

## STUDY OF MEMBRANE-STABILIZING AND ANTI-INFLAMMATORY ACTIVITY OF TINCTURE FROM HERBA BIDENS TRIPARTITA L.

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### Abstract

*Bidens tripartita* L. is herbaceous plant of the Asteraceae family, which is widely used in medicine as an anti-inflammatory, diuretic, choleric, diaphoretic, antimicrobial, antifungal, immunomodulatory agent and improve metabolism. The chemical composition of the herb is represented by phenolic compounds (butein, marein, isocoreopsin, flavanomarein, sulfuretin, sulfurein, marimetimetin, maritimein, luteolin, cinaroside), hydroxycinnamic acids (chlorogenic, caffeine, umélefene) tannins, polysaccharides, macro- and micro elements, fatty acids. The aim of the work is to establish the anti-inflammatory and membrane-stabilizing activity of the tincture from the herb of *Bidens tripartita*.

Tincture from the herb of *Bidens tripartita* was prepared by the classical method of maceration at room temperature, the ratio of plant raw materials to the finished product 1:5, extractant 50% ethyl alcohol. Studies of the effect of tincture on the state of cell membranes were performed under conditions of spontaneous hemolysis of rat erythrocytes in Jager F. C. Influence of tincture doses of 0.1 and 0.2 ml/kg, which were administered in a maintenance regimen for 7 days, with an experimental mechanism of inflammation, mainly carried out in the model of acute carrageenan edema of the foot in white rats weighing 180-200 g according to the method. The presence of antiexudative activity of *Bidens* herbs tincture is due to the composition and complex effect of biologically active substances of the studied *Bidens* tincture. According to the manifestation of membrane stabilizing activity in the model of spontaneous hemolysis of erythrocytes, it was found that the use of *Bidens* herbs tincture at a dose of 0.1 ml/kg was not characterized by a significant manifestation of membrane stabilizing activity, while the administration of *Bidens* herbs tincture at a dose of 0.2 ml/kg contributed to the manifestation of membrane-stabilizing activity.

**Keywords:** *Bidens tripartita* L, membrane-stabilizing, anti-inflammatory activity.

## Introduction

*Bidens tripartita* L. is herbaceous plant of the Asteraceae family, which is widely used in medicine as an anti-inflammatory, diuretic, choleric, diaphoretic, antimicrobial, antifungal, immunomodulatory agent and improve metabolism [1, 2, 3]. Medicinal plant raw materials are herbs, the monograph of which is available in the State Pharmacopoeia of Ukraine 2.0, the State Pharmacopoeia of the Russian Federation XIV edition [7, 8]. Decoctions and infusions are obtained from herbs, which are used to treat various skin diseases [9]. The chemical composition of the herb is represented by phenolic compounds (butein, marein, isocoreopsin, flavanomarein, sulfuretin, sulfurein, marimetimetin, maritimein, luteolin, cinaroside), hydroxycinnamic acids (chlorogenic, caffeine, umélefene) tannins, polysaccharides, macro- and micro elements, fatty acids [10, 11, 12, 13]. The herb of *Bidens tripartita* is a part of plant collections "Nephrophyte", "Phytocystol", "Detoxify", "Elekasol", "Elekasol", "Brusniver", "Brusnilan", "Elakosept", "Elekasol" and elixir "Kliofit" [9]. The herb is used to make a dietary supplement "Bidens Extract", which has anti-allergic, anti-inflammatory, diuretic and emollient effects and is recommended for use in allergies, inflammation, liver, gallbladder and all metabolic disorders [10, 11] and biologically active liquid "Bidens" (Danicapharm) [11].

There were obtained various aqueous solutions with different nonionic surfactants with using an ultrasonic auxiliary micelle-mediated extraction method, which enhanced the transition of polyphenols and flavonoids from plant raw materials to solution, which also increased the antioxidant effect of the solution [13].

Aqueous, 50% methanol and methanol extracts and diethyl ether, ethyl acetate and butanol fractions were obtained from the herb of *Bidens tripartita* in which the relationship between the component composition of phenolic compounds and pharmacological activity was determined [1]. In vivo studies have shown that all extracts stimulate gastrointestinal motility and can be used to treat some possible clinical symptoms, including abdominal discomfort, bloating, constipation,

irritable bowel syndrome, gastritis, gastroparesis, and other functional dyspepsia. [1].

We obtained a tincture of the herb of *Bidens tripartita* and determined the quantitative content of polyphenolic compounds (not less than 0.07 mg/ml, in terms of pyrogallol), hydroxycinnamic acids (not less than 0.001 mg/ml, in terms of chlorogenic acid), organic acids (not less than 1.0 mg/ml, in terms of malic acid) and macro- and micronutrients [14].

ince, according to the literature, the herb of *Bidens tripartita* and tincture from it contain phenolic compounds that exhibit a wide range of pharmacological activity: anti-inflammatory, diuretic, choleric, antimicrobial, antioxidant [15, 16, 17], so the study of anti-inflammatory and membrane-stabilizing activity is actual.

The aim of the work is to establish the anti-inflammatory and membrane-stabilizing activity of the tincture from the herb of *Bidens tripartita*.

## Materials and Methods

Tincture from the herb of *Bidens tripartita* was prepared by the classical method of maceration at room temperature, the ratio of plant raw materials to the finished product 1:5, extractant 50% ethyl alcohol.

Studies of the effect of tincture on the state of cell membranes were performed under conditions of spontaneous hemolysis of rat erythrocytes in Jager F. C. The method of Jager F. C. [18] is based on photoelectrocolorimetric determination of non-erythrocyte hemoglobin, which is got into the environment as a result of spontaneous lysis of the erythrocyte membrane caused by lipid peroxidation with oxygen. The white rats were selected into 4 groups for the experiment: 1 group - intact control (IC) includes 6 rats, which were given an equivalent amount of pure water; 2 group - 6 rats injected with tincture at a dose of 0.1 ml/kg; 3 group - 6 rats treated with tincture at a dose of 0.2 ml/kg. After randomization, animals of groups 2, and 3 for 2 weeks intragastrically injected tincture at a dose of 0.1 and 0.2 ml/kg at a dose of 1 mg/kg. The intact control group during this period received an equivalent amount of solvent intragastrically. After 2 weeks, all rats were bled from the tip of the tail and the degree of hemolysis of erythrocytes

was determined according to the method [18]. The membrane-stabilizing effect of tincture was determined by the ability to prevent damage to erythrocyte membranes and was assessed by the change in the number of hemolyzed erythrocytes in animals of groups 2, 3 and 4 compared to rats of intact control group 1 and expressed as %.

Influence of tincture doses of 0.1 and 0.2 ml/kg, which were administered in a maintenance regimen for 7 days, with an experimental mechanism of inflammation, mainly carried out in the model of acute carrageenan edema of the foot in white rats weighing 180-200 g according to the method [19]. In the process of creating a model of acute carrageenan inflammation of rat paws, characterized by the ability to study the dynamics of the process in the pathogenesis of inflammation in the first 30-90 minutes with serotonin and histamine, after 1.5-2.5 hours - kinins, and after 2.5-5.5 hours - prostaglandins, which indirectly suggested that the mechanisms of anti-inflammatory action of these substances [20, 21]. There is also a direct correlation between the effectiveness of the drug in a model of acute inflammation of the feet of rats with carrageenan and its effectiveness in the clinic.

For the experiment, white root rats were similarly selected into 3 groups: 1 group - IC includes 6 rats, which were introduced an equivalent amount of pure water; 2 group - 6 rats, which received a dose of tincture of 0.1 ml/kg; 3 group - 6 rats given a tincture dose of 0.2 ml/kg. After randomization, animals of groups 2 and 3 received intragastric tincture at a dose of 0.1 and 0.2 ml/kg for three weeks. The intact control group 1 during this period received an equivalent amount of solvent intragastrically. Subplantation of 0.1 ml of 1% carrageenan solution in one of the hind limbs of experimental and control rats 1 hour after administration of doses of 0.1 and 0.2 ml/kg of tincture caused inflammation. The development of edema was judged by the increase in foot volume, which was measured in dynamics 1; 2; 3; 4 and 5 hours after introduction of phlogogen by means of a mechanical oncometer according to a technique. The anti-inflammatory activity of the drugs was expressed in % and was determined by the ability to reduce edema in animals of groups 2 and 3 compared with intact control group 1 [22, 23].

During the experiment, the animals were kept in a vivarium with natural light regime "day-night" at a temperature of 18-24°C and a relative humidity of 50-60%. Rats were kept in plastic cages on a balanced diet in accordance with current regulations. The research was conducted in accordance with the National Recommendations "General Ethical Principles for Animal Experiments" (Ukraine, 2001), which are consistent with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) [31].

### Results and Discussion

The model of carrageenan edema of the paw in rats is classic model of acute inflammatory process [24]. Tissue volume increased depending on the dynamics of edema and reached its maximum at 4 and 5 hours (by 2.14 and 2.23 times more than intact control,  $p < 0.05$ ) (Table 1). In the first hours after the administration of carrageenan increases vascular permeability due to biogenic amines: histamine and serotonin [25, 26], in the second hour with kinins due to activation of the kallikrein-kinin system [27]. The latter promotes the local release of hydrolytic enzymes of lysosomes, which stimulate the formation of prostaglandins, that mediate the late phase of inflammation, which then develops with carrageenan edema after 3 hours. Nitric oxide is also released in the third hour [28]. Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and nitric oxide are formed by induction of cyclooxygenase (COX-2) and inducible NO-synthase. PGE<sub>2</sub> synergistically with histamine and bradykinin cause increased inflammation, edema, exudate, erythema, redness, pain and fever. Some scientists distinguish two phases of carrageenan edema [29, 30]: the first is due to histamine, serotonin and bradykinin, and the second (3-5 hours) due to prostaglandins. There is a fairly high swelling of the paw due to the peak concentration of prostaglandin E<sub>2</sub>, which is observed exactly 24 hours after administration of carrageenan.

The use of Bidens herb tincture in two experimental doses of 0.1 and 0.2 ml/kg allowed to establish the following: the most significant antiexudative effect of the studied tincture was observed at 1st and 2nd hours of carrageenan

edema, as evidenced by anti-inflammatory activity at the level of 30.36-33.93% ( $p < 0.05$ ) (Table 1). After 3rd hour of administration of carrageenan, the use of Bidens herbs tincture at a dose of 0.1 ml/kg was not characterized by any significant manifestation of antiexudative activity. The administration of Bidens herbs tincture at a dose of 0.2 ml/kg was characterized by a significant manifestation of antiexudative activity for 4 hours ( $p < 0.05$ ) of administration of carrageenan, which was characterized by a anti-inflammatory activity value of 30.83% ( $p < 0.05$ ).

The well-known dynamics of the release of mediators in the model of carrageenan edema, suggests the mechanism of activity of the studied means by the amount of edema suppression for a certain period of its development. Given the above, we can conclude that the studied Bidens herbs tincture inhibits the mediators of the early phase of the inflammatory process (histamine, serotonin, kininins).

The presence of antiexudative activity of Bidens herbs tincture is due to the composition and complex effect of biologically active substances of the studied Bidens tincture.

Membrane-stimulating and anti-inflammatory activity of tincture of the herb of Bidens tripartita is due to the presence of polyphenolic compounds, hydroxycinnamic acids and organic acids, the quantitative content of which was determined by us earlier [14].

Based on literature data and data from our own research, we can assume that the antiexudative effect of Bidens herbs tincture is due to the depressant effect of BAS tinctures and is realized through the destructive effect of polyphenols on the release of inflammatory mediators: histamine, kinins and prostaglandins.

According to the manifestation of membrane stabilizing activity in the model of spontaneous hemolysis of erythrocytes, it was found that the use of Bidens herbs tincture at a dose of 0.1 ml/kg was not characterized by a significant manifestation of membrane stabilizing activity, while the administration of Bidens herbs tincture at a dose of 0.2 ml/kg contributed to the manifestation of membrane-stabilizing activity.

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**Table 1.** Influence of Bidens herbs tincture on spontaneous hemolysis of erythrocytes in intact animals  
by F. C. Jager method

Experimental conditions	Severity of hemolysis, %	Membrane stabilizing activity, %
CP	16,54	
Bidens herbs tincture, 0,1 ml/kg	17,4	5
Bidens herbs tincture, 0,2 ml/kg	13,88	16,00*

**Table 2.**

Experimental conditions	1 hour $\Delta V$ , conventional units	AIA, 1 hour, %	2 hour	AIA, 2 hour, %	3 hour	AIA, 3 hour, %	4 hour	AIA, 4 hour, %	5 hour	AIA, 5 hour, %	Average value of AIA for 5 hours
CP	11,20	-	16,20	-	19,80	-	24,00	-	25,00	-	-
Bidens herbs tincture, 0,1 ml/kg	7,4	33,93 *	11,3	30,25 *	16,00	19,19	18,00	25,00 *	20,00	20,00	23,61
Bidens herbs tincture, 0,2 ml/kg	7,8	30,36 *	11,00	32,10 *	14,20	28,28 *	16,60	30,83 *	19,00	24,00	29,00*