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Study of the content of biologically active substances and anti-inflammatory activity of phyto collection 'Dorusim'

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ABSTRACT

Introduction: The use of anti-inflammatory drugs in chronic diseases often leads to various side effects. Therefore, there is a need to develop new drugs from medicinal plants that are less toxic and have higher biological activity compared to synthetic drugs. This study aims to investigate the chemical composition and therapeutic effectiveness of 'Dorusim', a plant-based complex containing dry extracts of *Herba alhagi, Folium Uvaeursi, Fructus Rosae, Glycyrrhiza glabra,* and *Flores chamomillae,* in treating chronic autoimmune inflammation.

Materials and methods: Dry powder from different parts of medicinal plants was extracted using ethanol and phytochemicals contents were determined by high performance chromatography. A model of chronic autoimmune arthritis induced by Freund's complete adjuvant was used to determine the anti-inflammatory activity.

Results: Analysis of the complex composition of the dry extract of Dorusim showed the presence of rutin (0.008%), quercetin (0.042%), apigenin (0.87%), luteolin (2.63%) and glycyrrhizic acid (0.0031%). Dorusim had a distinct anti-inflammatory activity, which was statistically significantly manifested in the change of haematological parameters, as well as the level of pro-inflammatory and anti-inflammatory interleukins objectively reflecting the direction and degree of chronic immune inflammation.

Conclusion: The findings suggest that Dorusim, a plant extract, has anti-inflammatory activity and further comprehensive pharmaco-toxicological studies are needed to explore its potential pharmacotherapeutic properties.

Keywords: medicinal plants, diclofenac, Canephron, adjuvant arthritis, anti-inflammatory effects, interleukins, haematological parameters

Introduction

The healing effect of medicinal plants on living organism is explained by the presence of various biologically active substances in their composition. Plants produce a huge number of complex chemical compounds[1,2,3]. However, a significant disadvantage of these remedies is the low concentration of the active ingredient in the recommended dosage forms and, consequently, low efficacy in clinical practice.

Development of effective methods of isolation of biologically active components from plant raw materials and obtaining extracts with maximum content of active substances is one of the most important tasks of pharmaceutical technology[4]. Phytocomposition consisting of dry extracts of plants: *Herba alhagi*, *Folium Uvaeursi, Fructus*

Rosae, *Glycyrrhiza glabra* and *Flores chamomillae* (Dorusim) has pronounced anti-exudative, antiproliferative and anti-alternative properties[5,6]. At the same time, it has low toxicity[7,8] and also has antipyretic and analgesic activity[9]. The mentioned properties of Dorusim were established in animals on the model of acute inflammatory processes. Its efficacy in chronic inflammatory processes remained unexplored. The latter, as literature data show, is a rather common pathology[10]. The latter includes rheumatoid arthritis (RA), affecting from 0.5 to 1% of the world population[10], and up to 5% in the elderly[12]. This pathology is a chronic immunoinflammatory (autoimmune) disease manifested by progressive destruction of joints, systemic inflammation of internal organs and a wide range of comorbid pathologies associated with chronic inflammation[13]. Despite great successes in early diagnosis and pharmacotherapy of RA, the problem of therapy of this pathology is far from being resolved[14]. This is largely due to the lack of specific biomarkers at the early stage of the disease, heterogeneity of the mechanism of immunopathogenesis, which makes it difficult to personalise therapy[13,15,16,17]. It has become evident that selective blocking of inflammatory mediators using innovative drugs is not infrequently associated with 'primary' ineffectiveness, 'secondary' resistance, development of generalised immunosuppression leading to paradoxical activation of autoimmune process and aggravation of associated comorbid diseases[13]. This circumstance quite often leads to disability of patients with RA[18]. It is believed that the choice of drugs in RA is often limited due to the development of adverse reactions to drug therapy in patients, poor tolerability, and insufficient efficacy of the drugs used[19,20]. Therefore, the development of new drugs in RA therapy is currently one of the urgent tasks of biomedical sciences. In view of the above, the aim of the present work is to quantify the biologically active substances in the dry extract of 'Dorusim' and to study the pharmacotherapeutic efficacy in chronic autoimmune inflammation.

Materials and methods

Plant raw materials

On the basis of previously conducted research we have created a combined plant raw material consisting of a mixture of dry extracts of the following 5 medicinal plants*: Herba alhagi*, *Folium Uvaeursi, Fructus Rosae*, *Glycyrrhiza glabra* and *Flores chamomillae.* This phyto composition was given the conventional name 'Dorusim'.

Extraction of plant material

To obtain dry extracts, each plant material is ground separately and sieved through a sieve for leaves and herbs to a size of 7 mm, for tubers, roots to a size of 5 mm, for fruits to a size of 0.5 mm.

To 50 g of a mixture of plant raw materials (herbs *Jantaka* false 13.7 g, rosehip fruit 10.22 g, chamomile flowers 12.64 g, bearberry leaves 8.24 g, licorice roots 5.22 g) loaded into the extractor pour 2500 ml of 70% ethyl alcohol. Extraction is carried out at a temperature of 55 0C for 6 hours with constant stirring. The obtained extract is filtered off. The obtained liquid extract in the amount of 2010 ml is concentrated by distillation under vacuum, the residue is dried in a vacuum-drying cabinet at 60-65 0C to constant weight. The yield of the dry extract was 23 g (46 %).

Determination of the content of biologically active substances.

For identification and quantitative determination of flavonoids, glycyrrhizic acid in the dry extract of 'Dorusim' was used the method of high-performance liquid chromatography (HPLC).

The analysis was performed on an Agilent-1260 chromatograph (USA), with 'Chemstation 09.03.a' software, equipped with spectrophotometric detector and gradient pump[20]. The separation of flavonoids was carried out on a 4.6 x150 mm column, with Zorbax Eclipse C-18 sorbent, with a particle size of 5 μm. For quantitative determination of rutin and quercetin a mobile phase consisting of orthophosphoric acid 0.3 % (A) and methanol (B) in the ratio 80:20 was used. The volume flow rate of the eluent was 1.0 ml/min and the volume of sample injected was 10 μl. Detection was carried out at wavelength of 370 nm (quercetin), 357 nm (rutin), temperature 40ºC. The time duration of the analysis was 25 minutes.

For quantitative determination of apigenin, a mobile phase consisting of a mixture of methanol (A) and trifluoroacetic acid (B) in a ratio of 80:20 was used. Detection was carried out at a wavelength of 269 nm (apigenin), temperature 25 oC. The time duration of the assay was 15 min.

Quantitative determination of luteolin was carried out on a chromatograph with a 250x4.6 mm column, sorbent Zorbax Eclipse C-18, with a particle size of 5 μm. The mobile phase was acetonitrile (A) and 0.2% orthophosphoric acid (B), in a ratio of 28:72. Detection was performed at a wavelength of 350 nm (luteolin), temperature 30oC. The time duration of the analysis was 20 min.

For the quantitative determination of glycyrrhizic acid, a chromatograph with a 3.0 x150 mm column

was used. The mobile phase consisted of acetic acid 0.1 % (A) and acetonitrile (B) in the ratio 70:30. The volume flow rate of the eluent was 1.0 ml/min and the volume of sample injected was 10 µl. Detection was carried out at a wavelength of 254 nm (glycyrrhizic acid), temperature 40ºC. The time duration of the analysis was 25 min.

Preparation of the test solution: 0.1 g of the dry extract 'Dorusim' was placed in a measuring flask with a capacity of 100 ml, dissolved in ethyl alcohol and the volume of the solution was brought to the mark with the same solvent.

Preparation of working standard sample solution (WSS) of rutin. 0.05 g (exact weight) WSS rutin, dried at 130-140 ° C, dissolved by heating on a water bath in 80 ml of 70% ethyl alcohol in a measuring flask with a capacity of 100 ml and brought the volume of the solution with the same solvent after cooling to the mark. Shelf life of the solution is 1 month.

Preparation of the solution of WSS of apigenin. 0.05 g (exact weight) of WSS apigenin dried at 130-140°C was dissolved on heating in 80 ml of 70% ethyl alcohol in a 100 ml measuring flask in a water bath and the volume of the solution was brought to the mark with the same solvent after cooling. Shelf life of the solution is 1 month.

Preparation of quercetin WSS solution. 0.05 g (exact weight) of quercetin WSS dried at 130-140°C was dissolved by heating on a water bath in 80 ml of 70% ethyl alcohol in a 100 ml measuring flask and the volume of the solution was brought to the mark with the same solvent after cooling. Shelf life of the solution is 1 month.

Preparation of luteolin WSS solution. 0.05 g (exact weight) of WSS luteolin dried at 130-140°C was dissolved by heating on a water bath in 80 ml of 70% ethyl alcohol in a 100 ml volumetric flask and the volume of the solution was brought to the mark with the same solvent after cooling. Shelf life of the solution is 1 month.

Preparation of WSS solution of glycyrrhizic acid. 0.05 g (exact weight) of WSS of glycyrrhizic acid, dried at 130-140°C, was dissolved by heating on a water bath in 80 ml of 70% ethyl alcohol in a 100 ml volumetric flask and the volume of the solution was brought to the mark with the same solvent after cooling. Shelf life of the solution is 1 month.

A reproduction model of chronic autoimmune artiritis.

The study of therapeutic and prophylactic effect of Dorusim on the course of chronic arthritis was carried out on male sexually mature outbred rats with an initial body weight of 185-200 g. The animals were obtained from the nursery of sanitary and epidemiological supervision of the Main Medical Department under the Administration of the President of the Republic of Uzbekistan. The animals were obtained from the nursery of sanitary-epidemiological supervision of the Main Medical Department at the Administration of the President of the Republic of Uzbekistan. Before the experiment all laboratory animals were carefully examined, their age, body weight, sex, and motor activity were taken into account. During the whole period of preparation for the experiment and during its performance, laboratory animals were kept in standard conditions of vivarium at temperature 20-25°C, humidity not less than 50%, in well-ventilated room and day/night light regime, in plastic cages of 6 animals each, free access to standard diet. The study was conducted in accordance with the 'Rules for Work Using Experimental Animals' and the rules adopted in the European Convention for the Protection of Vertebrate Animals Used for Experimental Research or Other Scientific Purposes (Treaty adopted in Strasbourg on 18 March 1986, entered into force on 1 January 1991).

A model of chronic arthritis (CA) in laboratory animals was created by subplantar injection into the right hind foot of 0.1 ml of Freund's Complete Adjuvant (FCA) (Chondrex, Inc., USA), which contains killed mycobacteria H37RA at a concentration of 2 mg/ml suspended in oil designed to reproduce adjuvant induced arthritis (AIA) in rats[22]. Rats without experimental pathology served as control animals (n=6). The severity of inflammation was evaluated in dynamics by the increase of symptoms of primary reaction (swelling of the right hind paw) and secondary immunological reaction (swelling of the front and left hind paws) to FCA injection. When studying the prophylactic effect of drugs after FCA injection, animals were divided into several groups, which were injected once daily intragastrically with Dorusim, Canephron in doses of 100 mg/kg, and Diclofenac sodium in a dose of 10 mg/kg for 14 days. The choice of comparison drugs is that Canephron ('Bionorica', Germany) is a well-known herbal medicine with anti-inflammatory activity[23], Diclofenac sodium ('Merckle GmbH', Germany) is a well-known non-steroidal anti-inflammatory drug, which is a gold standard NSAID and is widely used as a comparison drug[24,25]. To study the therapeutic effect of the above-mentioned drugs in separate series of the experiment after FCA injection starting from the 15th day the treatment was carried out in similar doses up to the 30th day. Before and after 7, 10, 14 days (prophylactic), as well as 15, 20, 25 and 30 days (therapeutic) after the drugs injection,

oncometric measurements of the affected paws were made using a plethysmometer (Ugo Basile Srl, Italy). The value of anti-inflammatory activity (AIA) of the drugs was calculated according to the formula:

AIA = $V_{control}$-V_{drug}/$V_{control}$ x 100 = %.

where, $V_{control}$ - average limb volume growth in control, V_{drug} - average limb volume growth in experiment.

For haematological and biochemical studies, blood was collected 24 hours after the last manipulation under ether anaesthesia. Blood parameters were determined on a haematological analyser 'Mindray BC-20s', China. The level of pro- (IL-1β) and anti-inflammatory (IL-10) interleukins, tumour necrosis factor (TNF-α) and C-reactive protein (CRP) was determined in blood serum samples on an immunoenzyme analyser AT-858 from 'Shenzhen Mindray Bio-Medical' using commercial ELISA kits produced by Human Diagnostics and Vector-Best (Russia).

The results of experimental studies were processed using Biostat 2009 software package. Data are presented as mean value (M) and standard error of the mean value (m). Differences at a probability level of 95% or more ($p < 0.05$) were considered statistically significant.

Results.

Identification of the separated substances was performed by comparing the retention times of the peaks obtained on the chromatogram of the components of the dry extract 'Dorusim' with the retention times of the standard samples (Figures 1-10).

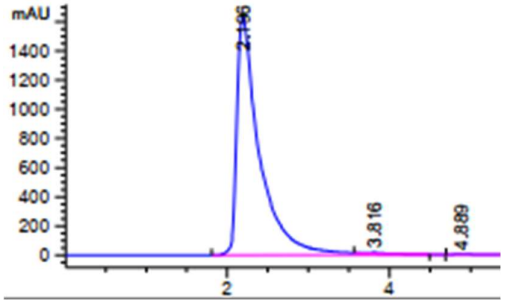


Fig.1. Chromatogram of "Dorusim"

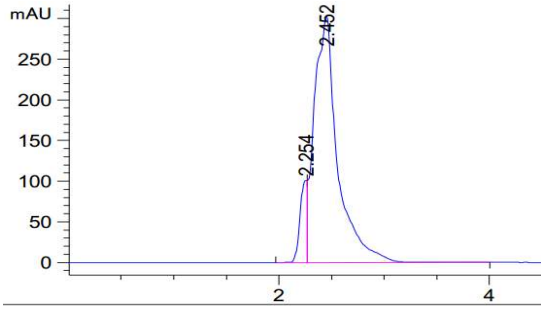


Fig.2.Chromatogram of standard sample of apeginine (retention time 2.45 min)

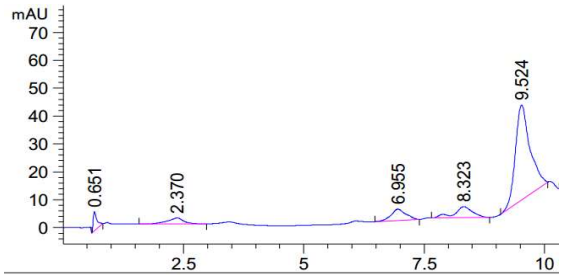


Fig.3. Chromatogram of "Dorusim"

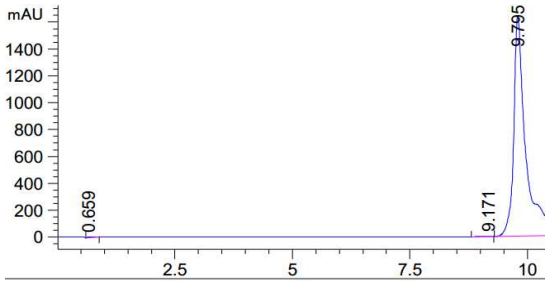
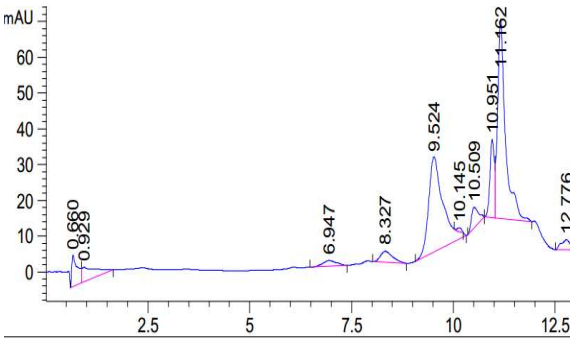


Figure 4. Chromotogram of a standard sample of rutin
(retention time 9.795 min)



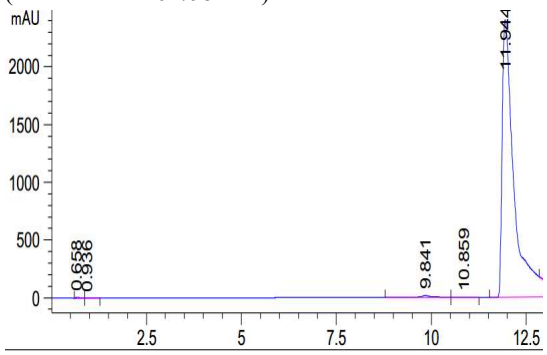


Figure 5. Dorusim chromatogram

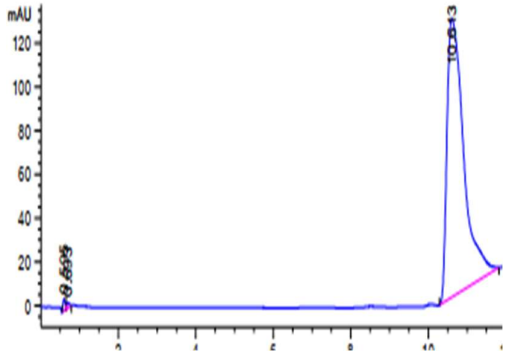


Fig. 7. Chromatogram of "Dorusim"

Figure 6. Chromatogram of a standard sample of quercetin
(retention time 11.944 min)

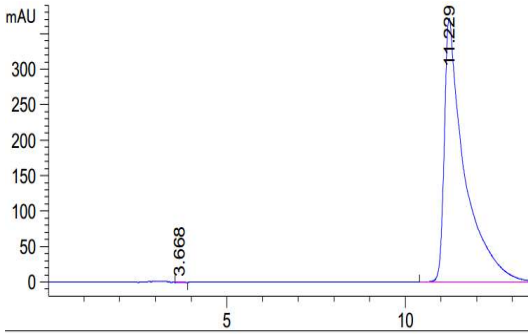


Figure 8. Chromatogram of a standard sample of luteolin
(retention time 11.229 min)

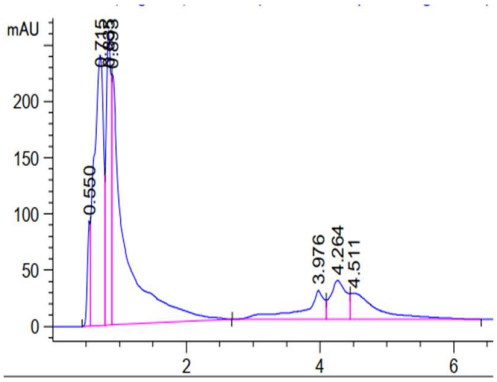


Fig. 9. Dorusim chromatogram

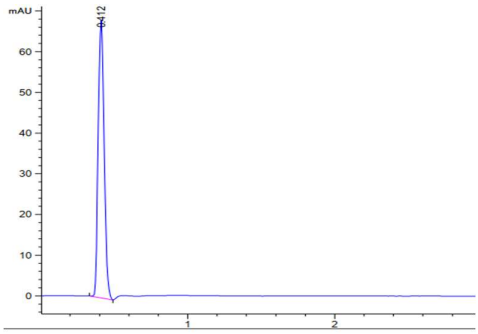


Figure 10: Chromatogram of a standard sample of glycyrrhizic acid (retention time 0.412 min)

The results of HPLC analysis show the presence of apigenin, rutin, quercetin, luteolin and glycyrrhizic acid in the dry extract of 'Dorusim'.

Figure 2 shows the chromatogram of standard samples of apigenin, rutin (Figure 4), quercetin (Figure 6), luteolin (Figure 8) and glycyrrhizic acid (Figure 10). The retention time of the standard sample of apigenin 2.45 min, rutin 9.795 min, quercetin 11.944 min, for luteolin 11.229 min and for glycyrrhizic acid 0.412 min.

Chromatograms of solutions of the dry extract 'Dorusim' (Fig.1,3,5,7,9) differ from the standard samples in which the following are clearly identified: apigenin (Fig.1), rutin (Fig.3), quercetin (Fig.5), luteolin (Fig.7) and glycyrrhizic acid (Fig.9).

Complex composition analysis of the dry extract of Dorusim shows the presence of a characteristic peak at a holding time of 9.79 min of rutin with a quantitative content of 0.008 %. At holding time of 11.944 min the presence of a peak characteristic for quercetin with the quantity of 0.042 % was found, also the chromatogram of dry extract 'Dorusim' revealed characteristic peaks for apigenin with holding time of 2.404, luteolin with holding time of 11.229 and glycyrrhizic acid with holding time of 0.412 min and the quantitative content of these substances reaches up to 0.87 %, 2.63 %, 0.0031 %.

Adjuvant-induced arthritis (AIA) is widely used in preclinical studies of new compounds for the treatment of arthritis[26,27]. This model is characterised by a reliable rapid onset of arthritis and progression with persistent polyarthritis, bone resorption and increased periosteal proliferation. The development of severe polyarthritis is observed rapidly, approximately 7-10 days after injection of Freund's complete adjuvant (FCA)[22,28,29]. Analyses of the results of these studies showed that subplantar injection of FCA leads to an increase in paw volume by

221.2, 251.5 and 300% compared to baseline after 7, 10 and 14 days, respectively. The marked effect was clearly maintained and by the end of the 20th, 25th and 30th day of the experiment the paw paw volume was increased by 284.0, 290.0 and 303.3%.

The model of chronic immunoinflammation induced by FCA injection into the hind paw of rats is considered as a model of RA in which joint damage develops in other limbs (including the tail) besides the one to which FCA is injected. Indeed, the development of oedema of the paws and other limbs was observed in experimental rats. This secondary immunological reaction developed in varying degrees of severity in 40-47% of animals.

Consequently, FCA injection leads to the development of chronic arthritis stable for more than 1 month. According to the literature, FCA injection leads to impaired limb function manifested by changes in the coordination of animal movement in the test (rotating rod)[19].

In the group of animals prophylactically treated with Diclofenac sodium, Dorusim and Canephron the development of paw oedema was lower by 40.4, 25.7 and 12.8% on the 7th day of the experiment and by 50.0, 30.0 and 14.2% on the 14th day of the experiment, respectively. The data in Table 1 show that diclofenac sodium surpasses other phytopreparations and especially Canephron in its pharmacological activity.

Table 1

Effect of Dorusim, Canephron and Diclofenac sodium on the course of adjuvant induced arthritis during preventive application (M±m, n=6).

Groups	Paw volume, cm3 (days)			
	Initial	7	10	14
Control	0,99 ± 0,05	3,18 ± 0,09*	3,48 ± 0,22*	3,96 ± 0,23*
Dorusim	0,84 ± 0,06	2,46 ± 0,18*	2,63 ± 0,19*	2,91 ± 0,28*
Canephron	0,94 ± 0,05	2,85 ± 0,18*	3,10 ± 0,15*	3,48 ± 0,14*
Diclofenac	0,86 ± 0,04	2,16 ± 0,06*	2,25 ± 0,06*	2,34 ± 0,05*

Note. * - *reliable difference with baseline values of the corresponding groups of animals.*

Practically the same direction of changes was established in the experiment with therapeutic application of the drug. Thus, after 5, 10 and 15 day treatment with Diclofenac sodium the degree of oedema decreased by 14.2, 27.3 and 37.3%, respectively, and in the group of animals treated with Dorusim it was equal to 9.2, 17.3 and 30.7%, respectively. At the same time, as can be seen from the data of Table 2, treatment with Canephron did not lead to statistically significant changes in the investigated parameter of inflammation.

Table 2

Study of the therapeutic effect of Dorusim, Canefron and Diclofenac sodium on the course of adjuvant induced arthritis (M±m, n=6).

Groups	Paw volume, cm3 (days of treatment)			
	Initial	5	10	15
Control	3,77 ± 0,28	3,80 ± 0,27	3,88 ± 0,26	3,99 ± 0,25
Dorusim	3,64 ± 0,19	3,49 ± 0,21	3,33 ± 0,22	3,08 ± 0,21*
Canephron	3,71 ± 0,23	3,53 ± 0,22	3,48 ± 0,22	3,52 ± 0,26
Diclofenac	3,81 ± 0,12	3,29 ± 0,11	2,98 ± 0,18*	2,76 ± 0,19*

Note. * - *reliable difference with baseline values of the corresponding groups of animals.*

Consequently, the dry extract 'Dorusim' in animals with AIA shows a distinct pharmacotherapeutic effect both in prophylactic and therapeutic application. 'Dorusim' by its pharmacological activity, although somewhat inferior to Diclofenac sodium, but significantly superior to Canephron - a phytopreparation with anti-inflammatory activity[23,30,31].

To objectively assess the severity of the inflammatory process and the level of immune reactivity, we studied the effect of Dorusim on some haematological parameters. It was noted that among all form elements the values of leucocyte count, in particular lymphocytes, are most influenced by the form of treatment and its duration. It is known that chronic inflammation is reflected by the increase of leucocytes in peripheral blood. And indeed, as can be seen from the data of Table 3, in rats two weeks after FCA injection there is an increase in the number of leucocytes by 231.2%, lymphocytes by 135.6% against which there is an increase in the number of granulocytes

by 85.3%, and the absolute mixture of monocytes, eosinophils and basophils by 235.8%. Along with this, the number of platelets in peripheral blood increased by 84.9% and the index of thrombocrit by 43.4%.

Consequently, the studied haematological indices indicate the development of a distinct inflammatory process, the values of which increased as the observation period increased. Indeed, after 30 days from the beginning of the experiment the number of leukocytes, lymphocytes, granulocytes and absolute content of monocytes, eosinophils and basophils increased by 364.9%, 199.2%, 103.7% and 344.6%, respectively. Meanwhile, platelet count increased by 105.7% and thrombocrit increased by 93.5%. The above data confirm the validity of the conclusion that haematological changes are an objective and reliable indicator of RA severity and course[32]. In contrast to untreated animals, animals receiving Dorusim, Canephron and Diclofenac sodium both for prophylactic and therapeutic purposes showed a decrease in the degree of haematological changes. Thus, at prophylactic administration of Dorusim the total number of leukocytes, lymphocytes and absolute content of monocytes, eosinophils and basophils in comparison with control decreased more than 2 times, and granulocytes by 24,2%. To a similar extent, there was a decrease in platelet indices. In contrast, preventive application of Canephron did not lead to a statistically significant decrease in the studied parameters in comparison with untreated animals, whereas in animals the application of Diclofenac sodium caused a decrease in the number of leucocytes by 64, 8%, lymphocytes 52,6%, absolute content of monocytes, eosinophils and basophils - by 64,4%, granulocytes - by 46,4% against which there was a decrease in platelets by 36,7% and thrombocrit - by 21,8%.

Since the overwhelming majority of patients seek medical help after the pathology has developed, where the use of pharmacological agents is therapeutic in nature, we further studied the effect of the therapeutic use of Dorusim and Diclofenac sodium on haematological parameters in chronic inflammation induced by FCA, Canephron and Diclofenac sodium on haematological indices in chronic inflammation induced by FCA, Thus, in those treated with Canephron the number of leukocytes and lymphocytes decreased by 30,9% and 18,5%, Diclofenac sodium by 60,5% and 44,9%, and Dorusim - by 52,2 and 33,8% respectively. At the same time in animals treated with Canephron other studied haematological parameters did not undergo statistically significant changes in comparison with untreated animals, whereas in animals treated with Diclofenac sodium and Dorusim the absolute content of monocytes, eosinophils and basophils decreased by 58.3 and 47.7%, and granulocytes by 42.5 and 35.9%, respectively. Table 3

Comparative study of the effects of Dorusim, Canefron and Diclofenac sodium on haematological parameters of rats in adjuvant induced arthritis

Indicators / Groups	Leucocytes, 10^9 /l	Absolute lymphocyte count, 10^9/l	Absolute count of a mixture of monocytes, basophils, eosinophils, 10^9/l	Granulocyte count, 10^9/l	Platelets count, 10^9 /l	Plateletcrit, %
Intact	6,47±0,51 6,05±0,47	7,93±0,54 7,33±0,51	1,48±0,12 1,66±0,10	6,07±0,34 6,37±0,38	312,83±20,22 300,62±24,86	0,369±0,038 0,362±0,037
Control	21,43±1,72* 28,13±1,92*	18,68±1,58* 21,93±1,65*	4,97±0,36* 7,38±0,44*	11,25±0,89* 12,98±1,21*	578,72±30,62* 618,58±17,91*	0,528±0,052* 0,707±0,047*
Dorusim	11,37±1,15*,x 13,15±1,23*,x	10,77±1,08x 14,51±1,54*,x	2,41±0,21*,x 3,86±0,32*,x	8,53 ±0,93 8,31 ±0,69x	438,53±30,24*,x 430,21±38,53*,x	0,458±0,042 0,446±0,043x
Canephron	17,25±1,41* 19,43±1,29*,x	15,33±1,30* 17,87±1,67*	3,62±0,38*,x 6,48±0,65*	9,88±0,64* 10,43±0,88*	510,13±45,57* 522,18±29,57*,x	0,505±0,049 0,653±0,037*
Diclofenac	7,53±0,48x 11,12±0,86*,x	8,85±0,59x 10,98±0,76*,x	1,77±0,14x 3,08±0,27*,x	7,15±0,69x 7,46±0,46x	366,48±18,57x 383,33±32,91x	0,413±0,030 0,396±0,035x

Note: in the numerator blood indices of the preventive effect of the drugs, and in the denominator of the therapeutic effect after phlogogen administration., *- differences statistically significantly different from intact, x - differences statistically significantly different from controls

Against this background, the level of platelets decreased by 38.0 and 30.4%, respectively. Almost the same degree of changes was observed in plateletcrit indices.

Consequently, the studied preparations show a distinct AIA, both in prophylactic and therapeutic application, which is statistically significantly manifested in the change of haematological indicators, objectively reflecting the direction and degree of the pathological process. The given material confirms the results of the conducted studies of anti-inflammatory activity of Dorusim in chronic arthritis. The drug is superior in its activity to Canephron and is not significantly inferior to Diclofenac sodium.

One of the leading links in the chain of pathogenesis of chronic inflammatory process is the induction of cytokines. It has been established that such cytokines as IL-1β, TNFα stimulate inflammation and degradation of bone and cartilage (proinflammatory cytokines). Along with this, a number of cytokines have anti-inflammatory effects (IL-10)[33,34,35]. The imbalance between the content and activity of pro- and anti-inflammatory cytokines leads to multisystem immune complications. In this regard, the level of serum or plasma cytokines in patients with RA allows us to judge the severity of the disease[12]. This fact determined the purpose of further research in which we studied the content of proinflammatory cytokines (IL-1β, TNFα) and anti-inflammatory cytokine (IL-10) during prophylactic and therapeutic use of Dorusim in comparison with Diclofenac sodium and Canephron. It should be noted that cytokines - polypeptide mediators are involved in the formation and regulation of defence reactions of the organism and carrying out communication between neuroendocrine, immune, hematopoietic, as well as other systems in order to involve them in the organisation of a single regular defence reaction. The results of the conducted studies showed that in 2 weeks after the injection of FCA lead to statistically significant increase in the level of IL-1β more than 5 times, and TNFα by 85.9% against the background of which there was almost twofold decrease in the content of IL-10. The marked effect remained somewhat high even by the end of the 30th day of the study (see Tables 4).

Table 4

Content of cytokines IL-1β, IL-10 and TNF-α, as well as CRP in peripheral blood of rats with chronic arthritis under the therapeutic and preventive effect of Dorusim, Canephron and Diclofenac sodium (M±m, n=6).

Показатели / Группы	IL-1β, pg/ml	IL-10, pg/ml	TNF-α, pg/ml	CRP mg/l
Intact	2,45 ± 0,13	2,73 ± 0,25	2,41 ± 0,11	0,563 ± 0,051
Control	12,53 ± 1,03* 13,69 ± 1,28*	1,41 ± 0,13* 1,22 ± 0,11*	4,48 ± 0,37* 5,20 ± 0,43*	3,293 ± 0,201* 3,681 ± 0,291*
Dorusim	4,38 ± 0,49*,x 4,67 ± 0,45*,x	3,29 ± 0,29x 3,12 ± 0,31x	3,22 ± 0,25*,x 2,92 ± 0,28x	1,511 ± 0,161*,x 2,007 ± 0,098*,x
Canephron	8,72 ± 0,74*,x 11,39 ± 0,97*	1,64 ± 0,11* 1,52 ± 0,12*	3,91 ± 0,28* 4,62 ± 0,33*	2,846 ± 0,314* 3,183 ± 0,199*
Diclofenac sodium	2,92 ± 0,24x 3,02 ± 0,26x	4,52 ± 0,49*,x 4,41 ± 0,40*,x	2,73 ± 0,24x 2,54 ± 0,21x	1,035 ± 0,099*,x 1,160 ± 0,108*,x

Note: *in the numerator are the results of experiments in the preventive regime application, and in the denominator are the results of experiments conducted in the therapeutic mode. mode, * -reliable difference in comparison with intact animals, x -reliable difference in comparison with the control of the corresponding terms studies.*

Consequently, in chronic arthritis induced by FCA there is a significant increase in proinflammatory and a decrease in the level of anti-inflammatory cytokines. It is known that IL-1β is a pleiotropic cytokine that has an effect on almost all cells of the body: it increases the synthesis of 'acute phase', activates the proliferation of fibroblasts, their production of prostaglandins, increases procoagulant activity, causes vasodilation, and in neutrophils oxygen burst[33,36,37,38]. As it was mentioned, TNFα belongs to the main cytokines of the inflammatory

process, which have a powerful pro-inflammatory effect, causes the induction of IL-1β, acute-phase proteins, inflammatory mediators, activating the vascular endothelium, increases vascular permeability, activates collagenase, and stimulates granulomatosis[32,39]. On this basis, in order to suppress inflammation, it is pathogenetically justified to reduce the level of proinflammatory and increase the level of anti-inflammatory cytokines. Indeed, in animals receiving preventively a classical representative of NSAIDs - Diclofenac sodium - compared to the control the level of IL-1β decreased by 77,7%, TNFα by 39,1%, and at the therapeutic application 77,9 and 51,1% respectively. At the same time, the concentration of IL-10 increased by 220.5% in prophylactic and 261.5% in therapeutic application. The same direction of changes was observed in animals with AIA, receiving Dorusim for prophylactic purpose. Thus, the level of IL-1β decreased by 65,0%, and TNFα - by 28,1%, on this background the level of IL-10 increased by 133,3%. At the therapeutic application this phyto preparation reduced the level of IL-1β by 65.9%, and TNFα by 42.5%, while the level of IL-10 increased by 155.5%. In contrast, in animals treated with Canephron we did not find statistically significant changes, although there was some decrease in pro-inflammatory and increase in anti-inflammatory cytokines.

Summarising the results of studies of the quantitative level of cytokines during the therapeutic and prophylactic use of Dorusim, we can conclude that the effect on the cytokine status of the organism in chronic adjuvant arthritis is important in the mechanism of its anti-inflammatory action. In terms of its pharmacological activity, Dorusim is not noticeably inferior to Diclofenac sodium, but significantly superior to Canephron. It should be noted that IL-10 is the leading anti-inflammatory pleiotropic cytokine, which inhibits the migration of monocytes, macrophages, mast cells into the focus of inflammation and leads to the control of the inflammatory process, inhibits the synthesis of anti-inflammatory cytokines, chemokines, adhesive molecules, inhibits the effector function of macrophages, natural killer cells, neutrophils, protects vascular endothelium by weakening the effects of angitensin II and restoring the activity of nitric oxide synthetase[28,30]. Taking into account the above material on the role of cytokines in the process of chronic inflammation, we can state that the anti-inflammatory effect of Dorusim is largely associated with its corrective effect on the cytokine status of the organism.

In clinical practice CRP as a laboratory indicator of inflammation severity is considered to be an objective and reliable parameter of the pathological process. Therefore, in order to objectify the assessment of anti-inflammatory effect of Dorusim, we studied the level of CRP in animals with AIA. The results of this series of experiments showed that by the end of the 15th day of FCA administration the level of CRP increased more than 5 times, and by the end of the 30th day more than 6.5 times, which indicates not only a decrease in pathology over time, but, on the contrary, its progression. Preventive application of Canephron, Dorusim and Diclofenac sodium resulted in reduction of CRP levels by 13.6, 54.1 and 68.5%, respectively. The therapeutic application of the drugs resulted in a 13.5, 45.4 and 60.0% decrease in blood CRP concentrations compared to untreated animals, respectively. It can be seen that Dorusim by its pharmacodynamic effects more than three times exceeds the activity of phytocomplex Canephron and is not noticeably inferior to Diclofenac sodium.

Conclusion.

Analyses of the results of chemical studies showed different contents of apigenin, rutin, quercetin, luteolin and glycyrrhizic acid in the alcoholic extract of the phyto preparation Dorusim consisting of plants: *Herba alhagi*, *Folium Uvaeursi*, *Fructus Rosae*, *Glycyrrhiza glabra* and *Flores chamomillae*, which exhibited distinct anti-inflammatory activity in animals with chronic 'adjuvant arthritis', both in preventive and curative applications. Dorusim eliminated disorders of haematological parameters, objectively reflecting the degree of autoimmune inflammation. The drug decreased the quantitative level of pro-inflammatory interleukins and increased the level of anti-inflammatory interleukins. Thus, Dorusim is a phytocomplex possessing anti-inflammatory action that finds its objective evaluation in the reduction of C-reactive protein in blood in animals with adjuvant induced arthritis. By its pharmacological activity Dorusim clearly surpasses Canephron and is not noticeably inferior to Diclofenac sodium. The results highlight the need for further comprehensive pharmaco-toxicological study for its potential pharmacotherapeutic properties.

Conflict of interests.

The authors declare that they have no conflict of interest.

Ethical considerations.

This Research was approved by The Ethical Committee of the Ministry of Health of the Republic of Uzbekistan (Protocol Number: 3/20-1422/12.06.2023).

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