Hedera helix L. (English ivy, Common ivy) is an evergreen climbing plant of Araliaceae family, with deep green, glossy, coriaceous leaves, whose shape depends on the type of a branch (flowering or non-flowering) and the location on stems. English ivy naturally grows in Western, Central and Southern Europe and was also introduced in North America and Asia.

The biologically active compounds, mainly responsible for the medicinal application of the H. helix extracts, in medicine are triterpene saponins, particularly hederacoside C (not less than 3.0% in dried herbal substance, according to European Pharmacopoeia (1)) with a small amount of α-hederin, hederagenin (which can be developed by hydrolysis during the drying process), Phenolics (flavonoids, coumarins and phenolic acids), steroids, volatile oil, and polyacetylenes are also present in leaves (2).

The Hederas leaves extracts exhibit spasmyloytic, anti-inflammatory, antimicrobial, analgesic, antimitogenic and antioxidant activities, and are used as an expectorant in case of a productive cough, for catarrhs of the respiratory tract, and in case of the symptoms of chronic inflammatory bronchial conditions (2-7).

**ANTI-INFLAMMATORY, ANTIMICROBIAL ACTIVITY AND INFLUENCE ON THE LUNGS AND BRONCHUS OF HEDERA HELIX LEAVES EXTRACTS**

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**Abstract:** Hedera helix leaves extracts are traditionally used in medicine as an expectorant in case of a productive cough associated with a cold. The aim of the study was to investigate anti-inflammatory and antimicrobial activity of the Hedera leaves extracts obtained using water as well as 30% and 70% ethanol. The most potent extract (70% ethanol) with the highest concentration of hederacoside C was investigated on its influence on the lungs and airways in an experimental model of bronchiolitis. The influence of the extract on the lung evaluated histopathologically, and as a result, a reduction of dystrophy, a correction of hemodynamic disorders and a decrease in reactivity of bronchus-associated lymphoid tissue were observed. The pharmacological effect of this extract can be explained by an increased exudation with the release of fluid and erythrocytes from the microvasculature. The inhibition of reactions in the hyperplastic lymphoid tissue may have here an indirect effect. Concluding, 70% ethanolic extract of Hedera leaves should be recommended to produce effective mucolytic preparations for the treatment of acute and chronic inflammation-associated bronchopulmonary infections.

**Keywords:** Hedera helix extracts, antimicrobial activity, anti-inflammatory activity, experimental bronchiolitis

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The aim of the study was to determine the hederacoside C content in three different extracts from the leaves of *H. helix*, to evaluate the antimicrobial activity *in vitro*, and the anti-inflammatory activity in the carrageenan-induced paw edema test as well as the effects on bronchial and lung tissue of the most potent H70 extract.

**MATERIALS AND METHODS**

**Plant material**

The leaves of *H. helix* were collected in the autumn in the Botanical Garden of Danylo Halytsky Lviv National Medical University, Lviv, Ukraine. The plant was authenticated by dr. O. Cherpak (Department of Pharmacognosy and Botany of the same University). A voucher specimen No. LVMU 13209/6 was deposited in the herbarium of the same department.

**Preparation of the extracts**

The air-dried leaves of *H. helix* were powdered, then extracted with water, 30% ethanol and 70% ethanol separately (6 × 30 min) in a water bath under reflux. The extracts were combined and evaporated to the soft residue under reduced pressure and re-dissolved in water, then lyophilized to attain dry amorphous yellowish to yellow-green powders with a light aromatic odor and a specific bitter taste, and marked as H, H30 and H70. The procedure of preparation of the extracts according to the scheme was repeated three times and yield (mean ± SEM) was calculated as a percentage regarding the plant material.

**TLC analysis of the extracts**

10.0 mg of each extract (H, H30 and H70) was dissolved in 1 mL of 70% ethanol and analyzed by TLC chromatography applying specific spray reagents to detect the presence of the selected saponins, flavonoids and phenolic acids.

Phenolic acids and flavonoids were analyzed by TLC on cellulose plates with the mobile phase: ethyl acetate – anhydrous formic acid – glacial acetic acid – water (100 : 11 : 11 : 26). The plates were viewed under UV-254 and 365 nm light and sprayed before and after with Naturstoffreagenz A (β-aminoethyl ester of diphenylboric acid) (NA) (0.1% in ethanol). The analysis of saponins was performed by TLC on silica gel plates in the mobile phase: anhydrous formic acid – ethanol – acetone – ethyl acetate (4 : 20 : 20 : 30) and after that sprayed with an alcoholic solution of sulfuric acid, heated for 10 min at 110°C, and observed at daylight and UV-365 nm (8).

**Quantification of triterpene saponins (hederacoside C) by HPLC**

An assay of hederacoside C in the plant material and the extracts was carried out using HPLC-method according to European Pharmacopoeia (1) for Ivy leaf.

**Pharmacological tests**

**Animals**

Adult (3–4 month old) Swiss albino mice (25 – 30 g) and Wistar albino rats (180 – 280 g) of both sexes were obtained from the animal house of the Department of Pharmacology, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine. The animals were allowed to acclimatize for 7 days. They were maintained in steel cages under standard laboratory conditions (temperature 25 ± 2°C in a natural light-dark cycle). Standard laboratory chow and water were supplied *ad libitum*. The ethical guidelines for investigations using conscious animals were obeyed; the procedures were approved by the Danylo Halytsky Lviv National Medical University’s Ethics Committee and complied with international guidelines.

**Acute toxicity test**

The acute toxicity (LD₅₀) of the extracts (H, H30 and H70) was determined in Swiss albino mice according to the procedures outlined by the Organization for Economic Co-operation and Development (OECD) (9, 10).

The animals were randomly divided into 10 groups, each of them with 6 male mice. Groups 1 – 5 were intraperitoneally administered the aqueous solutions of the dry extracts at a dose of 0.5, 1.0, 1.5, 2.0, and 2.5 g/kg body weight, respectively; groups 7 – 9 were intragastrically administered the extracts at a dose of 5.0, 7.5 and 10.0 g/kg, respectively. The animals of group 6 received water intraperitoneally and group 10 – water intragastrically (control groups). The animals were followed for any signs of toxicity and/or death within 24 h and then observed for 14 days.

**Antimicrobial activity**

The antimicrobial activity of the H, H30 and H70 extracts was tested by the disc diffusion method against standard strains: Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228; Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Proteus vulgaris* ATCC 4636 and yeast: *Candida albicans* ATCC 885653, by the procedure according to European Pharmacopoeia (1).
The surface of a culture medium (sterile nutrient agar for bacteria and Sabouraud agar for yeast) was inoculated with 1 mL of suspension cultures adjusted to a microorganism concentration of $1 \times 10^9$ colony forming units (CFU)/mL. After the incubation, known amounts (0.5, 1.0 and 1.5 mg) of each dry extract were dissolved in distilled water (50 µL), applied on sterile paper discs (6 mm in diameter, paper chromatography Whatman No.1), and allowed to dry at room temperature. The discs were applied to the surface of the culture medium with inoculated strains and incubated at 37°C for 24 and 72 hours for bacteria and yeast, respectively. The antimicrobial activity was determined through the zone of the microorganisms growth inhibition around the disk in mm. The mean values were calculated for five parallel experiments.

**Anti-inflammatory activity**

The anti-inflammatory activity of the H, H30 and H70 extracts was investigated in an aseptic edema model, which was induced by carrageenan (7, 9, 11). Rats (250-280 g) of both sexes were divided into five groups of six animals each. The extracts dissolved in water were administrated intragastrically at a dose of 200 mg/kg once a day for four days, while sodium diclofenac as a reference drug was given at a dose of 8 mg/kg in water solution. The control group (negative control) received only vehicle (1 mL of distilled water by the same scheme). On the fifth day of the experiment, 0.1 mL (2%, w/v) of carrageenan solution in water was subcutaneously injected into the plantar surface of the right hind paws of all the animals. The paw volume was measured with a plethysmometer two times: once before the injection of carrageenan and then after 3 h following the carrageenan administration.

The ratio of the anti-inflammatory effects of the *Hedera* extracts was calculated by the following expression: the anti-inflammatory activity (%) = $(1 - \frac{D}{C}) \times 100$, where $D$ represents the percentage difference in paw volume after carrageenan was administered to the rats, and $C$ – the percentage difference of volume in the control group.

**Influence on the lungs and bronchus**

The animals (rats, 180-220 g) were divided into three groups, each of them with 6 rats: 1 – the intact animals; 2 – the control group, with intranasally administered Sephadex G-50 suspension (50 mg/mL) at a dose of 2.0 mL/kg of body weight once a day for 5 days; 3 – the animals which were intragastrically administered an aqueous solution of the *Hedera* leaves dry extract (H70 at a dose of 200 mg/kg twice a day) and sephadex for 5 days (12, 13).

The body weight of each rat was assessed using a sensitive balance during the acclimatization period, once before the beginning of dosing, next weekly during the dosing period and finally on the day of sacrifice.

All the animals were euthanized by ether on the sixth day of the experiment and bronchus and lung tissue fixed in 10 % formalin solution was evaluated by histopathological examination; sections of tissue were stained by hematoxylin and eosin (HE stain) (14).

**Statistical analysis**

The results were expressed as a mean ± standard error of a mean (SEM). Student’s $t$-test was used to analyze the data and $p < 0.05$ was considered to be statistically significant.

**RESULTS AND DISCUSSION**

**Phytochemical analyses**

The H, H30 and H70 extracts were obtained (with yield 23.0 ± 0.06; 24.5 ± 0.07 and 24.6 ± 0.07%, respectively) from the *Hedera helix* leaves. Phytochemical screening of the extracts by TLC showed the presence of saponins ($\alpha$-hederin and hederacoside C), flavonoids (rutin), and phenolic acids (isochlorogenic, chlorogenic and rosmarinic acid).

The hederacoside C content in the extracts H, H30 and H70, determinated by HPLC analysis according to European Pharmacopoeia (1) was 1.71 ± 0.04; 8.95 ± 0.05; 13.95 ± 0.06%, respectively.

**The acute toxicity ($LD_{50}$)** of all the extracts was determined in mice according to the procedures outlined by the Organization for Economic Co-operation and Development (OECD) (9, 10). The $LD_{50}$ of the intraperitoneally administered H, H70 and H30 extracts was 1.0, 1.5 and 2.5 g/kg respectively; the $LD_{50}$ of the intragastrically administered extracts was more than 10 g/kg. At a single intragastric dose of 10 g/kg and up to 1.0 g/kg of the intraperitoneally administered extracts of *Hedera* leaves no signs of toxicity or death in mice within the first 24 h and during the 14-day observation period were observed when compared with the control group. Thus, the extracts considered to be non-toxic (9).

**Antimicrobial activity**

The *Hedera* leaves dry extracts demonstrated dose-dependent antimicrobial activity against all the
The obtained results are presented in Table 1.

The H70 extract at a concentration of 1.0 and 1.5 mg/disk against \textit{Pseudomonas aeruginosa} with 22 mm of the growth inhibition zone showed the highest antibacterial activity. The H and H30 extracts at all the analyzed concentrations and the H70 extract at a concentration of 0.5 mg/disk showed insignificant antimicrobial activity which rose slightly with increasing concentration of the extracts in the samples.

Antimicrobial activity of saponin-containing extracts of \textit{H. helix} leaves were previously observed against different strains of bacteria and yeast (15-19).

The in-vitro experiments were carried out for extracts by different methods. Water, ethanol, methanol, hexane, chloroform, and ethyl acetate were used as solvents to prepare the extracts (2). The antibacterial activity of saponins from \textit{Hedera helix} were tested against 22 strains of bacteria and one against yeast species (\textit{Candida albicans}). At 10 and 5 mg/mL concentration of the saponin, the solution was bactericidal against all the 23 tested strains. Generally, the saponins were more active against the Gram-positive (MIC was 0.312 to 1.250 mg/mL) than against the Gram-negative bacteria (MIC was 1.25 to 5.0 mg/mL). An ethanolic extract of \textit{Hedera} leaves completely inhibited the growth of \textit{Staphylococcus aureus} and \textit{Pseudomonas aeruginosa} and partially inhibited the growth of \textit{Escherichia coli} (16). Hederagenin derivatives inhibited yeast species (\textit{Candida albicans}, \textit{C. krusei}, \textit{C. tropicalis}, \textit{C. pseudotropicalis} and \textit{C. glabrata}) at 50 µg/mL or less. The MIC for the dermatophytes were within the range 5-100 µg/mL (17).

Monodesmosidic hederagenin derivatives were shown to exhibit a broad spectrum of activity against yeast as well as dermatophyte species in-vitro by the agar diffusion assay. α-Hederin was the most active compound and \textit{Candida glabrata} was the most susceptible strain (MIC 6.7 µM) (18). The ethyl acetate and methanol extracts of \textit{Hedera helix} showed the antibacterial activity against three Gram-positive and two Gram-negative selected bacteria strains at a concentration of 22 mg/mL (19).

**Anti-inflammatory activity**

The results of the anti-inflammatory activity of the \textit{Hedera} extracts in the carrageenan-induced paw edema test in rats are presented in Table 2. The extracts H, H30 and H70 at a dose of 200 mg/kg (corresponding to 3.42, 17.9 and 27.9 mg hederacside C, respectively) suppressed the development of inflammation. The H70 extract was the most effective with 20.61% of the anti-inflammatory activity, but less than the reference substance (sodium diclofenac) with 53.76% activity.

As H70 with the highest content of hederacside C showed the highest anti-inflammatory activi-

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**Table 1. Determination of antimicrobial activity* of \textit{Hedera} leaves extracts.**

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>H</th>
<th>H30</th>
<th>H70</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Pseudomonas aeruginosa}</td>
<td>0</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>\textit{Escherichia coli}</td>
<td>0</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>\textit{Proteus vulgaris}</td>
<td>0</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>\textit{Staphylococcus aureus}</td>
<td>0</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>\textit{Staphylococcus epidermidis}</td>
<td>0</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>\textit{Candida albicans}</td>
<td>0</td>
<td>1.0</td>
<td>1.5</td>
</tr>
</tbody>
</table>

* - mean diameter of five replicates.

**Table 2. Anti-inflammatory activity of the \textit{Hedera} extracts in carrageenan-induced acute paw edema.**

<table>
<thead>
<tr>
<th>Biological tests</th>
<th>Control group water 1 mL</th>
<th>H 200 mg/kg</th>
<th>H30 200 mg/kg</th>
<th>H70 200 mg/kg</th>
<th>Sodium diclofenac 8 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edema rate (%)</td>
<td>131.5 ± 6.22</td>
<td>116.5 ± 2.13*</td>
<td>109.87 ± 3.90*</td>
<td>104.40 ± 2.77*</td>
<td>60.81 ± 5.69*</td>
</tr>
<tr>
<td>Anti-inflammatory activity (%)</td>
<td>–</td>
<td>11.41 ± 2.80</td>
<td>16.45 ± 2.96</td>
<td>20.61 ± 2.11</td>
<td>53.76 ± 4.33</td>
</tr>
</tbody>
</table>

*p < 0.05 significantly differed in comparison with the control group, \(n = 6\).
ty, some relationship between level of this saponin and activity can be suggested.

Other authors previously reported the anti-inflammatory activity of *H. helix* extracts and its individual compounds.

The anti-inflammatory effects of a crude saponin extract and saponin’s purified extracts of *Hedera helix* in carrageenan- and cotton-pellet-induced acute and chronic inflammation models in rats were investigated by Soleyman. In carrageenan-induced acute inflammation model in rats, the crude saponin extract administered orally at 100 and 200 mg/kg body weight was the most potent extract with 77% anti-inflammatory effects, but less active than indomethacin (89.2%). The saponin’s purified extract of *H. helix* was more potent than the crude saponin extract in its chronic anti-inflammatory effect (60% and 49%, respectively), whereas indomethacin was more active (66%) (6).

α-Hederin and hederacoside C isolated from *Hedera* leaves administrated orally at concentrations of 0.02 mg/kg body weight were ineffective in the first phase of inflammation in carrageenan-induced acute paw oedema in rats, whereas hederacoside C showed the anti-inflammatory effect in the second phase of acute inflammation. Hederacoside C may exert its anti-inflammatory activity by blocking bradykinin or other inflammation mediators (7).

The ethanol extract from fresh *H. helix* leaves was tested for its anti-inflammatory properties in formalin-induced paw edema in mice (20). Intraperitoneal injections of 7.5 mL/kg body weight of ethanol extract showed anti-inflammatory activity with 88.89% inhibition, compared to diclofenac, as a reference drug, which showed 94.44% inhibition.

The methanol extract of the leaves of *H. helix* showed significant dose-depended analgesic and anti-inflammatory activities in the tail flick/hot-plate test and egg albumen-induced rat paw edema tests that were comparable to the test drugs (morphine 20 mg/kg and indomethacin 50 mg/kg respectively (21). The extract administered orally at a dose of 500 mg/kg showed the highest percentage of the inhibition of oedema (44.50%) at 90 minutes and was more potent than a reference drug - indomethacin (50 mg/kg) with 34.65% inhibition.

The previous results of investigations showed that anti-inflammatory activity of *H. helix* depends on the methods of preparing extracts (raw or dried plant material, method of extractions, etc.), so *Hedera* extracts may indeed vary with respect to their effectiveness.

**Influence on the lungs and bronchus**

In the following study of anti-inflammatory activity, it was shown that the H70 extract was twofold/twice as effective as an aqueous extract and exhibited stronger activity than the H30 extract, so the H70 extract was selected for further study of the influence on the lungs and airways. An experimental model of bronchiolitis was induced by an intranasal administration of Sephadex 50, which, in contact with the airways, causes proliferation of bronchus epithelium, increasing its reactivity and interstitial edema (12, 13). This model of lung injury by instillation of xenobiotic particles is responsible for bronchiolitis in humans and has been used previously to demonstrate the anti-inflammatory properties of some compounds based upon their ability to modulate lung edema (13).

The variable changes in the body weight of rats in all the groups were observed during the experiment. The intact rats gained weight throughout the duration of the experiment, whereas weight loss was
observed in rats of the control group. The group administered Sephadex 50 and treated with the H70 extract at a dose of 200 mg/kg showed no significant changes in animals’ weight.

The histological examination of bronchus and lung tissue of the intact animals (Fig. 1), bronchus and lung tissue in the control group of the animals (Fig. 2) and those treated with the *Hedera* extract (Fig. 3) were evaluated.

The microscopic examination in the animals’ lung tissue of the control group (Fig. 2) in comparison with the intact animals (Fig. 1) showed the complexity of changes such as dystrophy, hemodynamic disorders, and the reaction of peribronchial lymphoid tissue in various degrees of severity. There were mild focal degenerative changes – vacuolization of the epithelium of the bronchi and endothelium of small vessels. Hemodynamic disorders were manifested by unevenly pronounced plethora of the microvasculature, interstitial edema of interalveolar membranes, and focal inside alveolar hemorrhages. Pronounced changes were observed in peribronchial lymphoid tissue such as hyperplasia of T- and B-dependent areas, sometimes with the formation of secondary lymphoid follicles with germinal centers. Proliferation of lymphoid elements led to bronchoconstriction and a decrease in vessels in diameter, sometimes at 1/3 ñ 2/3 of lumen (Fig. 2).

In the group of the animals treated with the dry extract of *Hedera* leaves (H70), vacuoles in epithelial cells of some bronchi and bronchioles were detected in the tested material; it may be a manifestation of dystrophy or an increase in secretory activity of cells (moderate severity) (Fig. 3a). Stenosis of the lumen of the bronchi was insignificant. Hemodynamic disorders were observed in some areas of lung tissue only. Peribronchial lymphoid proliferates were more significant than in the intact animals, but less pronounced than in the control group (Fig. 3b).

As a result of the investigation of the *Hedera* extracts action, the reduction of dystrophy, correction of hemodynamic disorders, such as edema and hemorrhage, and a decrease in reaction of bronchus-
associated lymphoid tissue were observed. It can be explained by the decreasing immunoreactivity of macrophage cells. The mucolytic effect of the extract can be explained by an increase in the bronchial secretory activity as well as an increase in exudation with the release of fluid and erythrocytes from the microvasculature. The mucolysis can be caused by reducing the surface tension of the mucus thanks to the surface-active effect of the saponins. The inhibition of reactions in hyperplastic lymphoid tissue may have an indirect effect, because it suppresses the bronchial spasm and results in the absence of mucus retention in bronchus. Therapeutic effects of the H70 extract on the bronchi and lung tissue are supported by the antibacterial and anti-inflammatory activity.

Herbal preparations containing extracts from the Hedera leaves are popular in many European countries. The effectiveness of the treatment of a productive acute cough in the upper respiratory tract infections, bronchial asthma or a chronic obstructive pulmonary disease was established in clinical studies (2, 22-25).

The results of the research using an experimental model of bronchiolitis suggest that the H70 extract with the highest content of hederacoside C and with the strongest antimicrobial and anti-inflammatory activity in comparison with H and the H30 extracts should be recommended for making effective expectorant preparations.

CONCLUSIONS

The H70 extract obtained of H. helix leaves with the highest hederacoside C content showed the most potent anti-inflammatory and antibacterial activity, in comparison with the H and H30 extracts. It can be concluded, that there is some relation between the hederacoside C content and investigated activity. In the literature there is a lack of data comparing biological activity of the extracts prepared in this manner.

The reduction of dystrophy, correction of hemodynamic disorders, and the decrease in reactivity of bronchus-associated lymphoid tissue of the lung by the H70 extract were proved histopathologically.

The results of the research have provided additional evidence considering the effectiveness of H. helix leaves in the lung and bronchus infections. Concluding, the H70 extract is considered to be promising in the bronchopulmonary diseases treatment.

Conflict of interest

The authors have declared no conflict of interest.

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