Experimental study of the effectiveness of the Capsicum annuum L. extracts for treatment of the rheumatoid arthritis

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ABSTRACT

The treatment of rheumatoid arthritis with the use of alcohol extracts of Capsicum annuum L. is widely practiced in traditional medicine on the territory of southern Ukraine. The purpose of this study was the experimental evaluation of the effectiveness of these traditional methods. The inflammatory process induced by administration of Freund's complete adjuvant into the young male rats was used as the experimental model of rheumatoid arthritis. Ibuprofen was applied as a reference drug of official medicine. The treatment effectiveness was assessed by changes in the morphological parameters of inflammation, as well as changes in the differential white blood cell count, serum concentration of alpha-1-acid glycoprotein, and acetylcholinesterase activity. It was demonstrated that the use of alcohol extracts of Capsicum annuum L. reduced the severity of nonspecific inflammation - edema decreased (by 50-60% in relation to the control group of animals), white blood cell left shift decreased, and the amount of alpha-1-acid glycoprotein in the blood decreased. Treatment of animals with extracts of Capsicum annuum L. prevented the development of autoimmune inflammation. The result of work was experimental evidence of significant therapeutic effectiveness of alcohol extracts of Capsicum annuum L. in case of rheumatoid arthritis.

Keywords: Capsicum annuum L., Freund’s adjuvant, rheumatoid arthritis

INTRODUCTION

The joint diseases are widespread and significant medical and social problem which affects all age groups of patients. All lesions of the joints can be divided into two large groups: dystrophic and inflammatory [1]. The dystrophic joint diseases (osteoarthritis) are one of the most common local chronic lesions, which are accompanied by injury of all parts of the joint and, in particular, articular cartilage [2, 3]. Inflammatory diseases of the joints are acute or chronic pathologies which have an autoimmune, infectious-allergic or infectious genesis [4-6].

The rheumatoid arthritis refers to inflammatory lesions of the joint tissue and is, in fact, incurable disease. The therapeutic interventions applied in case of rheumatoid arthritis are symptom-oriented and, in many cases, are ineffective, which requires the selection of new therapeutic approach [7, 8]. All this indicates that the modern rheumatology requires the development of fundamentally new approaches to the treatment of rheumatoid joint lesions. One of such approaches may be the use of drugs based on biologically active substances of natural origin. Currently, the substances most demanded in this aspect are compounds derived from plant raw materials [9-11]. A large variety of anti-inflammatory substances of plant origin enables them to influence on different stages of the inflammatory process, which is manifested not only in the form of a symptomatic, but pathogenetic therapeutic effect also.

In the southern regions of Ukraine, the widespread means of traditional medicine, which are used in case of arthritic injuries, including rheumatoid ones, are alcohol extracts of Capsicum annuum L. (chili pepper). Extracts of chili pepper are a very popular anti-arthritis remedy both among the local population and specialists practicing the traditional medicine [12]. The main anti-inflammatory substances of the chili pepper fruits are capsaicin [13]. Despite widespread use of Capsicum annuum L. fruits and related species as antirheumatic, antipyretic, anti-inflammatory remedies in traditional practices of different countries, the classical medicine considers the capsaicin mostly as analgesic agent [13, 14].
Aim and objectives

The aim of this work was the experimental determination of the anti-inflammatory activity of alcohol extracts of Capsicum annuum L. of local varieties on the model of adjuvant-induced arthritis. The data obtained in the course of study could be used to evaluate the effectiveness of traditional remedies for treatment of rheumatoid arthritis, and would reveal the additional pharmacological effects of the action of chili pepper extracts in addition to analgesic action.

MATERIALS AND METHODS

Plant materials

The fruits of chili pepper were bought in large local markets (Odessa city, Ukraine). The most widespread in the southern region of Ukraine variety of Capsicum annuum L. – “Ukrainian bitter” was used. The varietal identification was defined with the use of variety catalogs.

Animals

The animals raised and kept in the vivarium of Odessa National Medical University were used for studies. 30 animals were randomly selected for experimental work.

Studies were conducted on male Wistar rats, body weight 180-220 g, 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 (Strasbourg, 1986) [15] and the principles of the National Ukrainian Bioethics Congress (Kyiv, 2001) [16].

Preparation of extracts of plant raw materials

Chili pepper pods were pre-dried in air until achievement of constant weight. After drying, the pods were crushed, the kernel and the green part were removed. The crushed mass of the fruits was poured into 96° ethyl alcohol in a ratio of 1:4 and left in a dark place for 1 day at a temperature of 20-22°C. Thereafter, the liquid portion was drained and filtered through a gauze layer.

Anti-inflammatory analysis

Experimental groups of animals

In order to induce the inflammatory arthritis, all experimental animals were injected with 0.1 ml of Freund’s complete adjuvant underneath the plantar aponeurosis of the right limb. Whereupon the animals were divided into 3 groups, 10 rats in each group. The first group (control group) of animals was used as a control, the treatment was carried out with the use of pure 96° ethyl alcohol. The second group (group 2) of animals was experimental group and was treated with earlier prepared alcohol extracts of chili pepper. Treatment of the third group (group 3) of animals was carried out with the use of commercial formulation, which was an ointment with ibuprofen with a concentration of 50 mg/g of the preparation.

Treatment procedure

The treatment was started in 1 day after the injection of the adjuvant and development of the inflammatory reaction. The therapeutic intervention was implemented once a day, on a daily basis. In case of animals of the first and second groups, the injured limb was immersed in ethyl alcohol or chili pepper extract, so that these liquids completely cover the area of inflammation. The limb was immersed for 20 minutes. ~ 50 mg of ointment with ibuprofen was applied to the area of inflammation and slightly embrocated for several minutes in animals of third group.

Anti-edema action

The dynamics of changes in the thickness and volume of the limb in the inflammation area were studied for 28 days in order to assess the anti-edema action. The volume and thickness of the opposite (left) limb were measured from 9th day in order to assess the severity of autoimmune lesion. The thickness of the limb in the area of inflammation was measured by means of digital calipers YT-7201, manufactured by YATO, Poland. The limb volume in the area of inflammation was measured by plethysmometer 37140, UgoBasile, Italy.

Serum acetylcholinesterase activity and serum alpha-1-acid glycoprotein concentration

Measurement of the acetylcholinesterase activity and concentration of alpha-1-acid glycoprotein in the blood serum of experimental animals was carried out with the use of commercial test systems for rapid analysis manufactured by “Scientific Industrial Enterprise “Filisit-Diagnostika” Ltd., Ukraine.

Total count of white blood cells and differential white blood cell count

The total count of white blood cells of the peripheral blood was determined microscopically, using the Gorjaev’s chamber. The differential white blood cell count was generated by counting the individual white blood cells in a peripheral blood smear, the color was defined according to Romanovski. The light microscopy with x900 immersion lens was used for counting.

Statistical analysis

The preliminary distribution of animals into the experimental groups was made randomly. The 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

The injection of Freund’s complete adjuvant caused prominent signs of the inflammatory process (Figure 1). In 1 day after the inflammatory process induction, as a result of the developed edema, the volume and thickness of the affected limb increased by 70-75% in all groups of animals. The maximum edematous manifestations were fixed on the 3rd and 12th days after the injection of phlogogen. Reliable decreases in edematous manifestations was not observed for the entire treatment period. In contrast to two other groups of animals, the animals of group 2 had no repeated increase in edema in the inflammation area on 12th day. Autoimmune inflammation (Table 1) assessed by the severity of the edema of the opposite limb developed in 9-10 days after the phlogogen administration at the animals of the control group and group 3. On average, an increase in the width and volume of the opposite limb in the area of metatarsal joint for animals of the control group and group 3 was 12-15% compared to the initial figures. The animals of group 2 had no significant changes in thickness and volume of the opposite limb.

The serum acetylcholinesterase activity increased after the induction of the inflammatory process (Figure 2). The highest figures of acetylcholinesterase activity, three times higher than intact values, were recorded in all animal groups on the 3rd, 5th day of inflammation. On the
12th day of the inflammatory process, the repeated increase in the acetylcholinesterase activity was observed in animals of the control group and group 3. After the 20th day of inflammation, the acetylcholinesterase activity in animal groups 3 and 2 decreased by 15% and 50%, respectively, in comparison with the animals in the control group. On the 28th day of the inflammatory process, the acetylcholinesterase activity in the control group exceeded intact values by 3.2 times, in group 3 - by 2.2 times, in group 2 – by 1.8 times.

For serum alpha-1-acid glycoprotein (Figure 3), as with other studied parameters, the significant increase in blood concentrations (~ 3 times higher than intact values) was observed on the 3rd - 5th day of the inflammatory process. Subsequently, the control group of animals didn’t demonstrate significant decrease in the amount of alpha-1-acid glycoprotein during the entire period of observation. In groups of animals with administered ibuprofen and pepper extract, the amount of alpha-1-acid glycoprotein decreased in 5 days of inflammation. The concentration of alpha-1-acid glycoprotein in the blood serum of animals of group 2 was 1.3-1.4 times lower than in animals of group 3 from 9th to 28th day of inflammation.

Data on the change in the total count of white blood cells and differential white blood cell count are given in Table 1. In all groups of animals, the increase in the count of white blood cells was progressive throughout the entire period of observation. The highest values of the total count of white blood cells were recorded on the 28th day of inflammation in all groups of animals. In animals of group 2, the total count of white blood cells on the 28th day of inflammation was less than in the animals of the control group and group 3. During the period from 3rd to 7th days, there was neutrophil shift in the differential white blood cell count towards the young forms, which was subsequently replaced by lymphocytosis. The number of neutrophils and monocytes decreased from the 12th day of inflammation and reached a minimum value on 28th day. These changes were observed in all groups of animals, but in animals of group 2 they were significantly less than in the other groups.

**Table 1**: Dynamics of changes in morphological signs of autoimmune inflammation of the opposite limb (thickness and volume of edema) in rats with adjuvant-induced arthritis treated with *Capsicum annuum* L. and ibuprofen.

| Swelling volume, ml          | Inflammation day |
|-----------------------------|------------------|
|                             | 0    | 9    | 12   | 15   | 20   | 28   |
| Control                     | 0.80 ± 0.03     | 0.86 ± 0.02 | 0.93 ± 0.02 | 0.92 ± 0.03 | 0.9 ± 0.03 | 0.91 ± 0.01 |
| Extract of *Capsicum annuum* L. | 0.81 ± 0.005   | 0.83 ± 0.01 | 0.84 ± 0.005** | 0.83 ± 0.009** | 0.83 ± 0.01* | 0.82 ± 0.01* |
| Ibuprofen                   | 0.82 ± 0.05     | 0.88 ± 0.04 | 0.95 ± 0.03 | 0.93 ± 0.03 | 0.93 ± 0.06 | 0.90 ± 0.07 |

| Swelling thickness, mm      | Inflammation day |
|-----------------------------|------------------|
|                             | 0    | 9    | 12   | 15   | 20   | 28   |
| Control                     | 5.35 ± 0.04     | 5.67 ± 0.21 | 5.96 ± 0.14 | 6.02 ± 0.12 | 5.91 ± 0.19 | 5.97 ± 0.10 |
| Extract of *Capsicum annuum* L. | 5.35 ± 0.04   | 5.48 ± 0.03 | 5.53 ± 0.09** | 5.50 ± 0.08** | 5.49 ± 0.01** | 5.42 ± 0.07** |
| Ibuprofen                   | 5.40 ± 0.08     | 5.75 ± 0.16 | 6.08 ± 0.10 | 5.99 ± 0.19 | 5.96 ± 0.13 | 5.90 ± 0.20 |

**Figure 1**: Dynamics of changes in morphological signs of inflammation (thickness of edema A and volume of edema B, in the area of adjuvant injection) in rats with adjuvant-induced arthritis treated with *Capsicum annuum* L. and ibuprofen.

**Figure 2**: Dynamics of changes in the acetylcholinesterase activity in the serum of rats with adjuvant-induced arthritis treated with *Capsicum annuum* L. and ibuprofen.
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**Figure 3:** Dynamics of changes in the alpha-1-acid glycoprotein concentration in the serum of rats with adjuvant-induced arthritis treated with *Capsicum annuum* L. and ibuprofen.

**Table 2:** Dynamics of changes in the total count of white blood cells and differential white blood cell count in the blood of rats with adjuvant-induced arthritis treated with *Capsicum annuum* L. and ibuprofen.

| Group                      | WBC                            | Inflammation day |
|----------------------------|-------------------------------|-----------------|
|                            | 0            | 3         | 7         | 12        | 20        | 28        |
| Control                    | Leuc, G/l   | 12.2 ± 0.16 | 16.7 ± 1.0 | 19.5 ± 0.6 | 22.0 ± 0.8 | 24.5 ± 0.4 | 30.1 ± 0.7 |
|                            | Mon, %       | 4.6 ± 0.15  | 4.7 ± 0.13  | 4.4 ± 0.05  | 4.2 ± 0.11 | 2.5 ± 0.27 | 2.2 ± 0.13  |
|                            | Lym, %       | 60.3 ± 2.9  | 54.3 ± 3.3  | 52.6 ± 1.5  | 72.4 ± 2.5 | 78.6 ± 2.2 | 83.2 ± 4.8  |
|                            | Neu stab, %  | 22.3 ± 1.5  | 22.5 ± 1.6  | 19.5 ± 0.8  | 9.3 ± 0.8  | 9.6 ± 0.4  | 6.1 ± 0.9   |
|                            | Neu seg, %   | 6.8 ± 0.34  | 10.6 ± 0.3  | 12.4 ± 0.7  | 5.6 ± 0.9  | 3.3 ± 0.7  | 2.8 ± 0.9   |
|                            | Met, %       | 0 ± 0       | 2.4 ± 1.0   | 4.3 ± 0.6   | 1.0 ± 0.5  | 0 ± 0      | 0 ± 0       |
|                            | Eos, %       | 5.5 ± 0.15  | 5.0 ± 1     | 6.1 ± 0.1   | 6.7 ± 0.6  | 5.4 ± 1.1  | 5.5 ± 1.3   |
|                            | Bas, %       | 0.5 ± 0.23  | 0.5 ± 0.44  | 0.7 ± 0.46  | 0.8 ± 0.19 | 0.6 ± 0.37 | 0.2 ± 0.15  |
| Extract of *Capsicum annuum* L. | Leuc, G/l   | 11.9 ± 0.27 | 16.8 ± 0.2  | 17.9 ± 0.9  | 19.4 ± 0.1 | 19.9 ± 0.7 | 22.5 ± 0.9* |
|                            | Mon, %       | 4.3 ± 0.13  | 4.1 ± 0.41  | 4.7 ± 0.37  | 4.1 ± 0.22 | 3.6 ± 0.4*  | 3.7 ± 0.23*  |
|                            | Lym, %       | 62.3 ± 3.4  | 58.7 ± 2    | 53.1 ± 4.3  | 69.8 ± 2.8 | 73.4 ± 3.3 | 74.9 ± 3.9* |
|                            | Neu stab, %  | 18.2 ± 1.6  | 18.1 ± 1.5**| 17.1 ± 0.5**| 12.4 ± 1.5**| 11.3 ± 0.5 | 11 ± 1.1**  |
|                            | Neu seg, %   | 8.8 ± 0.6   | 10.3 ± 1.0* | 13.7 ± 0.2* | 7.4 ± 0.6* | 6.1 ± 0.1*  | 5.4 ± 0.2*  |
|                            | Met, %       | 0 ± 0       | 3 ± 0.3     | 4.7 ± 0.7   | 0 ± 0*     | 0 ± 0      | 0 ± 0       |
|                            | Eos, %       | 5.5 ± 0.25  | 5.2 ± 1     | 6.3 ± 1.2   | 5.7 ± 0.4  | 5.1 ± 0.8  | 4.6 ± 0.7   |
|                            | Bas, %       | 0.9 ± 0.1   | 0.6 ± 0.32  | 0.4 ± 0.18  | 0.6 ± 0.26 | 0.5 ± 0.32 | 0.4 ± 0.23  |
| Ibuprofen                  | Leuc, G/l   | 12.3 ± 0.15 | 15.3 ± 0.8  | 19.6 ± 0.4  | 20.8 ± 0.9 | 22.2 ± 0.7 | 25.7 ± 0.7  |
|                            | Mon, %       | 4.4 ± 0.2   | 4.5 ± 0.15  | 4.9 ± 0.36  | 4 ± 0.18   | 2.3 ± 0.29 | 2.4 ± 0.16  |
|                            | Lym, %       | 59.4 ± 2.4  | 54.2 ± 1.4  | 50.4 ± 3.0  | 70.7 ± 4.0 | 75.5 ± 4.7 | 80.6 ± 2.2  |
|                            | Neu stab, %  | 23.2 ± 1.8  | 23.0 ± 0.8  | 22.5 ± 1.7  | 9.6 ± 0.8  | 10.2 ± 0.4 | 6.7 ± 1.4   |
|                            | Neu seg, %   | 6.9 ± 0.76  | 7.9 ± 0.1   | 10 ± 0.2    | 9.2 ± 0.5  | 5.6 ± 0.4  | 4.2 ± 0.9   |
|                            | Met, %       | 0 ± 0       | 3.5 ± 0.7   | 3.6 ± 0.5   | 0 ± 0      | 0 ± 0      | 0 ± 0       |
|                            | Eos, %       | 5.6 ± 0.33  | 6.2 ± 0.5   | 7.7 ± 1.0   | 5.9 ± 0.2  | 5.8 ± 0.6  | 5.3 ± 0.2   |
|                            | Bas, %       | 0.5 ± 0.24  | 0.7 ± 0.54  | 0.9 ± 0.61  | 0.6 ± 0.20 | 0.6 ± 0.28 | 0.8 ± 0.53  |

Leuc – leucocytes, Mon – monocytes, Lym – lymphocytes, Neu stab – stab neutrophils, Neu seg – segmented neutrophils, Met – metamyelocytes, Eos – eosinophils, Bas – basophils.

* - p ≤ 0.05, compared to control group, # - p ≤ 0.05, compared to ibuprofen treated group

**DISCUSSION**

Demonstrated therapeutic effect, which was expressed in a significant decrease in edema, decrease in soreness (palpatory tenderness), as well as restoration of the joint’s functional activity indicates the availability of pronounced anti-inflammatory properties in the extract of *Capsicum annuum* L. By virtue of the fact that the rheumatoid arthritis is primarily a consequence of autoimmune lesion, the effect of the extract of chili pepper on this link of pathogenesis is of particular interest. In classical methods, the anti-arthritic effect is assessed on the basis of changes in the joints of the opposite limbs taking into account the beginning of treatment not earlier than in 10-14 days after the administration of Freund’s complete adjuvant. Such conditions are considered objective for assessing the anti-rheumatic activity of the studied drugs. The purpose of this work is to determine the effectiveness of traditional methods of treatment when using the extracts of chili pepper by patients
The mechanisms of pharmacological action of *Capsicum annum* L. alcohol extracts are of particular interest. The majority of the therapeutic effects of these agents are associated with a high content of capsainoids, among which the main one is capsaicin [12]. The analgesic activity of capsaicin is well described in the literature [14] and is associated with its ability to interact with transient receptor potential vanilloid 1 (TRPV1), which in the course of prolonged use causes desensitization of painful vanilloid-sensitive nerve endings, and also reduces the number of neuropeptides released by them. [19]. Local inflammatory processes cause a systemic response of the body, which is manifested in appearance of a significant amount of acute phase proteins, one of which is the alpha-1-actin glycophorin. A sharp decrease in the content of alpha-1-actin glycophorin in the blood of animals treated with *Capsicum annum* L. extracts indicates a decrease in the overall inflammatory response. We attribute this effect to a decrease in the number of inflammatory mediators in the lesion focus in case of application of the extracts. As already mentioned above, the capsaicin can reduce the release of neuropeptides by nerve endings, in particular, substance P and calcitonin gene-related peptide. However, in case of Freund’s adjuvant injection the inflammatory response involves not only nerve fibers, but also other tissue structures (immunocompetent cells, capillary endothelocytes, and connective tissue elements). In our opinion, the reduced manifestations of the inflammatory reaction in case of application of the *Capsicum annum* L. is associated not only with capsainoids, but also with other active substances contained in pepper fruits. Such substances include carotenoids, vitamins C and E [21]. These compounds are antioxidants that have a normalizing effect on biochemical processes in the inflammatory focus, especially at the first, alternative stage of the inflammatory process [22]. Decreased level of cellular damage, as well as decreased number of inflammatory mediators and pro-inflammatory cytokines promote not only decrease in chemotaxis of white blood cells in the inflammatory focus, but also result in specific changes in the differential white blood cell count which were shown for animals in group 2.

One of the factors which reduce the severity of inflammatory phenomena in case of rheumatoid arthritis is the cholinergic anti-inflammatory pathway [23]. Acetylcholine, of non-neuronal origin, by interaction with α7-subunit of nicotinic acetylcholine receptor (α7-nAChR) of macrophages, reduces the production of pro-inflammatory cytokines in case of rheumatoid arthritis [23]. When hydrolyzing acetylcholine, the acetylcholinesterase restricts its biological activity, thereby affecting the activity of the cholinergic anti-inflammatory pathway. In the course of development of the experimental rheumatoid arthritis, the use of ibuprofen and *Capsicum annum* L. extract as medicines caused a decrease in the activity of serum acetylcholinesterase. Based on this fact, we can assume an increased effect of the cholinergic anti-inflammatory pathway on the induced pathological process.

CONCLUSION

In the course of the experimental study it was demonstrated that treatment of rheumatoid arthritis with the use of alcohol extracts of *Capsicum annum* L. widely practiced in traditional medicine on the territory of southern Ukraine has considerable therapeutic effectiveness. The ability of alcohol extracts of *Capsicum annum* L. to prevent the development of autoimmune inflammatory process in rats with injection of Freund’s complete adjuvant is also shown. The healing properties of the studied extracts are related to the content of a number of biologically active substances, the most important of which are capsainoids. Application of *Capsicum annum* L. alcohol extract as a therapeutic agent in the case of developed adjuvant-induced inflammatory process resulted both in decrease in the morphological signs of inflammation (edema, redness, pain) and decrease in the response from the blood system (changes in differential white blood cell count, decrease in the number of acute phase proteins). Thus, having confirmed the effectiveness of this traditional remedy for the treatment of rheumatoid arthritis experimentally, we can recommend it for use in official medicine.

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