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Influence of polysaccharide fraction C isolated from *Caltha palustris* L. on T and B lymphocyte subsets in mice

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Abstract

Extracts from a diverse range of plants have been shown to possess immunomodulatory properties. Caltha palustris L. (Ranunculaceae) is a plant widely known and distributed in Europe, Asia and North America. The extracts from Caltha palustris have been used in traditional Canadian and Asian medicine to treat arthritis rheumatism, gonorrhea and a variety of skin diseases. The effects of polysaccharide fraction C from Caltha palustris L. extract (0.1, 1 and 10 mg/kg) on the total number of lymphocytes in the thymus, spleen and mesenteric lymph nodes and the percentage and the absolute number of T cells ($CD4^-CD8^-$, $CD4^+CD8^+$, $CD4^+$, $CD8^+$) in the thymus and T cells ($CD3^+$, $CD4^+$, $CD8^+$) and B ($CD19^+$) lymphocytes in the spleen and mesenteric lymph nodes in mice were studied. The investigated substance was administered intraperitoneally once or five times to mice. The measurements were determined twice: on days 1 and 3 after last administration of fraction C. It was found that five times administration of fraction C from Caltha palustris L. extract significantly increased the absolute count and the percentage of $CD4^+$ thymic cells irrespective of the dose applied. Moreover, five exposures to fraction C (1 and 0.1 mg/kg) increased percentage of $CD4^+$ splenocytes. Multiple administration of examined fraction C increased the B lymphocyte population ($CD19^+$ cells) in spleen and mesenteric lymph nodes. The results of the study showed that fraction C from Caltha palustris extract is able to change the percentage and absolute number of T and B lymphocytes in lymphatic organs. The effect of the examined substance depends on the number of consecutive doses applied.

Key words: *polysaccharide, Caltha palustris L., extract, B and T lymphocyte subsets, mice.*

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Introduction

Natural products have been shown to be an excellent and reliable source for the development of new drugs [1]. A large number of plant extracts used in traditional medicine have been shown to possess nonspecific immunomodulating activities [2]. Thus, they can be an alternative source of bioactive agents of medical significance. Remedies derived from *Echinacea* sp. might serve as an example. They have been widely used as commercial formulations, but their immunomodulatory properties are still under investigation [3].

Caltha palustris L. (Ranunculaceae) is a plant widely known and distributed in Europe, Asia and North America. The extracts obtained from *Caltha palustris* have been used in traditional medicine: it has been used to treat arthritis [4] leprosy, rheumatism, gonorrhea [5], and a variety of skin lesions [6]. Some studies demonstrated that water extract of *Caltha palustris* presents slight oncostatic activity against some mice tumors (Sarcoma S-180, Ehrlich carcinoma) [7]. Isolation of the polysaccharide fractions of tested plant and their chemical and physical properties were described [8].Also, some of their biological effects were studied *in vitro* [9]. It was demonstrated that fraction C

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stimulated both lymphocyte and the granulocyte activation, whereas it did not exert the effect on the proliferation of human leukemic cell cultures. Fraction C from *Caltha palustris* L. extract enhanced *in vitro* proliferative activity of PHA-induced human lymphocyte, and elevated the granulocytes H_2O_2 production and release in the cultures without the addition of PHA.

This study examined the *Caltha palustris* polysaccharides fraction C in *in vivo* tests. Specifically, the immunomodulatory effect on T cell subsets in thymus and T and B lymphocytes in the spleen and mesenteric lymph nodes were examined and characterized.

Material and methods

Plant material and preparation of polysaccharide fraction

The plant material was collected near Trzebnica (West Poland). The dried, powdered material (1000 g) was extracted with methanol (5L) for 48 h and afterwards, the solution was discarded. After evaporation of the dissolvent, the residual herb was extracted with distilled water to obtain water extract (A) and residue. Then, the residue was extracted with 0.1 M NaOH to obtain extract B and another residue which was extracted with 5% CH_3COOH to obtain extract C. Extract C was reduced *in vacuo* to 2L without heating. Next, it was centrifuged and the supernatant was treated with TCA and then with acetone. Fraction C, obtained from 5% CH_3COOH extract tested positive for saccharides in reaction with phenol reagent [10] and was found to contain polysaccharides of molecular weight 3,6 × 104 Da and 2.5 ×10^4 Da (Biogel-P10 – gel permeation chromatography). Total sugar content (39%) was determined as anhydroglucose after Dubois *et al*. [10], uronic acids (4.8%) by the Blumenkrantz method [11]. Investigated fraction also contained a significant quantity of ash –24%.

A stock solution of the plant extract fraction was prepared *ex tempore* by dissolving 2 mg of fraction C in phosphate-buffered saline solution (PBS, Institute of Immunology and Experimental Therapy, Wrocław, Poland).

Animals

The studies were conducted on male and female Balb/c mice (8-10 weeks of age), each weighing 20-22 g. The mice were obtained from a breeding laboratory at the Medical University, Wrocław, Poland. The animals were fed a commercial granulated food and water *ad libidum* and kept under the conventional conditions. The Local Ethics Committee approved the studies (No. 22/2008).

Drugs and treatment

The solution of fraction C from *Caltha palustris* extract at doses of 10, 1, and 0.1 mg/kg, was administered intraperitoneally, once or five times at 24 h intervals. The volume of each dose was 0.2 ml/mouse. The trials in control group were conducted in parallel. The mice in the control group were treated with phosphate buffered saline – PBS (0.2 ml/mouse). Each control and experimental group consisted of seven mice.

Measurements

The following measurements were taken:

- the total number of thymocytes, splenocytes and lymphocytes of the mesenteric lymph nodes,
- the weight ratio of the thymus, spleen, mesenteric lymph nodes calculated according to the following formula: weight of organ (g)/body weight of mouse (g) × 100,
- the percentage and count of CD subsets ($CD4^-CD8^-$, $CD4^+$ $CD8^+$, $CD4^+$, $CD8^+$ in thymus, $CD19^+$, $CD3^+$, $CD4^+$, $CD8^+$ in spleen and mesenteric lymph nodes).

The measurements were determined at two time points: 24 (day 1) and 72 h (day 3) after last administration of fraction C from *Caltha palustris* extract.

Assay of thymocyte, splenocyte and lymphocyte of mesenteric lymph node subpopulations

The mice were anesthetized with halothane, (Narcotan, Zentiva, Prague, Czech Republic), and killed by cervical dislocation 24 and 72 h after last administration of fraction C from *Caltha palustris* extract. Thymus, spleens and mesenteric lymph nodes were removed and placed in disposable Petri dishes containing sterile, ice-cold PBS. The suspended cells were removed from the lymphatic organs by gentle passage through a nylon mesh and then centrifuged (2250 × g, 15 min, 4°C) on a layer of Ficoll 400 (Pharmacia, Fiene Chemicals AB, Sweden)/Uropolinum 75% (diatrizoate sodium and meglumine diatrizoate; Polpharma, Poland) in a 1 : 3 ratio at a density of 1.071. After centrifugation, the cells were collected from the interphase and washed twice (375 × g, 8 min, 4°C) with PBS supplemented with 1% bovine serum albumin (BSA, Sigma, USA) at 4°C. After the second wash, the cells were resuspended in PBS with 1% BSA at 1 × 10^7 cells/ml. The viability of each cell suspension, as determined by trypan blue dye exclusion, was 90-95%. The cells were resuspended in 100 µl PBS solution containing 1% BSA. The thymocytes, splenocytes and lymphocytes of mesenteric lymph nodes were stained with the monoclonal antibodies: Rat Anti Mouse CD4 : FITC/CD8 : RPE dual color reagent (Serotec, Kidlington UK), at the dilutions recommended by the manufacturer. The splenocytes and the lymphocytes of the mesenteric lymph nodes were also stained with the antibodies: Rat Anti Mouse CD19 : FITC/CD3 : RPE dual color reagent (Serotec, Kidlington. UK), according to the manufacturer's instruction.

Cells were incubated at 4°C for 30 min and then washed 3 times with ice-cold PBS. Fluorescence was analyzed using

a flow cytometer (FACS Calibur; Becton Dickinson, Germany). Lymphocyte marker distribution was analyzed using the Cell Quest 3.1f software.

Statistical analysis

The data obtained in the study were analyzed statistically using a t-test. The differences were considered significant at $p < 0.05$.

Results

As can be seen in Table 1, a single administration of fraction C from *Caltha palustris* extract at all three doses (0.1 mg/kg, 1 mg/kg, 10 mg/kg), on day 1 after injection, temporarily but significantly decreased the percentage and the absolute count of mature $CD8^+$ thymic cells. Whereas 3 days after a single administration, the significant increase of the percentage of the $CD8^+$ cells in thymus at the doses of 10 and 0.1 mg/kg was found (Table 1). Further, 1 day after the administration of a single dose of 10 mg/kg, the decrease of the total number of thymocytes was observed. It was followed by the decrease of absolute count of immature $CD4^+$ $CD8^+$ thymocytes (double-positive cells).

As can be seen in Table 1, five times administration of fraction C from *Caltha palustris* extract increased significantly the absolute count and the percentage of $CD4^+$ thymic cells, irrespective of the dose applied. Additionally, the increase of total number of thymocytes was observed on day 1 after the last administration of five injections at doses 1 and 0.1 mg/kg of fraction C.

No changes in the absolute count and the percentage of immature $CD4^-$ $CD8^-$ (double-negative cells) thymocytes were observed.

Single administration of fraction C from *Caltha palustris* extract at a dose of 10 mg/kg increased the percentage and absolute count of $CD3^+$ (Pan-T cells), which corresponded with a decreased percentage of the $CD8^+$ lymphocytes in the spleen (Table 2).

However, five exposures to fraction C (1 and 0.1 mg/kg) increased percentage of $CD4^+$ splenocytes, as can be seen in Table 2. Multiple administration of examined fraction C at the doses of 0.1 and 10 mg/kg increased the percentage of $CD19^+$ (B lymphocytes) in the spleen but a single administration did not exert such influence.

As can be seen in Table 3, five times administration of fraction C from *Caltha palustris* extract at all doses under investigation augmented the absolute count and the percentage of $CD19^+$ (B lymphocytes) in the mesenteric lymph nodes. The strongest stimulating effect on B lymphocytes of mesenteric lymph nodes was observed after five injections of fraction C at a dose of 0.1 mg/kg. A decrease in the percentage of $CD4^+$ and $CD3^+$ lymphocytes at all doses and in the percentage of $CD8^+$ at the doses of 1 and 10 mg/kg was also demonstrated (Table 3). However, after 3 days from last administration of the fraction C at the dose of 1 mg/kg, the increase in the percentage and in the absolute count of $CD3^+$ mesenteric lymphocytes, with an accompanying decrease in B lymphocytes, was found (Table 3).

Discussion

Polysaccharide compounds in other plants (such as *Solanum nigrum*, *Opilia celtidifolia*, *Potentilla anserina*) have been reported to have immunomodulating activities [12-14]. In the present study, we confirmed these properties also in polysaccharide fraction C of *Caltha palustris*.

The number and ratio of two main lymphocyte T subsets ($CD4^+$ cells-T helpers, and $CD8^+$ cells-T cytotoxic/suppressors) have been recognized as the most meaningful parameters for evaluating the balanced state of immunomodulation and homeostatic responses of the intrinsic immune system [15]. $CD4^+$ T cells recognize antigens presented by the major histocompatibility complex class II (MHC II) proteins and mediate both cellular immune responses through Th1 cells and humoral immune responses through Th2 cells. The recognition of antigens presented by MHC I molecules and mediation of cellular immune responses through cytotoxic T cells are the domain of $CD8^+$ cells.

In the present study, the increase of the $CD4^+$ lymphocyte population (thymus, spleen) after administrating fraction C from *Caltha palustris* extract was reported. This effect was similar to the results reported by Wu *et al*. [16], who investigated the traditional Chinese medicine, Chi-Shie-Shuang-Bu-An-Shen-Tang (CST). The oral administration of sterilized CST for 3 weeks caused an increase in the population of $CD4^+$ T cells in spleen. The authors suggest that probably polysaccharide component was responsible for this activity. According to tha data obtained in this study, the increase in $CD4^+$ population may be potentially benefitial, especially in the states of $CD4^+$ lymphopenia. Such conditions may result from systemic diseases, i.e. systemic lupus erythrematosus [17] or action of drugs, i.e. cyclophosphamide [18], and may increase the risk of infection.

$CD4^+$ and $CD8^+$ are also two subpopulations which, in peripheral blood, play a crucial role in adult host defense toward cancer [19]. Li *et al*. [12] demonstrated the protective effect of fraction 1a of polysaccharides, isolated from Solanum nigrum Linne (SNL-P1a), on thymus in tumor-bearing mice. They studied the profile of $CD4^+$ and $CD8^+$ in PBMC (peripheral blood mononuclear cell) and showed that treatment with SNL-P1a following tumor implantation caused a significant increase in the number of $CD4^+$ T-lymphocyte and a decrease in the number of $CD8^+$ T-lymphocyte in peripheral blood of tumor-bearing mice.

In our study, a significant decrease in the number of single-positive $CD8^+$ thymocytes after administration of fraction C from *Caltha palustris* extract was found.

Our study showed that multiple administration of examined fraction C increased the $CD19^+$ B^- lymphocyte

Table 1. Percentage, absolute count of thymocytes subpopulations, total number of thymocytes and weight ratio of thymus in mice treated once and five times with fraction C of *Caltha palustris* extract. The mean values (n = 7) and standard deviations are presented

Index		**Day**	**Control**	**Fraction C of *Caltha palustris* extract**		
				1 × 10 mg/kg	**1 × 1 mg/kg**	**1 × 0.1 mg/kg**
Thymocytes						
Weight ratio of thymus		1	0.207 ±0.035	0.244 ±0.065	0.202 ±0.055	0.220 ±0.080
		3	0.226 ±0.058	0.168 ±0.047*	0.202 ±0.022	0.203 ±0.056
The total number		1	40.86 ±6.25	35.34 ±4.81*	36.51 ±6.01	38.03 ±7.93
		3	28.94 ±10.15	22.03 ±6.47	19.03 ±2.97*	24.94 ±5.65
$CD4^-CD8^-$	(%)	1	3.34 ±1.26	3.46 ±1.84	2.60 ±0.20	2.75 ±1.05
	(× 10^6)		1.39 ±0.61	1.23 ±0.70	0.95 ±0.20	1.06 ±0.48
	(%)	3	3.19 ±1.09	3.92 ±0.79	4.44 ±1.76	8.81 ±4.00*
	(× 10^6)		0.84 ±0.16	0.86 ±0.30	0.82 ±0.30	2.12 ±0.75*
$CD4^+CD8^+$	(%)	1	82.79 ±1.98	79.24 ±8.54	84.30 ±1.22	83.51 ±1.23
	(× 10^6)		33.77 ±4.89	27.88 ±4.24*	30.76 ±4.98	31.74 ±6.58
	(%)	3	68.99 ±22.01	75.19 ±2.29	76.18 ±2.91	72.37 ±3.43
	(× 10^6)		19.53 ±9.70	16.54 ±4.88	14.50 ±2.34	18.12 ±4.39
$CD4^+$	(%)	1	11.36 ±1.28	11.72 ±2.10	10.84 ±1.09	11.68 ±0.78
	(× 10^6)		4.66 ±0.94	4.13 ±0.86	3.98 ±0.88	4.45 ±1.05
	(%)	3	16.55 ±2.35	17.30 ±1.25	16.26 ±1.81	15.63 ±1.25
	(× 10^6)		4.65 ±1.36	3.83 ±1.18	3.11 ±0.68*	3.92 ±1.01
$CD8^+$	(%)	1	2.50 ±0.27	2.08 ±0.18*	2.25 ±0.18*	2.06 ±0.33*
	(× 10^6)		1.03 ±0.23	0.74 ±0.13*	0.82 ±0.12*	0.78 ±0.18*
	(%)	3	2.70 ±0.54	3.64 ±0.62*	3.13 ±0.87	3.18 ±0.47*
	(× 10^6)		0.74 ±0.15	0.81 ±0.30	0.60 ±0.24	0.79 ±0.16
			Control	**5 × 10 mg/kg**	**5 × 1 mg/kg**	**5 × 0.1 mg/kg**
Weight ratio of thymus		1	0.230 ±0.044	0.232 ±0.042	0.245 ±0.042	0.242 ±0.052
		3	0.238 ±0.038	0.212 ±0.032	0.193 ±0.042*	0.224 ±0.048
The total number		1	35.60 ±6.84	38.57 ±6.95	42.14 ±3.46*	43.89 ±6.32*
		3	35.00 ±3.01	37.77 ±8.48	35.03 ±4.65	31.49 ±4.77
$CD4^-CD8^-$	(%)	1	10.92 ±16.83	3.80 ±1.82	2.77 ±0.49	2.39 ±0.50
	(× 10^6)		3.47 ±4.98	1.40 ±0.53	1.17 ±0.27	1.04 ±0.21
	(%)	3	2.34 ±1.99	2.81 ±2.18	3.56 ±1.32	2.74 ±1.97
	(× 10^6)		0.84 ±0.75	1.13 ±1.04	1.28 ±0.60	0.62 ±0.16
$CD4^+CD8^+$	(%)	1	72.75 ±15.29	78.38 ±1.67	79.52 ±1.94	79.82 ±0.98
	(× 10^6)		26.24 ±8.33	30.29 ±5.86	33.46 ±2.06*	35.03 ±5.13*
	(%)	3	77.54 ±10.87	78.64 ±1.05	79.30 ±2.20	79.25 ±3.15
	(× 10^6)		26.93 ±2.85	29.69 ±6.69	27.79 ±3.90	24.95 ±3.97
$CD4^+$	(%)	1	13.56 ±3.36	15.34 ±0.89	15.49 ±1.57	15.65 ±0.59
	(× 10^6)		4.92 ±1.86	5.92 ±1.14	6.57 ±1.19*	6.87 ±1.00*
	(%)	3	13.96 ±1.91	17.37 ±0.69*	14.86 ±1.57	16.05 ±1.94*
	(× 10^6)		4.93 ±1.02	6.56 ±1.47*	5.17 ±0.60	5.04 ±0.98
$CD8^+$	(%)	1	2.63 ±0.25	2.49 ±0.46	2.18 ±0.27*	2.15 ±0.49*
	(× 10^6)		0.93 ±0.15	0.96 ±0.24	0.92 ±0.17	0.95 ±0.26
	(%)	3	2.33 ±0.59	2.04 ±0.34	2.28 ±0.63	1.96 ±0.39
	(× 10^6)		0.82 ±0.22	0.77 ±0.23	0.79 ±0.21	0.62 ±0.18*

$p < 0.05$ as compared to the control group

Table 2. Percentage, absolute count of splenocytes subpopulations, total number of splenocytes and weight ratio of spleen in mice treated once and five times with fraction C of *Caltha palustris* extract. The mean values (n = 7) and standard deviations are presented

Index		**Day**	**Control**	**Fraction C of *Caltha palustris* extract**		
				1 × 10 mg/kg	**1 × 1 mg/kg**	**1 × 0.1 mg/kg**
Splenocytes						
Weight ratio of spleen		1	0.589 ±0.124	0.626 ±0.067	0.642 ±0.100	0.584 ±0.123
		3	0.547 ±0.063	0.623 ±0.106	0.642 ±0.187	0.636 ±0.104*
The total number		1	58.91 ±11.10	62.26 ±5.69	60.71 ±7.21	59.29 ±8.38
		3	63.37 ±8.42	63.97 ±9.85	61.40 ±10.25	55.83 ±6.49*
$CD3^+$	(%)	1	33.36 ±3.60	38.27 ±1.87*	37.26 ±5.76	35.99 ±5.07
	(× 10^6)		18.42 ±3.86	23.86 ±2.80*	22.61 ±4.36*	21.52 ±5.30
	(%)	3	33.52 ±4.40	37.57 ±5.35	30.34 ±6.38	33.01 ±3.32
	(× 10^6)		21.05 ±2.81	24.46 ±7.08	19.04 ±7.05	18.27 ±1.31
$CD4^+$	(%)	1	26.20 ±3.50	27.88 ±3.91	29.39 ±2.13*	26.67 ±2.70
	(× 10^6)		15.64 ±4.63	17.31 ±2.75	17.84 ±2.41	15.89 ±3.23
	(%)	3	27.77 ±3.00	27.68 ±3.36	24.47 ±4.91	24.13 ±3.07*
	(× 10^6)		17.49 ±2.25	17.92 ±4.50	15.24 ±4.88	13.37 ±1.54*
$CD8^+$	(%)	1	7.15 ±1.39	5.14 ±0.87*	6.58 ±1.65	6.10 ±1.76
	(× 10^6)		4.26 ±1.41	3.19 ±0.60	4.04 ±1.28	3.68 ±1.45
	(%)	3	7.04 ±1.45	8.10 ±1.71	5.84 ±2.22	7.13 ±0.95
	(× 10^6)		4.43 ±1.06	5.31 ±1.78	3.74 ±2.08	3.94 ±0.36
$CD19^+$	(%)	1	53.61 ±3.43	50.61 ±2.88	51.36 ±3.19	55.03 ±4.94
	(× 10^6)		31.31 ±4.56	31.45 ±2.65	31.13 ±3.66	32.40 ±3.32
	(%)	3	55.97 ±4.29	52.71 ±5.91	57.61 ±6.50	56.60 ±5.58
	(× 10^6)		35.62 ±6.65	33.28 ±2.66	34.98 ±4.28	31.89 ±6.49
			Control	**5 × 10 mg/kg**	**5 × 1 mg/kg**	**5 × 0.1 mg/kg**
Weight ratio of spleen		1	0.714 ±0.225	0.843 ±0.124	0.765 ±0.122	0.679 ±0.069
		3	0.628 ±0.163	0.779 ±0.073*	0.597 ±0.115	0.626 ±0.112
The total number		1	71.14 ±6.65	69.71 ±9.33	67.20 ±10.07	65.94 ±10.81
		3	64.66 ±6.26	67.11 ±8.50	62.23 ±7.48	63.63 ±5.55
$CD3^+$	(%)	1	33.92 ±3.55	31.07 ±3.63	32.91 ±3.70	33.23 ±3.18
	(× 10^6)		24.30 ±4.60	21.54 ±2.91*	22.10 ±4.26	21.73 ±3.01
	(%)	3	34.86 ±4.05	36.96 ±2.40	35.33 ±3.57	41.79 ±1.54*
	(× 10^6)		22.63 ±4.12	24.87 ±4.06	21.87 ±2.34	27.13 ±1.45*
$CD4^+$	(%)	1	24.61 ±2.37	23.31 ±3.29	28.40 ±3.57*	30.60 ±4.78*
	(× 10^6)		17.60 ±3.14	16.14 ±2.30	19.13 ±4.32	19.84 ±2.27
	(%^{6})	3	24.61 ±2.95	27.37 ±1.48	24.32 ±3.29	29.75 ±2.18*
	(× 10^6)		15.94 ±2.71	18.37 ±2.52	15.09 ±2.45	19.26 ±0.80*
$CD8^+$	(%)	1	5.76 ±1.55	3.98 ±1.09*	4.86 ±0.60	5.05 ±0.91
	(× 10^6)		4.12 ±1.29	2.76 ±0.80	3.25 ±0.53	3.35 ±0.93
	(%)	3	5.55 ±0.90	5.85 ±0.69	5.27 ±1.22	7.90 ±0.74*
	(× 10^6)		3.61 ±0.83	3.93 ±0.70	3.24 ±0.63	5.12 ±0.42*
$CD19^+$	(%)	1	53.51 ±4.18	58.36 ±3.98*	55.18 ±5.03	55.73 ±4.74*
	(× 10^6)		37.95 ±3.30	21.54 ±2.91	37.07 ±6.42	37.45 ±6.34
	(%)	3	59.42 ±3.92	57.38 ±2.62	59.44 ±4.09	52.91 ±1.62*
	(× 10^6)		38.31 ±3.08	38.43 ±4.37	37.12 ±6.08	34.44 ±3.30*

**$p < 0.05$ as compared to the control group*

Table 3. Percentage, absolute count of mesenteric lymph node cells subpopulations, total number of mesenteric lymph node cells and weight ratio of lymph nodes in mice treated once and five times with fraction C of *Caltha palustris* extract. The mean values (n = 7) and standard deviations are presented

Index		Day	Control	Fraction C of *Caltha palustris* extract		
				1 × 10 mg/kg	1 × 1 mg/kg	1 × 0.1 mg/kg
Mesenteric lymph node cells						
Weight ratio of mesenteric lymph node		1	0.445 ±0.085	0.526 ±0.063*	0.501 ±0.043	0.564 ±0.089*
		3	0.483 ±0.084	0.467 ±0.061	0.537 ±0.063	0.428 ±0.178
The total number		1	38.69 ±7.72	32.51 ±5.64	24.94 ±6.71*	36.14 ±7.92
		3	37.20 ±6.72	33.49 ±4.13	36.31 ±3.66	32.46 ±5.95
$CD3^+$	(%)	1	49.91 ±7.99	50.39 ±3.23	58.24 ±4.36*	50.47 ±4.99
	($\times 10^6$)		17.43 ±3.13	16.44 ±3.31	13.50 ±3.79*	18.00 ±3.42
	(%)	3	51.36 ±4.57	51.15 ±1.15	44.51 ±7.49*	49.60 ±6.08
	($\times 10^6$)		19.14 ±4.07	17.12 ±2.08	16.11 ±2.88	16.32 ±4.63
$CD4^+$	(%)	1	44.70 ±3.80	41.43 ±9.09	45.17 ±2.33	40.07 ±3.17*
	($\times 10^6$)		17.42 ±4.47	12.58 ±3.57*	11.35 ±3.54*	14.38 ±3.03
	(%)	3	43.15 ±5.52	43.03 ±1.26	38.75 ±6.50	40.61 ±4.13
	($\times 10^6$)		16.14 ±4.12	14.43 ±1.98	14.01 ±2.41	13.28 ±3.33
$CD8^+$	(%)	1	9.43 ±2.84	7.47 ±2.41	13.14 ±3.08*	10.82 ±2.81
	($\times 10^6$)		3.75 ±1.61	2.43 ±0.91*	3.31 ±1.20	3.81 ±1.08
	(%)	3	7.95 ±2.58	8.28 ±1.18	5.26 ±0.80*	6.71 ±1.12
	($\times 10^6$)		2.99 ±1.22	2.79 ±0.64	1.90 ±0.31*	2.21 ±0.71
$CD19^+$	(%)	1	41.02 ±8.06	43.54 ±3.66	37.07 ±4.65	45.77 ±5.29
	($\times 10^6$)		15.58 ±2.88	14.13 ±2.51	9.03 ±1.66*	16.78 ±4.77
	(%)	3	44.21 ±7.09	45.56 ±1.86	52.22 ±7.63*	46.13 ±5.91
	($\times 10^6$)		16.33 ±3.45	15.27 ±2.16	19.03 ±3.60	14.75 ±1.93
			Control	**5 × 10 mg/kg**	**5 × 1 mg/kg**	**5 × 0.1 mg/kg**
Weight ratio of mesenteric lymph node		1	0.564 ±0.094	0.573 ±0.107	0.544 ±0.064	0.683 ±0.230
		3	0.599 ±0.101	0.547 ±0.097	0.544 ±0.081	0.572 ±0.042
The total number		1	37.69 ±6.25	41.26 ±4.96	42.54 ±5.04	36.77 ±8.80
		3	38.66 ±6.36	38.06 ±5.01	38.40 ±3.09	35.11 ±4.64
$CD3^+$	(%)	1	55.13 ±2.87	49.92 ±4.41*	44.68 ±5.78*	44.92 ±6.24*
	($\times 10^7$)		20.74 ±3.36	20.71 ±3.78	18.79 ±1.20	16.46 ±4.55*
	(%)	3	45.39 ±4.87	45.41 ±4.88	59.42 ±14.62*	50.44 ±5.74
	($\times 10^7$)		17.70 ±4.05	17.29 ±3.05	22.90 ±6.18*	17.85 ±4.01
$CD4^+$	(%)	1	47.51 ±1.96	44.19 ±3.32*	40.63 ±3.14*	39.72 ±5.60*
	($\times 10^7$)		17.89 ±3.00	18.29 ±2.91	17.18 ±1.17	14.56 ±4.11
	(%)	3	38.88 ±2.81	38.41 ±3.15	45.10 ±5.28*	41.97 ±3.73
	($\times 10^7$)		16.00 ±2.28	14.59 ±2.05	17.35 ±2.67	14.83 ±3.00
$CD8^+$	(%)	1	7.34 ±1.76	5.31 ±0.72	5.42 ±1.03*	6.14 ±1.59
	($\times 10^7$)		2.76 ±0.78	2.18 ±0.33	2.28 ±0.32	2.23 ±0.64
	(%)	3	5.69 ±2.11	4.83 ±1.61	11.04 ±7.30	7.32 ±1.46
	($\times 10^7$)		1.92 ±0.66	1.86 ±0.76	2.61 ±0.51*	2.60 ±0.77
$CD19^+$	(%)	1	41.23 ±3.14	46.88 ±4.13*	50.27 ±6.55*	50.32 ±6.95*
	($\times 10^7$)		15.57 ±3.09	19.23 ±1.85*	21.63 ±5.35*	18.59 ±5.26
	(%)	3	52.23 ±5.11	52.77 ±5.09	37.79 ±15.80*	47.00 ±5.88
	($\times 10^7$)		20.02 ±2.63	20.08 ±3.41	14.43 ±5.97*	16.36 ±1.95*

$p < 0.05$ as compared to the control group

population. Stimulating activity of other polysaccharide extracts was demonstrated in the studies on polysaccharide isolated from the radix of *Platycodon grandiflorum* (PG) [20]. *Platycodon grandiflorum* was found to markedly increase polyclonal IgM antibody production and the proliferation of B cells. Moreover, the intraperitoneal administration of PG in mice immunized by using T-dependent antigen, sheep red blood cells (SRBCs), resulted in increased IgM antibody production in B cells.

The present study demonstrated that fraction C from *Caltha palustris* extract can change the percentage and absolute number of T cell subsets in thymus, and T and B lymphocytes in the spleen and mesenteric lymph nodes. The effect of the examined substance depended on the number of consecutive doses applied. However, more experiments should be carried out to explain the detailed mechanism that induced the immunomodulatory activity.

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