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Study of anti-inflammatory activity of *Verbena officinalis* L. extracts on the models of carrageenic and formalin edemas

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Abstract

Verbena officinalis L. relates to the herbs which have many-centuries experience of use in medicine for treatment of inflammatory diseases. This herb is famous for its therapeutic effect for inflammatory diseases, gastrointestinal tract diseases, liver and spleen diseases, jaundice. We studied the anti-inflammatory activity of *Verbena officinalis* L. extracts on the models of carrageenic and formalin edemas.

Anti-exudative activity of *Verbena officinalis* L. extracts VLT-0 and VLT-7 was determined as a result of research of their anti-inflammatory activity under the conditions of pathology of different genesis on two models of aseptic inflammation (carrageenic and formalin edemas) recommended by the Government Expert Center of Ukraine for preclinical research.

Keywords: Anti-inflammatory activity, *Verbena officinalis* L., carrageenic edema, formalin edema

Introduction

Pharmacotherapy of inflammatory process remains an actual problem of modern medicine. Among medicines which are widely used for the treatment of inflammatory processes in the organism there is a group of non-steroidal anti-inflammatory drugs. Despite their doubtless efficiency, they have a set of side effects, which restrict their use in clinic. Typical side effects of non-steroidal anti-inflammatory drugs are connected with a mechanism of their pharmacological effect^[1 - 6]. The use of phytomedicines gives an opportunity to reduce or completely avoid traditional side effects.

Verbena officinalis L. relates to the herbs, which have a many-centuries experience of use in non-traditional medicine for treatment of inflammatory diseases^[7]. *Verbena officinalis* L. is a perennial herb that grows on the whole territory of Ukraine in weeded places, roadsides and wet sands^[7]. *Verbena officinalis* L. contains iridoids, terpenoids (lupeol, ursolic acid), steroids (β -phytosterol), tannins, essential oils, vitamin C. This herb is famous for its therapeutic effect for treatment of inflammatory diseases, gastrointestinal tract diseases, liver and spleen diseases, jaundice^[7].

The aim of our research was to study the anti-inflammatory activity of extracts of *Verbena officinalis* L. on the models of carrageenic and formalin edemas.

Anti-exudative effect is one of the main criteria for studying anti-inflammatory properties. Drugs with anti-inflammatory properties influence on patho-physiological and biochemical links of inflammation^[8]. The inflammation caused by different alternative factors differs not only by its peculiarities of development but by the factors taking part in its genesis. Therefore, according to Methodical Recommendations of Preclinical Research of Anti-Inflammatory Drugs^[9] we have chosen two models of inflammation as model pathology:

- Research of anti-inflammatory activity on the model of exudative inflammation – carrageenic edema, which characterizes cyclooxygenase way of inflammation, by the methods of F. P. Trynus and co-authors. In the mechanism of development of carrageenic edema different mediators of inflammation are involved: during the first 30 – 90 min – mostly serotonin and histamine, during next 1.5 – 2.5 hours – kinins, and during 2.5 – 5 hours – prostaglandins^[9, 10].
- Research of anti-inflammatory activity on the model of aseptic formalin inflammation – 2% solution of formalin, which ability to cause a destruction of membrane albumins is proved, was used as a phlogogen^[9].

Inflammation caused by formalin leads to both local and systematic changes, because mediators of inflammation including prostaglandins are exuded. Pathogenesis of formalin edema is characterized by a significant destruction of membrane albumins. The peak of inflammatory reaction usually takes place at the third hour after using phlogogen [9].

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Materials and Methods: All results of experimental research obtained during the experiment were processed on personal computer. Methods of comparison of the average values in temporal HP Spectre 13-AE050A with the package of applied and statistic programs Microsoft Office 2010 and the package Statistica 6.0 (Stat-Soft 2001) were used to compare the indicators received in control and experimental groups. The authenticity of all values compared in experimental and control groups of animals was determined using the Student's criterion (St). A possibility of divergences of samples (p) and authentic interval of an average value was calculated using the tables of Student's division; level of probability is $p_{St} < 0,05$ [11].

The research and analysis of the obtained experimental data were conducted in comparison with a reference drug from the group of non-steroidal anti-inflammatory drugs – diclofenac sodium, which was taken in the conventional effective dose ED_{50} (8 mg/kg) [12].

Experimental investigations were conducted on white male rats with the weight of 180 - 250 g, which were on a normal diet, 6 animals in each group.

In the first series of experiments the inflammatory edema was caused by the injection of 0.1 ml of 1% carrageen solution under aponeurosis of sole of the rat's back paw. 2 hours before the injection of phlogogen agent and right after the injection animals of the first and second groups were injected the extracts of *Verbena officinalis* L. VLT-0 (extractant – purified water) and VLT-7 (extractant – 70% ethanol) relatively in the dosage of 10 mg per 100 g of body weight of

the animal. Animals of the third (control) group were injected 0.1 ml of 1% carrageen solution. Diclofenac sodium was injected to the animals of the fourth group.

0.1 ml of 2% water solution of formalin was injected subplantar under aponeurosis of sole of the rat's back paw to study the anti-inflammatory activity on the model of aseptic formalin inflammation. 2 hours before the injection of 2% formalin solution and right after the injection *Verbena officinalis* L. extracts VLT-0 (extractant – purified water) and VLT-7 (extractant – 70% ethanol) were administered orally to animals of the first and second groups in the dosage of 10 mg per 100 g of the animal body weight. 0.1 ml of 2% water solution of formalin was injected to the animals of the third (control) group [9].

In each series of experiment as a reference drug was used the non-steroidal anti-inflammatory drug – diclofenac sodium in the dosage of ED_{50} (0.8 mg per 100 g of the animal body weight), which is considered to be a standard anti-inflammatory drug (the fourth group of animals) [12, 13].

The size of the rat's paw was measured oncometrically before the experiment, in 1, 3 and 5 hours after the injection of the phlogogen agent. Maximum edema occurred during 3-5 hours and lasted until the completion of the experiment (Table 1, 2) [14].

The influence of the extracts researched was assessed according to their ability to depress the development of carrageenic and formalin edemas of the rat's paw in comparison with the animals of the control group.

Depression of inflammatory reaction was calculated by the formula:

$$AA = ((V_c - V_e) / V_c) \times 100,$$

where AA – anti-exudative activity, %;

V_c – growth in size of the swollen paw of animals in control group;

V_e – growth in size of the swollen paw of animals in the experimental group.

Results and Discussions

Values of growth in size of the rat's paw depending on the effect of *Verbena officinalis* L. extract in carrageenic edema are shown in Table 1.

Table 1: Anti-exudative activity of extracts of *Verbena officinalis* L. in carrageenic edema

Group of animals	Dosage mg/100g	Growth in size of the rat's paw: $\bar{x} \pm \Delta\bar{x}$, n=6		
		in 1 hour	in 3 hours	in 5 hours
Control	-	115.11 ± 0.81	133.12 ± 0.85	144.15 ± 1.10
VLT-0	10	102.22 ± 1.16*	113.42 ± 1.38*	101.92 ± 1.08*
VLT-7	10	100.61 ± 0.92*	111.69 ± 1.18*	97.30 ± 1.27*
Diclofenac sodium	0.8	89.90 ± 1.16*	82.93 ± 1.46*	85.19 ± 1.46*
Group number	Name of drug	Anti-exudative activity, %		
		in 1 hour	in 3 hours	in 5 hours
1	VLT-0	11.2	14.8	29.3
2	VLT-7	12.6	16.1	32.5
3	Diclofenac sodium	21.9	37.7	40.9

Note. * - deviation of an indicator is authentic in relation to the data of the control pathology ($p \leq 0,05$).

The results obtained (Table 1) show that extracts of *Verbena officinalis* L. VLT-0 and VLT-7 reveal anti-exudative activity. Edema of the rat's paw in experimental groups has been growing during 3 hours since the injection of a

phlogogen agent. In 5 hours a maximum depression of inflammation was observed. The anti-exudative effect of *Verbena officinalis* L. was revealed in an hour after the beginning of treatment as compared to the animals of the

control group. Anti-exudative activity differed depending on the type of the injected drug. Edema was growing during 5 hours in the control group of animals, which has not been treated at all.

During the first and third hours of the experiment the highest activity was shown by the extract of *Verbena officinalis* L. VLT-7, which reduced the edema by 12.6% during 1 hour, by 16.1% during 3 hours and by 32.5% in 5 hours after the injection of a phlogogen agent.

In the group of animals treated by the extract VLT-0 the rat's paw size was reduced by 11.2% during 1 hour, by 14.8% in 3 hours and by 29.3% in 5 hours after the injection of a phlogogen agent in relation to the group of the control pathology.

At the end of the test the extract VLT-7 turned out to be the most active (32.5%) but it conceded to the effect of diclofenac sodium (40.9%). By the average anti-exudative activity the substances researched can be placed in the following row: diclofenac sodium (40.9%) > VLT-7 (32.5%) > VLT-0 (29.3%).

The results of research indicate that the extracts of *Verbena officinalis* L. have anti-inflammatory activity in carrageenic edema. Extract of *Verbena officinalis* L. VLT-7 showed more distinctive anti-exudative activity by depressing the inflammatory reaction by 32.5%.

The anti-inflammatory activity of the extracts of *Verbena officinalis* L. in formalin edema is shown in Table 2.

Table 2: Anti-exudative activity of *Verbena officinalis* L. extracts in formalin edema

Group of animals	Dosage mg/100 g	Growth in size of the rat's paw: $\bar{x} \pm \Delta\bar{x}$, n=6		
		in 1 hour	in 3 hours	in 5 hours
Control	-	118 ± 1.93	138 ± 1.61	159 ± 0.96
VLT-0	10	106 ± 1.13*	116 ± 0.81*	123.23 ± 1.19*
VLT-7	10	99 ± 1.13*	103 ± 0.81*	104.5 ± 1.29*
Diclofenac sodium	0.8	97 ± 1.61*	101 ± 1.29*	103.19 ± 1.31*
Anti-exudative activity, %				
Group number	Name of drug	in 1 hour	in 3 hours	in 5 hours
1	VLT-0	10.40	15.90	22.20
2	VLT-7	18.50	26.90	34.50
3	Diclofenac sodium	19.50	28.40	35.10

Note. * - deviation of an indicator is authentic in relation to the data of the сштежид pathology ($p \leq 0,05$).

Analysis of data obtained during the experiment (Table 2) indicate that extracts of *Verbena officinalis* L. VLT-0 and VLT-7 show rather distinctive anti-inflammatory effect on the model of formalin edema. Extract VLT-7 revealed an anti-exudative effect in 3 hours (26.9%). Its effect was close to the reference drug – diclofenac sodium (28.4%). After 5 hours of the test in the group of animals which were previously injected VLT-7 the anti-exudative effect was 34.50% and it was close to the anti-exudative effect of the reference drug – diclofenac sodium (35.10%).

Complex of biologically active substances of *Verbena officinalis* L. extracts consists of hydroxycinnamic acids, tannins, iridoids, (aucubin, verbenene, verbenalin), triterpenoids (lupeol, ursolic acid), steroids (β - phytosterol), flavonoids, carotene, essential oils, organic acids, vitamin C [15]. Obviously, all these substances make synergic effect and cause a set of anti-exudative effects defined during the experiment.

Therefore, we conducted a comparative research of anti-inflammatory activity of *Verbena officinalis* L. extracts on two models of inflammation caused by different alternative factors. It was determined that in carrageenic edema, which is characterized by a cyclooxygenase way of inflammation, extract VLT-7 revealed an anti-exudative effect during the test. The highest level of edema depression was observed 5 hours after the injection of a phlogogen agent, which demonstrates the influence of extract VLT-7 on the mediators of inflammation – prostaglandins. In formalin edema destruction of membrane albumins takes place and mediators of inflammation – prostaglandins – are exuded. Extract VLT-7 shows distinctive anti-inflammatory effect 3 hours after the beginning of the experiment influencing on the inflammation mediators.

We determined the distinctive anti-inflammatory effect of the

extract VLT-7, which influences on the mediators of inflammation – prostaglandins, and may be used as an anti-inflammatory drug for inflammatory processes of pancreas and gallbladder.

Conclusion

1. The anti-inflammatory activity of *Verbena officinalis* L. extracts VLT-0 and VLT-7 under the condition of pathology of different genesis on experimental models of carrageenic and formalin edemas in rats was studied.
2. The anti-exudative activity of *Verbena officinalis* L. extracts on the model of carrageenic edema was determined. Extract VLT-7 showed the most distinctive activity and reduced edema by 12.6% during 1 hour, by 16.1% in 3 hours and by 32.5% in 5 hours after the injection of the phlogogen agent. The anti-inflammatory activity of extract VLT-7 was lower than the effect of diclofenac sodium.
3. The obvious anti-exudative activity of *Verbena officinalis* L. extracts was determined on the model of the formalin edema. Extract VLT-7 revealed the anti-exudative activity close to the activity of the reference drug (diclofenac sodium) and it was 26.9% – in 2 hours, and 35.1% – in 5 hours after the beginning of experiment.

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