

# Study of Anti-Inflammatory Activity of Fatsiphloginum™ (*Fatsia japonica*) and a New Purified Triterpene-Rich Extract of Saponins (PS-551) in Experimental Model of Arthritis

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## Summary

In this study, two extracts from *Fatsia japonica*-Fatsiphloginum™ (extract of triterpene glycosides containing 45-50 % of fatsiosides (FS)) and purified triterpene-rich extract of saponins with code name PS-551 (PS) were administered in combination with methotrexate (MTX) and in monotherapy to rats suffering adjuvant arthritis (AA). The anti-inflammatory activities of extracts were evaluated as monotherapies in comparison with untreated AA. PS administered in higher dose showed on day 28 effective decrease of hind paw volume (HPV), decreased activity of gamma-glutamyl transferase (GGT) in joints, and also interleukin-17A was decreased significantly on day 14. The higher dose of PS was more effective than both doses of FS. Further, we evaluated the higher doses of PS and FS in combination with MTX. PS improved the effect of MTX in combination more effective than FS (HPV, body weight and activity of GGT in joint). However, FS was more effective in reducing the level of IL-17A on day 14 and activity of GGT in spleen than PS. In conclusion, our study showed that generally FS has higher anti-arthritis activity comparing to PS. Thus, the novel combination of Fatsiphloginum™ and methotrexate could be interesting for future clinical studies in patients suffering auto-immune diseases.

## Key words

Arthritis • Methotrexate • Saponins • Terpenes • *Fatsia japonica*

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## Introduction

Rheumatoid arthritis (RA) is an autoimmune disease manifested as chronic, progressive and invasive arthritis. It is characterized by synovial hyperplasia, inflammation, destruction of articular tissues and restricted movement of the joints (Fournier 2005). The pathogenesis of RA has not been fully understood yet. It has been suggested that the abnormal activation of inflammatory signaling pathways (Sglunda *et al.* 2014) and subsequently excessive oxidative stress play a critical role in the development of RA (Chen *et al.* 2017).

To control inflammatory symptoms and pain, disease modifying antirheumatic drugs are used; they are associated with certain undesirable side effects. Many clinical studies show that combination therapy is usually more effective than a single medicine alone (monotherapy) to control the inflammation in RA. A popular combination for relieving symptoms and preventing long-term joint damage and disability of arthritis is methotrexate – major immunosuppressive substance and anti-inflammatory compound (Smolen *et al.* 2010, Hazlewood *et al.* 2016, Koziolová *et al.* 2018).

Herbal anti-inflammatory substances allocate a wide-ranging mechanism of action including interaction

with the inflammatory cascade, cytokine production, increased anti-oxidative effectiveness and other still unspecified effects that may contribute to joint protective effects (Maroon *et al.* 2010).

Triterpenoid glycosides (triterpenes) belong to the group of saponins, widely distributed in the plant kingdom and contain a large number of biologically active components. These compounds consist of a hydrophobic part, called aglycone, which is linked to one or several sugar chains that build the hydrophilic part of the molecule. Because of their amphiphilic nature, saponins possess also surface-active properties and are able to efficiently solubilize hydrophobic molecules (Wu *et al.* 2014, Mesgarzadeh *et al.* 2017, Walthelm *et al.* 2001).

It has been proven that triterpenes are characterized by a wide spectrum of pharmacological activities, including anti-arthritic and anti-inflammatory actions (Sultana and Saify 2012). These effects have been described as a result of mediators of inflammation inhibition with its antioxidant property to suppress the formation of reactive oxygen species (ROS) which also play a major role in inflammation (Desai *et al.* 2010, Wang *et al.* 2008, Sparg *et al.* 2004).

*Fatsia japonica* (Thunb.) Decne et Planch. (Araliaceae) is an evergreen plant growing in the regions around Black Sea. At the Institute of Pharmacology, Tbilisi State Medical University (TSMU) in the Laboratory of Terpene Compounds ethanolic extract of the leaves of *Fatsia japonica* (formulated into para-medical preparation – Fatsiphloginum™) was obtained. Five triterpene glycosides (fatsiosides) were also identified in the ethanolic extract of leaves of *Fatsia japonica*. The original para-medical preparation Fatsiphloginum™ is available as 25 mg and 50 mg, coated tablets. The active substance of Fatsiphloginum™ possesses anti-rheumatic, anti-inflammatory and analgesic activities and it is an original, nonsteroidal para-medical preparation used in Georgia (Getia *et al.* 2017). The active substance of Fatsiphloginum™ is hygroscopic dry powder which represents the crude extract of triterpene glycosides containing 45-50 % of fatsiosides (FS) – derivatives of hederagenin and oleanic acid (Kemertelidze *et al.* 2001, Shalamberidze *et al.* 1998, Tsiklauri *et al.* 2004). The Laboratory of Terpene Compounds of TSMU has continued in the research of this ethanolic extract and recently more purified triterpene-rich extract of saponins with code name PS-551 (PS) was obtained from *Fatsia japonica*. This extract – PS-551 is non-hygroscopic dry powder and

contain oleanic fatsiosides.

The aim of this study was to evaluate the anti-inflammatory activity of orally administrated herbal preparation with code name PS-551 and compare it with the active substance, which is the main part of the original para-medical preparation Fatsiphloginum™. Moreover, the effect of combination of PS or FS with methotrexate on the adjuvant-induced arthritis (AA) progression was evaluated. For this purpose, the paw volume (HPV), change of body weight (CBW), arthritic score, along with biochemical and immunological markers in plasma and tissues were assessed.

## Methods

### Drugs and chemicals

The crude extract of triterpene glycosides, active substance of original drug – Fatsiphloginum™ (FS) – and purified triterpene-rich extract of saponins with code name PS-551 (PS) used in this experiment were prepared in the laboratory of Terpene Compounds of Kutateladze Pharmacology Institute. The stock suspension of compounds tested was prepared on daily basis, as *vehiculum aqua pro injectione* was used (B. Braun Melsungen AG, Germany). Incomplete Freund's adjuvant was obtained from MERCK (Sigma Aldrich, Germany) and heat-inactivated *Mycobacterium butyricum* was obtained from Difco Lab (USA). Methotrexat (Ebewe® 20 mg/ml), heparin (HEPARIN LÉČIVA® 50 K) and anesthesia (Zoletil® 50 and Xylarium® 20 mg/ml) were obtained directly from public pharmacy. Chemicals for GGT: buffer (2.6 mM NaH<sub>2</sub>PO<sub>4</sub>, 50 mM NaH<sub>2</sub>PO<sub>4</sub>, 15 mM EDTA, 68 mM NaCl; pH 8.1); 8.7 mM  $\gamma$ -glutamyl-p-nitroaniline, 44 mM methionine) 65 % isopropylalcohol, methanol were obtained from MERCK (Sigma Aldrich, Germany). All chemicals were in pharmaceutical quality, purity and analytical grade.

### Animals

In this experiment Male Lewis rats were used, weighing 160-180 g, obtained from the breeding station Dobrá Voda (Slovakia). Rats had free access to standard pelleted diet and tap water *ad libitum*. The animal facilities comply with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes. The experimental protocol was approved by the Ethics Committee of the Institute of Experimental Pharmacology and Toxicology and by the State Veterinary and Food Administration of the Slovak Republic.

### Induction of adjuvant arthritis (AA)

After 7-days quarantine, the animals were allocated to 9 groups of eight-ten rats in each group. Group one was used as healthy control. Group two were untreated AA rats. In group three to nine AA rats were treated as given in experimental design below (Table 1). Rat's adjuvant arthritis is an experimental model of

inflammation with proven track record of predictability (Bendele 2001). Adjuvant arthritis was induced by means of intradermal injection at the base of the rat's tail, with 0.1 ml suspension of 12 mg/ml heat-killed *Mycobacterium butyricum* (MB) suspended in incomplete Freund's adjuvant (Bauerova *et al.* 2011).

**Table 1.** Experimental design.

Group	Treatment and active substance	Posology
Group 1: Healthy controls (HC)	vehiculum	0.5 ml
Group 2: AA untreated	vehiculum	0.5 ml
Group 3: AA + treatment	methotrexate (MTX)	0.3 mg/kg of b.w.
Group 4: AA + treatment	PS-551 (PS5)	5 mg/kg of b.w.
Group 5: AA + treatment	PS-551 (PS50)	50 mg/kg of b.w.
Group 6: AA + treatment	PS50 + MTX	50 mg/kg + 0.3 mg/kg of b.w.
Group 7: AA + treatment	ac. sub. of Fatsiphloginum™ (FS5)	5 mg/kg of b.w.
Group 8: AA + treatment	ac. sub. of Fatsiphloginum™ (FS50)	50 mg/kg of b.w.
Group 9: AA + treatment	ac. sub. of Fatsiphloginum™ (FS50) + MTX	50 mg/kg + 0.3 mg/kg of b.w.

AA – adjuvant arthritis, MTX – methotrexate, b.w. – body weight.

Anti-rheumatic medicine MTX was administrated orally twice a week during 28 days; all substances tested were administered daily orally from day 1 to day 28. After all animals were sacrificed under deep anesthesia, heparinized blood for plasma preparation and tissues of spleen and hind paw joint were taken on day 28. The plasma samples were stored at -70 °C until biochemical and immunological analysis.

### Dose selection

Two doses of 5 mg/kg and 50 mg/kg of body weight were selected based on preliminary studies (data not published) which have showed better response for doses above 5 mg/kg of body weight for anti-inflammatory effect.

### Clinical parameters – hind paw volume (HPV), body weight of animals and arthrogram

Hind paw volume (HPV) was calculated as the percentage increase of the hind paw of each animal, compared to the HPV measured of the beginning of the experiment. HPV was recorded on days 1, 14, 21 and 28 by means of an electronic water plethysmometer (UGO BASILE, Comerio-Varese, Italy). The body weight of the animals (g) was measured on days 1, 14, 21 and 28. The

arthritic score was measured as the total score of HPV (ml, max. points 8) + paw diameter of forelimb (mm, max. points 5) + diameter of scab, in the site of MB application, measured parallel to the spinal column (mm, max. points 5) for each animal. The more points, the more severe the inflammation was.

### Activity of $\gamma$ -glutamyl transferase (GGT) in spleen and hind paw joint tissue

The activity of cellular  $\gamma$ -glutamyltransferase (GGT) in hind paw joint and spleen tissue homogenates was measured at the end of the experiment (on day 28) by the method of Orłowski and Meister (1970) and modified by Ondrejickova *et al.* (1993). Samples were homogenized by UltraTurax TP 18/10 (Janke&Kunkel, Germany) for 1 min at 0 °C in phosphate buffer (2.6 mM of NaH<sub>2</sub>PO<sub>4</sub>, 50 mM of Na<sub>2</sub>HPO<sub>4</sub>, 15 mM of EDTA, 68 mM of NaCl; pH 8.1) at 1:9 (w/v). Substrates (8.7 mM of  $\gamma$ -glutamyl-p-nitroaniline, 44 mM of methionine) were added to 65 % isopropylalcohol to final concentrations of 2.5 mM and 12.6 mM, respectively. After incubation for 60 min at 37 °C, the reaction was stopped with 2.3 ml of cold methanol and the tubes were centrifuged for 20 min at 5,000 rpm (Centrifuge Eppendorf). Absorbance of supernatant was measured on spectrophotometer

Specord 40 (Jena, Germany) in a 0.5 cm cuvette at 406 nm. Reaction mixtures in the absence of either substrate or acceptor were used as reference samples.

#### *Measurement of interleukin 17A in plasma*

For the determination of interleukin 17A (IL-17A) concentrations level in plasma, an ELISA kit from eBioscience® (Waltham, MA, USA) was used. The assay procedures were applied as described in the product manuals. The results were calculated from the standard calibration curves based on internal standards.

#### *Statistical analyses*

The mean values and  $\pm$  SEM were calculated in each group for particular parameter. Statistical significance among treated groups, untreated AA group and HC group were tested using parametric Analysis of Variance (ANOVA). After *post hoc* Dunnett test, significance designations were specified as follows: extremely significant ( $***p < 0.001$ ), highly significant ( $**p < 0.01$ ), significant ( $*p < 0.05$ ), and not significant ( $p > 0.05$ ).

## Results

#### *Change of the body weight (CBW)*

A significant decrease in change of the body weight on day 14, 21 and 28 was observed in AA group compared to HC animals ( $**p < 0.01$  AA vs. HC, Fig. 1). The AA group treated with MTX was observed as highly significant on day 14 and 21 compared to the untreated AA group ( $^{++}p < 0.01$  AA-MTX vs. AA). This effect of MTX continued till the end of the experiment, but not significant. Groups administered with both active substances (PS and FS 5 mg/kg) showed not significant change of the body weight on day 14, 21 and 28. We observed a non-significant increase in higher dose of both substances (PS and FS 50 mg/kg) for this parameter when administered alone. Both substances also demonstrated the dose dependent trend on day 14 and 21. Highly significant result showed the combination of FS in a dose of 50 mg/kg and MTX on day 14 ( $^{++}p < 0.01$  AA-FS50+MTX vs. AA) and the combination of PS in a dose of 50 mg/kg and MTX ( $^{+}p < 0.05$  AA-PS50+MTX vs. AA, Fig. 1).

#### *Hind paw volume (HPV)*

Hind paw swelling reveals both inflammatory and arthritic alterations occurring in adjuvant arthritic

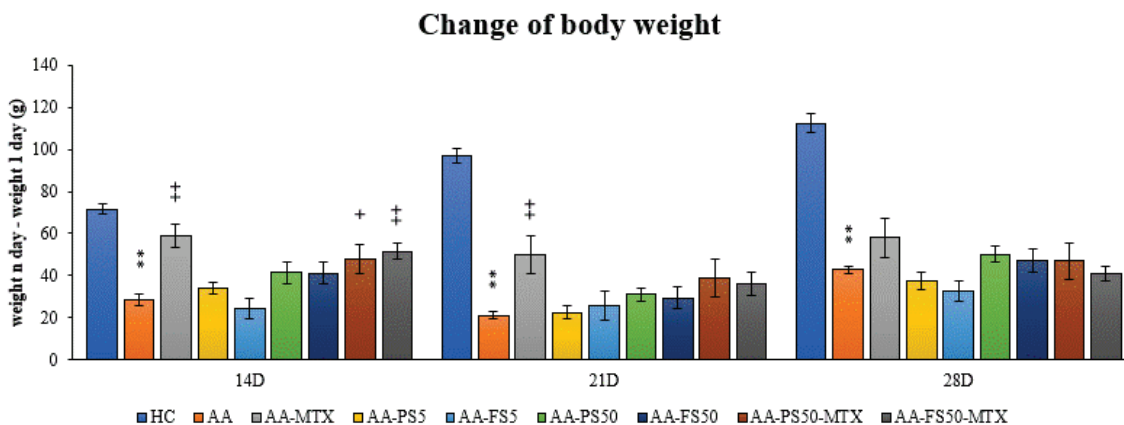
rats. The results in Table 3 indicate that from day 14, 21 and 28 HPV reached a highly significant value in untreated AA groups comparing to healthy controls ( $**p < 0.01$  AA vs. HC, Table 2). Therapy with MTX reduced swelling on all days monitored comparing to untreated AA group; on days 14 and 21 this effect was highly significant ( $^{++}p < 0.01$  AA-MTX vs. AA). At the end of the experiment, the effect of MTX alone decreased the HPV by 28.55 % compared to untreated AA group. The combination of PS in a dose of 50 mg/kg and MTX showed highly significant decrease of HPV on day 14 and 21 ( $^{++}p < 0.01$  AA-PS50+MTX vs. AA) comparing to untreated AA animals and this similar effect was also observed on day 28. The highly significant effect of FS in the dose of 50 mg/kg and MTX was showed on day 14 ( $^{++}p < 0.01$  AA-FS50+MTX vs. AA, Table 2) comparing to untreated AA animals. However, this effect was not significant on days 21 and 28.

#### *Arthritic score*

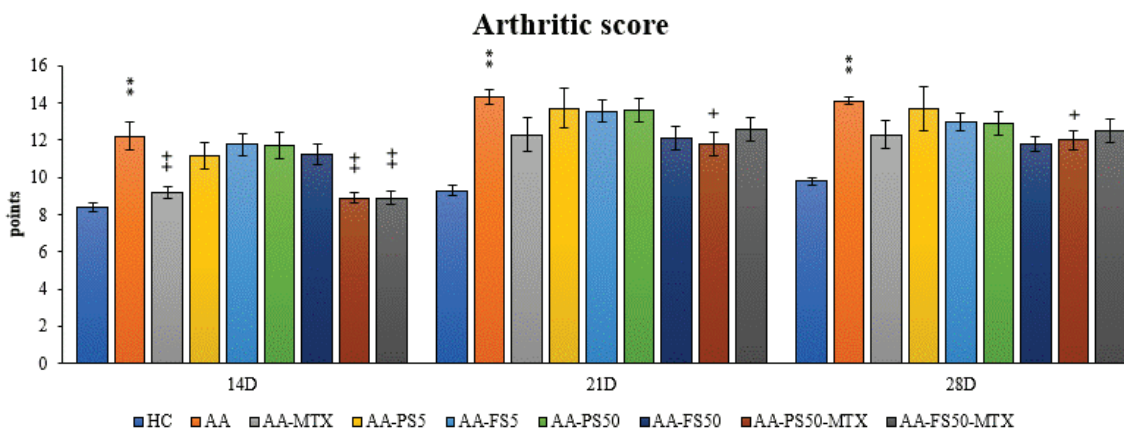
The untreated AA group showed highly significant manifestation in arthritic score on days 14, 21, and 28 by physical observation compared to healthy rats ( $**p < 0.01$  AA vs. HC, Fig. 2). The AA group treated with MTX in comparison to untreated AA animals showed highly significant arthritic score decrease on day 14 ( $^{++}p < 0.01$  AA-MTX vs. AA). Similar effect of MTX was also observed on day 21 and 28 without significance. The application of PS and FS showed no differences comparing to untreated AA rats. When MTX was in combination with PS or FS in higher dose, the effect observed was highly significant on day 14 comparing to untreated AA rats ( $^{++}p < 0.01$  AA-PS50+MTX vs. AA;  $^{++}p < 0.001$  AA-FS50+MTX vs. AA). The combination of PS in higher dose and MTX was observed significant also on days 21 and 28 comparing to untreated AA rats ( $^{+}p < 0.05$  AA-PS50+MTX vs. AA, Fig. 2).

#### *Activity of $\gamma$ -glutamyl transferase in joint and spleen tissue*

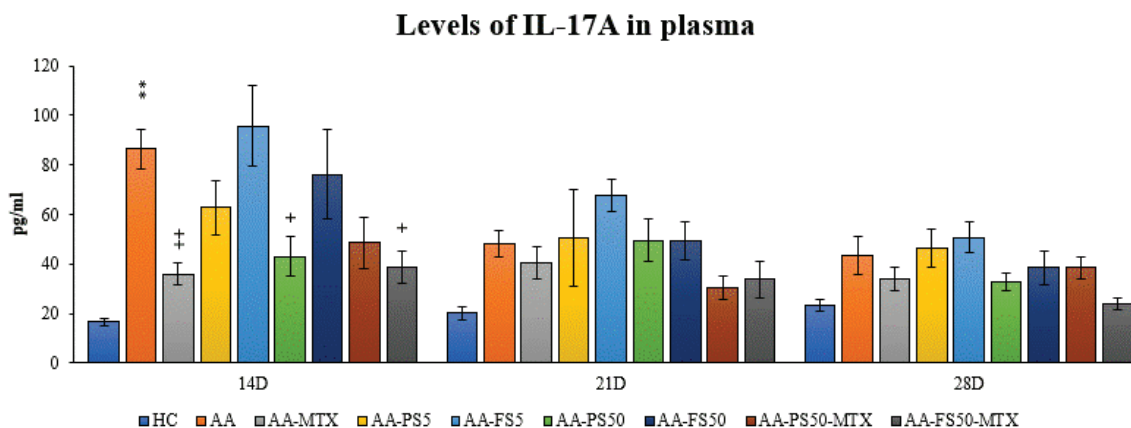
The increased activity of GGT was highly significant in joint tissues and also in spleen observed in an untreated AA group; this activity was approximately 1.46 times higher comparing to the healthy controls in the joint ( $**p < 0.01$  AA vs. HC, Table 3) and 3.60 higher in the spleen ( $**p < 0.01$  AA vs. HC). Application of MTX led to decreased activity of GGT in the joint and spleen



**Fig. 1.** Effect of PS, FS and MTX administered alone or in combination on change of the body weight on experimental day 14, 21 and 28. HC – control group, AA – adjuvant arthritis group, AA-MTX – adjuvant arthritis group administered Methotrexate 0.3 mg/kg twice a week, AA-PS5/AA-FS5 – adjuvant arthritis group administered PS/FS at dose of 5 mg/kg, AA-PS50/AA-FS50 – adjuvant arthritis group administered PS/FS at dose of 50 mg/kg, AA-PS50+MTX and AA-FS50+MTX – adjuvant arthritis group administered PS or FS (50 mg/kg) and Methotrexate 0.3 mg/kg twice a week. Results are expressed as mean ± SEM, n=8-10. Significant difference: \*\*p<0.01 vs. HC, \*\*p<0.01 vs. AA, \*p<0.05 vs. AA.



**Fig. 2.** Arthritic score in the model of adjuvant arthritis treated with PS, FS and MTX administered alone or in combination on the experimental day 14, 21 and 28. HC – control group, AA – adjuvant arthritis group, AA-MTX – adjuvant arthritis group administered Methotrexate 0.3 mg/kg twice a week, AA-PS5/AA-FS5 – adjuvant arthritis group administered PS/FS at dose of 5 mg/kg, AA-PS50/AA-FS50 – adjuvant arthritis group administered PS/FS at dose of 50mg/kg, AA-PS50+MTX and AA-FS50+MTX – adjuvant arthritis group administered PS or FS (50 mg/kg) and Methotrexate 0.3 mg/kg twice a week. Results are expressed as mean ± SEM, n=8-10. Significant difference: \*\*p<0.01 vs. HC, \*\*p<0.01 vs. AA, \*p<0.05 vs. AA.



**Fig. 3.** Effect of PS, FS and MTX administered alone or in combination on the levels of IL-17A in plasma on experimental days 14, 21 and 28. HC – control group, AA – adjuvant arthritis group, AA-MTX – adjuvant arthritis group administered Methotrexate 0.3 mg/kg twice a week, AA-PS5/AA-FS5 – adjuvant arthritis group administered PS/FS at dose of 5 mg/kg, AA-PS50/AA-FS50 – adjuvant arthritis group administered PS/FS at dose of 50 mg/kg, AA-PS50+MTX and AA-FS50+MTX – adjuvant arthritis group administered PS or FS (50 mg/kg) and Methotrexate 0.3 mg/kg twice a week. Results are expressed as mean ± SEM, n=8-10. Significant difference: \*\*p<0.01 vs. HC, \*\*p<0.01 vs. AA, \*p<0.05 vs. AA.

**Table 2.** Effect of PS, FS and MTX administered alone or in combination on HPV on experimental day 14, 21 and 28.

Groups	Treatment	Change of hind paw volume (HPV) [%]		
		Day 14	Day 21	Day 28
1	HC	9.80 ± 1.10	15.46 ± 0.77	20.12 ± 0.88
2	AA	48.10 ± 7.62**	65.71 ± 3.55**	61.12 ± 2.13**
3	AA-MTX	16.27 ± 3.04 <sup>++</sup>	42.95 ± 8.47 <sup>++</sup>	43.67 ± 6.53
4	AA-PS5	44.58 ± 7.68	67.53 ± 8.12	59.48 ± 13.26
5	AA-FS5	38.24 ± 6.24	60.44 ± 4.75	45.37 ± 3.49
6	AA-PS50	15.13 ± 2.22 <sup>++</sup>	39.74 ± 5.15 <sup>++</sup>	42.52 ± 3.68
7	AA-FS50	49.00 ± 5.20	62.81 ± 6.04	54.00 ± 4.85
8	AA-PS50-MTX	38.06 ± 6.75	53.43 ± 5.10	47.03 ± 2.47
9	AA-FS50-MTX	17.04 ± 5.14 <sup>++</sup>	54.77 ± 7.70	49.03 ± 6.25

HC – control group, AA – adjuvant arthritis group, AA-MTX – adjuvant arthritis group administered Methotrexate 0.3 mg/kg twice a week, AA-PS5/AA-FS5 – adjuvant arthritis group administered PS/FS at dose of 5 mg/kg, AA-PS50/AA-FS50 – adjuvant arthritis group administered PS/FS at dose of 50 mg/kg, AA-PS50+MTX and AA-FS50+MTX – adjuvant arthritis group administered PS or FS (50 mg/kg) and Methotrexate 0.3 mg/kg twice a week. Results are expressed as mean ± SEM, n=8-10. Significant difference: \*\*p<0.01 vs. HC, <sup>++</sup>p<0.01 vs. AA.

**Table 3.** Effect of PS, FS and MTX administered alone or in combination on the activity of  $\gamma$ -glutamyltransferase (GGT) in joint and spleen on the experimental day 28.

Activity of GGT [nmol p-nitroaniline/ min/g of tissue]	Joint	Spleen
AA	21.17 ± 1.34**	50.95 ± 2.33**
AA-MTX	18.77 ± 1.10	44.81 ± 4.30
AA-PS5	18.78 ± 1.41	53.82 ± 2.38
AA-FS5	20.35 ± 2.13	54.97 ± 2.83
AA-PS50	18.04 ± 0.94	55.38 ± 3.56
AA-FS50	18.34 ± 0.80	51.30 ± 3.15
AA-PS50-MTX	18.04 ± 1.16	51.85 ± 2.43
AA-FS50-MTX	18.77 ± 1.11	44.99 ± 5.16

HC – control group, AA – adjuvant arthritis group, AA-MTX – adjuvant arthritis group administered Methotrexate 0.3 mg/kg twice a week, AA-PS5/AA-FS5 – adjuvant arthritis group administered PS/FS at dose of 5 mg/kg, AA-PS50/AA-FS50 – adjuvant arthritis group administered PS/FS at dose of 50 mg/kg, AA-PS50+MTX and AA-FS50+MTX – adjuvant arthritis group administered PS or FS (50 mg/kg) and Methotrexate 0.3 mg/kg twice a week. Results are expressed as mean ± SEM, n=8-10. Significant difference: \*\*p<0.01 AA vs. HC

without statistical significance (Table 3). Application of PS and FS did not have such significant impact; however, application of PS in higher dose (50 mg/kg) showed

the most potent effect in joint and FS in higher dose (50 mg/kg) showed the most potent effect in spleen tissue.

#### Interleukin-17A in plasma

The level of IL-17A in untreated AA group increased approximately 5.24 times and was highly significant when compared to a group of healthy control animals on day 14 (\*\*p<0.01 AA vs. HC, Fig. 3). This trend was also observed on day 21 and 28, where the levels of IL-17A were increased in untreated AA group comparing to HC but without statistical significance.

On day 14 in the group treated with MTX alone the plasmatic levels of this cytokine decreased highly significant comparing to untreated AA group (<sup>++</sup>p<0.01 AA+MTX vs. AA); however, without significant effect on days 21 and 28.

Further, in the group of animals administered with PS alone in higher dose (50 mg/kg) the level of IL-17A was significantly decreased on day 14 (<sup>+</sup>p<0.05 AA+PS50 vs. AA) and remarkably decreased on days 21 and 28. This effect of PS50 administration alone was comparable with the effect of MTX monotherapy. On day 14, the combination treatment of FS50 and MTX significantly reduced the plasmatic levels of IL-17A compared to the untreated AA group (<sup>+</sup>p<0.05 AA-FS50+MTX vs. AA, Fig. 3) and not significantly during next following experimental days 21 and 28.

## Discussion

Adjuvant arthritis (AA) is one of animal models widely used to study the mechanisms of active substances for preclinical testing of potential anti-inflammatory drugs (van Eden *et al.* 1996, Poništ *et al.* 2015). In the present study, we used AA to test anti-inflammatory properties of herbal preparation with code name PS-551 (PS) administered alone and in combination with Methotrexate (MTX). Then we compared the activity of PS with active substance of herbal para-medical product Fatsiphloginum™ (FS). To evaluate the effectiveness of the substances tested, MTX was chosen as the treatment standard. In our previous experiments, MTX has been established as a good tool to evaluate the efficacy of experimental treatments (Bauerova *et al.* 2015, Drafi *et al.* 2012). Moreover, MTX is the most popular substance used worldwide as either in monotherapy or in combination with other drugs for the effective therapy of autoimmune disease (ACR 2002). The mechanism of action by which MTX exerts its anti-inflammatory and immunosuppressive effects were investigated according to the hypothesis that MTX exerts its effects *via* the production of reactive oxygen species (Phillips *et al.* 2003).

The substances tested (PS and FS) were obtained at the IPK from the plant *Fatsia japonica* cultivated in Georgia. Acute toxicity studies were not conducted with the purified extract in this study as the safety has been established up to 7,440 mg/kg by toxicological experiment (data not published). Two doses 5 mg/kg and 50 mg/kg were selected for PS and FS based on preliminary researches which have showed better response for doses above 5 mg/kg for anti-inflammatory activity.

In the AA model, systemic inflammation is manifested in increased hind paw volume, arthritic score and body weight loss. These parameters start to develop from day 10 after single intradermal injection of suspension of heat-inactivated *Mycobacterium butyricum* in incomplete Freund's adjuvant at the base of the tail (Bauerova *et al.* 2008b). In our experiment, we observed less body weight gain of untreated AA rats compared to healthy control rats, because of cachexia caused by autoimmune processes (Fig. 1). The previous experiments reported that basic parameters, such as HPV and body weight, became markedly worsened due to arthritis and were efficiently decreased by administration of substances with anti-oxidative and/or anti-inflammatory

properties (Bauerova *et al.* 2008a, Bauerova *et al.* 2008b, Bauerova *et al.* 2009). When PS was administered at the dose of 50 mg/kg daily in monotherapy, it did not affect these parameters (HPV, CBW, arthritic score) in comparison to untreated AA animals and for administration of FS it was a similar situation (Fig. 1, Fig. 2, Table 2). The combination of PS and FS on day 14 was as effective as MTX alone in the following parameters CBW, HPV, and particularly the arthritic score.

AA is not only an experimental model of polyarthritis, but it also induces pathological changes in a variety of other tissues. 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 (Bevaart *et al.* 2010).

GGT is an important component of inflammatory processes since its activity is closely connected with the overall antioxidant status of the organism. Elevated expression and activity of GGT in joint tissue is a good marker for synovial inflammation, thus molecules able to reduce the activity of GGT could be promising in therapy. The activity of GGT is considered as a reliable biochemical merit of inflammation and oxidative stress; its increased action in organs such as spleen and joint is believed to be a good indicator of AA development at the systemic and local level (Bauerova *et al.* 2006).

In our study, we observed increased activity of GGT in joint tissues and also in the spleen in untreated animals with induced AA. The activity of GGT was measured in the tissue homogenates of joint and spleen on experimental day 28 (Table 3). Administration of MTX led to slightly decreased values of GGT activity in the spleen and joint. This effect of MTX was reported also in previous experiments with a similar experimental design and setting (Bauerova *et al.* 2010, Drafi *et al.* 2012, Feketeova *et al.* 2012). The results of GGT activity measured in tissues of spleen and joint tissue after treatment with both tested compounds were not significantly affected. For GGT activity in spleen we observed that the combination of FS in dose 50 mg/kg and MTX was more potent than PS. However, in monotherapy PS in dose 50 mg/kg decreased the activity of GGT on day 28 in joint tissue homogenate (Table 3).

Previously, we have shown a good correlation between the GGT activity in joint tissue and HPV of arthritic animals (Bauerova *et al.* 2006). Similarly, as it is shown on Table 3, our measurements are in a good agreement with changes in HPV (Table 2).

In recent years, many researchers have focused on imbalances within the immune system to explain the therapy of rheumatoid arthritis DMARDs (Disease Modifying Anti-rheumatic Drugs), including methotrexate. T-helper cells and their cytokines are important factors in regulating immune balance. Interferon- $\gamma$  (IFN- $\gamma$ ), interleukin-4 (IL-4), and interleukin-17A (IL-17A) represent the hallmarks for Th1, Th2, and Th17 cells, respectively (Mosmann *et al.* 1989, Ivanov *et al.* 2006, Mucida *et al.* 2007, Bettelli *et al.* 2008). MTX has been found to influence cytokine production and inhibits the up-regulations of IL-17A in the co-culture of T cells and fibroblasts (Miranda-Carus *et al.* 2004). Hou *et al.* (2011) hypothesized that MTX could ameliorate pristane-induced arthritis (PIA) by regulating immune balance. Arthritis in Dark agouti (DA) rats was induced by pristane and then treated with MTX. The authors studied the arthritis severity macroscopically and microscopically, and measured the gene expressions of IFN- $\gamma$ , IL-4, IL-17A, transforming growth factor  $\beta$ , and tumor necrosis factor- $\alpha$  in the rat spleens. The authors have found that MTX can reduce the arthritis severity and decrease the mRNA expressions of IFN- $\gamma$  and IL-17A in pristane-induced arthritis of rats. In our study, we also decided to investigate the effect of our substances on the level of IL-17A in plasma during the development of AA. Significantly elevated plasmatic levels of this interleukin were detected on day 14, 21 and 28. One of the mechanisms of MTX anti-rheumatic activity can be explained by its ability to inhibit the production of pro-inflammatory cytokine – IL-17 in AA and RA (Ganesan and Rasool 2017). Signs of the onset of AA are followed by an acute intra-articular elevation of IL-17A, what probably means that IL-17A is more involved in the progression of the disease than in its early phase (Bush *et al.* 2001, Stolina *et al.* 2009). In our study, PS at higher concentration (50 mg/kg) significantly decreased the level of IL-17A on day 14. This effect was comparable with MTX treatment, which was highly significant on day 14 (Fig. 3). We hypothesize, that the effect of PS in higher dose towards IL-17A level in plasma on day 14 could be explained also by possible anti-inflammatory active property of the active substances – triterpene saponins, mostly derivatives of hederagenin and oleanic acid. Similarly to hederagenin molecule (hederagenin is the aglycone part of numerous saponins, e.g.  $\alpha$ -hederin) beneficial effect of  $\alpha$ -hederin (a water-soluble pentacyclic triterpenoid saponin) in ovalbumin-sensitized rats was described, suggesting that  $\alpha$ -hederin affects the IL-17

secretion pathways, altering miRNA-133a expression (Ebrahimi *et al.* 2016). To support our hypothesis of the possible effect of terpenes, it was described, that synthetic triterpenoids also inhibit IL-17 and could improve autoimmune disease in mice (Fitzpatrick *et al.* 2014). Moreover, it was also demonstrated, that oleanic acid (one of the component of PS-551) treatment inhibits dextran sodium sulfate-induced colitis, thus the induced expression of tumor necrosis factor- $\alpha$ , interleukin-1 $\beta$ , and IL-17 *in vivo*. Oleanic acid also inhibited the activation of NF- $\kappa$ B and expression of proinflammatory cytokines in LPS-stimulated peritoneal macrophages *in vitro*. These findings suggest that oleanic acid may ameliorate inflammatory diseases such as colitis by inhibiting Th17 cell differentiation and increasing T-reg cell differentiation (Kang *et al.* 2015). These triterpene saponins were previously described to have anti-inflammatory activities (Kemertelidze *et al.* 2001, Shalamberidze *et al.* 1998). Our results indicate that PS-551 in higher doses in monotherapy could prevent systemic inflammation partly through blocking the production of IL-17 in acute stage of inflammation.

## Conclusions

Based on the results of current study it can be concluded that PS and FS administrated in dose of 5 mg/kg alone daily did not affect the parameters selected during the whole experiment. On the other hand, PS in its highly pure composition administrated alone in dose 50 mg/kg daily had shown a therapeutic effect on the plasmatic level of IL-17A in early phases of AA. However, FS administrated alone in dose 50 mg/kg daily had no significant effect on HPV and arthritic score. The present study showed that generally FS has higher anti-arthritic activity in comparison to PS. In this study, we also tested the novel combination of the active substance of Fatsiphloginum<sup>TM</sup> as well as the extract PS-551 with MTX on the AA model. The combinations of PS and FS in higher doses improved its effect together with MTX in early stages of AA, which was demonstrated on the change of body weight, arthritic score and change of HPV. Moreover, FS in dose 50 mg/kg daily in combination with MTX significantly showed its anti-inflammatory properties on plasmatic level of IL-17A. Our findings indicate that in experimental model of AA higher doses than 50 mg/kg are required to achieve a better anti-rheumatic effect. To have a more explicit understanding of anti-inflammatory and anti-arthritic



activities of substances derived from *Fatsia japonica* further experimental studies with broader spectrum of immunological parameters will be required.

### Conflict of Interest

There is no conflict of interest.

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