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Effects of iridoid-anthocyanin extract of *Cornus mas L.* on hematological parameters, population and proliferation of lymphocytes during experimental infection of mice with *Trichinella spiralis*

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Abstract

The influence of iridoid-anthocyanin aqueous extract of cornelian cherry fruits (CM) on hematological parameters, lymphocyte subsets and proliferation during *Trichinella spiralis* infection in mice was investigated. CM (100 mg/kg) was administered orally to *T. spiralis*-infected mice six times within a period encompassing three days prior to the infection and three days after the infection (dai).

CM increased the percentage of $CD3^+$, $CD4^+$ cells and $CD4^+/CD8^+$ ratio and decreased total count of $CD8^+$ and $CD19^+$ splenocytes (5^{th} dai). An increase in total count of $CD4^+$, $CD3^+$, $CD19^+$ splenocytes was observed (21^{st} dai). CM elevated the percentage of $CD4^+$ cells (7^{th} dai) and $CD4^+/CD8^+$ ratio (21^{th} dai) in MLN. CM increased (14^{th} dai) and then reduced (21^{st} dai) the percentage of $CD8^+$ MLN lymphocytes and decreased total count of MLN $CD8^+$ cells (21^{st} dai) and B cells (14^{th} dai). An activation of lymphocyte proliferation in spleen and simultaneous decrease in MLN on 5^{th} dai was observed. An increase in red blood cells parameters (5^{th} dai) and in leukocyte count (7^{th} dai) was found. A rise in platelet count was noticed both on 5^{th} and 7^{th} dai. Moreover, the number of adult *T. spiralis* on 5^{th} dai in mice receiving CM extract was lower than in the control mice.

These results suggested that iridoid-anthocyanin aqueous extract of CM stimulated murine immune response during *T. spiralis* infection.

Keywords: *Trichinella spiralis*, *Cornus mas* L., lymphocyte subsets, lymphocyte proliferation, mice

1. Introduction

Trichinellosis is a widely spread parasitic zoonosis caused by nematodes of *Trichinella* genus. *Trichinella spiralis*, the causative agent of human trichinellosis, is also a huge

economic problem in porcine animal production and food safety. The presence of adult nematodes in a small intestine and larvae in the muscle of the same host modulate the host's immunological pathways, promoting survival of parasites by limiting effector immune mechanism. During the intestinal phase and muscle infection with *T. spiralis* Th2 response is maintained but it is preceded by a short Th1-polarized reaction (Bruschi and Chiumiento 2012).

In recent years, edible fruits of cornelian cherry (*Cornus mas L.*) have gained a lot of attention from researchers, who began to describe the fruit qualities not only in terms of their taste but also health benefits. Positive effects of these fruits were shown to be due to the presence of biologically active substances, such as vitamin C, anthocyanins, ursolic acid or loganic acid (Seeram et al., 2002; Yayaprakasam et al., 2006; Zhang et al., 2006; Kucharska et al., 2015). Anthocyanins from cornelian cherry act as modulators of immune processes and exhibit e.g. antitumor and antioxidant properties (Wang et al., 1999; Seeram and Nair, 2002; Wang et al., 2006; Haghi et al., 2014). Iridoids (including loganic acid and cornuside), present in *Cornus mas L.* fruits have antibiotic, anti-inflammatory or hypertensive properties (Asgary et al., 2013). Another study showed significant preventive effects of cornelian cherry regarding high fat diet-induced hypertriglyceridemia and development of atherosclerosis in rabbits. Anthocyanins and iridoids from cornelian cherry fruits modulated both the redox system and proinflammatory cytokines (Sozanski et al., 2014).

The aim of this study was to assess the immunomodulatory effects of iridoid-anthocyanin extract of *Cornus mas L* on blood parameters, T and B lymphocytes in the spleen and mesenteric lymph nodes in the course of experimentally-induced trichinellosis in mice.

2. Material and methods

2.1. Plant Material

Cornelian cherry fruits were harvested in the Arboretum and Institute of Physiography in Bolestraszyce (22° 51′ N, 49° 49′ E), near Przemyśl, Poland. The plant material was authenticated by Professor Jakub Dolatowski (Arboretum and Institute of Physiography in Bolestraszyce, Poland), and the adequate voucher specimens ('Bolestraszycki' – BDPA 3951) were deposited at the Herbarium of Arboretum and Institute of Physiography in Bolestraszyce, Poland.

Chemicals

Acetonitrile, formic acid, and methanol were purchased from Sigma-Aldrich (Steinheim, Germany). Cyanidin 3-*O*-glucoside (C 3-glc) and loganic acid were purchased from Extrasynthese (Genay, France).

Extraction of anthocyanins and iridoids

Juice extracted from frozen ripe fruits of cornelian cherry (*C. mas* L.) was purified by removing sugars and organic acids on Amberlite XAD-16 resin column (Rohm and Haas, Chauny Cedex, France). Anthocyanins and iridoids were eluted with 80% ethanol. The eluate was concentrated under vacuum at 40°C. The solvent was evaporated using Rotavapor (Unipan, Warsaw, Poland).

Quantitative determination of anthocyanins and iridoids by HPLC-DAD

The methodology was previously described by Sokół-Łętowska et al., (2014). HPLC analysis was performed using a Dionex (Germering, Germany) system equipped with a diode array detector model Ultimate 3000, a quaternary pump LPG-3400A, an autosampler EWPS-3000SI, a thermostated column compartment TCC-3000SD, and controlled by Chromeleon v.6.8 software. Cadenza Imtakt column C5-C18 (75 x 4.6 mm, 5 µm) was used. The mobile

phase was composed of solvent A (4.5% aq. formic acid, v/v) and solvent B (100% acetonitrile). The elution system was as follows: 0-1 min 5% B in A, 20 min 25% B in A, 21 min 100% B, 26 min 100% B, 27 min 5% B in A. Flow rate of the mobile phase was 1.0 mL/min and the injection volume was 20 µL. The column was operated at 30°C. Anthocyanins and iridoids were detected at 520 nm and 245 nm, respectively. Anthocyanins were expressed as mg of cyanidin 3-*O*-glucoside equivalents (Cy 3-glcE) per g of dry mass (DM), iridoids as loganic acid equivalents (LAE) per g of DM (Table 1).

2.2. Experimental animals

The experiment was carried out using BALB/c mice (male and female; between 8 and 10 weeks old), each weighing between 20 and 22 grams. The mice were orally infected with 200 *T. spiralis* larvae. All animals were maintained under standard environmental conditions and fed with a rodent diet. The study protocol was approved by the II Local Ethics Committee in Wroclaw, Poland (No. 43/2015).

2.3. Parasitological material

The strain of *T. spiralis* (T1, ISS1820, Poland) was identified at the Istituto Superiore di Sanita, Rome, Italy and maintained in the Department of Parasitology, Wroclaw Faculty of Veterinary Medicine, by serial passage in CFW inbred mice. The larvae used in the infection were recovered from muscle tissue of the mice that had been infected two to three months earlier. The parasites were released from the muscle tissue via digestion with 1% pepsin/HCl solution at 37ºC. BALB/c mice were infected with 200 larvae of *T. spiralis*/mouse.

2.4. Administration of iridoid–anthocyanin extract of *Cornus mas* L.

Iridoid-anthocyanin aqueous extract of cornelian cherry fruits (CM) was administered

orally (using a stomach tube) at a dose of 100 mg/kg b.w. for six days: three days prior to the infection and three days after the infection (dai) with *T. spiralis*. The volume of each dose was 0.2 ml per animal. The experiments in the control group were conducted simultaneously using water instead of CM (0.2 ml/mouse).

The experiment was carried out using 60 mice divided into two groups:

Group T+CM: infected with *T. spiralis* larvae and receiving CM (30 mice),

Group T (Control): infected with *T. spiralis* larvae (30 mice).

2.4 .1. Hematological analyses

On days 5, 7, 14 and 21 after infection, six mice from each group were anesthetized with isoflurane (Forane, Aesica Queenborough Limited, Queenborough, UK). Blood samples were taken from each animal by cardiac puncture and were transferred into tubes with hematology anticoagulant ethylenediaminetetraacetic acid (EDTA). Hematological parameters were explored by the hematology analyzer (PE-6800 Procan Electronics Inc., Chine) that perform complete blood cells and platelet counts, as well as specific leukocyte count. As the apparatuses differentiated only three white blood cell populations, manual morphology was performed calculating the absolute values obtained from the WBC. A total of 200 cells were counted.

2.4.2 Assay of lymphocyte subsets from spleen and mesenteric lymph nodes.

On 5^{th}, 7^{th}, 14^{th}, and 21^{st} dai spleens and mesenteric lymph nodes (MLN) were removed and the lymphocyte isolation was performed as described previously (Szczypka and Obmińska-Mrukowicz, 2010). The lymphocytes in a suspension (4×10^6 cells/ml) were stained with a monoclonal rat anti-mouse CD19:FITC/CD3:RPE dual color reagent (Serotec, Kidlington, UK) or a monoclonal rat anti-mouse CD4:FITC/CD8:RPE dual color reagent

(Serotec, Kidlington, UK), according to the manufacturer's protocol. After incubation (4ºC, 30 min), the lymphocytes were washed and centrifuged (380 g, 8 min, 4ºC) two times with ice-cold PBS. Fluorescence was measured using a flow cytometer (BD FACSCalibur, BD Biosciences, San Jose, CA, USA). Lymphocyte marker distribution was analyzed using CellQuest Pro software. CD subsets (percentage and total lymphocyte count) of $CD19^+$, $CD3^+$, $CD4^+$ and $CD8^+$ cells in spleens and mesenteric lymph nodes and $CD4^+/CD8^+$ ratio were determined.

2.4.3 Lymphocyte culture and proliferation assay

The spleen and mesenteric lymph node lymphocytes at a concentration of 4×10^5 cells per 150 µl final volume were plated into 96-well U-bottom culture plate (Costar 3596, Corning Incorporated, USA). The culture was maintained in RPMI 1640 Medium (Sigma-Aldrich, USA) supplemented with $NaHCO_3$, gentamycin to a concentration of 50 mg/l (Polfa, Tarchomin, Poland) and new-born calf serum (10%) (Gibco, No 26010074, New Zealand) and incubated with 0.9, 0.45, and 0.225 µg/ml of concanavalin A - Con A (Sigma-Aldrich, USA) at 37ºC in 5% CO_2 for 72 h. Cell proliferation was estimated with MTT test. At 4.5 h before the end of the incubation, 25 µl MTT (5 mg/ml; Sigma-Aldrich, USA) were added to each well and left at 37ºC in 5% CO_2 humidified atmosphere. Then, 125 µl of sodium dodecyl sulfate/dimethylformamide (SDS/DMF) extraction 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 2 hours. After that, optical density (OD) at 540 nm with a reference filter at 620 nm was measured in a spectrophotometer µQuant (Biotek Instruments, Inc.).

Proliferation index (PI) was calculated by dividing average optical densities (OD) for mitogen-stimulated cells by the average OD for the control (non-stimulated cells).

2.5 Determination of the parasite burden

Adult parasites were isolated and counted on 5th, 7th, 14th and 21st dai by incubation of small intestines in 0.9% NaCl at 37ºC in Baermann funnels overnight. The number of muscle larvae was examined on 60th dai. Whole eviscerated carcasses were minced and artificially digested according to above mentioned method.

2.6 Statistical analysis

The data were subjected to t-Student's test to determine their significance. P-value ≤ 0.05 was considered significant. Results were shown as means ± SD (standard deviation). Numerical calculations were carried out using STATISTICA ver. 11.0 software package.

3. Results

3.1 *Effects of iridoid-anthocyanin aqueous extract from Cornus mass L. on the hematological parameters*

A significant increase in red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW-SD), and packed cell volume (RDW-CV) was observed on 5th dai. There was no change in mean corpuscular volume (MCV) of erythrocytes (Table 2A).

An increase in the number of leukocytes (WBC) on 7th dai was noticed. This change was accompanied by an increase in the number and percentage of segmented neutrophils (S).

There was no significant difference in the number and percentage of banded neutrophils (P) and monocytes (M), and the number of lymphocytes (L) and eosinophils (E).

The percentage of lymphocytes (L) and eosinophils (E) was reduced on 7th dai and 14th dai, respectively (Table 2B).

A rise in platelet count (PLT) was noticed both on 5th and 7th dai, with accompanying increase

196 in PCT on 5th dai. Mean platelet volume (MPV), platelet distribution width (PDV), and
197 platelet large cell ratio (P-LCR) were not affected in the mice treated with CM as compared
198 with the control group (Table 2C)
199
200 ***3.2.*** **Flow cytometry analysis**
201 ***Effects of iridoid-anthocyanin aqueous extract from Cornus mass L. on the subpopulations***
202 ***of splenocytes and lymphocytes of mesenteric lymph node in T. spiralis infected mice.***
203 In the spleen, administration of iridoid-anthocyanin aqueous extract from *Cornus mass L*. at
204 100 mg/kg b.w. significantly increased the percentage of CD3$^+$, CD4$^+$ T cells and CD4$^+$/CD8$^+$
205 ratio measured on day 5th after infection with *T. spiralis* ($p<0.05$). No difference in the
206 percentage of CD8$^+$ T cells and B lymphocytes (CD19$^+$) was observed between Group T+CM
207 and Group T (Table 3A). At the same time, a significant decrease in the absolute count of
208 CD8$^+$ and CD19$^+$ splenocytes was seen. An increase in the absolute count of CD4$^+$, CD3$^+$ and
209 CD19$^+$ spleen cells appeared only on 21st dai ($p<0.05$) (Table 4A).
210 In MLN, administration of iridoid-anthocyanin aqueous extract triggered a growth in the
211 percentage of CD4$^+$ cells for all sampling times and CD8$^+$ T lymphocyte on 7th and 14th day
212 after infection with *T. spiralis*. The greatest impact on CD4$^+$ subpopulation was observed on
213 7th dai ($p<0.01$), on CD8$^+$ lymphocytes on 14th and 21st dai ($p<0.01$), and on CD4$^+$/CD8$^+$ ratio
214 on 21st dai (Table 3B). Significant decrease in absolute count of MLN T CD8$^+$ cells was found
215 on 21st dai and for B cells (CD 19$^+$) on 14th dai ($p<0.05$) (Table 4B).
216
217 **3.3. Effects of iridoid-anthocyanin aqueous extract from *Cornus mass* L. on lymphocyte**
218 **proliferation**
219 In the spleen, a significant increase in lymphocyte proliferation in T+CM group was observed
220 only on 5th dai in the presence of 0.225 µg/ml of Con A ($p<0.01$). Otherwise, MLN

lymphocytes in *T. spiralis* infected mice that received iridoids and anthocyanins revealed a significant decrease in proliferation on 5th dai in the presence of 0.225 µg/ml of Con A ($p < 0.01$) (Table 5A and B).

3.4 Parasite burden – numbers of adults and muscle larvae

A significant reduction of intestinal parasites in T+CM group occurred only on 5th dai. (Table 6 A). There was no significant difference in the number of muscle larvae in both examined groups of mice (Table 6B).

4. Discussion

During the course of *T. spiralis* infection, $CD4^+$ and $CD8^+$ T lymphocytes are involved in the regulation of the immune response both in the intestinal and muscular phase of the disease (Karmańska et al., 1995). In the intestinal phase, Th 2 cytokine response (IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13) arranged by $CD4^+$ T cells is mobilized in the mesenteric lymph nodes, and subsequently recruited to the intestinal mucosa (Ashour, 2013). Th2 lymphocytes play also a crucial role in the regulation of long-lasting infection of muscles with *Trichinella*.

The results of our study indicated that in the mice infected with *T. spiralis*, administration of iridoid-anthocyanin aqueous extract of cornelian cherry fruits altered the proportions of splenocytes and MLN lymphocytes. A significant increase in T lymphocytes ($CD3^+$ cells), especially $CD4^+$ T cells, was observed on 5th dai in the spleen and on 14th dai in MLN of the infected mice. Furthermore, the proportion of $CD8^+$ lymphocytes significantly changed on 14th and 21st dai in MLN. CM also selectively altered the absolute count of $CD3^+$, $CD4^+$, $CD8^+$, and $CD19^+$ in the spleen and of $CD8^+$ and $CD19^+$ in MLN. The mechanism of action of the administered extract is not yet understood, but its modulatory activity could be attributed

246 to the presence of biologically active loganic acid, cornuside and five active compounds of
247 anthocyanins (Table 1).

248 Immunomodulatory effect of iridoids on phagocytic activity of macrophages and other
249 immune competent (T and B) cells was observed during *Candida albicans* and *Salmonella*
250 *typhimurium* infections in mice (Sidiq et al., 2011; Ghule and Yeole, 2012). Furthermore,
251 antiparasitic properties of iridoids were reported during *Schistosoma mansoni* infection in
252 mice. Iridoid mixture blocked cercarial penetration and caused significant reduction in worm
253 burden. Moreover, mice treated with iridoids exhibited a significant increase mainly in
254 $CD4^+$T thymocytes, and an increase in $CD4^+$T lymphocytes of MLN similar to that found the
255 present study (Bahgat et al., 2005). Anti-inflammatory properties of iridoids were confirmed
256 by their strong inhibitory effect on the expression, maturation, and secretion of IL-1β
257 cytokine, which are essential for creating a specific mucosal environment that promote long
258 lasting infection of helminths (Zaiss et al., 2013; Zhu et al., 2014).

259 Anthocyanins are also known as modifiers of inflammatory processes and as compounds with
260 antitumor and antioxidant properties (Dinda et al., 2016; Thomasset et al., 2009; Wang et al.,
261 1999). Some of them also exhibit antiparasitic activity. Sorghum bicolor red-leaf 3-
262 deoxyanthocyanidins showed a strong inhibitory effect against the proliferative stage of *T.*
263 *gondii* in *in vitro* conditions (Abugri et al., 2016). However, the impact of some anthocyanins
264 on T cell response is questionable. Graf et al. (2013) claimed that anthocyanin-rich grape-
265 bilberry juice (anthocyanin intake-15 mg/day, for 10 weeks) did not affect the number of T
266 lymphocytes, the number and activity of NK cells, or cytokine production by lymphocytes
267 (IFN-γ, TNF-α, IL-10) in healthy rats. On the other hand, in a study conducted in mice with
268 collagen-induced arthritis (CIA), Min et al. (2015) found that anthocyanin extracted from
269 black soybean seed coats reduced the concentration of proinflammatory cytokines in affected

joints, diminished the number of Th17 cells in the spleen, and consequently improved clinical symptoms of CIA.

Cyanidin-3-glucoside chloride downregulated Th2 cytokine synthesis (IL-4, IL-13), but it did not affect Th1 cytokine production (IL-2, IFN-γ, IL-12) (Pyo et al., 2014). In this study, the proliferative response of the spleen and MLN lymphocytes in mice infected with *T. spiralis* and treated with CM was evaluated. Significant changes in the proliferation index of Con A-stimulated lymphocytes were observed in both lymphoid organs only on 5th dai, with the activation in the spleen and inhibition in MLN. Moreover, on 5th dai in mice infected with *T. spiralis* and receiving CM a significant reduction of intestinal parasites occurred. Different proliferative activity observed in the spleen and MLN could be explained by different type of stimulation in the peripheral and local lymphoid organs in the course of trichinellosis. A similar relationship in proliferative response in spleen and MLN was observed by Kato et al. (2005a; 2005b) and Piekarska et al. (2011). Th2 lymphocyte response that predominates in MLN activates the mechanisms of the parasite elimination by affecting a defense reaction (mastocytosis in intestinal mucosa, local and peripheral eosinophilia, increase in IgE and IgG1 in serum). On the other hand, Th1 lymphocyte response that predominates in the spleen via secreted cytokines (IFN-γ and IL-2) may control Th2 reaction and successfully modulate the immune response of the host (Grencis, 1997; Sofronic-Milosavljevic et al., 2015).

Results of our study demonstrated a significant CM-caused increase in all examined red blood cells parameters on 5th dai in *T. spiralis* infected mice. Significant elevation in leukocyte count on 7th dai was observed. Furthermore, a rise in platelet count with accompanying increase in PCT parameter both on 5th and 7th dai were noticed. Similarly, Abdollahi et al. (2014) proved that high doses of hydro-methanolic extract of *Cornus mas* affected the hematological parameters of male rats resulting in significant elevation particularly in mean corpuscular hemoglobin concentration (MCHC), mean platelet volume (MPV), and total

platelet mass (PCT).

This study showed that in the course of experimentally-induced trichinellosis in mice, iridoid-anthocyanin aqueous extract of cornelian cherry fruits (CM) apart from affecting hematological parameters and proliferative activity of lymphocytes was capable of altering the percentage and absolute number of T cells subpopulations and B lymphocytes in the spleen and MLN. This modulatory effects of CM on immune response finally contributed to a decrease of the intestinal parasite burden. Further studies on iridoids and anthocyanins from cornelian cherry fruits are now needed to better understand their mechanisms of action in the course of trichinellosis and to elucidate details of the host–parasite response.

Conflict of Interests

The authors declare that there is no conflict of interests to report.

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Table 1. Content (mg/g DM) of iridoids and anthocyanins in the extract from cornelian cherry fruits

Compound	mg/g		
Iridoids			
Loganic acid	198.25	±	31.43
Cornuside	9.71	±	4.02
Total	**207.96**		
Anthocyanins			
Delphinidin 3-*O*-galactoside	0.77	±	0.03
Cyanidin 3-*O*-galactoside	17.12	±	0.72
Cyanidin 3-*O*-robinobioside	7.08	±	0.37
Pelargonidin 3-*O*-galactoside	32.74	±	1.50
Pelargonidin 3-*O*-robinobioside	6.51	±	0.35
Total	**64.22**		

Table 2A.

Red Blood Cell (RBC) parameters in the mice infected with *T. spiralis* (Group T) and infected with *T. spiralis* and receiving *Cornus mass* iridoid-anthocyanin aqueous extract (Group T+CM). Mean values (n = 6) and standard deviations are presented. *p<0.05 **p<0.01

Parameter	5 dai		7 dai		14 dai		21 dai	
	T+CM n = 6	T n = 6	T+CM n = 6	T n = 6	T+CM n = 6	T n = 6	T+CM n = 6	T n = 6
RBC (10^6/μl)	*8.37 ±0.55	7.55 ±0.35	8.16 ±0.54	7.66 ±0.71	7.11 ±0.56	6.92 ±0.52	8.52 ±0.51	8.37 ±0.44
HGB (g/dL)	**16.95 ±1.15	14.97 ±0.84	16.53 ±1.32	15.48 ±1.62	14.27 ±1.34	13.93 ±1.03	17.63 ±1.38	17.15 ±1.07
HCT (%)	*47.80 ±3.29	43.10 ±2.30	46.83 ±3.26	44.17 ±4.17	41.73 ±2.98	40.35 ±3.55	51.75 ±5.19	50.88 ±3.44
MCV (fL)	56.40 ±1.87	57.18 ±0.52	57.47 ±0.87	57.78 ±1.52	58.80 ±1.43	58.35 ±1.20	60.65 ±2.31	60.85 ±1.11
MCH (pg)	*20.18 ±0.28	19.77 ±0.24	20.20 ±0.41	20.15 ±0.41	20.00 ±0.47	20.10 ±0.49	20.63 ±0.43	20.45 ±0.40
MCHC (g/dL)	*35.42 ±0.56	34.68 ±0.32	35.23 ±0.55	34.98 ±0.84	34.07 ±0.90	34.53 ±1.31	34.08 ±0.95	33.67 ±0.37
RDW-SD (fL)	*24.10 ±0.00	23.20 ±0.99	23.82 ±1.39	24.12 ±1.17	24.43 ±1.40	23.82 ±1.39	26.32 ±2.74	27.25 ±1.52
RDW-CV (%)	*14.25 ±0.12	13.67 ±0.56	13.98 ±0.70	14.08 ±0.59	14.05 ±0.67	13.78 ±0.84	14.72 ±1.33	15.17 ±1.03

Abbreviations: RBC- red blood cell count; HGB- hemoglobin; HCT- hematocrit; MCV- mean corpuscular volume; MCH- mean corpuscular hemoglobin; MCHC- mean corpuscular hemoglobin concentration; RDW-SD- red cell distribution width; RDW-CV- packed cell volume;

Table 2B.

White Blood Cell (WBC) parameters in the mice infected with *T. spiralis* (Group T) and infected with *T. spiralis* and receiving *Cornus mass* iridoid-anthocyanin aqueous extract (Group T+CM). Mean values (n = 6) and standard deviations are presented. *p<0.05 **p<0.01

Parameter	5 dpi		7 dpi		14 dpi		21 dpi	
	T+CM n = 6	T n = 6	T+CM n = 6	T n = 6	T+CM n = 6	T n = 6	T+CM n = 6	T n = 6
WBC (10^3/μl)	11.37 ±3.79	10.03 ±2.20	*12.63 ±2.18	8.78 ±2.11	11.85 ±2.53	12.08 ±3.41	11.65 ±2.92	11.97 ±3.15
P (10^3/μl)	0.35 ±0.31	0.16 ±0.11	0.30 ±0.12	0.25 ±0.15	0.31 ±0.15	0.28 ±0.16	0.21 ±0.09	0.23 ±0.15
P (%)	2.92 ±2.08	1.42 ±0.74	2.33 ±0.88	2.75 ±1.33	2.67 ±1.13	2.17 ±0.82	1.83 ±0.82	1.83 ±0.98
S (10^3/μl)	4.38 ±2.74	2.82 ±1.17	*4.44 ±0.79	2.12 ±0.90	3.47 ±1.14	2.88 ±1.25	2.86 ±1.72	2.72 ±1.45
S (%)	36.25 ±9.63	27.08 ±6.45	*35.83 ±8.50	23.67 ±7.61	29.17 ±8.16	23.33 ±3.54	23.33 ±8.84	21.33 ±7.26
Eos (10^3/μl)	0.09 ±0.08	0.09 ±0.09	0.16 ±0.11	0.17 ±0.10	0.24 ±0.11	0.54 ±0.33	0.82 ±0.53	1.04 ±0.44
Eos (%)	0.83 ±0.88	0.92 ±0.86	1.25 ±0.99	1.92 ±0.86	*2.00 ±0.84	4.58 ±3.09	6.75 ±3.49	9.08 ±4.64
Lymp (10^3/μl)	6.47 ±1.18	6.88 ±1.02	7.65 ±2.08	6.17 ±1.38	7.49 ±1.86	8.23 ±2.09	7.66 ±1.14	7.82 ±1.79
Lymp (%)	59.25 ±9.31	69.58 ±6.82	*59.75 ±8.17	70.83 ±7.33	63.50 ±9.00	68.50 ±4.97	67.17 ±8.09	66.50 ±8.43
M (10^3/μl)	0.07 ±0.04	0.09 ±0.09	0.09 ±0.11	0.07 ±0.06	0.33 ±0.19	0.16 ±0.10	0.10 ±0.12	0.16 ±0.12
M (%)	0.75 ±0.42	1.00 ±1.10	0.83 ±1.13	0.83 ±0.61	2.67 ±1.17	1.42 ±0.97	0.92 ±1.20	1.25 ±0.76

Abbreviations: WBC- white blood cell count; P- banded neutrophils; S- segmented neutrophils ; Eos- eosinophils; Lymp - lymphocytes; M- monocytes;

Table 2C.

Platelet parameters in the mice infected with *T. spiralis* (Group T) and infected with *T. spiralis* and receiving *Cornus mass* iridoid-anthocyanin aqueous extract (Group T+CM). Mean values (n = 6) and standard deviations are presented. *P<0.05 **P<0.01

Parameter	5 dpi		7 dpi		14 dpi		21 dpi	
	T+CM n = 6	T n = 6	T+CM n = 6	T n = 6	T+CM n = 6	T n = 6	T+CM n = 6	T n = 6
PLT (10^3/μl)	*820.83 ±201.18	617.83 ±62.82	*826.00 ±153.65	626.67 ±88.29	682.00 ±111.81	569.00 ±99.96	559.03 ±258.00	720.50 ±138.37
MPV (fL)	8.30 ±1.17	7.25 ±0.26	8.47 ±1.31	7.77 ±1.07	7.82 ±1.32	7.52 ±1.20	8.33 ±1.46	7.15 ±0.23
PDW (%)	8.52 ±1.06	7.90 ±0.00	8.72 ±0.91	8.17 ±0.65	8.58 ±0.89	8.17 ±0.65	8.30 ±0.73	7.90 ±0.00
PCT (%)	*0.69 ±0.26	0.44 ±0.03	0.71 ±0.23	0.48 ±0.09	0.53 ±0.14	0.42 ±0.04	0.55 ±0.10	0.51 ±0.09
P-LCR (%)	6.72 ±10.43	0.00 ±0.00	9.28 ±10.23	3.35 ±8.21	6.15 ±9.59	3.23 ±7.92	7.00 ±10.86	0.00 ±0.00

Abbreviations: PLT – platelet count; MPV- mean platelet volume; PDW- platelet distribution width; PCT- total platelet mass; P-LCR- platelet large cell ratio

Table 3A.
The percentage of splenocyte subpopulations in the mice infected with *T. spiralis* (Group T) and infected with *T. spiralis* larvae and receiving *Cornus mass* iridoid-anthocyanin aqueous extract (Group T+CM). Mean values (n = 6) and standard deviations are presented. *p<0.05 **p<0.01

	Day after infection	n	CD4$^+$		CD8$^+$		CD3$^+$		CD19$^+$		CD4$^+$/8$^+$	
			$\bar{x}$	±SD	$\bar{x}$	±SD	$\bar{x}$	±SD	$\bar{x}$	±SD	$\bar{x}$	±SD
Group T+CM	5	6	*29.4	±3.49	6.3	±1.57	*40.7	±4.42	56.7	±4.78	*4.9	±0.89
	7	6	33.0	±6.81	8.3	±2.23	57.0	±10.8	42.0	±10.5	4.2	±0.98
	14	6	21.9	±4.33	8.3	±1.13	31.5	±4.50	60.3	±5.30	2.7	±0.52
	21	6	25.9	±3.23	6.2	±1.74	36.7	±5.07	56.3	±4.35	4.2	±0.92
Group T	5	6	23.5	±4.54	6.7	±1.11	34.3	±3.25	61.8	±4.89	3.5	±0.53
	7	6	28.3	±5.26	6.6	±0.95	49.0	±6.59	49.3	±7.29	4.3	±0.24
	14	6	22.6	±5.38	7.0	±1.09	33.2	±3.03	58.9	±4.66	3.2	±0.65
	21	6	26.4	±2.10	6.7	±0.80	38.3	±2.55	57.4	±5.53	4.0	±0.77

Table 3B.
The percentage of mesenteric lymph nodes (MLN) lymphocyte subpopulations in the mice infected with *T. spiralis* (Group T) and infected with *T. spiralis* larvae and receiving *Cornus mass* iridoid-anthocyanin aqueous extract (Group T+CM). Mean values (n = 6) and standard deviations are presented. *p<0.05 **p<0.01.

	Day after infection	n	CD4$^+$		CD8$^+$		CD3$^+$		CD19$^+$		CD4$^+$/8$^+$	
			$\bar{x}$	±SD	$\bar{x}$	±SD	$\bar{x}$	±SD	$\bar{x}$	±SD	$\bar{x}$	±SD
Group T+CM	5	6	54.3	±2.12	12.0	±1.33	69.6	±2.41	29.3	±2.00	4.6	±0.67
	7	6	*68.6	±2.13	13.6	±5.55	77.4	±12.6	22.8	±13.0	5.6	±1.72
	14	6	55.0	±6.93	*17.1	±2.35	69.7	±8.33	26.5	±8.08	3.3	±0.63
	21	6	62.6	±6.49	*13.6	±1.05	79.0	±6.01	18.4	±5.57	*4.6	±0.60
Group T	5	6	53.2	±3.70	13.6	±2.34	68.3	±5.35	30.8	±5.13	4.0	±0.53
	7	6	59.5	±4.20	11.8	±1.13	77.7	±6.32	22.0	±6.32	5.1	±0.45
	14	6	48.8	±9.19	13.7	±2.55	64.2	±10.6	31.1	±8.74	3.6	±1.00
	21	6	59.4	±3.02	16.9	±2.88	77.6	±5.08	19.9	±4.01	3.6	±0.72

Table 4A.
The absolute numbers of splenocyte subpopulations in the mice infected with *T. spiralis* (Group T) and infected with *T. spiralis* larvae and receiving *Cornus mass* iridoid-anthocyanin aqueous extract (Group T+CM). Mean values (n = 6) and standard deviations are presented. *p<0.05 **p<0.01

	Day after infection	n	CD4$^+$		CD8$^+$		CD3$^+$		CD19$^+$	
			$\bar{x}$	±SD	$\bar{x}$	±SD	$\bar{x}$	±SD	$\bar{x}$	±SD
Group T+CM	5	6	51.2	±11.0	*10.9	±3.02	70.8	±14.0	*97.8	±13.3
	7	6	49.2	±20.3	12.7	±6.82	86.5	±40.6	68.2	±39.7
	14	6	57.9	±23.1	21.4	±6.53	82.8	±30.3	158.4	±54.9
	21	6	*80.0	±21.3	18.4	±2.74	*112.1	±26.4	175.3	±52.3
Group T	5	6	57.8	±12.7	16.5	±2.81	84.8	±14.7	155.9	±39.6
	7	6	49.9	±17.7	11.7	±4.17	85.8	±24.9	89.8	±38.0
	14	6	55.2	±11.7	17.0	±1.48	81.1	±8.58	145.2	±25.0
	21	6	54.8	±12.7	13.9	±4.04	76.2	±19.1	112.6	±25.7

Table 4B.
The absolute numbers of mesenteric lymph nodes (MLN) lymphocytes subpopulations in the mice infected with *T. spiralis* (Group T) and infected with *T. spiralis* larvae and receiving *Cornus mass* iridoid-anthocyanin aqueous extract (Group T+CM). Mean values (n = 6) and standard deviations are presented . *p<0.05 **p<0.01

	Day after infection	n	CD4$^+$		CD8$^+$		CD3$^+$		CD19$^+$	
			$\bar{x}$	±SD	$\bar{x}$	±SD	$\bar{x}$	±SD	$\bar{x}$	±SD
Group T+CM	5	6	31.6	±9.42	7.1	±2.61	40.5	±14.5	16.8	±5.24
	7	6	20.6	±3.75	4.1	±1.79	22.8	±5.89	6.5	±3.49
	14	6	36.5	±8.91	11.6	±4.03	46.5	±12.6	*17.6	±5.51
	21	6	22.6	±6.96	*5.0	±1.86	28.8	±9.25	6.9	±3.64
Group T	5	6	34.0	±14.8	8.9	±4.98	41.3	±19.2	17.4	±4.86
	7	6	24.4	±16.7	4.7	±2.95	30.9	±20.1	8.9	±7.12
	14	6	50.6	±16.9	14.1	±4.38	66.0	±19.6	31.0	±9.18
	21	6	34.9	±14.1	10.0	±4.97	45.9	±19.5	10.8	±2.22

Table 6A. *T. spiralis* adult count in the small intestine
Mean values (n = 6) and standard deviations are presented. **p<0.01

	Day after infection	Group T+CM		Group T	
		$\bar{x}$	±SD	$\bar{x}$	±SD
No of adult *T. spiralis*	5	**33.33	±10.8	52.67	±12.2
	7	23.83	±8.3	34.33	±10.5
	14	1.17	±1.2	2.16	±1.7
	21	0.00	±0.0	0.50	±0.8

Table 6B. *T. spiralis* total larval count in the muscles
Mean values (n = 6) and standard deviations are presented.

	Group T+CM		Group T	
	$\bar{x}$	±SD	$\bar{x}$	±SD
No of larvae *T. spiralis* in 60 dai	8791.7 ± 2507		10458.3 ± 5173	

Table 5A. Proliferative response of splenocytes to ConA. Mean values (n = 6) and standard deviations are presented. *p<0.05 **p<0.01

Spleen			Index 0.9	Index 0.45	Index 0.225
Group	Day after infection	n	x ± SD	x ± SD	x ± SD
Group T+CM	5	6	1.41 ±0.439	1.32 ±0.433	1.30* ±0.294
	7	6	2.87 ±1.652	2.33 ±0.903	1.80 ±0.757
	14	6	2.56 ±1.246	1.93 ±0.879	1.81 ±0.776
	21	6	2.08 ±0.502	1.74 ±0.290	1.64 ±0.221
Group T	5	6	0.85 ±0.400	0.80 ±0.230	0.87 ±0.117
	7	6	2.08 ±1.207	1.79 ±1.060	1.70 ±0.961
	14	6	2.18 ±1.074	1.89 ±0.928	1.79 ±0.846
	21	6	2.96 ±0.985	2.55 ±0.898	2.01 ±0.335

Table 5B . Proliferative response of MLN cells to ConA. Mean values (n = 6) and standard deviations are presented. *p<0.05 **p<0.01

MLN			Index 0.9	Index 0.45	Index 0.225
Group	Day after infection	n	x ± SD	x ± SD	x ± SD
Group T+CM	5	6	1.26 ±0.338	1.04 ±0.233	0.97** ±0.142
	7	6	4.55 ±1.910	3.73 ±1.788	3.20 ±1.077
	14	6	2.49 ±0.487	2.28 ±0.976	1.95 ±1.031
	21	6	2.44 ±0.942	2.00 ±0.570	1.35 ±0.421
Group T	5	6	1.65 ±0.720	1.47 ±0.503	1.98 ±0.414
	7	6	3.45 ±1.364	2.69 ±1.004	1.64 ±0.924
	14	6	3.21 ±1.137	2.31 ±0.510	1.46 ±0.263
	21	6	2.11 ±0.570	1.61 ±0.296	1.54 ±0.258

Highlights

- The immunomodulatory properties of iridoid-anthocyanin aqueous extract of *Cornus mas* L were studied in the course of experimentally-induced trichinellosis in mice.
- Iridoid-anthocyanin extract of cornelian cherry fruits affected hematological parameters and proliferative activity of lymphocytes in *T. spiralis* infected mice.
- Iridoid-anthocyanin extract of cornelian cherry altered the percentage and absolute number of T cells subpopulations and B lymphocytes in the spleen and mesenteric lymph nodes in *T. spiralis* infected mice.