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Study of anti-inflammatory effect of liquid *Capsicum annuum* L. extracts in specific and non-specific adjuvant-induced arthritis

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Abstract
Purpose: The work is devoted to studying the anti-inflammatory properties of carotenoids extracted from the *Capsicum annuum* L. fruits. Such a choice is due to their high antioxidant activity and membrane-stabilizing effect. Of particular interest is the anti-inflammatory activity of carotenoids under conditions of specific autoimmune inflammation.
Materials and Methods: The fruits of *Capsicum annuum* L. variety "Ukrainian bitter" were used as plant raw material. The total carotenoid and capsaicinoid concentration was determined in the extracts after the extraction process. The anti-inflammatory properties of the obtained extracts were studied using an experimental model of carrageenan-induced inflammation (specific and nonspecific inflammatory processes). The anti-edema effect and changes in the total count of white blood cells in the peripheral blood were used as criteria for the efficacy of anti-inflammatory therapy.
Results: The therapeutic effect of the plant extract application was recorded on the 15th day in the case of nonspecific inflammation. On the 25th day of treatment, the anti-edema effect of the plant extract was significantly higher than that of the reference ibuprofen drug (Δ 24.4%) in the case of non-specific inflammation. The extractive substances of *Capsicum annuum* L. fruits had a pronounced anti-inflammatory effect in the case of autoimmune inflammation of the contralateral limb, this effect is accompanied by a decrease in white blood cell count in peripheral blood in experimental animals.

Keywords: adjuvant, inflammation, *Capsicum annuum* L.

Introduction
Plants are a rich source of biologically active compounds. Therefore, plant matter may be used as a basis for searching for new substances with medicinal properties and their possible precursors, as well as a cheap and practical material for creation of already known pharmacologically active compounds [1, 2]. Fruits of *Capsicum annuum* L. (also known as pepper) contain large amounts of capsaicinoids and carotenoids [3, 4]. These substances have a wide range of biological effects [5, 6], including anti-inflammatory and analgesic effects. Capsaicinoid compounds have a stronger effect on chronic inflammations and pain syndromes than on acute processes [7]. These properties match rheumatoid arthritis - a chronic autoimmune progressive disease accompanied by articular and extra-articular damage and excruciating pain syndrome [8]. Hence, study of therapeutic effects of *Capsicum annuum* L. extracts for treatment of this disease is of interest. Purpose of the paper was to study anti-inflammatory activity of liquid *Capsicum annuum* L. alcohol extracts in non-specific and specific (autoimmune) arthritis induced by administration of the Freund's complete adjuvant.

Materials and Methods
Plant material
Plant matter used in the study was ripe fruits of *Capsicum annuum* L. of the brand "Ukrainian bitter pepper". In order to prepare extracts, pepper was chopped, put into 96º ethanol in 1:3 ratio and allowed to infuse for 72 hours. Content of capsaicinoids and carotenoids was controlled by photometrical method [9, 10]. Concentration of capsaicinoids in extracts was 0.05%, carotenoids - 0.03%.

Experimental animals
The study was performed on male Wistar rats with body weight 180-220 g kept in a standard animal facility with free access to food and water. The study was performed in compliance with recommendations of the European Convention for Protection of Experimental Animals

and approved by Bioethics Committee of Odessa National Medical University (minutes No. 84
dated October 10, 2008). When working with laboratory animals, the researchers also complied with the Rules for Use of Experimental Animals, approved by Order of the Ministry of Health of Ukraine No. 249 dated March 1, 2012 and the Law of Ukraine "Protection against Animal Cruelty" No. 3447-IV (as amended on December 15, 2009 and October 16, 2012).

Experimental procedure

0.1 ml of the Freund's complete adjuvant was administered under the plantar fascia to all animals to induce inflammatory arthritis. Animals were under light ether anaesthesia during administration. The animals were further divided into 3 groups, 10 animal units in each. The first group was a test group, which received pepper extracts for treatment. Second group was used as a positive control and received ibuprofen ointment (50 mg per 1 g of ointment) for treatment, and the third group was used as a negative control and received no treatment. Treatment included daily single application of liquid extracts on the adjuvant injection site by submerging the animal's paw into a container with the extract for 15 minutes. In case of the positive control group ibuprofen ointment was applied onto the adjuvant injection site. Half of the animals in the test group and positive control group received treatment from day 1 after inflammatory response induction. The rest of the animals received treatment from day 10 after adjuvant injection (study of specific inflammatory response, both hind paws were subject to therapeutic procedure). Therapeutic effect was evaluated in view of dynamics of morphological changes (range of the oedema), as well as changes in cellular composition of blood. Range of the oedema was measured with a digital plethysmometer (37140, Ugo Basile, Italy). Anti-oedemateous effect was calculated by formula:

$$AoE\ (\%) = 100\% - (V_t/V_c * 100\%)$$

Where
AoE - anti-oedemateous effect in percentage
V_t - mean difference in size of the inflamed and intact paw in the test group animals, ml
V_c - mean difference in size of the inflamed and intact paw in the control group animals, ml
Leukocytes were counted in Goryaev chamber.
Relative change in peripheral WBC count in comparison with values in the control group animals was calculated by formula:

$$CL\ (\%) = 100\% - (L_t/L_c * 100\%)$$

Where
CL - relative change in peripheral WBC (leukocyte) count in comparison with values in the control group animals in percentage
L_t - mean difference in WBC (leukocyte) count, when inflamed and intact, in the test group animals, 10^9/L
L_c - mean difference in WBC (leukocyte) count, when inflamed and intact, in the control group animals, 10^9/L

Statistical analysis

Preliminary distribution of animals into the experimental groups was made randomly. Distribution of the data obtained during the studies was checked for normality by means of Shapiro-Wilk test. By virtue of the fact that all the received data had a normal distribution, ANOVA was carried out using the Student's t-test with Bonferroni correction. The calculations were performed with the use of MS Office Excel software.

Results and Discussion

Data on anti-inflammatory efficiency of *Capsicum annuum* L. extract in non-specific inflammation induced by administration of the Freund's complete adjuvant are given in Table 1.

Table 1: Changes in size of the paw in the inflamed site and anti-oedemateous efficiency of medications used in non-specific inflammation (M ± m, n = 5)

Time after phlogogen administration (days)	Difference (Δ) in size of the inflamed and intact paw, ml			Anti-oedemateous effect, %	
	Control group	Group treated with *Capsicum annuum* L. extract	Group treated with Ibuprofen	Control group	Group treated with *Capsicum annuum* L. extract
1	0,82 ± 0,11	0,85 ± 0,07	0,8 ± 0,12	-3,66	2,44
2	0,93 ± 0,13	0,87 ± 0,14	0,95 ± 0,15	6,45	-2,15
3	1,19 ± 0,09	1,17 ± 0,19	1,3 ± 0,15	1,68	-9,24
5	1,31 ± 0,16	1,27 ± 0,1	1,35 ± 0,17	3,05	-3,05
7	1,39 ± 0,06	1,29 ± 0,13	1,3 ± 0,1	7,19	6,47
10	1,05 ± 0,15	0,88 ± 0,09	0,94 ± 0,14	16,19	10,48
12	1,25 ± 0,16	0,9 ± 0,1	1 ± 0,14	28,00	20,00
15	1,23 ± 0,12	0,77 ± 0,13*	0,97 ± 0,15	37,40	21,14
20	1,39 ± 0,11	0,69 ± 0,08**	1,12 ± 0,16	50,36	19,42
25	1,35 ± 0,09	0,69 ± 0,07**#	1,02 ± 0,1	48,89	24,44
30	1,31 ± 0,14	0,61 ± 0,08**#	0,89 ± 0,07*	53,44	32,06

* - significance of differences between the test group and the control group p≤0.05; ** - significance of differences between the test group and the control group p≤0.01; # - significance of differences between the group treated with *Capsicum annuum* L. extract and the group treated with ibuprofen p≤0.05.

Administration of the adjuvant triggered severe inflammatory oedema in all animal groups on day 1 after injection (Δ0.8-0.85 ml). Inflamed site grew to its maximum on day 5-7 after injection (Δ1.3-1.39 ml). Maximum size of the oedematous site was achieved again on day 20 after inflammation in the control group (Δ1.39 ml). Therapeutic application of *Capsicum annuum* L. extract resulted in definite decrease in oedematous manifestations by 37.4% in comparison with the control group animals starting from the 15th day of treatment. Definite difference in anti-inflammatory efficiency between the extract studied and the reference drug ibuprofen was

registered after 25 days of treatment and constituted 24.4% in favour of the herbal extract.

Specific autoimmune inflammation induced by administration of the Freund's complete adjuvant developed on day 10-12 after phlogogen injection. Data on anti-inflammatory efficiency of *Capsicum annuum* L. extract are given in Table 2.

Table 2: Changes in size of the contralateral metatarsal joint and anti-oedemateous efficiency of medications used in specific inflammation (M ± m, n = 5)

Time after phlogogen administration (days)	Difference (Δ) in size of the inflamed and intact paw, ml			Anti-oedemateous effect, %	
	Control group	Group treated with *Capsicum annuum* L. extract	Group treated with Ibuprofen	Control group	Group treated with *Capsicum annuum* L. extract
10	0,03 ± 0,02	-0,01 ± 0,01	0,02 ± 0,008	133,33	33,33
12	0,06 ± 0,03	0,03 ± 0,015	0,03 ± 0,02	66,67	50,00
15	0,1 ± 0,04	0,04 ± 0,008	0,04 ± 0,01	90,00	60,00
20	0,11 ± 0,03	0,02 ± 0,009	0,06 ± 0,025	100,00	45,45
25	0,1 ± 0,05	0,02 ± 0,01	0,06 ± 0,02	90,00	40,00
30	0,12 ± 0,04	0,02 ± 0,012*	0,05 ± 0,018	83,33	58,33

* - significance of differences between the test group and the control group p≤0.05

Accumulative growth in size of the contralateral metatarsal joint in the control group animals was registered throughout the entire study and constituted +0.12 ml on the 30th day of the inflammation process. In the animals treated with *Capsicum annuum* L. extract maximum growth in size of the contralateral joint was 0.04 ml and Δ decreased down to 0.02 by the 30th day of treatment. In animals treated with ibuprofen, an interim dynamics of changes was noted in comparison with the control group and the test group.

One of the objective features of any inflammatory process is changes in the WBC count. Data on WBC dynamics in adjuvant inflammation are given in Table 3 and Table 4.

Table 3: Dynamics in peripheral WBC count in non-specific inflammation (M ± m, n = 5)

Time after phlogogen administration (days)	Difference (Δ) in peripheral WBC count, when inflamed and intact, 10^9/L			Relative change in WBC count, %	
	Control group	Group treated with *Capsicum annuum* L. extract	Group treated with Ibuprofen	Control group	Group treated with *Capsicum annuum* L. extract
1	1,4 ± 0,12	1,1 ± 0,1	2 ± 0,1	21,43	-42,86
2	4,3 ± 0,35	3,4 ± 0,31	3,7 ± 0,23	20,93	13,95
3	4,8 ± 0,26	4,5 ± 0,29	5,7 ± 0,32	6,25	-18,75
5	5,9 ± 1,3	4,1 ± 0,46	6,5 ± 0,69	30,51	-10,17
7	5,7 ± 0,39	6,4 ± 0,88	7,9 ± 0,75	-12,28	-38,60
10	16,9 ± 1,2	13,6 ± 1,7	20,7 ± 1,9	19,53	-22,49
12	19 ± 2,0	18,1 ± 2,8	22,1 ± 1,9	4,74	-16,32
15	19,9 ± 1,8	15,1 ± 1,7	23,4 ± 2,0	24,12	-17,59
20	23,2 ± 2,2	14,4 ± 2,4*	18,9 ± 2,0	37,93	18,53
25	25,5 ± 2,1	12,1 ± 2,0**	14,3 ± 1,0**	52,55	43,92
30	27,9 ± 3,1	13,9 ± 1,8*	15,6 ± 2,3*	50,18	44,09

* - significance of differences between the test group and the control group p≤0.05; ** - significance of differences between the test group and the control group p≤0.01.

Table 4: Dynamics in peripheral WBC count in specific inflammation (M ± m, n = 5)

Time after phlogogen administration (days)	Difference (Δ) in peripheral WBC count, when inflamed and intact, 10^9/L			Relative change in WBC count, %	
	Control group	Group treated with *Capsicum annuum* L. extract	Group treated with Ibuprofen	Control group	Group treated with *Capsicum annuum* L. extract
10	17 ± 2,0	18,7 ± 1,4	18,5 ± 1,9	-10,00	-8,82
12	19,5 ± 2,7	21 ± 2,6	20,9 ± 1,3	-7,69	-7,18
15	24,1 ± 2,6	19,4 ± 1,1	19 ± 2,7	19,50	21,16
20	23,9 ± 2,6	14,1 ± 2,7	18,1 ± 3,4	41,00	24,27
25	25,1 ± 2,2	15,9 ± 2,4*	16,7 ± 3,0	36,65	33,47
30	25,6 ± 2,3	17,9 ± 1,9*	19,6 ± 2,7	30,08	23,44

* - significance of differences between the test group and the control group p≤0.05.

WBC count grows in parallel with accumulation of morphological signs of inflammation (size of the oedema). WBC count slowly drops in the treatment groups starting from day 15 (treatment with *Capsicum annuum* L. extract) and day 20 (treatment with ibuprofen) in case of non-specific inflammation. In the course of autoimmune response development, ibuprofen did not have any definite effect on dynamics of changes in total WBC count. Unlike ibuprofen, application of *Capsicum annuum* L. extract resulted in definite decrease in WBC count (by 36.65%) after the 25th day of treatment.

We believe that anti-inflammatory effect of *Capsicum annuum* L. extract noted in case of adjuvant-induced inflammation is attributed to capsaicinoids and carotenoids, large content of which is found in pepper. In case of capsaicin-induced non-specific inflammation (major capsaicinoid contained in fruits of *Capsicum annuum* L.), gradual desensitization of small capsaicin-sensitive type C pain fibres occurs, which leads to decrease in production of p-substance and calcitonin-gene-related peptide [11]. Being powerful antioxidants, carotenoids reduce intensity of alterative processes, attributed to release of reactive oxygen

intermediates and peroxide compounds in the inflammation site [6, 12].

Specific autoimmune inflammation developing after administration of the Freund's complete adjuvant has a complex pathogenesis, leading components of which are CD4+ T-cells, synovial macrophages and fibroblast-like synoviocytes, as well as a large amount of cytokines secreted by them. It is known for a fact that capsaicin can reduce bone resorption during inflammation [13] and macrophage expression of pro-inflammatory cytokines [14], based on which we can make a careful assumption about direct inhibitory effect of capsaicin on autoimmune inflammatory cascades of articular tissue.

Conclusions

As a result of the work performed, we can conclude that *Capsicum annuum* L. extract has a definite anti-inflammatory effect on both non-specific and specific stages of inflammation induced by the Freund's complete adjuvant. We are inclined to explain such effect by high content of capsaicinoids and carotenoids in fruits of *Capsicum annuum* L.

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