INVESTIGATION OF THE ANTI-INFLAMMATORY EFFECT OF THE DRY EXTRACT FROM THE HERB OF STACHYS SIEBOLDII MIQ.

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Abstract

A detailed investigation on the anti-inflammatory activity of an ethanol extract of the herb of Stachys sieboldii was undertaken to find the pharmacological basis for the ethnomedical use of the plant. The study of anti-inflammatory action in the model of carrageenan edema in the foot of rats was performed on 30 white male rats weighing 180–220 g. The volume of paws was measured using a mechanical oncometer, anti-exudative activity of the studied extracts was determined by the degree of swelling subsidence in experimental animals compared with control. With prophylactic administration of an extract from Stachys sieboldii herb in the dose range of 5–25 mg/kg, a decrease in paw edema was observed in rats only when using the test sample at a dose of 10 mg/kg. Analysis of the obtained data showed that the studied extract of the herb of Stachys sieboldii at a dose of 10 mg/kg had a pronounced anti-inflammatory effect. The effectiveness of the extract only in the first hours of the dynamics of inflammation suggests its effect on the mediators of the acute phase: histamine, serotonin, and leukotrienes. The results obtained that there is some rationale behind the ethnomedical use of the plant for treating inflammatory disorders.

Keywords: dry extract, herb, Stachys sieboldii, anti-inflammatory activity, carrageenan
Introduction

Today in Ukraine, as in most countries near and far abroad, there is a tendency to expand research on the study of medicinal plants and herbal medicines and scientific and methodological justification for the introduction of phytotherapy in modern pharmacy and medicine [1, 2]. Pharmacotherapy increasingly takes into account the centuries-old knowledge of folk medicine with the use of phytopreparations as monotherapy and in combination with synthetic drugs [3, 4]. The appearance of synthetic drugs, which mainly imitate the biologically active substances of plants, has not reduced the role of natural drugs [5, 6]. Plants are exceptional sources of healing compounds biologically active substances that are used both for the prevention and treatment of various diseases of the human body [7-9]. Combinations of various medicinal plants need special attention as such herbal mixtures have a variety of biologically active substances [10, 11].

According to the literature, plants of the genus Stachys have been used as herbal medicines in all cultures around the world. Despite the fact that some species have a very unpleasant taste and smell – most plants of the genus Stachys are used to make tea, which in the Mediterranean and Iran is called “mountain tea”. The literature sources contain information about the use of plants of the genus Stachys as antioxidant, antibacterial, antiulcer, sedative, anti-inflammatory agents [12, 13].

Stachys sieboldii (Japanese artichoke) has long been used in Tibetan and Chinese medicine in the treatment of hypertension, tuberculosis, senile dementia, and ischemic stroke [14]. Biologically active substances of Stachys sieboldii show a wide range of pharmacological properties in the complete absence of toxicity [15, 16]. Its tubers lower cholesterol, regulates metabolic processes, and strengthens the immune system [13, 17].

There is information about the study of essential oils of Japanese artichoke in the sources of scientific literature. It was found that they contain germacrene D, caryophyllene, cadinene. It is believed that β-caryophyllene and hermacrene D are the main components of the essential oil of Japanese artichoke characterized by moderate antibacterial activity [12, 18].

Japanese scientists have found that methanolic tuber extract of Japanese artichoke, which contains glycosides, including acteoside and stachysosides C, significantly inhibits induced mortality from potassium cyanide poisoning in mice [19]. This extract inhibits hyaluronidase activity, has anti-inflammatory action, and is effective in kidney disease [15].

Mi Ra Yang et al. studied the antimicrobial activity of methanol and ethanol extracts from the leaves, herb and root tubers of Stachys sieboldii [20]. It was found that methanol extract from the leaves and root tubers and ethanol extract from the root tubers of Stachys sieboldii have a pronounced antibacterial effect on the culture of Salmonella typhimurium. In addition, methanol extract from the leaves of Stachys sieboldii showed a significant antibacterial effect on the culture of Bacillus cereus. It is believed that the antibacterial effect of Japanese artichoke is associated with the total content of polyphenols and flavonoids contained in the plant and which are extracted with methanol and ethanol [20].

Pharmacological study of the anti-inflammatory activity of the studied plant is relevant, taking into account a significant content of compounds of phenolic nature has been found in Stachys sieboldii herb.

Methods

Plant Materials

The herb of Stachys sieboldii was collected on research grounds of Educational and Scientific Centre “Institute of Biology and Medicine”, Taras Shevchenko National University of Kyiv in November 2017. A voucher specimen was deposited in the laboratory herbarium of the Department of Pharmacognosy and Medical Botany (TSMU, Ternopil, Ukraine) [21, 22]. The study plant material was dried using the conventional method and stored in paper bags in a dry, protected from direct sunlight place [23, 24].
Preparation of extract

About 500 g of dried Stachys sieboldii herb were powdered with the help of a suitable crusher. It was taken in an extractor and extracted using 70 % ethanol as a solvent. The extract was concentrated under vacuum to half under volume and dried at a temperature of 50±2º C [25].

Animal models

The experiments were performed on 30 white Wistar rats weighing 180–220 g. All animals were kept on a standard I. Horbachevsky Ternopil National Medical University (TNMU), vivarium diet [26]. The animals were kept in room having temperature 22 ± 2 º C, and relative humidity of 44-55 % under 12/12 hour light and dark cycle with standard laboratory diet and water given ad libitum [27].

Pharmacological studies have been conducted in accordance with the rules and requirements of the “General Principles for the Work on Animals” approved by the I National Congress on Bioethics (Kyiv, Ukraine, 2001 and agreed with the provisions of the “European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes” (Council of Europe No 123, Strasbourg 1985), and the Law of Ukraine “On the Protection of Animals from Cruelty” of 26.02.2006 [28-32]. The removal of animals from the experiment was carried out under light inhalation (ether) anesthesia by decapitation.

Acute toxicity studies

Studies of acute toxicity of dry extracts from the herb of Stachys sieboldii were performed V. B. Prozorovskyi method [33] on white nonlinear mice of both sexes weighing 18–24 g. Animals were divided into groups of 6 animals each. Animals were injected intragastrically with test extracts in the dose range of 500 mg/kg, 1000 mg/kg, 3000 mg/kg, and 5000 mg/kg. If the size of the extract exceeded 5 ml, the administration was performed in a fractional manner during the day [34]. At the end of the experiment (14 days), the mortality rate was determined in each group to calculate the average lethal dose (LD50) [35].

Intragastric administration through a metal probe of the studied extracts of Stachys sieboldii was performed after night (8–12 h) fasting of animals [34, 35].

Throughout the experiment, the survival of animals, consumption of food and water by them, as well as clinical manifestations of intoxication: general condition, changes in body position, skin condition, the color of mucous membranes, and individual symptoms (lacrimation, diarrhea, changes in the color of urine and feces, drowsiness, convulsions) were observed. In case of death of animals, their autopsy was performed and macroscopic analysis of abdominal organs was performed in order to establish that the lethal outcome of the animal did not occur due to manipulation errors, as well as to determine the probable cause of death.

Study of the anti-inflammatory effect of the dry extract from the herb of Stachys sieboldii

The study of anti-inflammatory effect of extract of Stachys sieboldii herb was carried out in accordance with the guidelines of the State Expert Center of the Ministry of Health of Ukraine [36].

The study of anti-inflammatory action in the model of carrageenan edema in foot of rats was performed on 30 white male rats weighing 180–220 g, the study design is shown in Table 1.

Acute exudative inflammation was caused by subplantar administration of 1 % carrageenan solution manufactured by Sigma (USA). The test samples were administered once intragastrically 1 hour before the administration of phlogogen.

The volume of paws was measured using a mechanical oncometer, anti-exudative activity of the studied extracts was determined by the degree of swelling subsidence in experimental animals compared with control, calculated by the formula:

\[ AIA = \frac{\Delta V_K - \Delta V_E}{\Delta V_K} \times 100\% \]

where: AIA – anti-inflammatory activity in %;
\( \Delta V_K \) – the average difference in volume between the paws with edema and without edema in the positive control group;
$\Delta V_E$ – the average difference in volume between the paws with edema and without edema in the experimental group.

The development of edema was observed in the dynamics of 1, 2, 3, 4, 5, 6 and 24 hours after administration of the test sample. The effectiveness of the extract from the herb of Stachys sieboldii was evaluated by the severity of the suppression of edema in foot in comparison with animals from the control pathology group and from the group of animals administered the comparison drug “Orthofen” tablets (OJSC "Vitamins", Uman, Ukraine) – at a dose of 8 mg/kg.

To obtain statistical conclusions when comparing samples of relative variables, we used the Newman-Keuls method, or the Mann-Whitney test. To assess the statistical significance of differences between groups, a significance level of $p < 0.05$ was established [37]. Standard packages of statistical programs Statistica 8.0 and Excel were used for mathematical calculations [38].

Results and Discussion

Study of acute toxicity of a dry extract from the herb of Stachys sieboldii

The results of observations of animals, which were carried out within two weeks after the introduction of the studied extracts of Stachys sieboldii showed that during this period there were no cases of mortality of animals in the experimental groups.

A short-term decrease in locomotor activity of mice was observed in the assessment of acute toxicity 30 min after administration of extracts at a dose 1000 times higher than the conventionally effective (5000 mg/kg). This effect is probably due to the overload of phytoextract solutions. No other symptoms of poisoning, such as incoordination, blepharoptosis, hypersalivation, and lateral position, were observed. In the study of low and medium doses, no visible signs of effects on the appearance, appetite or behavior of mice were recorded.

Given that no mortality was observed in animals at high doses of the extract, it can be assumed that the LD50 value at enteral administration of the extract exceeds the maximum dose used in the experiment, ie in mice LD50 > 5000 mg/kg. Thus, the results of the conducted research testify that dry extract of Stachys sieboldii herb, according to K. K. Sydorov classification can be attributed to class V of toxicity – almost harmless substances [33].

Investigation of the anti-inflammatory activity of a dry extract from the herb of Stachys sieboldii

Given that Stachys sieboldii herb contains a significant amount of compounds of phenolic nature, the anti-inflammatory activity of the obtained dry extract from the studied herb was determined. The results of the study are shown in Table 2.

As a result of the experiment, it was found that the comparison drug – “Orthofen” tablets had a stable anti-inflammatory effect, starting from the first hour of the experiment. The most expressed anti-inflammatory activity was observed in 3–5 hours during the release of prostaglandins, which confirmed the anticyclooxygenase mechanism of action of the drug. On average, the anti-inflammatory activity of the comparison drug was 50 % (Table 2).

With prophylactic administration of extract from Stachys sieboldii herb in the dose range of 5–25 mg/kg, a decrease in paw edema was observed in rats only when using the test sample at a dose of 10 mg/kg (Table 2). Moreover, a pronounced anti-inflammatory effect was observed in the first 2 hours of the experiment – at the level of 48 %. Efficiency of a sample decreased in the subsequent terms of research (Table 2). The dynamics of edema caused by carrageenan depends on the action of different mediators, the release of which occurs at different times of the experiment [36, 39]. Thus, biogenic amines, kinins are released in the first 30 minutes of carrageenan inflammation, histamine and serotonin – in 1–1.5 hours, leukotrienes – within 1.5–2 hours, prostaglandins – 2–5 hours of the experiment [36]. However, the leading role in the mechanism of development of acute inflammation in this model is played by the release of prostaglandins due to the activation of the cyclooxygenase way of arachidonic acid oxidation.
Analysis of the obtained data showed that the studied extract at a dose of 10 mg/kg had a pronounced anti-inflammatory effect during the release of histamine, serotonin and leukotrienes.

The test extract had no anti-inflammatory effect at doses of 5 and 25 mg/kg (Table 2).

Conclusions

The acute toxicity of the dry extract from the herb of Stachys sieboldii was studied. The test extract was determined according to K. K. Sydorov classification class V of toxicity of compounds (almost harmless substances, LD₅₀ ≥ 5000 mg/kg). Statistically significant data on the pharmacological anti-inflammatory activity of dry extract from Stachys sieboldii herb in the experiment on rats were determined. Thus, a dry extract of Stachys sieboldii herb at a dose of 10 mg/kg had a pronounced anti-inflammatory effect in a model of acute paw inflammation in rats caused by carrageenan. The effectiveness of the extract only in the first hours of the dynamics of inflammation suggests its effect on the mediators of the acute phase: histamine, serotonin and leukotrienes.

References


Table 1. Design of research of anti-inflammatory activity of dry extract from *Stachys sieboldii* herb on model of carrageenan edema in foot of rats

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Doses, mg/kg</th>
<th>Number of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control pathology</td>
<td>–</td>
<td>1–6</td>
</tr>
<tr>
<td>Dry extract from <em>Stachys sieboldii</em> herb</td>
<td>5</td>
<td>7–12</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>13–18</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>19–24</td>
</tr>
<tr>
<td>Ortofen tablets</td>
<td>8</td>
<td>25–30</td>
</tr>
</tbody>
</table>

Table 2. Dynamics of anti-inflammatory effect of dry extract from *Stachys sieboldii* herb on the model of carrageenan edema in rats, (n=6) M±m

<table>
<thead>
<tr>
<th>Observation time</th>
<th>Increase in foot volume, conventional units</th>
<th>“Ortofen” tablets, 8 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control pathology</td>
<td>Dry extract from <em>Stachys sieboldii</em> herb</td>
</tr>
<tr>
<td></td>
<td>5 mg/kg</td>
<td>10 mg/kg</td>
</tr>
<tr>
<td>1 h</td>
<td>7.5 (6.0; 9.0)</td>
<td>7.5 (5.0; 10.0)</td>
</tr>
<tr>
<td>2 h</td>
<td>7.0 (7.0; 8.0)</td>
<td>11.5 (6.0; 16.0) α</td>
</tr>
<tr>
<td>3 h</td>
<td>8.0 (8.0; 9.0)</td>
<td>16.5 (15.0; 20.0) α*#</td>
</tr>
<tr>
<td>4 h</td>
<td>11.5 (9.0; 12.0)</td>
<td>18.0 (11.0; 24.0) α*#</td>
</tr>
<tr>
<td>5 h</td>
<td>8.0 (5.0; 12.0)</td>
<td>19.0 (17.0; 25.0) α*#</td>
</tr>
<tr>
<td>6 h</td>
<td>11.5 (9.0; 14.0)</td>
<td>19.5 (13.0; 21.0) α*#</td>
</tr>
<tr>
<td>24 h</td>
<td>4.5 (3.0; 7.0)</td>
<td>4.0 (2.0; 10.0)</td>
</tr>
</tbody>
</table>

Notes: p – the level of statistical significance when comparing samples using the Kruskal-Wallis test;
* – the level of statistical significance when compared with the positive control group using the Mann-Whitney test, p<0.05;
# – level of statistical significance when compared with a group of rats treated with “Orthofen” tablets using the Mann-Whitney test, p<0.05;
α – the level of statistical significance when compared with a group of rats treated with dry extract at a dose of 10 mg/kg, using the Mann-Whitney test, p<0.05.