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Original article

Effect of aqueous extract from *Scutellaria baicalensis* Georgi roots on CD4$^+$ and CD8$^+$ T cell responses during experimental infection with *Trichinella spiralis* in mice

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Abstract

The aim of this study was to investigate the effect of aqueous extract from *Scutellaria baicalensis* Georgi roots (SB) on blood parameters and immune response during an experimental trichinellosis. A total of 60 mice infected with 200 *Trichinella spiralis* larvae were assigned into two groups. One of them served as a control and the second received SB extract orally from day 5 before infection to day 28 after infection (dpi). Blood was sampled at 7, 14, 21 dpi. Lymphocytes obtained from the spleen and mesenteric lymph nodes (MLN) at 7, 14, 21, and 28 dpi were counted, CD4$^+$ and CD8$^+$ subpopulations were analyzed by flow cytometry, and lymphocyte proliferation was estimated with colorimetric (MTT) assay. The intensity of intestinal and muscle invasion was also studied. SB caused a remarkable elevation of banded neutrophils in the blood at 7 dpi. SB increased ConA-stimulated splenocyte proliferation and CD4$^+$ and CD8$^+$ splenocyte subsets at 14 and 21 dpi, whereas MLN lymphocyte subset stimulation involved only CD4$^+$ at 14 dpi. After administration of SB a downward trend in the number of *T. spiralis* larvae in the muscle was observed. These results suggest that *Scutellaria baicalensis* root extract stimulates murine cellular immune response during intestinal phase of *T. spiralis* infection.

Keywords: *Scutellaria baicalensis*, *Trichinella spiralis*, immunomodulation

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Introduction

Trichinellosis is a food-borne parasitic disease occurring worldwide and triggered by eating raw or undercooked meat containing infective larvae of *Trichinella* nematodes. Trichinellosis involves a presence of adult forms of the parasite in a host intestine and larval forms in their muscles. This presence is accompanied by structural, cellular and physiological changes that initiate an acute inflammatory response. Lymphocytes play an essential role both in an early host response and in subsequent pathogenesis of organ changes. The activation of a cellular or humoral response depends on the activation of a specific subpopulation of T lymphocytes (Th1 or Th2) and the profile of secreted cytokines. Cell composition and activation can be regulated by parasitic factors (Bruschi and Chiumiento 2012).

Scutellaria baicalensis Georgi (Baikal skullcap, SB), a popular Chinese herb, is used in Asia as a food ingredient and a traditional herbal medicine for the treatment of various inflammatory diseases. It contains many active compounds with the most important flavonoids baicalin, baicalein, and wogonin. Baikal skullcap and its ingredients are investigated owing to their anti-inflammatory, anti-allergic, antioxidant, antimicrobial and anticancer properties (Orzechowska et al. 2014, Chu et al. 2015, Liu et al. 2015, Jung et al. 2017, Xiao et al. 2017, Xu et al. 2017, Grzegorczyk-Karolak 2019, Lu et al. 2019). Some of these properties, such as anti-inflammatory and anti-allergic ones stem to a large extent from the effect of Baikal skullcap active ingredients on the immune system. Most of these studies concerning the effect of Baikal skullcap on the immune system focused on lymphocytes and their activity. Some authors concluded that Baikal skullcap and its active flavonoids predominantly inhibit Th2 lymphocyte response (especially through decreasing Th2 cytokine production) and stimulate Th1-mediated response (Shin et al. 2014a, Jung et al. 2017). However, other researchers demonstrated SB-derived inhibition of both Th1 and Th2-mediated response (Liu et al. 2015).

Th2 response is maintained during the intestinal phase and muscle infection by *T. spiralis* and is preceded by a short stimulation of Th1-mediated reaction (Bruschi and Chiumiento 2012). Therefore, the factors that change the Th1/Th2 balance may affect the course of the infection. There is no literature available on the effect of Baikal skullcap on *Trichinella spiralis* infection.

The purpose of our study was to estimate whether the administration of Baikal skullcap aqueous root extract affects $CD4^+$ and $CD8^+$ T cells response in the course of experimentally induced trichinellosis in mice. Moreover, our study may contribute to develop prevention and treatment strategies concerning trichinellosis in humans and animals.

Materials and Methods

Plant material

Scutellaria baicalensis Georgi roots were obtained from an experimental field of the Garden of Medicinal Plants Herbarium at the Medical University of Wrocław, Poland. Plants were grown from authenticated seeds. In the spring, the seeds were sowed in light well-drained sandy soil in partial shade and then were watered weekly. The roots were harvested from 2-year-old plants. Then were thoroughly washed in distilled water and dried under controlled humidity at room temperature (±25°C) until a moisture content 5% of roots. The dried roots were then precisely crushed using a laboratory mill and stored at –20 °C as was described by Króliczewska et al. (2017). Before the study, 5 gram of dried, milled SB roots were lightly boiled in 1000 ml of distilled water for 45 min and then filtered using a 0.45-µm filter. Analysis of polyphenols of the aqueous extract of *Scutellaria baicalensis* Georgi roots was done at the Department of Fruit, Vegetable and Cereal Technology, Wrocław University of Environmental and Life Sciences.

Chemical analysis of aqueous extract of *Scutellaria baicalensis* Georgi roots

All analyzes of polyphenols in the aqueous extract were carried out using an ACQUITY Ultra Performance LC system (UPLC) equipped with a binary solvent manager (Waters Corp., Milford, MA, USA), a UPLC BEH C18 column (1.7 µm, 2.1 mm × 50 mm, Waters Corp., Milford, MA, USA), and a Q-Tof Micro mass spectrometer (Waters, Manchester, UK) with an ESI source operating in negative and positive modes. During the extraction and determination of phenolic compounds, we used protocol previously described by Lachowicz et al. (2019). The mobile phase consisted of solvent A (0.1% formic acid, v/v) and solvent B (100% acetonitrile). The chromatogram was recorded at 280 nm, 320 nm, and 340 nm and spectral data for all peaks were accumulated in the range of 200–600 nm in steps of 2 nm. All of the results were expressed as mg/L. Leucine enkephalin was used as the reference compound at a concentration of 500 pg/µL, at a flow rate of 2 µL/min. The analysis was carried out using full-scan, data-dependent MS scanning from *m*/*z* 100 to 1500. The data obtained from UPLC−MS were subsequently entered into the MassLynx 4.0 ChromaLynx

Table 1. Chemical analysis of aqueous extract of *Scutellaria baicalensis* Georgi roots.

Compound name	Retention time (min)	λ (nm)	MS-MS fragments	Concentration (mg/L)
Chrysin-7-O-glu-8-C-glu	5.26	272/ 313	577/267	4.12
Apigenin-6-C-glu-8-C-ara	5.98	272/330	563/297	0.95
Chrysin-6. 8-di-C-glu	6.2	272/314	577/267	2.62
6-C-ara-8-C-glu chrysin	6.59	272/314	547/457/367	17.89
6-C-glu-8-C-ara chrysin	6.7	272/314	547/457/367	17.36
6-C-glu-8-C-ara chrysin	7.1	272/314	547/457/427/367	2.53
Baicalin	9.03	273/313	445/269	512.39
Wogonin 5-O-d-glu	9.31	273	445/269	33.99
Wogonoside	9.48	273	459/283	4.40
Wogonin-7-O-glu-glucoronide	9.86	273	621/268	0.23
Oroxylin A-7-O-β-D-glucuronide	10.1	271/ 311	/919/651/459/283/267	39.63
Wogonoside	10.55	273	/919/459/283/267	176.44
Norwogonin	11.78	273	269	1.16
Baicalein	12.15	273/313	269	14.97

Application Manager software (Waters Corp., Milford, MA, USA). The results concerning polyphenolic compounds in the aqueous extract are summarized in Table 1.

Animals and parasite

The experiment involved male and female CFW mice (n=60), 8-10 weeks old, weighing approximately 25-30 g, derived from a mouse breeding laboratory at the Faculty of Veterinary Medicine (Wrocław University of Environmental and Life Sciences, Poland). The animals had unlimited access to food and tap water. They were housed in an air-conditioned room (23±2°C) with a 12 h light/12 h dark cycle. The mice were orally infected (by stomach-tube) with 200 *T. spiralis* larvae.

The *Trichinella spiralis* isolate (T1, ISS1820, Poland) was maintained by serial passages in CFW inbred mice at the Division of Parasitology, Wroclaw Faculty of Veterinary Medicine. The infective larvae were recovered from the muscle tissue of mice infected two to three months earlier via digestion with 1% pepsin/HCl solution for 1 hour at 37ºC.

All efforts were made during the experiments to minimize animal suffering. The minimum number of animals was involved to produce statistically reproducible results of our study. The European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, as well as national and institutional guidelines for the care and use of laboratory animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the Wroclaw University of Environmental and Life Sciences were the studies were conducted. These experimental procedures were approved by the II Local Ethics Committee for Experiments on Animals in Wrocław, Poland (approval number 61/2013).

Administration of aqueous extract of *Scutellaria baicalensis* roots and experimental design

The freshly prepared aqueous extract of *Scutellaria baicalensis* Georgi roots (as was described in "Plant Material" subsection) was administered orally (*ad libitum*, instead of drinking water), daily starting 5 days before infection and ending 28 days post infection (dpi) with *T. spiralis*.

This study included two groups of mice (30 mice in each) infected by *T. spiralis* larvae.

Group T: infected untreated (control group) and group T+SB: infected and receiving SB.

Six mice of each group were killed on 7, 14, 21, 28 and 60 dpi.

Determination of lymphocyte subsets from spleen and mesenteric lymph nodes

At 7, 14, 21 and 28 dpi the lymphoid organs (spleen and mesenteric lymph nodes - MLN) were removed and lymphocytes were isolated as described previously (Szczypka and Obmińska-Mrukowicz 2010). The lymphocytes in a suspension (at 1×10^7 cells/ml) were stained with monoclonal rat anti-mouse CD4:FITC/CD8:RPE dual color antibodies (Serotec, Kidlington, UK), according to the manufacturer's protocol. Then they were incubated (4ºC, 30 min), washed and centrifuged (380 xg, 8 min, 4ºC) twice with ice-cold PBS. Fluores-

Table 2A. The percentage of $CD4^+$ and $CD8^+$ T cells in the spleen of untreated mice infected with *T. spiralis* (Group T) and mice receiving aqueous extract of *Scutellaria baicalensis* root infected with *T. spiralis* larvae (Group T+SB). Mean values (n=6) and standard deviations are presented.

	Day after infection	$CD4^+$	$CD8^+$	$CD4^+/8^+$
		$\bar{x}$±SD	$\bar{x}$±SD	$\bar{x}$±SD
Group T+SB	7	19.5±6.77	5.2±1.66	3.7±0.23
	14	**27.3±5.00	**8.2±1.75	3.5± 0.98
	21	*38.9±2.38	11.3±260	3.6±1.04
	28	37.8±2.03	10.4±1.15	3.7±0.35
Group T	7	16.1±2.10	4.6±1.10	3.6±0.70
	14	14.7±0.95	5.4±0.42	2.7±0.22
	21	33.2±3.88	9.8±2.50	3.5±0.82
	28	38.4±2.65	10.3±1.15	3.8±0.34

* p<0.05, ** p<0.01

Table 2B. The absolute count (cells x10^6) of $CD4^+$ and $CD8^+$ T cells in the spleen of untreated mice infected with *T. spiralis* (Group T) and mice receiving aqueous extract of *Scutellaria baicalensis* root infected with *T. spiralis* larvae (Group T+SB). Mean values (n=6) and standard deviations are presented.

	Day after infection	$CD4^+$	$CD8^+$
		$\bar{x}$±SD	$\bar{x}$±SD
Group T+SB	7	36.9±17.3	9.9±4.2
	14	**87.9±23.4	27.2±11.4
	21	82.9±10.7	24.4±6.8
	28	94.0±17.6	26.2±7.4
Group T	7	37.9±4.0	10.9±2.2
	14	45.0±8.0	16.4±2.0
	21	91.1±24.5	27.7±12.4
	28	101.8±18.3	27.3±5.6

* p<0.05 ** p<0.01

cence was measured with BD FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA). The acquired flow-cytometric data were analyzed using CellQuest Pro software. $CD4^+$ and $CD8^+$ lymphocyte subsets (percentage and total lymphocyte count from individual mice) as well as $CD4^+/CD8^+$ ratio in the spleen and mesenteric lymph nodes were determined.

Lymphocyte proliferation studies

The spleen and mesenteric lymph node lymphocytes at a concentration of 4 x 10^5 cells per 150 µl final volume were plated into 96-well microtiter plate (Costar 3596, Corning Incorporated, USA). The culture was maintained in RPMI 1640 Medium (R6504 Sigma-Aldrich, USA) supplemented with $NaHCO_3$, HEPES 5 mM, sodium pyruvate 1 mM, gentamycin (50 mg/l, Polfa, Tarchomin, Poland) and heat-inactivated fetal bovine serum (10%) (Gibco, No 26010074, New Zealand). Concanavalin A (Con A; Sigma-Aldrich, USA) at the final working concentrations of 0.9 and 0.45 µg/mL was added and cultured for 72 h at 37ºC and 5% CO_2. Colorimetric lymphocyte proliferation was evaluated using MTT (3-(4, 5-dimethyl thiazol--2-yl) 2, 5-diphenyl tetrazolium bromide) method. At 3 h before the end of the incubation, 25 µl MTT (5 mg/ml; Sigma-Aldrich, USA) were added to each well and plates were further incubated at 37ºC in 5% CO_2 humidified atmosphere. Then, 125 µl of lysis buffer (13% SDS, 40% N,N-DMF, pH 4.7) were added, and the entire sample was incubated under the same conditions for the next two hours. Absorbance was then measured at 540 nm against reference wavelength of 620 nm and proliferation index (PI) was determined. The PI is expressed with average optical densities (OD) value for mitogen-stimulated cells by average OD for the control (non-stimulated cells).

Hematological analyses

The mice were anesthetized with isoflurane (Forane, Aesica Queenborough Limited, Queenborough, UK) at 7, 14 and 21 dpi. Blood samples were taken from each animal by cardiac puncture and transferred into

Table 3A. The percentage of $CD4^+$ and $CD8^+$ T cells in the mesenteric lymph nodes (MLN) of untreated mice infected with *T. spiralis* (Group T) and mice receiving aqueous extract of *Scutellaria baicalensis* root infected with *T. spiralis* larvae (Group T+SB). Mean values (n=6) and standard deviations are presented.

	Day after infection	$CD4^+$	$CD8^+$	$CD4^+/8^+$
		$\bar{x}$±SD	$\bar{x}$±SD	$\bar{x}$±SD
Group T+SB	7	43.7±8.10	8.5±1.85	5.3±1.42
	14	**58.4±2.36	13.6±1.81	**4.3±0.51
	21	58.3±7.09	14.6±1.26	4.0±0.23
	28	61.3±2.43	15.1±0.89	4.1±0.23
Group T	7	35.3±1.38	8.9±1.53	4.1±0.68
	14	40.2±6.75	16.1±2.22	2.6±0.66
	21	56.7±5.48	14.5±1.71	3.9±0.22
	28	60.8±2.27	15.6±0.99	3.9±0.28

* p<0.05, ** p<0.01

Table 3B. The absolute count (cells x10^6) of $CD4^+$ and $CD8^+$ T cells in the mesenteric lymph nodes (MLN) of untreated mice infected with *T. spiralis* (Group T) and mice receiving aqueous extract of *Scutellaria baicalensis* root infected with *T. spiralis* larvae (Group T+SB). Mean values (n=6) and standard deviations are presented.

	Day after infection	$CD4^+$	$CD8^+$
		$\bar{x}$±SD	$\bar{x}$±SD
Group T+SB	7	**37.4±5.3	7.5±2.4
	14	39.8±4.5	*9.2±0.7
	21	29.8±8.1	7.5±2.0
	28	*25.9±4.2	*6.4±1.0
Group T	7	27.7±3.7	6.9±1.2
	14	40.3±9.8	16.6±5.6
	21	35.7±6.2	9.1±1.3
	28	41.0±13.0	10.6±3.8

* p<0.05, ** p<0.01

tubes with hematology anticoagulant ethylenediaminetetraacetic acid (EDTA). Hematological parameters were explored with a hematology analyzer (PE-6800 Procan Electronics Inc., China) that determined red blood cell (RBC) count, hemoglobin (HGB) concentration, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC) count, platelet (PLT) count, as well as individual leukocyte counts. As the apparatus differentiated only three white blood cell populations, manual morphology was performed in blood smears, calculating the percentages and absolute values obtained from the WBC. A total of 200 cells in the blood smear were counted and differentiated to classify individual leukocyte types like neutrophils, eosinophils, lymphocytes and monocytes.

Parasitological studies

At 7, 14 and 21 dpi adult parasites were isolated and counted by incubation of small intestines in 0.9 % NaCl at 37°C in Baermann funnels overnight. At 60 dpi the number of muscle larvae was examined by artificial digestion of the whole eviscerated and minced mice carcasses (according to above mentioned method).

Statistical analysis

The data were subjected to t-Student's test or Mann-Whitney U test to analyze and determine their statistical significance. P-values <0.05 were considered significant. Results were shown as means ± SD (standard deviation). Calculations were carried out using STATISTICA ver. 13.0 software package.

Results

Effects of aqueous extract of Baikal skullcap roots on the subpopulations of splenocytes and lymphocytes of mesenteric lymph nodes

SB administration increased the percentage and absolute count of $CD4^+$ splenocytes as well as the per-

Table 4A. Proliferative response of splenocytes to ConA. Mean values (n=6) and standard deviations are presented.

Spleen		Index 0.9	Index 0.45
Group	Day after infection	$\bar{x}$±SD	$\bar{x}$±SD
Group T+SB	7	2.76±0.95	2.57±0.78
	14	**3.31±0.84	**2.80±0.92
	21	**2.11±0.31	**1.16±0.24
	28	1.97±0.39	1.59±0.34
Group T	7	3.54±2.08	2.84±1.28
	14	1.96±0.59	1.64±0.47
	21	1.82±0.39	1.48±0.22
	28	2.37±0.82	1.77±0.54

*p<0.05, **p<0.01

Table 4B. Proliferative response of MLN cells to ConA. Mean values (n=6) and standard deviations are presented; nt = not tested.

MLN		Index 0.9	Index 0.45
Group	Day after infection	$\bar{x}$±SD	$\bar{x}$±SD
Group T+SB	7	5.87±0.70	4.67±1.89
	14	6.97±1.4	3.29±1.08
	21	6.45±2.07	5.21±1.84
	28	5.87±1.83	3.96±1.23
Group T	7	nt	nt
	14	9.49±4.02	4.28±1.57
	21	5.91±1.72	4.19±1.64
	28	7.0±1.56	4.42±1.16

* p<0.05, ** p<0.01

centage of $CD8^+$ cells as compared with the group infected by *T. spiralis* that did not receive SB. These changes were present at 14 dpi. This stimulating effect of SB on the percentage of $CD4^+$ splenocytes persisted up to 21 dpi. No differences in the splenocyte subpopulations were observed between Group T and Group T+SB at 7 and 28 dpi (Tables 2A and 2B).

In MLN, an increase in the absolute count and the percentage of $CD4^+$ cells was observed at 7 and 14 dpi, respectively. A rise in the percentage of $CD4^+$ cells with a simultaneous decrease in $CD8^+$ lymphocyte subset (better visible as a decrease in total $CD8^+$ cell count) caused an increase of $CD4^+/CD8^+$ ratio (14 dpi). No changes in MLN lymphocyte subsets were observed at 21 dpi. However, at 28 dpi, contrary to 14 dpi, a decrease in the absolute count of $CD4^+$ as well as $CD8^+$ cells was noticed (Tables 3A and 3B).

Effects of aqueous extracts of Baikal skullcap roots on lymphocyte proliferation

Administration of SB increased proliferation of ConA-stimulated splenocytes in mice infected with *T. spiralis* at 14 and 21 dpi in the presence of all used mitogen (ConA) concentrations.

There were no significant differences in MLN cell proliferation index following exposure to SB preparation (Tables 4A and 4B).

Effects of aqueous extracts of Baikal skullcap roots on hematological parameters

Table 5 presents assessed hematological parameters. We found no significant differences between groups for most of the examined blood cell indicators. At 7 dpi a significant SB-caused increase occurred both for the percentage and absolute number of banded neutrophils in *T. spiralis* infected mice.

Parasite burden – number of adults and muscle larvae

There was no significant difference in the number of intestinal parasites between the examined groups (Group T+SB and Group T) (Table 6). Due to high individual variability, no significant differences in the number of muscle larvae were found, but a downward trend can be perceived in Fig. 1.

Table 5. Blood parameters in the mice infected with *T. spiralis* (Group T), and infected with *T. spiralis* and receiving the root of *Scutelaria baicalensis* aqueous extract (Group T+SB). Mean values (n=6) and standard deviations are presented.

Parameter	7 dai		14 dai		21 dai	
	T+SB	T	T+SB	T	T+SB	T
RBC (10^6/µl)	8.40±0.39	7.89±0.65	7.34±0.33	7.34±0.41	8.50±0.54	8.55±0.14
HGB (g/dL)	17.25±1.03	16.78±1.20	15.40±0.75	15.18±0.80	17.43±1.21	17.78±0.29
HCT (%)	51.23±2.81	48.18±3.35	48.53±1.99	46.50±1.59	53.40±2.78	56.05±1.17
MCV (fL)	61.02±0.78	61.23±3.71	66.13±1.01	63.52±2.70	62.98±2.11	65.63±1.10
MCH (pg)	20.47±0.57	21.23±0.64	20.90±0.40	20.67±1.00	20.47±0.53	20.77±0.25
MCHC (g/dL)	33.62±1.04	34.83±1.60	31.70±0.26	32.60±0.73	32.57±0.80	31.68±0.30
WBC (10^3/µl)	14.77±4.04	14.93±4.73	12.10±1.51	13.93±4.09	10.70±1.80	9.32±1.32
B (%)	**4.75±3.22	0.67±0.82	1.33±1.44	1.42±0.66	1.42±1.11	1.67±1.37
B (10^3/µl)	**0.78±0.68	0.08±0.10	0.16±0.17	0.21±0.13	0.16±0.13	0.16±0.14
S (%)	31.00±16.86	42.00±17.15	24.83±8.52	24.58±7.16	21.92±13.09	20.58±4.68
S (10^3/µl)	5.00±3.90	5.83±1.60	3.05±1.34	3.22±0.44	2.47±1.75	1.96±0.67
Eos (%)	1.50±0.84	0.83±0.61	3.33±1.61	1.42±1.02	10.83±4.18	10.50±3.32
Eos (10^3/µl)	0.25±0.19	0.14±0.12	0.39±0.17	0.22±0.21	1.20±0.65	0.95±0.23
Lymp (%)	61.33±20.26	55.92±17.56	68.17±5.58	71.50±6.58	65.67±15.04	66.75±3.11
Lymp (10^3/µl)	8.52±2.33	8.82±4.92	8.23±1.01	10.15±3.80	6.85±1.00	6.21±0.84
M (%)	1.42±0.38	0.58±0.74	1.67±1.15	0.75±0.42	0.17±0.26	0.33±0.41
M (10^3/µl)	0.22±0.11	0.07±0.09	0.19±0.11	0.11±0.08	0.02±0.03	0.03±0.03
PLT (10^3/µl)	688±197	697±120	574±119	725±195	711±110	709±43

Abbreviations: RBC – red blood cell count; HGB – hemoglobin; HCT – hematocrit; MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; WBC – white blood cell count; B – banded neutrophils; S – segmented neutrophils ; Eos – eosinophils; Lymp – lymphocytes; M – monocytes; PLT – platelet count;
* $p<0.05$, ** $p<0.01$

Table 6. Effect of SB on the number of adults of *Trichinella spiralis* in intestine of infected mice. Mean values (n=6) and standard deviations are presented.

	dai	T	T+SB
		$\bar{x}$±SD	$\bar{x}$±SD
No. of adults of *T. spiralis*	7	21.5±22.3	28.2±14.8
	14	3.3 0	0 0
	21	0 0	0 0

Discussion

Immune response to trichinellosis depends on T helper cells ($CD4^+$ lymphocytes) (Bruschi and Chiumiento 2012). Our study showed that aqueous extract of *Scutellaria baicalensis* roots may be beneficial for *T. spiralis* infected mice due to its stimulating effect on $CD4^+$ lymphocyte subset observed both in the spleen and MLN.

During *T. spiralis* infection, type 2 immune response predominates. It is mediated by Th2 lymphocytes that secrete cytokines (IL-4, 5, 9, 13) stimulating proliferation of B lymphocytes and immunoglobulin synthesis, whereas Th1-mediated reaction that promotes cellular immune response including cytotoxic T lymphocytes ($CD8^+$ cells) is suppressed (Bruschi and Chiumiento 2012). In this study, we demonstrated a drop in $CD8^+$ lymphocytes in MLN in *T. spiralis* infected mice treated with *Scutellaria baicalensis* root extract, which resulted in increased $CD4^+/CD8^+$ ratio. Given these *Scutellaria* induced changes, it seems likely that the plant extract may downregulate Th1-mediated response.

Many studies focused on the effect of skullcap (*Scutellaria baicalensis*) or its main active compounds, baicalin, baicalein and wogonin, on Th1/Th2 balance, and especially cytokine production by Th1 and Th2 lymphocytes. However, the studies brought about ambiguous conclusions. Some showed that skullcap and its active compounds, e.g. wogonin, suppress cytokine production by Th2 lymphocytes (IL-4, IL-5, IL-10, IL-13), hereby inhibiting Th2-mediated immune

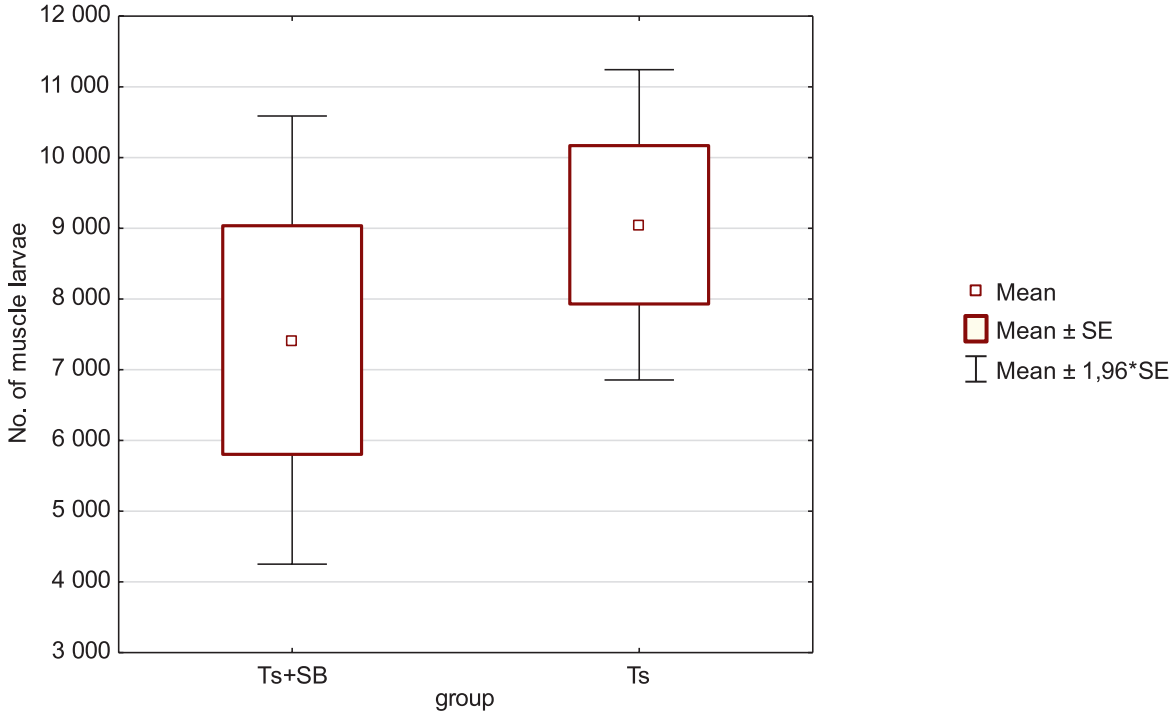

Fig. 1. Number of muscle larvae in the mice infected with *T. spiralis* (Group T), and infected with *T. spiralis* larvae and receiving aqueous extract of *Scutellaria baicalensis* root (Group T+SB).

response (Lim 2004, Shin et al. 2014a,b, Jung et al. 2017). Moreover, they were supposed to increase Th1 cytokine synthesis: IFN-γ, IL-2, IL-12 (Lim 2004, Chu et al. 2015, Jung et al. 2017). Other authors published contrary findings: a decrease in IFN-γ production and increase in Th2 cytokine synthesis (IL-4, IL-10) (Zeng et al. 2007, Błach-Olszewska et al. 2008, Zhu et al. 2012). Also Kim et al. (2013) demonstrated impaired activation of Th1-dependent immune response by baicalin-treated dendritic cells.

Results of our study allow us to notice that the inhibition of the Th1 immune response in mice treated with SB was accompanied by an elevation of the Th2 immune response at 7 dpi, which promoted adult worm expulsion at the intestinal stage. Regarding the splenocyte subpopulation, *Scutellaria* mediated stimulating effect on $CD4^+$ and $CD8^+$ splenocytes observed in our study in *T. spiralis* infected mice confirmed the findings of Zhu et al. (2012), who reported that baicalin increased the count of $CD4^+$ and $CD8^+$ T cells in the spleen in a murine model of polymicrobial sepsis.

The MTT assay is a commonly used method to detect cell proliferation. In this study, the administration of SB induced proliferative response of the spleen in mice infected with *T. spiralis*. Our results confirmed those obtained by Gong et al. (2011), who found in an *in vitro* study (MTT test) that baicalin increased proliferation of human T and B cells. Contrary to that, Zeng et al. (2007) demonstrated in MTT assay that baicalin suppressed proliferation of mononuclear cells from lymph nodes of mice with experimentally induced autoimmune encephalomyelitis.

Results of blood examination demonstrated a significant SB-caused increase in the percentage and absolute number of banded neutrophils in *T. spiralis* infected mice at 7 dpi. Neutrophils play a key role in the innate immune system responses including controlling of helminths infection levels. The functions of neutrophils is to recognize and phagocytose microbes, and then to kill pathogens through a combination of cytotoxic mechanisms (Mortan et al. 2018).

In the present study, the increase of neutrophils at 7 dpi may be associated with the expulsion of worms by killing newborn larvae in antibody-dependent cell-mediated cytotoxicity (ADCC) systems (Falduto et al. 2015). This indicated a modulatory effect of the administered SB-extract as compared to previous *in vitro* studies that confirmed the inhibitory effects of glycoprotein derived from *T. spiralis* on neutrophils (Bruschi et al. 2000).

The stimulatory effect of flavonoids from *S. baicalensis* was visible during bacterial infection. Baicalin, flavonoid compound isolated from *S. baicalensis*, protected mice from *Escherichia coli* infection (Zhang et al. 2017). Baicalein from Baikal skullcap roots significantly inhibited colonization and growth of *Listeria monocytogenes* (LM), prevented LM-induced cell injury and mice fatality, and might

be a safe and potentially efficient therapeutic for listeriosis (Lu et al. 2019).

Numerous studies demonstrated antiparasitic properties of aqueous extracts of *S. baicalensis* administered during protozoan infection. Treatment with the extract prolonged survival, reduced parasite burden, lowered liver histopathological score, and enhanced Th1 response in mice infected with *T. gondii* (Yang et al. 2012). Flavonol baicalein, the major bioactive component of *S. baicalensis*, significantly inhibited proliferation and viability of *Trypanosoma cruzi* and *Leishmania donovani* (Schinella et al. 2002, BoseDasgupta et al. 2008, Lacombe et al. 2014). There is no conclusive evidence of direct anthelmintic effect of Baikal skullcap and its ingredients. The results of our research indicated only a visible trend of downregulating the muscle larvae of *T. spiralis*. Baicalein is used for the treatment of eosinophilic meningitis induced by a nematode *Angistrongylus cantonensis*. The combinatory treatment of albendazole and baicalein exhibited synergistic beneficial effects in mice infected with this nematode that involved prolonged survival time, reduced worm burden and hypersensitivity reaction (He et al. 2011).

Conclusions

This study suggests that in the course of experimentally-induced trichinellosis in mice, aqueous extract of *Scutellaria baicalensis* roots (SB) may downregulate Th1-mediated response. *Scutellaria* plant extract caused a remarkable elevation of banded neutrophils in the blood at 7 dpi, altered the percentage and absolute number of $CD4^+$ and $CD8^+$ T cells in the spleen, and MLN and proliferative activity of splenocytes. The study results demonstrated a drop in T $CD8^+$ lymphocytes in MLN in *T. spiralis* infected mice treated with *Scutellaria baicalensis* root extract, which resulted in increased $CD4^+/CD8^+$ ratio. Although the effects of SB on immune response did not expressly affect the intensity of the parasitic invasion, its immunomodulatory properties could be used to alleviate symptoms associated with trichinellosis, which of course requires further research.

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