Effects of herbal preparation EM 1201 in adjuvant arthritic rats: comparison with diclofenac

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Abstract

The aim of this study was to investigate anti-inflammatory and antioxidant effects of preparation EM 1201 on adjuvant induced arthritis in rats.

Material and methods. Adjuvant arthritis (AA) was induced in 24 female Wistar rats by intradermal injection of complete Freund’s adjuvant into the left hind paw. The course of disease and pro-/antioxidant status of blood serum in response to the prophylactic treatment with EM 201 has been investigated in animals that received oral injection preparation of EM 1201 in dose 110 mg/kg 5 times a week and the effect was compared not only with the control AA group without the treatment but also with the group treated with diclofenac. Body weight, joint swelling, and the blood parameters like ESR, leukocyte and erythrocyte count were checked. Indices of pro-/antioxidant status of blood serum such as malondialdehyde (MDA), the activities of antioxidant enzyme catalase (CAT), total antioxidant activity (AOA), and histological changes in joints and the liver were investigated.

Results. Preparation EM 1201 showed anti-inflammatory and antioxidative effect, improved blood indices, significantly decreased joint swelling and histological changes in them. Joint swelling was suppressed by 29–46% and 18–37% in response to administration of EM 1201 and diclofenac during the all experiment. Any usuries, pannus formation and thinning of cartilage and significant decrease of erosion, inflammatory infiltration, edema, angiomatosis, synovial villy proliferation in the treated groups were observed. The effect of preparation EM 1201 was more potent than diclofenac in suppression of soft articular tissue edema and infiltration with macrophages. More effectively than diclofenac EM 1201 decreased MDA level and elevated CAT activity in the blood serum. Diclofenac increased only AOA.

A higher weight of the thymus and significantly lower dystrophic processes in the liver, the lower hypertovelia of V. centralis and inflammatory infiltration of hepatic stroma with lymphocytes showed the positive effect of treatment with EM 1201.

Conclusions. Treatment with EM 201 reduced multiple indices of arthritis and demonstrated its potential beneficiary effect. Anti-inflammatory and anti-oxidative effect of EM 1201 in rats with AA support the need of further investigations by using it as supplementary agent in the treatment of rheumatoid arthritis and other autoimmune diseases.

Key words:
herbal preparation EM 1201, rat’s adjuvant arthritis, diclofenac

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Introduction

For several years research in our laboratory has been directed towards the search for new preparations with anti-inflammatory and anti-oxidative activity in animals with arthritic models.

Rheumatoid arthritis (RA) is a chronic inflammatory disease of multifactorial etiology characterized by excessive synovial hyperplasia, vasculogenesis, cartilage and bone destruction and joint malformation [1, 2]. Due to the complex etiology of RA, identification of effective therapies has proven difficult. Although many of drugs used for the treatment of RA transiently suppress inflammation and ameliorate symptoms, they do not significantly improve the long-term disease and may result in serious side effects. So, many patients especially elderly tend to use a more effective and safe therapeutic strategy to treat RA and alleviate the adverse effects of chemotherapeutic drugs [3]. Therefore, efficacy of various natural products against inflammation and arthritis has been explored [4–6]. It is also known that oxygen metabolism and an increase in reactive oxygen species (ROS) have important roles in the pathogenesis of RA [7–9]. It has been suggested that the pro-oxidant/anti-oxidant imbalance in RA and arthritis models may be due to acceleration of some cellular reactions or insufficiency of the antioxidant defense system [10].

In the present study we investigated the effects of oral administration of EM 1201 on inflammation and antioxidant status in a rat adjuvant arthritis model, which is a T-cell dependent and immune-mediated condition characterized by hyperplasia of the synovial lining and inflammation in affected joints with pathogenesis similar to RA [11–13]. Although animal models may not reproduce all features of human RA, they can help understand normal inflammatory and immune responses during RA pathogenesis. Adjuvant arthritis (AA) has been widely used in studies of pathological mechanisms that underlie RA or serve as vehicle to test novel therapeutic agents [14].

Material and methods

Chemicals and drugs

Complete Freund’s adjuvant (CFA), 10% formalin, spirit-formol, hematoxylin, eosin, picrofuxin, toluidine blue, methyl-green-pyronin-y, acetic acid, trichloracetic acid, orthophosphoric acid, thiobarbituric acid, nitric acid, ferrous sulfate, ascorbic acid, ammonium molybdate, hydrogen peroxide were obtained from Sigma-Adamich Chemie and Fluka Chemie GmbH (Germany), ketamine and xylazine from Biowet (Poland) and diclofenac sodium from Ciba-Geigy Ltd (Switzerland). Tetrachloroauric acid (HAuCl4·3H2O) and tannic acid were obtained from Carl Roth GmbH & Co (Germany), sodium citrate – from Penta (Czech Republic).

Preparation of EM 1201

Herbal preparation with code name EM 1201 used in experiment was prepared in the Pharmaceutical company “Aconitum” and kindly proposed for investigation.

Animals

The study was carried out on 24 female albino rats of Wistar strain about 12 weeks of age and weighing approximately 195–200 g obtained from the vivarium of the State Research Institute Centre for Innovative Medicine, Department of Biomodels. The animals were fed with normal pellet diet and water ad libitum, and maintained in standard environmental conditions (temperature 20–22°C, relative humidity 50–70% and 12 hours light/dark cycle). Rats were housed in a group of 8 per cage and acclimatized to the laboratory condition 1 week prior to experiments. Throughout the study, the animals were cared for in accordance with the European Convention and Guide for the Care and Use of Laboratory Animals and with Lithuanian Laws. Study was approved by the Lithuanian Laboratory Animal Use Ethics Committee under the State Food and Veterinary Service (No 0207–2010).

Adjuvant-induced arthritis, its evaluation and treatment

Adjuvant arthritis (AA) was induced by injecting a 0.1 ml of complete Freund’s adjuvant (CFA) into the subplantar region of rat left hind paw on day 0. The animals were divided into three groups of eight animals each as follows: group I – control rats with AA, group II – AA + EM 1201 (110 mg/kg/d, orally by gastric intubation 5 times a week), Group III – AA + diclofenac (1 mg/kg/d, orally by gastric intubation 5 times a week). The treatment was performed since AA inducing day. Experiment lasted 18 days. The changes in body weight and joint swelling were recorded 3 times a week. The anti-arthritic effect of EM 1201 or diclofenac sodium was evaluated by measuring the paw volume plethysmometrically by using plethysmometer (PVP1001; Kent Scientific Corporation). On the 18th day, the rats were sacrificed by decapitation. Blood, liver and articular joint tissues were collected for the further investigation.
Blood and tissue collection

At the end of experiments animals were humanely killed by decapitation under ketamine-xylazine anesthesia. Their internal organs were examined macroscopically, weighed and the liver and injected joints were taken for histological analysis. The erythrocyte and leukocyte counts (made using a Picoscale, Hungary) and the erythrocyte sedimentation rate (ESR) were determined in their blood. Blood samples were centrifuged at 800 g for 10 min to obtain serum samples which were stored frozen at −20 °C until testing.

Lipid peroxidation (MDA), and catalase (CAT) and total antioxidant activity (AOA) level detection in blood serum

The extent of lipid peroxidation was estimated from the concentration of malondialdehyde (MDA), a thiobarbituric acid reactive substance, which is produced due to lipid peroxidation. MDA, the antioxidant enzyme catalase (CAT) and total antioxidant activity (AOA) were determined in the blood serum of all test groups. Measurement of the MDA levels in blood serum, expressed as nmol/ml, was determined by the thiobarbituric acid reaction at 535 nm and 580 nm by the method of Gavrilov and co-workers [15]. CAT activity, expressed in mmol/l/min, was measured at 410 nm as described by Koroliuk et al. [16]. The total AOA was determined in the reaction with thiobarbituric acid, described by Galaktionova et al. [17].

Histology

The liver and injected paws from AA rats were excised, followed by routine fixation, decalcification, and paraffin embedding. Histological 5 µm-thick tissue sections were stained with hematoxylin-eosin, picrofuxin, toluidine blue, methyl-green-pyronin-y and safranin O. Histological assessment of changes in the liver, synovium, soft periarticular tissues and cartilage was performed in a blinded manner using the 0–3 scale, where 0 indicates the absence of changes and 3 means the most severe expression of a particular sign.

Statistical analysis

Statistical evaluation of the results was done by one-way analysis of variance ANOVA using PRISM Software (GraphPad Software, San Diego, CA, USA) and Student’s t test. The nonparametric Mann-Whitney U test was used to evaluate the histological changes. All data were expressed as mean ± SEM and were considered to be statistically significant at P < 0.05.

RESULTS

Body and organ’s weight

The total body weight of all investigated groups increased during the experiment and from the beginning to the end of investigation it was higher by 11.8%, 16.1% and 21.6% in the control and both tested groups respectively, although no significant differences between the groups were observed (data not shown). At the end of experiment the body weight was higher by 8.9% in the group treated with EM 1201 and by 8.5% in the group treated with diclofenac than in the control group.

A postmortem examination of the internal organs (Table 1) revealed only a significantly higher weight of the thymus (P < 0.05) in the II experimental group of animals treated with EM 1201. Although the absolute and relative weight of the other internal organs did not significantly differ from the control, but relative weight of the liver was lower by 8.4% and 9.3%, relative weight of the spleen – by 10.0% and 5.7% in the groups respectively treated with EM 1201 and diclofenac. The absolute and relative weight of the thymus was higher by 31% (P < 0.05) and 26.3% in the group that received EM 1201 and by 21.4% and 15.8% in the group treated with diclofenac than in the control group, what showed a positive effect of preparations on the internal organs.

Blood indices and pro-/antioxidant activity in rats with AA treated with EM 1201 and diclofenac

Changes in the blood indices are shown in Fig. 1(A). The ESR and leukocyte count for both groups of rats after the treatment with EM 1201 and diclofenac was markedly lower than in the control group: ESR decreased by 55.8% (P < 0.001) and 62.25% (P < 0.001) and the amount of leukocytes – by 31.26% (P < 0.0001) and 21.4% and 15.8% in the control group treated with EM 1201 and diclofenac. The absolute and relative weight of the thymus was higher by 31% (P < 0.05) and 26.3% in the group that received EM 1201 and by 21.4% and 15.8% in the group treated with diclofenac than in the control group, what showed a positive effect of preparations on the internal organs.

Fig. 1(B) shows the free radical formation resulting in lipid peroxidation, measured as the MDA level, in rats with AA serum. It should be noted that MDA level in rats treated with of EM 1201 was found to be lower by 21.3% than in the control group (P < 0.01) and diclofenac treated group (P < 0.01). The treatment with diclofenac decreased MDA level by 11.3% only, although the difference compared with the control group was near to significant; (t = 2.05; P < 0.06). Serum antioxidant enzyme CAT activities were found to be significantly higher by
Preparation EM 1201 decreased hypervolemia of rats by 47.9% (P < 0.02) and suppressed the inflammatory infiltration of hepatic stroma with lymphocytes by 56.8% (P < 0.05), and penetration of inflammatory cells into the lobule by 63.8% (this decrease was near to significant in comparison with the control group (t = 2.13; P < 0.055). Although after the treatment with diclofenac infiltration of stroma and penetration of inflammatory cells into the lobule was also lower than in the control group, but no significant differences between these groups were found.

Table 1. Body and organ’s weight of rats with adjuvant arthritis treated with preparation EM 1201 and diclofenac

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Liver Absolute (g)</th>
<th>Liver Relative (g/kg)</th>
<th>Kidney Absolute (g)</th>
<th>Kidney Relative (g/kg)</th>
<th>Spleen Absolute (g)</th>
<th>Spleen Relative (g/kg)</th>
<th>Thymus Absolute (g)</th>
<th>Thymus Relative (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I AA control</td>
<td>218.87 ± 6.42</td>
<td>9.41 ± 0.50</td>
<td>4.30 ± 0.17</td>
<td>1.88 ± 0.04</td>
<td>0.86 ± 0.03</td>
<td>0.78 ± 0.06</td>
<td>0.35 ± 0.02</td>
<td>0.42 ± 0.03</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>II AA + EM 1201</td>
<td>238.41 ± 6.59</td>
<td>9.35 ± 0.24</td>
<td>3.94 ± 0.16</td>
<td>1.99 ± 0.04</td>
<td>0.83 ± 0.02</td>
<td>0.75 ± 0.037</td>
<td>0.31 ± 0.02</td>
<td>0.55 ± 0.05</td>
<td>0.24 ± 0.03</td>
</tr>
<tr>
<td>III AA + diclofenac (1 mg/kg)</td>
<td>237.40 ± 6.50</td>
<td>9.20 ± 0.34</td>
<td>3.90 ± 0.21</td>
<td>1.86 ± 0.04</td>
<td>0.79 ± 0.02</td>
<td>0.77 ± 0.06</td>
<td>0.33 ± 0.03</td>
<td>0.51 ± 0.06</td>
<td>0.22 ± 0.03</td>
</tr>
</tbody>
</table>

Note: Adjuvant arthritis (AA) was induced by a single injection of 0.1 ml complete Freund’s adjuvant (CFA) into the left hind paw. II and III tested groups were treated prophylactically (since AA inducing day) with 110 mg/kg of EM 1201 and 1 mg/kg of diclofenac which were injected orally 5 times a week. *The differences are significant in comparison with the control group.

Histological changes in the liver
Any toxic effect on the liver of rats with AA hasn’t been detected after the treatment with EM 1201 and diclofenac (Table 2). Both treatment decreased alteration of hepatic parenchyma in comparison with the control group. Dystrophic changes were significantly lower by 21.4% (P < 0.01) and 24.3% (P < 0.01) in the groups of rats respectively treated with EM 1201 and diclofenac. Preparation EM 1201 decreased hypervolemia of V. centralis by 47.9% (P < 0.02) and suppressed the inflammatory infiltration of hepatic stroma with lymphocytes by 56.8% (P < 0.05), and penetration of inflammatory cells into the lobule by 63.8% (this decrease was near to significant in comparison with the control group (t = 2.13; P < 0.055). Although after the treatment with diclofenac infiltration of stroma and penetration of inflammatory cells into the lobule was also lower than in the control group, but no significant differences between these groups were found.
The fibrotic processes decreased by 56.8% and 15.9% in the groups respectively treated with EM 1201 and diclofenac, but significant differences in comparison with the control group were not observed.

So, both preparation did not show toxic effects and actually improved the histological changes induced by AA in hepatic tissue. EM 1201 showed better effect on the liver than diclofenac, because significantly diminished not only dystrophic processes in hepatic parenchyma, but also hypervolemia of \( V. \) centralis and infiltration of hepatic stroma with lymphocytes.

**Joint swelling and incidence of polyarthritis development**

As is seen from Fig. 2, the administration of CFA into the subplantar region induced arthritis and increased the paw volume. The EM 1201 treated group showed significant reduction in joint swelling from 3rd day \((P < 0.01)\) till the end of experiment and it was lower than in the control group by 34–46.3% \((P < 0.001)\).

Diclofenac also significantly decreased joint swelling throughout the 18 days study \((P < 0.05–0.002)\), except the 12th day of experiment, but the reduction induced by diclofenac was something lower than after therapy with EM 1201 and at the end of experiment reached 37.5%. Comparison of the inhibition observed for the EM 1201 and diclofenac did not reveal any significant difference.

Polyarthritis did not develop in the animals of the treated groups, whether a slight polyarthritis damaging one non-injected limb and characterizing the generalization of the disease and exacerbation of the autoimmune process was observed in 37.5% animals of the control group.

### Table 2. Pathomorphological changes in the liver of Wistar rats with adjuvant arthritis treated with EM 1201 and diclofenac

<table>
<thead>
<tr>
<th>Index</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I (AA control)</td>
</tr>
<tr>
<td>Alteration of parenchyma</td>
<td>2.06 ± 0.06</td>
</tr>
<tr>
<td>Necrosis</td>
<td>1.25 ± 0.16</td>
</tr>
<tr>
<td>Hypervolemia of V. centralis</td>
<td>1.19 ± 0.16</td>
</tr>
<tr>
<td>Inflammatory infiltration</td>
<td></td>
</tr>
<tr>
<td>of hepatic stroma</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.44 ± 0.11</td>
</tr>
<tr>
<td>Macrophages</td>
<td>0.25 ± 0.09</td>
</tr>
<tr>
<td>General</td>
<td>0.69 ± 0.16</td>
</tr>
<tr>
<td>Penetration into the lobule</td>
<td>0.69 ± 0.16</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>0.44 ± 0.15</td>
</tr>
</tbody>
</table>

*Note: Adjuvant arthritis (AA) was induced by a single injection of 0.1 ml complete Freund’s adjuvant (CFA) into the left hind paw. II and III tested groups were treated prophylactically (since AA inducing day) with EM 1201 (100 mg/kg) and diclofenac (1 mg/kg). Preparations were injected orally 5 times a week. Hepatic tissue was fixed in spirit-formol (1:9), embedded in paraffin, and 3 μm-thick histological sections of liver were stained with haematoxylin-eosin for visualization of inflammation and inflammatory cell infiltration and hepatocytes necrosis) and microscopically (for determination of fibrotic processes). Each parameter was scored on a 0 to 3 point scale, where 0 means the absence of changes, 1 – traces of changes, 2 – minimal changes, 3 – moderate changes, 4 – heavy changes. The differences are significant in comparison with the control group.

**Fig. 2.** Joint swelling of rats with adjuvant arthritis treated with EM 1201 and diclofenac. Adjuvant arthritis (AA) was induced by a single injection of 0.1 ml complete Freund’s adjuvant (CFA) into the left hind paw. Tested groups were treated prophylactically (since AA inducing day) with 110 mg/kg of EM 1201 and 1 mg/kg of diclofenac which was injected orally 5 times a week. *The differences are significant in comparison with the control group.
**Histological features of arthritis**

The histological examination of the joints of the injected paw on day 18 after arthritis induction showed the most expressed soft tissue pathology in the control group of rats (Table 3). The treatment of AA with EM 1201 or diclofenac similarly suppressed inflammatory infiltration with lymphocytes by 58% and 54.3% (P < 0.001) respectively in comparison with the control group. None infiltration with leukocytes and 76% lower infiltration with macrophages (P < 0.001) were observed in the group treated with EM 1201. Diclofenac diminished the infiltration with macrophages by 32% (P < 0.05) compared with the control group and significant differences (P < 0.01) between the both treated groups were also observed. Only one animal in this group had an infiltration with leukocytes (+1). The general inflammatory reaction in the groups that received EM 1201 or diclofenac respectively decreased by 62.3% and 43.8% in the soft periarticular tissues and significantly differed from the control (P < 0.05; P < 0.001 respectively). Well-marked suppression of edema by 87.5% and 68.5% was observed after the treatment with EM 1201 and diclofenac (P < 0.0001) respectively, and a significant difference between the both tested groups was found (P < 0.05), what showed more potent action of EM 1201. Both preparations suppressed the soft tissues angiogenesis by 76% (P < 0.0001) and 57% (P < 0.01), what was not observed in the animals of the control group.

Both preparations equally diminished synovial villi proliferation (P < 0.001) and angiogenesis (P < 0.002; P < 0.001) and only minimal changes (score – ±) in all animals of the tested groups were found. One animal in the group treated with EM 1201 and two – with diclofenac had a minimal synovial edema and only one of 8 rats in every tested groups showed the disorganization of the connective tissue, what significantly differed from the control group of animals (P < 0.001 – edema; P < 0.05 – γ-metachromasia). Similar intensification of fibrotic processes (P < 0.001) was observed after the treatment with both preparations. EM 1201 decreased the infiltration

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Index</th>
<th>I</th>
<th>II EM 1201 (110 mg/kg)</th>
<th>III Diclofenac (1 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>0.20 ± 0.09*</td>
<td>0.19 ± 0.09*</td>
</tr>
<tr>
<td>Soft tissues</td>
<td>Inflammatory infiltration</td>
<td>Lymphocytes</td>
<td>2.19 ± 0.09</td>
<td>0.92 ± 0.20*</td>
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<tr>
<td></td>
<td></td>
<td>Plasma cells</td>
<td>0.13 ± 0.03</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leukocytes</td>
<td>2.00 ± 0.21</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Macrophages</td>
<td>1.75 ± 0.13</td>
<td>0.42 ± 0.15**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>General</td>
<td>2.44 ± 0.11</td>
<td>0.92 ± 0.20*</td>
</tr>
<tr>
<td>Synovium</td>
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<td>Edema</td>
<td>2.00 ± 0.13</td>
<td>0.25 ± 0.11**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Angiogenesis</td>
<td>1.75 ± 0.13</td>
<td>0.42 ± 0.08*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fibrosis</td>
<td>–</td>
<td>1.08 ± 0.27**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>g-metachromasia</td>
<td>0.73 ± 0.26</td>
<td>1.12 ± 0.28*</td>
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<tr>
<td></td>
<td></td>
<td>Villi pro.</td>
<td>1.38 ± 0.18</td>
<td>0.50 ± 0.00*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Edema</td>
<td>1.37 ± 0.18</td>
<td>0.08 ± 0.08*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>g-metachromasia</td>
<td>0.50 ± 0.16</td>
<td>0.08 ± 0.08*</td>
</tr>
<tr>
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<td></td>
<td>Inflammatory infiltration</td>
<td>Lymphocytes</td>
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<tr>
<td></td>
<td></td>
<td>Granulocytes</td>
<td>0.19 ± 0.09</td>
<td>–</td>
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<tr>
<td></td>
<td></td>
<td>Macrophages</td>
<td>0.25 ± 0.16</td>
<td>0.25 ± 0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>General</td>
<td>1.25 ± 0.09</td>
<td>0.33 ± 0.17*</td>
</tr>
<tr>
<td>Cartilage</td>
<td>Attention</td>
<td>Erosion</td>
<td>0.88 ± 0.12</td>
<td>0.17 ± 0.10*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Usura</td>
<td>0.50 ± 0.19</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fissure</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pannus</td>
<td>0.38 ± 0.18</td>
<td>–</td>
</tr>
</tbody>
</table>

Note: Adjuvant arthritis (AA) was induced in 24 rats by the foot paw method by injecting 0.1 ml of complete Freund’s adjuvant (CF A) into the left hind paw under light anaesthesia. Group I (control) – animals with induced AA. Groups II and III – rats with AA treated daily (except weekends) with EM 1201 and diclofenac. Joint tissues are fixed in spirit-formol (1:9) and embedded in paraffin. Histological 5 μm-thick tissue sections were stained with hematoxylineosin, picrofuchin, toluidine blue, methyl-green-pyronin-γ and safranin O. Assessment of changes in synovium, soft periarticular tissues and cartilage was performed in a blinded manner using the 0–3 scale, where 0 indicates the absence of changes and 3 means the most severe expression of a particular sign.

*The differences are significant in comparison with the control AA group. **The differences are significant between the both treated groups.
with lymphocytes by 73.6% ($P < 0.001$), and in the group that received diclofenac only one animal had a minimal infiltration. General inflammatory reaction was lower in diclofenac treated group ($P < 0.0001$), although EM 1201 also significantly suppressed it ($P < 0.001$).

It should be noted that changes in the cartilage are negligible. 7 of 8 animals of the control group had slight erosion and 4 – small usuries. Minimal erosion was found in the cartilage of two and three animals treated respectively with EM 1201 and diclofenac. In comparison with the control group the decrease of erosion reached 80.7% and 76.1% in the groups respectively received EM 1201 and diclofenac ($P < 0.001$), and any usuries, however pannus and thinning of cartilage in these groups were not found. In the control group small pannus (+1) was found in the cartilage of 3 of 8 animals, and minimal and slight thinning of cartilage in 2 animals.

**Discussion**

Concerns about the safety and efficacy of many drugs used for the treatment of autoimmune diseases have persisted for many years and researches put high efforts to found the new drugs with lower side effects. During the past years many research groups throughout the world have concentrated on finding biologically-active substances from various plants and efficacy of various natural products against inflammation and arthritis has been explored [4–6]. In our study we investigate the herbal preparation with code name EM 1201 prepared in the Pharmaceutical company “Aconitum” and compared its anti-inflammatory and anti-oxidative effect with diclofenac, which is widely used for the treatment of rheumatoid arthritis and other inflammatory diseases. Both preparations in animals were used in the doses that corresponded to the doses used for humans.

The obtained results indicated that preparation EM 1201 showed anti-inflammatory and antioxidative effect, improved blood indices, markedly decreased joint swelling and histopathological changes in them and was substantially more effective in comparison with diclofenac. A significant suppression of joint swelling by 29–46% in response to administration of EM 1201 was observed during the all experiment and it was more effective than diclofenac which decreased joint swelling by 18–37%. Preparation more potent than diclofenac suppressed peri-articular soft tissue infiltration with macrophages and edema. It is known, that macrophages play key roles in inflammation. During the onset of the inflammatory process, these phagocytic cells become activated and have destructive effects [18]. Antioxidant effectiveness of EM 1201 was confirmed by assessing blood serum lipid peroxidation value MDA which was markedly suppressed, and catalase activity that significantly increased in comparison with diclofenac treated group, although significant increase of AOA was observed in both treated groups. It is known that low antioxidant level is a risk factor of RA, and it worsens the severity of the condition. High lipid peroxidation levels were an indicator of reduced antioxidant capacity and increased oxidative stress in RA [19]. Our results revealed that MDA level was significantly lower in EM 1201-treated group than in the diclofenac-treated group and arthritic control.

Numerous studies have shown that in the course of AA not only joints with obvious signs of inflammation, but also visceral organs may be affected by the pathological process. Body and thymus weight is reduced in arthritic rats as compared to nonarthritic animals [20].

A higher weight of the thymus and significantly lower dystrophic processes in the liver, the diminished inflammatory infiltration of hepatic stroma with lymphocytes showed the positive effect of treatment with EM 1201.

In summary, results of the present study showed beneficial therapeutic effect of EM 1201, which reduced systemic and local indices of inflammation in affected joints and enhanced antioxidant activity of organism in rat model of RA.

**Conclusions**

Treatment with EM 1201 reduced multiple indices of arthritis and demonstrated the potential beneficiary effect of it as antioxidative agent on AA in rats. The obtained results support the need of further investigation by using this preparation as supplementary agent in the treatment of rheumatoid arthritis and other autoimmune diseases.

**References**


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PREPARATO EM 1201 POVEIKIS ŽIURKĖMS SU ADJUVANTINIU ARTRITU: PALYGINIMAS SU DIKLOFENAKU

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Santrauka

Darbo tikslas

Ištirti priešuždegiminio ir antioksidacinio preparato EM 1201 poveikį žiurkėms su adjuvantiniu artritu.

Medžiaga ir tyrimo metodai.

Adjuvantinis artritas (AA) buvo sukelta 24 Wistar populiacijos žiurkių patelėms sulydžiant 0,1 ml pilnojo Froindo adjuvanto į užpakalinės kairės kojos padą. Patologinio proceso vystymosi eiga ir pro-/antioksidacinė kraujo serumo būklė vertinta profilaktiškai gydant gyvūnus preparatu EM 1201 (dozė 110 mg/kg) suleidžiant jį per os 5 kartus per savaitę ir poveikis lygintas ne tik su kontrole AA grupe negavusia gydymo, bet ir su grupe, kuriai taikyta medikamentinė gydymas. Vertinta gyvūnų kūno masės pokyčiai, sąnarių patinimas ir kraujo rodikliai, tokie kaip eritrocitų nusėdimo greitis (ENG), leukocitai ir eritrocitai. Ištirta pro-/antioksidacinės sistemos būklė įvertinant maloniniodialdehido (MDA) kiekį, antioksidacinio fermento katalazės (KAT) aktyvumą ir bendrą antioksidantinį aktyvumą (AOA) kraujo serume, bei histologinius pokyčius sąnariuose ir kepenyse.

Rezultatai.


Išvados.

Teigiamą terapinį preparato EM 1201 poveikį rodo daugelio eksperimentinio artrito rodiklių sumažėjimas. Gautas priešuždegiminis ir antioksidacinis poveikis žiurkėms su AA patvirtina tolimesnių tyrimų reikiamybę, įvertinant EM 1201, kaip papildomo agento, poveikį reumatoidinio artrito ir kitų automuninių ligų gydymui.

Raktažodžiai:

žolinis preparatas EM 1201, žiurkių adjuvantinis artritas, diklofenakas