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STUDY OF CERTAIN MECHANISMS OF ANTI-INFLAMMATORY EFFECT OF *TANACETUM PARTHENIUM* EXTRACT ON ADJUVANT ARTHRITIS MODEL IN RATS

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Abstract

Aim of the study was to investigate the immunological properties of Tanacetum parthenium extract on a model of chronic inflammation in rats.

Materials and methods. Immunotropic and anti-inflammatory properties of thick water-alcohol extract of Tanacetum parthenium herb (ETPH) were studied on a model of adjuvant arthritis (AdA) (0.1 ml of complete Freund's adjuvant (CFA), once subcutaneously at the base of the tail of white nonlinear male rats weighing 200- 220 g) after 7 days, the administration of CFA was repeated (subplantar in the right hind limb of rats). Animals were divided into 4 groups of 9 rats each, 6 animals in intact. Animals of positive control (PC) on the background of pathology were injected with distilled water (1 ml / 100 g). ETPH (aqueous solution, 50 mg / kg) and diclofenac sodium (1% starch suspension, 8 mg / kg) were administered from day 1 of the experiment and for four weeks intragastrically once a day. The volume of the affected foot (V) was measured using a plethysmometer LE7500 (company «PANLAB», Italy) in the dynamics - for 1, 5, 10, 15, 20, 25 and 30 days after the last dose of CFA. Intensity of inflammation in rats was assessed by peripheral blood parameters, immunological markers of the inflammatory process. Statistical analysis of data was performed by usage of a standard software package «Statistica v. 6.0». Differences between groups were considered statistically significant at $p<0,05$.

Results. The course of AdA was characterized by the development of systemic inflammation, as evidenced by increased proinflammatory cytokines TNF-α and IL-1β, increased production of «acute phase» proteins (fibrinogen and CRP), accumulation of CIC and accompanied by a decrease in anti-inflammatory cytokine IL-10. The use of ETPH at a dose of 50 mg / kg decreased the level of CRP and fibrinogen, the content of anti-inflammatory cytokines and increased the content of IL-10 in positive control animals, which indicates a decrease in the severity of the pathological process. At the background of the comparison drug, diclofenac sodium, similar dynamics was observed in most of the studied indicators, but the CIC content remained at the level of PC.

Conclusions. On the AdA model in rats, ETPH at a dose of 50 mg / kg has a pronounced anti-inflammatory effect and is not inferior to diclofenac sodium in effectiveness. Based on the obtained data, we can conclude that the anti-inflammatory effect of the ETPH is based on pronounced immunomodulatory properties, which determines the prospects for further studies of maroon extract as an effective anti-inflammatory agent.

Keywords: *chronic inflammation, adjuvant arthritis, anti-inflammatory action, immunomodulatory properties, cytokines, extract of Tanacetum parthenium herb (ETPH), rats.*

Introduction. Rheumatic diseases (RD), in particular rheumatoid arthritis (RA), are chronic systemic diseases with progressive lesions of predominantly peripheral (synovial) joints by the type of erosive-destructive polyarthritis. These diseases significantly impair the quality of life of patients, lead to temporary and permanent disability and are associated with premature mortality [1, 2]. A significant share of RD is RA - one of the most disabling and common connective tissue diseases (about 1% - in the world, 0.4% - in Ukraine) [1, 3].

RA is based on complex disorders of the immune response, which result in the development of chronic inflammation of autoimmune origin. The imbalance of Th1 / Th2 lymphocytes involved in the processes of cellular and humoral immunity is one of the fundamental immunological disorders that characterize RA. Th1-lymphocytes promote the proliferation of autoreactive T-lymphocytes and the development of cellular autoaggression, and Th2-lymphocytes regulate the humoral response. T1-type T-lymphocytes are activated in the early stages of the disease, and the progression of the disease is determined by dysregulation of the synthesis of pro-inflammatory and anti-inflammatory cytokines [1, 4, 5].

Despite the fact that significant progress has been made in the treatment of RA in recent decades, pharmacotherapy of this disease remains the most pressing problem of clinical medicine and pharmacology.

One of the ways to solve this problem is to find and develop drugs with anti-inflammatory and chondroprotective properties based on plant materials. A promising source of biologically active substances (BAS) with potential anti-inflammatory and analgesic properties is feverfew (Tanacetum parthenium (L.) Schultz Bip.), of the Asteraceae family, from which a standardized extract was obtained. The chemical composition of *Tanacetum parthenium (L.)* is represented by phenolic compounds, flavonoids, sesquiterpene lactones, essential oils, etc [6]. Previous studies have shown clear anti-inflammatory and analgesic properties of extract of *Tanacetum parthenium* herb (ETPH) in models of acute inflammation. The aim of this study was to investigate some immunological aspects of the anti-inflammatory effect of ETPH in a model of chronic inflammation in rats modeled by complete Freund's adjuvant (CFA).

Materials and methods. The experiments were performed on white nonlinear male rats obtained from the vivarium of National university of Pharmacy in accordance with the "General ethical principles of animal experiments", consistent with the provisions of the "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" (Strasbourg, 1986) with changes made in 1998) [7, 8]. Animals were kept in accordance with the current rules for equipment and vivarium at a room temperature of 21-24°C, humidity not exceeding 60-65%, on a standard diet and water - ad libitum [9]. Rats were acclimatized for 7 days in the test room before the start of the experiment.

The anti-inflammatory properties of ETPH and its effect on the immune status of animals were studied in a model of adjuvant arthritis (AdA). As a comparison drug (CD) used known NSAID, non-selective inhibitor of COX-1 and COX-2 with pronounced analgesic and anti-inflammatory properties Diclofenac sodium (trade name "Diclofenac sodium", manufactured by "Pharmaceutical company Darnitsa" (p. EG30919)), which is widely used. Adjuvant arthritis in rats is the most common model of systemic inflammation in experimental pharmacology, which most closely reproduces the pathogenesis and morphologic and functional changes in collagen diseases in humans [10, 11, 12]. Model of AdA was caused by a single subcutaneous administration of 0.1 ml of CFA into the base of the tail of male rats (200-220 g body weight). CFA administration was repeated after 7 days in the right hind limb of rats [11].

The animals were divided into 4 groups of 9 rats each, there were 6 animals in the intact group: Group 1 – physiological control (intact animals that have not been exposed to any exposure (IC); Group 2 – positive control (PC) – animals, which on the background of pathology were injected with distilled water at a dose of 1 ml / 100 g; Group 3 – animals that were administered ETPH at a dose of 50 mg / kg on the background of pathology; Group 4 – animals on the background of pathology with injected CD diclofenac sodium in an average effective dose of 8 mg / kg. Given the severity of the pathology, the test extract was administered to the

experimental animals in the most effective dose of 50 mg / kg, which was determined by screening in a model of acute carrageenan inflammation (unpublished data). The studied drugs were administered from the 1st day of the experiment and for four weeks intragastrically once a day: ETPH in the form of an aqueous solution, the CB – in the form of a 1% suspension of starch to minimize local side effect on the gastrointestinal tract.

The volume of the affected foot (amount of extruded fluid (V, ml) when immersing the damaged paw of the animal in the flask of the device) was measured by usage of plethysmometer LE7500 (company "PANLAB", Italy) in the dynamics - 1, 5, 10, 15, 20, 25 and 30 days after the second dose of CFA in the right paw. For the overall assessment of the anti-inflammatory effect of the studied drugs used an integrated indicator of AUC (volume of the affected foot / day of observation) – the area under the curves «severity of edema / day of observation».

The immunological status of animals was assessed by the content of acute phase proteins in blood plasma: fibrinogen, C-reactive protein (CRP), circulating immune complexes (CIC), using sets of reagents produced by "Granum" (Kharkiv, Ukraine) [13]. The content of cytokines in blood serum: interleukins IL-1β, IL-10 and tumor necrosis factor (TNF-α), was evaluated according to the instructions for the manufacturer's kits «Vector-Best» (Novosibirsk, Russia) by enzyme immunoassay. Blood serum samples were obtained from experimental animals by the conventional method [14].

Statistical analysis of the obtained data was performed by usage of a standard software package "Statistica v. 6.0 " with use of parametric (Newman-Cayles method) and nonparametric methods (Kruskal-Wallis and Mann-Whitney criteria). The normality of the data distribution was checked using the Shapiro-Wilk test. Differences between groups were considered statistically significant at $p < 0.05$.

Results and discussion. Text According to the data obtained, treatment with ETPH and CD diclofenac sodium led to a decrease in the severity of edema caused by CFA. Comparison of the obtained data in the experimental groups showed some differences in the dynamics of the pharmacologic effect of the studied drugs. Diclofenac sodium, as a powerful anti-inflammatory agent, had a pronounced anti-exudative effect on the first day of observation: the volume of the paw in rats at this group was 22% lower than in the PC group (Fig. 1). The severity of the anti-exudative effect gradually increased and from 10-15 days remained sufficiently pronounced until the end of the experiment (Fig. 1). During the experiment, the effectiveness of diclofenac sodium was 33% on average.

The use of ETPH in the first 4 days did not register a statistically significant decrease in the severity of edema in contrast to CD, but in numerical terms the paw volume in rats of this group was slightly lower than in animals of the CD group – 6-12% lower than in the CD group. Starting from day 10 (Fig. 1), the effectiveness of ETPH increased almost to the level of CD and remained at a stable high level until the end of the experiment – an average of 32% (10-30 days).

An integrated indicator, the area under the curves of the dynamics of changes in paw volume during the experiment, showed no statistical differences between the study groups – with ETPH AUC was 74 ml/day, with PC diclofenac sodium slightly less – 69 ml/day (Fig. 2).

Inflammation as a typical pathological process occurs as a result of tissue damage, is manifested by a complex of structural, functional and metabolic disorders, microcirculation disorders and is mediated by a number of pro-inflammatory and anti-inflammatory mediators. Among the wide range of pro-inflammatory mediators involved in the development of RA, special attention is paid to TNF-α, which is considered the main cytokine that causes the development of inflammation of the synovial membrane and osteoblast-mediated bone destruction in arthritis [1, 4, 5]. The maximum concentration of TNF-α in RA is reached in the active stage of the disease. The main pathogenetic effects of TNF-α in RA are an increase in the production of osteoclast differentiation factor – osteoprotegerin ligand (RANKL), it is responsible for the resorption of bone tissue, as well as the induction of overexpression of adhesion molecules, metalloproteinases, collagenases, chemokines and prostaglandins [5, 15].

Another key pleiotropic proinflammatory cytokine that plays a fundamental role in the development of RA is interleukin 1 (IL-1β) [1, 5, 16]. It

is IL-1β that induces the synthesis of other "proinflammatory" cytokines, such as TNF-α and IL-6, low molecular weight mediators of inflammation (nitric oxide and prostaglandins), chemokines, matrix metalloproteinases and enzymes that attract neutrophils to the inflammatory zone. Adhesion molecules at leukocytes and endothelial cells contribute to the destruction of cartilage and bone tissue [1, 4, 5]. IL-1β is able to increase the expression of enzymes involved in the destruction of cartilage (collagenase 3, stromelysin 1, gelatinase A and B), inhibit the synthesis of cartilage matrix proteins – type II collagen and proteoglycan. In addition, due to the induction of nitric oxide synthesis and PGE2, IL-1β inhibits the mitogenic activity of cartilage chondrocytes and, through an autocrine mechanism, induces its own synthesis of IL-1β chondrocytes [1, 3, 4, 5]. Its production is carried out by macrophages, synoviocytes, chondrocytes and osteoclasts. In RA there is a significant increase in the production of IL-1β in synovial tissue, with a subsequent increase in its concentration in synovial fluid and serum, which correlates with disease activity [17]. IL-1β has been shown to enhance the effects of TNF-α on cartilage and joint destruction inducing IL-1β synthesis [1, 5].

One of the main anti-inflammatory cytokines is interleukin 10 (IL-10). Like IL-1β, it has multiple pleiotropic effects on the regulation of immune processes and inflammation. Interleukin 10 blocks the T-cell response to specific antigens, reduces the expression of cytokines, T-helper-1 lymphocytes (Th1), MHC class II antigens and co-stimulating molecules on macrophages, inhibits the induction of secretion of inflammatory cytokines TNF-α and IL-1 stimulates the production of antibodies [18, 19, 20]. IL-10 is produced by macrophages, CD5 + B cells, CD4 + T-cells and monocytes. This cytokine is able to inhibit the expression of IL-1, 6, 8, TNF-α and matrix metalloproteases. In RA, there is a deficiency of IL-10 [18].

In the study of the cytokine profile of rats from the PC group found a statistically significant increase in TNF-α in 4 times and IL-1β in 1.6 times (Table 1).

The effect of IL-1β on hepatocytes leads to a decrease in albumin synthesis and an increase in the production of «acute phase» proteins [21], which in our experiment was reflected in an increase in fibrinogen in rats by 1.7 times and CRP by 6 times, respectively, compared with intact animals (Table 2). The increase in pro-inflammatory cytokines was accompanied by a statistically significant decrease in the anti-inflammatory cytokine IL-10 by 1.6 times (Table 1).

Imbalance of pro/anti-inflammatory cytokines in the direction of increasing pro-inflammatory indicates the development of not only local inflammation but also the generalization of the inflammatory process, as evidenced by the formation of CIC medium weight - complexes consisting of antigen, antibodies produced by B-lymphocytes and related with them complement components C1q, C3, C4. According to RA CIC are deposited in the main target organs – articular cartilage and are markers of autoimmune processes. The relationship between CIC level, severity of extra-articular manifestations and the degree of disease activity is proved [15].

The content of CIC under the AdA condition in the blood of PC animals increases by 1.8 times compared with intact level (table. 2).

According to obtained data, the introduction of ETPH at a dose of 50 mg/kg contributed to the normalization of the cytokine profile: the content of IL-1β was reduced by 1.8 times, and TNF-α – 2.2 times relative to the values of intact control. At the same time, the level of IL-10 increased to the values of intact animals (Table 1). As a consequence of these changes, the content of CIC (Fig. 2) and the synthesis of fibrinogen and CRP decreased (Table 2). The positive dynamics of the studied indicators with the use of ETPH indicates a decrease in the severity of the pathological process.

Similar dynamics of the studied parameters was observed with the use of CD diclofenac sodium. There was a statistically significant decrease in the level of pro-inflammatory cytokines and an increase in IL-10. The content of fibrinogen decreased to the level of intact animals, CRP level also decreased statistically significantly, but did not reach physiological values (Table 1, 2). However, the content of CIC remained at the level of positive control (Fig. 2). The obtained results are probably explained by the proven chondrotoxicity of non-selective COX inhibitors, in particular diclofenac sodium, as evidenced by a sufficiently high content of CIC, which are formed due to the accumulation of

decay products of articular cartilage and synovial membrane [22].

Based on the obtained data, it can be assumed that an important role in the mechanism of anti-inflammatory action of ETPH belongs to the immunocorrective properties. Analysis of the literature on the pharmacodynamics of ETPH shows that the mechanism of anti-inflammatory action of ETPH is based on the ability of BAS, which is part of the medicinal plant, to block PG synthesis, probably due to inhibition of prostaglandin synthetase, indicating intervention in the final stages of PG synthesis [6, 23, 24]. Parthenolide, the main sesquiterpene lactone and the main biologically active component of Tanacetum parthenium, is able to specifically bind to the inhibitory IκB kinase complex (IKK) β, which plays an important role in cytokine-mediated signaling in inflammation [24].

Cell adhesion molecules-1 (ICAM-1) play a significant role in the pathogenesis of RA, they are expressed on the surface of leukocytes, endothelial cells and human synovial fibroblasts and mediate the penetration of leukocytes into tissues during inflammation. Parthenolide has been shown to inhibit the increased cytokine IL-1, TNF-α, and interferon-γ expression of ICAM-1 on human synovial fibroblasts [25, 26]. Recent data from several studies in vitro and in vivo human cell line models have demonstrated the ability of parthenolide to significantly reduce the production pathways of IL-1, IL-2, IL-6, IL-8 and TNF-α [27, 28].

In our study, under the ETPH action a statistically significant decrease in the severity of paw edema we observed only for 4-5 days, indicating a less pronounced effect of ETPH compared with diclofenac sodium on the formation of prostaglandins. Subsequently, the effectiveness of ETPH reached the level of CD, diclofenac sodium - a potent non-selective COX inhibitor. The mechanism of anti-inflammatory activity of the studied ETPH, in addition to inhibition of PG synthesis, includes the effect on proinflammatory cytokines, which is indirectly confirmed by other researchers [29], and the ability to restore the phagocytic activity of the macrophage-monocyte system, which requires additional research. There is an immunosuppressive effect of ETPH on cells inducers (effectors) of the development of autoimmune diseases [30], which is AdA, due to the production of IL-10 and possibly other cytokines.

Based on the obtained data, we can conclude that the anti-inflammatory effect of the studied ETPH is based on pronounced immunomodulatory properties. The use of ETPH at a dose of 50 mg/kg reduces the level of the main indicators of systemic inflammation: CRP and fibrinogen - by 2 and 1.8 times, IL-1β - 1.8, TNF-α - 2.2 times, increases the content of IL- 10 by 1.8 times, the CIC level is reduced by 1.6 times relative to PC animals. The effectiveness of ETPH at a dose of 50 mg/kg is not inferior to CD diclofenac sodium, which determines the prospects for further studies of Tanacetum parthenium extract as an effective anti-inflammatory agent.

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Table 1. The effect of ETPH and CD diclofenac sodium on the cytokine profile of rats on the background of experimental AdA, M ± m

Animal groups	Serum parameters		
	IL-1β, pkg/ml	TNF-α, pkg/ml	IL-10, pkg/ml
Intact control	17,17±0,93	1,12±0,15	219,50±28,70
Positive control	27,44±0,71*	4,62±0,55*	123,00±6,65*
ETPH, 50 mg/kg	15,11±0,22**	2,08±0,16**	197,22±18,5**
DN, 8 mg/kg	16,00±0,35**	1,64±0,15**	182,57±9,82**

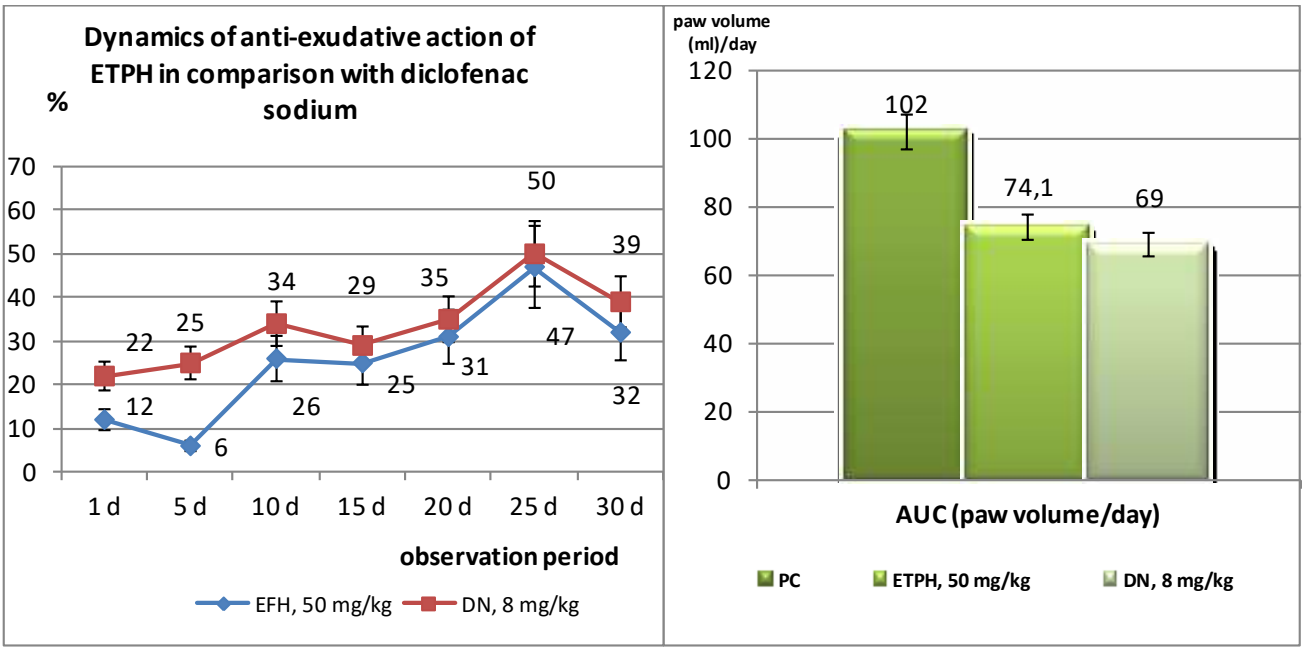
Notes: 1. * – differences are statistically significant relative to the IC group, p<0,05 (Newman-Keisle test); 2. ** – differences are statistically significant relative to the PC group, p<0,05 (Newman-Keisle test); 3. n – number of animals in each group.

Table 2. The effect of ETPH and CD diclofenac sodium on the content of acute phase proteins on experimental AdA (M ± m)

Animal groups	Studied indicators	
	Fibrinogen, g/l	CRP, g/l
Intact control	2,44±0,08	5,33±0,42
Positive control	4,08±0,20*	32,0±4,00*
ETPH, 50 mg/kg	2,75±0,14 **	14,67±2,47*/**
DN, 8 mg/kg	2,92±0,17**	12,00±3,79*/**

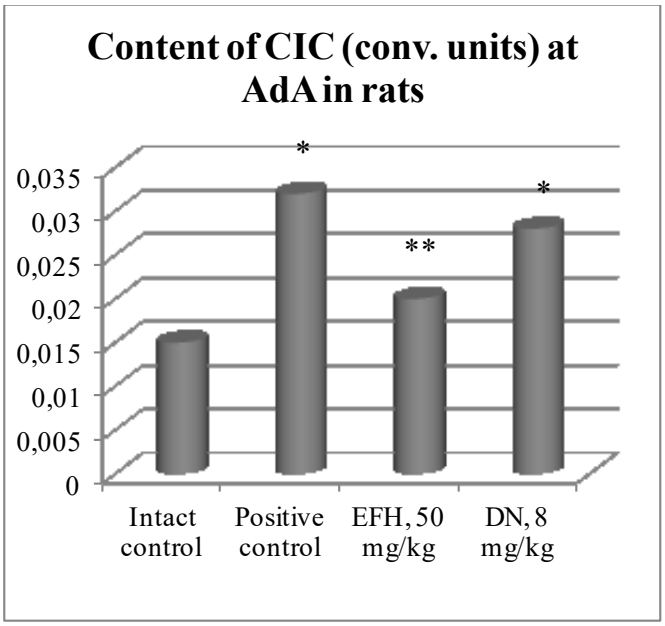
Notes: 1. * – differences are statistically significant relative to the IC group, p<0,05 (Newman-Keisle test); 2. ** – differences are statistically significant relative to the PC group, p<0,05 (Newman-Keisle test); 3. n – number of animals in each group.

Figure 1. Dynamics of anti-exudative effect of ETPH and AUC in comparison with diclofenac sodium in a model of adjuvant arthritis in rats



Note. % – the percentage of edema reduction under the action of ETPH at a dose of 50 mg/kg relative to the positive control.

Figure 2. Effect of ETPH (50 mg/kg) compared to diclofenac sodium (8 mg/kg) on the level of CIC under AdA in rats



Notes: 1. * – differences are statistically significant relative to the group IC, p<0,05 (Newman-Keisle test); 2. ** – differences are statistically significant relative to the PC group, p<0,05 (Newman-Keisle test); 3. n – number of animals in each group; 4. Conv. units – conventional units.