

dressings, histological, electron microscopic, morphometric research methods. In the postnatal period, there is a structural restructuring of the sinus system of the deep cervical lymph nodes. The first is

of formed under the capsular sinus, then the cerebral sinuses and, last of all, the intermediate ones, which reflects the dynamics of changes in the transport function of the lymph nodes.

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# СТРУКТУРНОЕ СТРОЕНИЕ ГЛУБОКИХ ШЕЙНЫХ ЛИМФАТИЧЕСКИХ УЗЛОВ В РАННЕМ ПОСЛЕНАТАЛЬНОМ ПЕРИОДЕ КРЫС, РОЖДЕННЫХ ОТ МАТЕРИ, НАХОДЯЩИХСЯ НА НОРМАЛЬНОМ ПИТАНИИ

**Ключевые слова:** морфология шейных лимфатических узлов, экспериментальные работы, экспериментальные животные, крысы.

В статье приведены данные изучения динамики структурно - клеточных преобразований глубоких шейных лимфатических узлов у крыс в неонатальном периоде (2-е, 10 суток). Материалом исследования, являются экспериментальные животные (крысы) постнатального периода развития (2-х, 10-ти дневные крысы. Авторами использована анатомическая приправка, гистологические, электронно-микроскопические,

морфометрические методы исследования. В постнатальном периоде происходит структурная перестройка синусной системы глубоких шейных лимфатических узлов. Первым формируется под капсульный синус, затем мозговые синусы и в последнюю очередь - промежуточные, что отражает динамику изменения транспортной функции лимфатических узлов.

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# ANTI-INFLAMMATORY ACTION OF DRY EXTRACT OF MEDICINAL PLANTS IN ADJUVANT INDUCED ARTHRITIS IN RATS

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The aim of study was to evaluate the anti-inflammatory effect of dry extract of medicinal plants (DEMP)-Glycyrhiza glabra L., Hypericum scabrum L., Mediasia macrophylla and Ziziphorapedicellata Pazij Vvedin complete Freund's adjuvant-induced arthritis in rats.

The preventative anti-inflammatory activity of dry extract of medicinal plants (50 mg/kg, 100 mg/kg) was studied in complete Freund's adjuvant induced arthritis model in albino rats in comparison with sodium diclofenac (10 mg/kg) and Articure (100 mg/kg). The induction of adjuvant induced arthritis (AIA) was carried out by subplanetary injection of 0.1 ml complete Freund's adjuvant.

It has been shown that DEMP had a significant anti-inflammatory effect on the development of adjuvant-induced arthritis in its preventive using. The right paw increased by 161,5 and 148,0% at the 3<sup>rd</sup> day of observation in animals treated with DEMP in doses 50 and 100 mg/kg, respectively. The percentage of inhibition in groups were 38,2% and 45,6%. At 14<sup>th</sup> day

experiment, the right paw increased by 197,4% and 169,3% in comparison with initial volume of paw, respectively. The percentage of inhibition were 27,5% and 39,8%, respectively. In its effectiveness in the prevention of the development of adjuvant-induced arthritis, DEMP is clearly superior to Articure and not inferior to sodium diclofenac. Inflammation, arthritis, dry extract, Freund's adjuvant

Rheumatic diseases aroused the interest of the medical community from ancient times and continue to occupy a significant place in modern medicine. Rheumatoid arthritis (RA) is an autoimmune disease characterized by the systemic inflammation accompanied by synovial tissue hyperplasia (proliferation and fibrosis of synovial cells), structural destruction of cartilage, bones and ligaments. It is considered that the main mediators of RA formation are pro-inflammatory cytokines, such as TNF- $\alpha$  IL-1 $\beta$  and there is no complete clarity on the aetiology and pathogenesis of this disease. It has not been discovered an effective therapeutic medicines with

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low toxicity [1]. The clinical course of the experimental model of RA in rats is similar to chronic autoimmune inflammation of human joints [2]. This model is characterized by reliable, rapid onset of arthritis and progression with persistent manifestations of polyarthritis, bone resorption and increasing of proliferation of the periosteal zone [1]. Earlier, we found that dry extract of medicinal plants (DEMP) consisting mix of *Hypericum scabrum* L., *Mediizia macrophylla*, *Glycyrrhiza glabra* L. and *Ziziphorapedicellata* Pazij Vved. had a high anti-inflammatory effect and its efficacy was not inferior comparing with diclofenac sodium which is a typical representative of the group of non-steroidal anti-inflammatory drugs [3,4,5]. However, studies on the effectiveness of DEMP in RA have not been investigated, which served as the basis for the present experimental study.

## Materials and methods Plant

Dry extract of medicinal plants was obtained from plants: *Hypericum scabrum* L., *Mediizia macrophylla*, *Glycyrrhiza glabra* L. and *Ziziphorapedicellata* Pazij Vved. Aerial parts of *Hypericum perforatum* L., *Ziziphorapedicellata* Pazij et Vved. and *Mediizia macrophylla* as well as root and rhizome parts of *Glycyrrhiza glabra* L. were collected in summer of 2018 from foothills to medium zones of mountains of Tashkent region, Fergana, Samarkand and Surkhandaryo regions of Uzbekistan by researchers Institute of Botany of Science Academy of Uzbekistan.

## Preparation of extract

Plant material was dried under dark conditions at room temperature for 10 days. Taking into account that the soil is contained various bacterial spores, raw material of plants were treated with special methods. The dry material was milled, obtaining 4-6 mm particles and mixed in proportion 1,25:1,0:1,25:1,5 (productivity of dried extract was higher than other proportions) then extracted by water at 93-95 °C temperature for 3 hours. The extract was then separated from the sample residue by filtration through filter paper. The resulting extracts were concentrated in vacuum until remaining a crude solid extract, which was then dried in a thermostat at temperature of 60 °C.

**Animals** mature albino rats - males with an initial weight of 140-160 g which were obtained from the animal organization. Animals were housed in standard vivarium conditions, quarantined for at least 12-14 days. The experiments were carried out on sexually

Each experimental group consisted of 6-7 animals. The house of Republican sanitary and epidemiology

rats were maintained on standard pelleted diet and water. Approval for the research was obtained from the Ethics Committee of Health Ministry of the Republic of Uzbekistan for experimental studies. All experiments were carried out in compliance with the requirements of

the European Convention "On Protection of vertebrate animals used for experimental and other scientific purposes" (Strasbourg 1986).

## Experiment

The induction of adjuvant induced arthritis (AIA) was carried out by subplanetary injection of 0.1 ml complete Freund's adjuvant (CAF) (Chondrex, Inc., USA), which contains killed mycobacteria H37RA at a concentration of 2 mg/ml suspended in oil designed to reproduce AIA in rats [6]. It is known that an adjuvant can be introduced into the base of the tail or into one of the paw pads [7], which makes possible to study the acute inflammatory reaction at the injection site, as well as the immunological reaction, which develops after about 9 days in the contralateral paw and various organs. The developed oedema in hind paw was monitored from the very first day to 15 days. In order to study the preventative effect of DEMP in AIA, the animals were divided into several groups and DEMP were administered orally once a day in doses of 50 and 100 mg/kg, diclofenac sodium -10 mg/kg and Articure -100 mg/kg for 14 days. Next day after the last administration, blood samples were taken for hematological studies.

Measurement of the volume of the hind paw of the animals was carried out by the oncometric method with plethysmometer (ugo basile, Italy) before and 3, 7, 10, 14 days after the injection of PAF. The percentage of inhibition of inflammation was calculated by formula as follows:

Percentage of inhibition =  $\frac{(V_0 - V_t)_{\text{control}} - (V_0 - V_t)_{\text{treated}}}{(V_0 - V_t)_{\text{control}}} \times 100 = \%$ .

Where,  $V_0$  is initial volume of paw and  $V_t$  volume of paw after injection CAF.

Along with this, the preventive effect of the studied medicines was evaluated in points at the indicated terms of study.

## Statistical analysis

The results were expressed as mean ± standard error of the mean ( $M \pm m$ ). Statistical processing performed by using standard software package Biostat 2009. Differences between means were assessed by the Student's t-test.  $P < 0.05$  was considered significant.

## Results

The results of experimental studies showed that the injection of Freund's adjuvant provoked an expressed inflammatory process in rats. Already on the 3rd day after immunization, the rats became lethargic, aggressive, inactive, the hair of animals became dull, disheveled, and feed intake decreased. A significant increase of the volume of rat's paw was observed. So, the volume of the right paw, into which

CAF was injected, increased by 268% on the 3rd day of the experiment, and the left paw increased only by 19.0%. The increase of observation days did not lead to a significant difference in the volume of the left paw compared with the 3rd day’s observation period. However, the volume of the CAF injected paw increased by 276% by the end of the 14th day of observation (table 1).

So, in rats treated with diclofenac sodium, the right paw increased by 117.0% after 3 days from the start of the experiment and the percentage of inhibition was 55,4%.At the end of the 14th day, it increased by 132.0% and the percentage of inhibition was 51.7%. The volume of the left paw (control) and intervertebral joints remained unchanged.

Table 1

Effect of DEMP on paw arthritis induced by complete Freund’s adjuvant

Values expressed as mean ± SEM, n= 6 in each group\*P<0.05compared with control.

| Groups              | leukocytes, 10 <sup>9</sup> /l | lymphocytes, 10 <sup>9</sup> /l | Absolute count of mix of monocytes, basophiles, eosinophiles 10 <sup>9</sup> /l | Granulocytes, 10 <sup>9</sup> /l | Hemoglobin, г/л | Erythrocytes, 10 <sup>12</sup> /l | Platelets, 10 <sup>9</sup> /l, PLT | Trombokrites %, PCT |
|---------------------|--------------------------------|---------------------------------|---------------------------------------------------------------------------------|----------------------------------|-----------------|-----------------------------------|------------------------------------|---------------------|
| Intact              | 16,17±0,78                     | 8,09±1,08                       | 1,43±0,36                                                                       | 5,71±0,16                        | 141,3±2,1       | 7,21±0,09                         | 419,7±60,2                         | 0,337±0,04          |
| Control             | 28,71±1,85                     | 15,61±1,40                      | 2,20±0,63                                                                       | 6,06±1,0                         | 138,3±4,1       | 6,72±0,17                         | 542,7±25,2                         | 0,387±0,034         |
| Diclofenac 10 mg/kg | 21,91±1,02*                    | 11,82±0,87*                     | 2,01±0,37                                                                       | 5,95±2,2                         | 142,8±4,5       | 6,08±0,2                          | 449,2±14,7*                        | 0,361±0,033         |
| DEMP 50 mg/kg       | 22,81±0,89*                    | 13,35±0,47                      | 2,13±0,16                                                                       | 6,1±1,2                          | 139,3±2,5       | 6,45±0,12                         | 472,7±15,5*                        | 0,377±0,02          |
| DEMP 100 mg/kg      | 21,61±0,89*                    | 12,15±0,47*                     | 2,01±0,16                                                                       | 5,99 ±1,2                        | 149,3±2,5       | 6,05±0,12                         | 492,5±15,5*                        | 0,347±0,02          |
| Articure 100 mg/kg  | 24,61±1,14                     | 13,97±0,37                      | 2,04±0,37                                                                       | 6,75±1,2                         | 147,7±4,6       | 6,91±0,45                         | 435,9±82,4*                        | 0,348±0,06          |

Table 2

Effect of DEMP on hemogram in rats with the Freund’s adjuvantinduced arthritis

Values expressed as mean ± SEM, n= 6 in each group\*P<0.05compared with control.

| Groups                     | Volume of paw, sm³ Absolute volume of paw/inflammation |           |                          |                          |                         |                          |                          |                          |                          |                         |
|----------------------------|--------------------------------------------------------|-----------|--------------------------|--------------------------|-------------------------|--------------------------|--------------------------|--------------------------|--------------------------|-------------------------|
|                            | Initial                                                |           | 3 <sup>rd</sup> day      |                          | 7 <sup>th</sup> day     |                          | 10 <sup>th</sup> day     |                          | 14 <sup>th</sup> day     |                         |
|                            | Right                                                  | Left      | Right                    | Left                     | Right                   | Left                     | Right                    | Left                     | Right                    | Left                    |
| Control                    | 0,76±0,02                                              | 0,75±0,02 | 2,8±0,36<br>2,04±0,35    | 0,89±0,05<br>0,15±0,04   | 2,45±0,19<br>1,68±0,18  | 0,88±0,03<br>0,13±0,03   | 2,61±0,13<br>1,84±0,11   | 0,83±0,03<br>0,08±0,03   | 2,86±0,11<br>2,11±0,10   | 0,98±0,04<br>0,23±0,04  |
| Diclofenac Sodium 10 mg/kg | 0,78±0,01                                              | 0,79±0,01 | 1,69±0,09*<br>0,91±0,10* | 1,55±0,07*<br>0,78±0,07* | 1,55±0,07*<br>0,77±0,07 | 0,81±0,02<br>0,02±0,02   | 1,81±0,02<br>1,02±0,02   | 0,87±0,03<br>0,09±0,03   | 1,81±0,07<br>1,02±0,07   | 0,79±0,01<br>0,01±0,01* |
| DEMP, 50 mg/kg             | 0,78±0,01                                              | 0,79±0,01 | 2,04±0,06*<br>1,26±0,06  | 0,81±0,010<br>0,01±0,005 | 2,13±0,10*<br>1,35±0,09 | 0,87±0,04<br>0,07±0,04   | 1,96±0,16*<br>1,18±0,15* | 0,78±0,02<br>0,02±0,02*  | 2,32±0,16<br>1,53±0,15*  | 1,10±0,21<br>0,30±0,21  |
| DEMP, 100 mg/kg            | 0,75±0,03                                              | 0,77±0,01 | 1,86±0,11*<br>1,11±0,09* | 0,82±0,02<br>0,05±0,02*  | 2,08±0,11*<br>1,33±0,12 | 0,79±0,020<br>0,03±0,007 | 1,92±0,21*<br>1,17±0,22* | 0,79±0,003<br>0,03±0,010 | 2,02±0,19<br>1,27±0,20*  | 1,01±0,14<br>0,24±0,13  |
| Articure, 100 mg/kg        | 0,73±0,01                                              | 0,75±0,01 | 2,01±0,07*<br>1,28±0,07  | 1,02±0,03<br>0,27±0,04   | 2,01±0,11*<br>1,28±0,11 | 0,92±0,05<br>0,17±0,06   | 2,0±0,14<br>1,27±0,14*   | 0,95±0,04<br>0,19±0,04   | 2,15±0,15<br>* 1,43±0,15 | 0,86±0,04<br>0,10±0,03  |

We noted a similar direction of changes in animals treated with DEMP in doses 50 and 100 mg/kg, in which the right paw increased by 161,5 and 148,0% at the 3rd day of observation,respectively. The percentage of inhibition in groups were 38,2% and 45,6%. At 14th day of experiment, the right paw increased by 197,4% and 169,3% in comparison with initial volume of paw, respectively. The percentage of

inhibition were 27,5%and 39,8%, respectively. There were minor changes in the left paw of rats. Unlike other preparations, in the group of rats treated with Articure, the increase of paw volume was preserved in a degree similar to animals of control group. It should be noted that effectiveness of DEMP was superior to Articure, especially at a dose of 100 mg/kg.

It is known that the presence of a chronic inflammatory process in animals is evidenced by the corresponding changes in hematological parameters. Indeed, it can be seen from the table 2 that there were marked leukocytosis with an increase of the absolute content of lymphocytes by 93.0%, a mixture of macrophages, eosinophils and basophils by 54.0% on 14th day of observation of the development of AIA. It is characteristic that the number of granulocytes did not undergo significant changes. However, the absolute platelet count increased by 30.0%. It is seen that with RA, the immune system undergoes significant changes, as indicated by lymphocytosis. In contrast, in animals preventively receiving diclofenac sodium, the number of leukocytes decreased by 24.0%, but remained high by 35.4% compared with intact animals. We noted similar changes in animals treated with DEMP, especially at a dose of 100 mg/kg. It should be noted that under the influence of Articure, a decrease of leukocytosis in treated animals was accompanied by a decrease of the absolute content of lymphocytes and a decrease of the mixture of macrophages, basophils and eosinophils. The data in the table 2 indicate that the effectiveness of DEMP and diclofenac sodium are not significantly different and their effect significantly exceed Articure. It was characteristic that the elimination of leukocytosis and lymphocytosis was accompanied by a decrease of the absolute number of platelets and thrombocritin treated rats in contrast to untreated animals. Given that leukocytosis, and with autoimmune diseases and lymphocytosis, are objective indicators of the inflammatory process, the results indicate a high efficiency of the studied drugs in preventing the development of AI.

## Discussion

Rheumatoid arthritis is very common (about 1% of the population suffers) chronic autoimmune, systemic disease with an unfavorable outcome. It is characterized by lesions of the synovial membranes of the joints, its hyperplasia and a rapid increase of the volume of synovial tissue with progressive destruction of cartilage and bone tissue. Adjuvant-induced arthritis (AIA) is one of the main experimental models used to test substances for the treatment of rheumatoid arthritis (RA). The similarities between AIA and human RA are the presence of edema on the joints, cartilage degradation, lymphocytic infiltration of the inflamed tissue of the joints, the loss of their function, in addition, bone and periosteum resorption is observed [8]. The AIA model is T-cell dependent and complement independent [9,10,11,12]. The increased content of

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TNF, INF $\gamma$ , and interleukins such as IL-1, IL-6, and IL-17A were found in the lymphatic nodes and (or) inflamed joints of rats with AIA [13,14]. The inhibition of TNF, IL-1, IL-21, and IL-17A in rats with AIA improves the course of the disease, which indicates the role of these cytokines in the pathogenesis of experimental arthritis [15,16,17]. Experimental models induced in laboratory animals are widely used to study new substances which is a valuable tool for studying potential medicines for treatment of this pathology [18,19,20].

Consequently, subplantary injection of CAF led to the development of a chronic inflammatory process of the joints, which manifested by the expressed increase of the volume of the injected paw, as well as other joints. The expressed oedema of the soft tissues of the paw were noted as well as there were swelling of the affected joints areas, such as interphalangeal, metatarsophalangeal, ankle and knee. Local temperature increased. A significant decrease of active and passive movements of knee was observed in experimental rats and there was severe pain on palpation (the animal was aggressive and pulled its paw).

The preventative administration of DEMP as well as diclofenac sodium had a noticeable effect on the course of arthritis. So, lesion of joints developed later in the experimental group of animals and the severity of the joint syndrome decreased. In this case, the increase of dose of the preparations led to the increase of the noted positive effect. Therefore, the preventive use of studied preparations have the same focus of effect- they prevent the development of autoimmune arthritis.

It should be noted that the anti-edematous effects of DEMP are comparable with the corresponding effect of diclofenac sodium.

From the above data, it is clear that there is a direct dependence of the severity of the joint syndrome on time of the onset of the disease. The preventative introduction of DEMP, as well as diclofenac sodium had a noticeable effect on the course of arthritis. So, arthritis in the treated group's animals started later, the severity of the articular syndrome was less, which was clearly noticeable with an increase of the dose of preparations.

To conclude, the presented data allow us to assume that DEMP has expressed positive effect on the course of AIA, especially at a dose of 100 mg/kg, while it was not inferior to the reference non-steroidal anti-inflammatory drug diclofenac sodium.

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