Original Article

Anti-Inflammatory Effect of Semisolid Dosage Forms Containing Capsaicin

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ABSTRACT

Background: Capsaicin is alkaloid found primarily in the fruit of the *Capsicum* genus. Capsaicin and its analogues have been used in topical creams and patches to treat chronic pain and inflammation.

Objectives: The paper deals with topical application of creams containing capsaicin to treat Freund's adjuvant-induced inflammation.

Materials and Methods: Anti-inflammatory activity was studied on the model adjuvant-induced inflammation with Freund's complete adjuvant. The study covered morphological changes in the inflamed area, the total number of white blood cells was studied by microscopic method in Gorjaev's chamber. The biochemical parameters of blood - cholinesterase activity and total number of alpha-1-acid glycoprotein in blood plasma were determined by the commercial test kits for rapid analysis

Results: It was shown that the cream containing *Capsicum annuum L.* extract proved to be more efficient for treatment of adjuvant-induced inflammation than the commercial drug Dolgit Cream. Application of therapeutic cream with *Capsicum annuum L.* extract reduced activity of plasma acetylcholinesterase and total WBC count on the 15 day of treatment to initial values.

Conclusion: The cream containing *Capsicum annuum L.* extract significantly inhibited inflammatory swelling, reduced WBC count and activity of plasma acetylcholinesterase.

Keywords: capsaicin, ibuprophen, Freund's adjuvant, inflammation.

Plant bioactive compounds have long been used as anti-inflammatory agents in the form of extracts, tinctures, poultices and the like. One of such compounds is capsaicin – an alkaloid giving hot spicy flavour to berries of *Capsicum annuum L.* (pepper) [1]. *Capsicum annuum*-based drugs have been widely used in folkmedicine as calefacient and anti-inflammatory agent. In traditional medicine capsicum is used in capsicum tincture (Tinctura Capsici), frostbite ointment (Unguentum contra congelationem), Capsitrinum – a mixture of *Capsicum annuum* tincture and *Hypericum* tincture, as well as in ammonia-capsicum liniment (Linimentum Capsiciammoniatum), capsicum-camphor liniment (Linimentum Capsicicamphoratum) and capsicum plaster (Emplastrum Capsici)[2].

Inflammatory model is convenient for studying the effect of capsicum containing drugs[3]. Steroidal and non-steroidal anti-inflammatory drugs are widely used for treatment of inflammatory state [4].

Capsaicin affects isolation of substance P and bradykinin[4], and also shows affinity for vanilloid receptors located in nociceptive nerve fibres and leukocyte surface. Normal intact tissues stimulated

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by capsaicin give reaction of inflammatory kind - redness, burning sensation and rise in local temperature. However, in case of developed inflammation tissues respond to application of capsaicin "paradoxically" – inflammation symptoms decrease. This is due to desensitization of nerve fibres to inflammatory factors, as well as increasing local microcirculation and decreasing porosity of blood vessels. We believe this should have a positive effect on inflammation progress induced by Freund's adjuvant.

MATERIALS AND METHODS:

Studies were conducted on male Wistar rats, body weight 180-220g, kept in a standard animal facility with free access to water and food, in compliance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Specific Purposes[6] (Strasbourg, 1986) and the principles of the National Ukrainian Bioethics Congress (Kyiv, 2003) [7].

Inflammatory model was morbidized by administering 0.1 ml Freund's complete adjuvant (solution of antigen (inactivated and dried *M. tuberculosis*) emulsified in mineral oil) in the metatarsal joint of the right lower limb [7].

All animals were divided into 4 groups, depending on the drug administered, 10 animals per group. Treatment consisted in application of semisolid dosage form in the inflamed area on the paw once a day, starting from the next day after administration of phlogogen and until complete recovery. The first test group received experimental semisolid dosage form containing capsaicin, and consisting of 1g semisolid base and 0.25mg capsaicin (0.025%) (Wako Pure Chemical Ind.). Semisolid base was obtained by mixing 1 part of Polyethyleneglycol (PEG)-1500 and 2 parts of Polyethylene oxide (PEO)-400. The second test group received cream-based semisolid dosage form identical to the one used in the first group, but capsaicin was substituted for *Capsicum annuum L.* extract in such a ratio, so as to obtain final concentration of capsaicin 0.025% in the final mixture, which would be equal to capsaicin concentration in the semisolid dosage form with synthetic alkaloid. The third test group received commercial drug Dolgit Cream (Ibuprophen Cream) containing ibuprophen (50mg per 1g cream). The fourth group was used as control.

Efficacy of treatment was evaluated based on changes in morphological symptoms of inflammation – width and volume of the paw in the affected area. Width of the paw was measured using electronic calliper YT-7201 (YATO, Poland), changes in paw volume were measured using digital plethysmometer 37140 (UgoBasile, China).

Changes in complete WBC count were studied using microscopic method in Goryaev chamber.

Changes in blood biochemistry - activity of acetylcholinesterase and total count of alpha-1-acid glycoprotein in blood plasma – were evaluated using commercial express-analysis test-systems (Filicit-Diagnostics Ltd., Ukraine).

RESULTS:

In the given model of adjuvant-induced inflammation we studied such morphological properties as swelling width and volume in the phlogogen application area. Based on time pattern of these changes we made a conclusion on efficacy of the drugs used for treatment. Data on changes in width and volume in the inflamed area are given in Figure (1).

Despite decrease in swelling width and volume in the group of rats receiving ibuprophen cream when compared with the control, still inflammation symptoms persisted even on day 20, and the size of the swollen paw was visibly larger than the initial size (before the adjuvant was

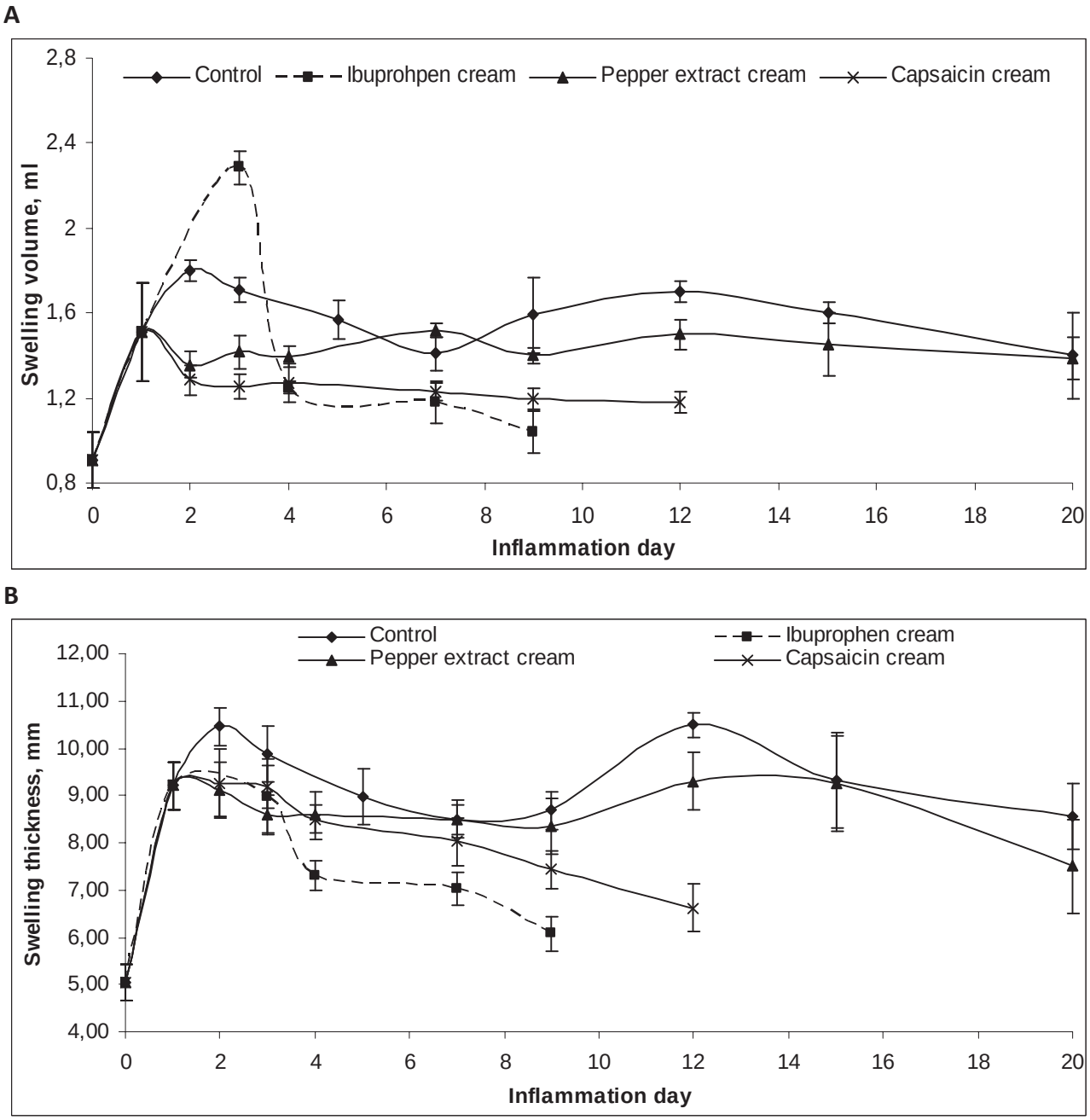

Figure (1): Changes in swelling volume (A) and thickness (B) when treating inflammation with drugs containing capsaicin

administered). The most probable explanation of such low efficacy of Ibuprophen Cream was the selected dosing schedule, which was identical to the dosing schedule for drugs containing capsaicin, and thus the desired therapeutic effect was not achieved.

When it comes to practical observation of shifts in blood cell composition, this aspect of pathogenesis manifests itself in appearance of a large number of young lymphocytes, which in case of rats, in whom lymphocytic fraction prevails among white blood cells (WBC), will lead to pronounced leukocytosis (Table 1).

Biochemistry of blood plasma is an extremely important factor reflecting general progress of inflammation.

Table (1): WBC count time pattern when treating inflammation with drugs containing capsaicin

Groups	Inflammation days (WBC count, 10^9/l)										
	0	1	2	3	4	5	7	9	12	15	20
Control	10.0 ±1.0	21.6 ±4.6	22.0 ±4.0	22.0 ±2.0	23.5 ±4.2	24.6 ±3.0	26.6 ±2.5	28. ±2.6	34.7 ±3.3	25.3 ±3.0	20.5 ±1.0
Pepper extract cream	10.0 ±1.0	21.6 ±4.6	22.8 ±2.4	24.7 ±1.6	16.3 ±2.0	16.0 ±1.8	15.5 ±1.0*	13. ±1.5*	13.7 ±2*	12.0 ±1.1*	10.5. ±2.1*
Capsaicin cream	10.0 ±1.0	21.6 ±4.6	20.3 ±3.0	24.0 ±2.1	22.8 ±2.0	21.3 ±1.1	21.1 ±2.5	22. ±3.0	24.0 ±2.4*	18 ±1.8*	13.3 ±1.5*
Ibuprophen cream	10.0 ±1.0	21.6 ±4.6	34.2 ±3.5	33.8 ±2.8	31.7 ±2.3	19.2 ±1.5	19.2 ±1.5	20.8 ±1.5	19.6 ±2.0*	18.6 ±2.1	16.25 ±1.8

*Significant differences from the control

Table (2): Serum acetylcholinesteraseactivity in case of adjuvant-induced inflammation, mcmol/sec*l

Inflammation day	Control	Ibuprophen Cream	Capsaicin cream	Pepper extract cream
0	31.8 ±4	31.8 ± 4	31.8 ± 4	31.8 ± 4
1	97.3 ± 7.2	97.3 ± 7.2	97.3 ± 7.2	97.3 ± 7.2
3	112.6 ±9.6	85.6 ± 12.3	105.6 ± 17.5	102 ± 16.9
7	145.7 ±19.6	94.3 ± 13.6	109 ± 13.7	111 ± 10.6
9	139.2 ±20.6	91.5 ± 9.4*	52.5 ± 12.8*	56.4 ± 4.7*
12	218.1 ±15.2	116.8 ± 13.7*	83.7 ±7*	42 ±8.1*
15	120.2 ± 14	80.5 ± 12.5*	65.6 ±10.8*	37.2 ±6*
20	110.4 ± 10.9	75.9 ±8.8	48.9 ± 8.3*	33±2.3*

*Significant differences from the control

Primarily, this refers to quantity and activity of plasma enzymes. Specific markers are also of interest. They are inherent to each type of pathological process. We have studied activity of plasma acetylcholinesterase and total count of plasma alpha-1-acid glycoprotein. Data on activity of cholinesterase in serum are given in Table 2. Significant increase in activity of cholinesterases is due of continuity in muscle fibres involved in inflammation, which leads to release of muscle acetylcholinesterase into the blood stream. In case of Ibuprophen Cream application activity of cholinesterase was significantly lower than in control, starting from day 9. However, increased activity of this enzyme was observed until day 20. In case of capsaicin cream, normalization of indicators of cholinesterase activity occurred faster than in case of ibuprophen cream. The best therapeutic effect was achieved when using semisolid dosage form based on chilli pepper extract. On

day 12 cholinesterase activity was 42 mcmol/sec*l, which is close to normal physiological range. Damaging effect of Freund's adjuvant is accompanied by destructive changes in connective tissue, leading to increase in concentration of serum fraction of alpha-1-acid glycoprotein. Indicators of concentrations of serum alpha-1-acid glycoprotein are given in Table 3. Drop in quantity of alpha-1-acid glycoprotein was observed in all groups. On day 20 ibuprophen reduced the level of alpha-1-acid glycoprotein down to 0.84 optical density units, which is still higher than the normal range. Capsaicin cream secured drop in quantity

Table (3): Concentration of serum alpha-1-acid glycoprotein in case of adjuvant-induced inflammation, optical density units

Inflammation day	Control	IbuprophenCream	Capsaicin cream	Pepper extract cream
0	0.6 ±0.11	0.6 ±0.11	0.6 ±0.11	0.6 ±0.11
1	1.22 ±0.1	1.22 ±0.1	1.22 ±0.1	1.22 ±0.1
3	1.3 ±0.18	1.0 ±0.07	1.28 ±0.03	0.89 ±0.09
7	1.6 ±0.2	1.24 ±0.12	1.02 ±0.15	1.07 ±0.07
9	1.55 ±0.25	1.1 ±0.22	0.81 ±0.05 *	0.73 ± 0.05 *
12	2 ± 0.3[1]	1.18 ±0.1 *	1.1 ±0.09*	0.62 ±0.12 *
15	1.8 ± 0.2[1]	1.05 ±0.08 *	0.9 ± 0.04*	0.63 ±0.08*
20	1.1 ±0.2	0.84 ± 0.03*	0.72 ±0.11*	0.61±0.1*

of serum alpha-1-acid glycoprotein on day 20 down to 0.78 optical density units. Application of pepper extract cream proved to be more therapeutically valid: alpha-1-acid glycoprotein were down to the normal physiological range by day 12.

DISCUSSION:

Semisolid dosage form containing capsaicin proved to be effective for treatment of adjuvant-induced inflammation. We believethis is due to the following factors: cream base ensured that a fair amount of capsaicin entered the skin in the inflamed area, and therapeutic concentrations of capsaicin are much lower than those of ibuprophen. Moreover, capsaicin improves microcirculation directly, while ibuprophen is cyclo-oxygenase inhibitor mediating its anti-inflammatory effect through decrease in the count of prostaglandins and thromboxanes, which in the given case proved to be less effective. Drug containing chilli pepper extract showed a more pronounced therapeutic effect. Width and volume of the paw in the phlogogen application area returned to normal as early as day 9. Further observation showed no recurrent inflammation symptoms, which usually manifest themselves by the end of the second week after adjuvant administration. We believe such high medicinal efficacy can be explained by other substances extracted together with capsaicin from chilli pepper and producing synergizing anti-inflammatory effect together with capsaicin, namely: a group of antioxidant compounds, such as flavonoids, anthocyanins, vitamins C and E, phenol derivatives and carotenoids[8]. The source[9] clearly states that the major antioxidant compounds in *Capsicum annuum L.* extract are various carotenoids. Namely, the following carotenoids were isolated and identified in that study: β-carotene, capsanthin, violaxanthin, β-cryptoxanthin, zeaxanthin, lutein epoxide,

capsorubin and neoxanthin. At the same time, anti-inflammatory analgesic effect of these substances in the acetic acid writhing test was demonstrated [9].

In case of this inflammation type B-lymphocytes get increasingly greater attention.

B-cells present antigens to T-lymphocytes, influence activation thereofand take part in synthesis of cytokines and autoantibodies[10, 11].

Under normal conditions B-lymphocytes have a complex development cycle ensuring formation of B-cell tolerance to self-antigens based on mechanisms of antigen-dependent and antigen-independent selection of autoreactive B-cell clones at specific stages of lymphopoiesis and immunogenesis. Progression of inflammation is characterized by loss of B-cell tolerance, leading to autoreactive B-cell survival and differentiation thereof into autoreactive plasma cells, which synthesize a broad autoantibody repertoire[12].

Ibuprophen's property to reduce total WBC count was reported by Lesko S.M. et al.[13], which we observed on day 5.

Chilli pepper extract based semisolid dosage form, used as an anti-inflammatory agent, contributed to pronounced drop in total WBC count on day 4 after administration of phlogogen. When comparing these values with data obtained for capsicum cream application, the efficacy of which was noticeably lower, we can draw a conclusion that this systemic impact is determined by some other extractive substances, having no relation to capsaicinoids.

The afferent and efferent limbs of the vagus nerve constitute the cholinergic anti-inflammatory pathway[14, 15] which acts as an interface between the immune system and the CNS. Most immune cells contain acetylcholinesterase and α-7-N-acetylcholine-receptors, the activation of which can reduce the release of pro-inflammatory cytokines. We can speculate that capsaicin decreased produce macrophage acetylcholinesterase and therefore capsaicin are potent inhibitor of pro-inflammatory mediators in murine macrophages.

Alpha1-acid glycoprotein is the acute phase protein. Its concentration is strongly increased under many pathological states, including inflammation. The alpha-1-acid glycoprotein measurements in clinical diagnostics to estimate of inflammation activity, monitored a patient's state and effects of anti-inflammatory therapy. The concentrations of alpha-1-acid glycoprotein were increased in rats inoculated with Freund's adjuvant. An elevation alpha-1-acid glycoprotein level is considered to be caused by pro-inflammatory cytokines (IL-1- α, IL-6, TNF- α). We suppose capsaicin can modulate production of proinflammatory molecules, as a result changes the level alpha-1-acid glycoprotein in plasma of blood.

CONCLUSION:

This study proves efficacy of semisolid dosage form based on chilli pepper extract for adjuvant-induced inflammation. By comparing pepper extract based dosage form to the cream containing synthetic capsaicin, one can draw a conclusion that this alkaloid plays an important role in anti-inflammatory effect of pepper extract.

The most probable among possible anti-inflammatory mechanisms of capsaicin efficacy are as follows: paradoxical activation of vanilloid receptors in nociceptive nerve endings (analgesic effect), increasing local microcirculation and decreasing porosity of blood vessels (anti-exudative effect). Anti-inflammatory activity of chilli pepper extract is also determined by carotenoids, which have a strong antioxidative effect.

CONFLICT OF INTERESTS AND ETHICAL CONSIDERATION:

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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AUTHORS CONTRIBUTION:

BoikoYu.A. – introduction, experimental part, analysis and discussion of results, conclusion, preparation for publication.
Kravchenko I. A. – analysis and discussion of results, conclusion.
Mohammad Ayat – experimental part.
Shandra A. A. – analysis and discussion of results, conclusion.

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