water-alcohol extract of Hypericum perforatum and Hypericum maculatum. Bacteriostatic effect of Hypericum maculatum extract is relatively higher than that of bacteria Pseudomonas aeruginosa compared with Hypericum perforatum extract.

*Table 4:* **The results of bacteriostatic activity of Hypericum perforatum and Hypericum maculatum to certain microorganisms investigation**

| № c/o | Notation extracts | Zone of stunted growth of microorganisms, mm | | | | |
|---|---|---|---|---|---|---|
| | | Pseudomonas aeruginosa | Escherichia coli | Proteus vulgaris | Staphylococcus aureus | Staphylococcus epidermidis |
| 1 | *HM-4* | 15 | 8 | 6 | 9 | 6 |
| 2 | HP-4 | 6 | 10 | 9 | 8 | 5 |

## CONCLUSION

While studying the acute toxicity of received extracts of Hypericum species it was established that all investigated extracts are nontoxic. Investigation of anti-inflammatory action showed that among all tested extracts the highest anti-inflammatory activity shows water-alcohol extract of Hypericum maculatum that inhibits the inflammatory response by 59,5 %. Investigation of antimicrobial activity of obtained extracts showed that the best antimicrobial activity exhibit water-alcohol extracts of Hypericum perforatum and Hypericum maculatum. Hypericum perforatum and Hypericum maculatum extracts are promising raw material for the development of drugs with antimicrobial and anti-inflammatory effect.

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