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Resveratrol reduces oxidative damage and inflammation in mice infected with *Trichinella spiralis*

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Research Paper

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Abstract

Trichinellosis is a serious food-borne zoonotic infection of cosmopolitan distribution. Currently, treatment for trichinellosis is far from ideal. Given the important role of oxidative stress and immune-mediated inflammation in the pathogenesis of trichinellosis, this study was designed to evaluate the possible protective effects of resveratrol (RSV) during the intestinal and muscular phases of *Trichinella spiralis* infection in mice. The oral administration of RSV at a dose of 20 mg/kg once daily for two weeks resulted in significant reductions in both adult and larval counts; significant improvements in the redox status of the small intestine and muscles; a significant reduction in interleukin 4, pentraxin 3 and vascular endothelial growth factor expression; and the mitigation of intestinal and muscular inflammation. In conclusion, this study identifies RSV as a promising agent for the treatment of experimental trichinellosis, and more studies in experimental animals and humans are worth consideration.

Introduction

Trichinella spiralis is the chief causative agent of trichinellosis (Murrell & Pozio, 2011). Humans become infected by eating raw or undercooked meat containing viable infective *T. spiralis* larvae (Pozio, 2007). Adult females of this parasitic species reside in the small intestinal epithelium of the host, leading to immune-mediated inflammation and hypersensitivity reactions that induce evident intestinal pathology (Khan, 2008). Moreover, *T. spiralis* has an exceptional relation with skeletal muscle owing to its distinctive ability to undergo intracellular growth in muscle cells. Once infected, muscle cells undergo extensive morphological and biochemical changes that allow them to develop into a protective refuge (a nurse cell) for the parasite (Wu *et al.*, 2008). The long-term infection of muscles with *Trichinella* results in a very powerful interaction with the host immune system, leading to marked inflammation of the affected muscles (Bruschi & Chiumiento, 2011).

Many factors other than direct injury induced by the parasite itself contribute to tissue damage in trichinellosis. One of the main causes of this damage is the oxidative stress state that accompanies *Trichinella* infection, as revealed by the increased production of various stress markers, such as GSTO-1, haem oxygenase I (Bruschi *et al.*, 2003), superoxide dismutase and malondialdehyde (MDA) (Mido *et al.*, 2012). Furthermore, inflammatory cells that produce excessive amounts of reactive oxygen species (ROS), nitrogen species and other free radicals upon activation are recruited (Chiumiento & Bruschi, 2009). Moreover, there is increased production of lipoperoxidized proteins in nurse cells (Bruschi *et al.*, 2003). Therefore, antioxidants and anti-inflammatory drugs are expected to help protect hosts against these injurious factors (Shimoni *et al.*, 2007; Kazemzadeh *et al.*, 2014). However, universally prescribed nonsteroidal or steroidal anti-inflammatory drugs possess many adverse effects that limit their usage (Barnes, 2014; Badri *et al.*, 2016; Oray *et al.*, 2016). Accordingly, there is a pressing need to examine new, safe and efficient compounds that have anti-inflammatory and anti-oxidant properties (Kunnumakkara *et al.*, 2018).

Resveratrol (RSV) is a polyphenolic stilbene abundant in the rinds of red fruits such as berries and grapes and in nuts (Szkudelska & Szkudelski, 2010). Plants produce it as a phytoalexin, and it makes them more resistant to stressful conditions and microbial and fungal infections (Chedea *et al.*, 2017). It has a wide safety margin and is commonly used as an over-the-counter nutraceutical (Vidavalur *et al.*, 2006). Accumulating data indicate its beneficial effects in many diseases, such as diabetes (Su *et al.*, 2006), muscular dystrophy (Hori *et al.*, 2011) and atherosclerosis (Wu & Hsieh, 2011). Additionally, it possesses neuroprotective effects (Sun *et al.*, 2010), cardioprotective effects (Bradamante *et al.*, 2004; Tanno *et al.*, 2010) and anti-inflammatory effects (Donnelly *et al.*, 2004). Furthermore, several studies have demonstrated that RSV has antimicrobial activities (Docherty *et al.*, 1999; Chan, 2002; Wang *et al.*, 2006).

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RSV is a potent anti-oxidant. It decreases intracellular ROS production through different mechanisms, including direct scavenging and activating enzymes, such as mitochondrial superoxide dismutase, that provide anti-oxidant defence (Leonard *et al.*, 2003; Robb *et al.*, 2008; Tanno *et al.*, 2010). Additionally, it acts as a modulator of several vital enzymes that play a role in cell survival, such as cyclooxygenases, protein kinase C, lipoxygenase, protein tyrosine kinase, ribonucleotide reductase, inducible nitric oxide synthase and P450 (Soliman *et al.*, 2017). Therefore, we aimed to explore the potential effects of RSV administration on the therapeutic outcome of the intestinal and muscular phases of experimental trichinellosis.

Materials and methods

Animals and parasites

Male Swiss albino mice aged 6–8 weeks and weighing 25–30 g were purchased from the Theodore Bilharz Research Institute (Giza, Egypt). They were maintained in accordance with institutional and national guidelines. The mice were acclimatized for seven days prior to being used in the experiments. The mice were infected with *T. spiralis* L1 larvae orally according to the method described by Dunn & Wright (1985). The *Trichinella* species used in this study was genotyped as *T. spiralis* (*Trichinella* - Istituto Superiore di Sanita code: ISS6158) by the European Union Reference Laboratory for Parasites, Superior Institute of Health, Rome, Italy. *Trichinella spiralis* larvae were orally inoculated at a dose of 200 larvae per infected mouse.

Experimental design

Animals were divided into four groups: the uninfected mouse group (20 mice; negative control); the infected mouse group, which did not receive any medication (40 mice; positive control); the early treatment group (20 mice), which received RSV for two weeks starting from the first day post-infection (p.i.); and the late treatment group (20 mice), which was treated with RSV for two weeks starting from the 28th day p.i. Additionally, seven mice received only the solvent carboxymethyl cellulose in distilled water.

On the 15th day p.i., ten mice from each of the control groups and from the early treatment group were sacrificed, and the small intestines were collected; one piece from each sample (1 cm from the jejunum) was used for histopathological examination, and another part was used for biochemical analysis. Another ten mice from the positive control group and from the early treatment group were sacrificed, and the small intestines were used to count adult worms.

On the 43rd day p.i., ten mice from the positive control group and from the late treatment group were sacrificed, and total larvae were counted in the muscles. Furthermore, ten mice from each of the control groups and from the late treatment group were sacrificed, and similar skeletal muscle samples were taken and used for the histopathological study, immunohistochemical study and biochemical analysis.

Drugs

Resveratrol powder was purchased from Sigma-Aldrich Chemie (Steinheim, Germany) and was administered orally as a freshly prepared suspension in 0.5% carboxymethyl cellulose in distilled water at a dose of 20 mg/kg once daily (Soliman *et al.*, 2017).

Parasitological assays

Isolation and counting of adult worms

Mice were euthanized, and the small intestines were separated. After the intestines were washed with physiological saline, they were divided into 1-cm portions and kept in 10 ml physiological saline for 2 h at 37°C. The saline was collected using a pipette, and the intestines were washed three times with physiological saline. All of the fluid was collected and centrifuged at 2000 rpm for 3 min. The supernatant was removed, the sediment was reconstituted in 3–5 drops of physiological saline and the number of adults was counted by examining the reconstituted sediment drop by drop at a magnification of 20× (Wakelin & Lloyd, 1976).

Total larval burden in muscles

Mice were euthanized on the 43rd day p.i. Muscle larval counts in whole carcasses were determined according to the method described by Dunn & Wright (1985). Briefly, each mouse was dissected and digested in 1% pepsin-hydrochloride in 200 ml distilled water. Following incubation of the mixture at 37°C for one hour with continuous stirring by means of an electric stirrer, encysted larvae were collected via the sedimentation technique and then washed several times in distilled water. The number of larvae was counted microscopically using a McMaster counting chamber (Lauda-Konigshofen, Germany).

Biochemical assays

Homogenates and protein concentration

Intestinal and muscle specimens were dissected and washed with ice-cold saline, cut into multiple small pieces, weighed, homogenized with 50 mM phosphate buffer (pH 7.4) and centrifuged at 12,000×g for 20 min at 4°C. Then, the resultant supernatant (free of insoluble materials) was frozen at −80°C until analysis. Tissue protein content was determined by the method described by Lowry *et al.* (1951).

Assessment of oxidant/anti-oxidant status in intestinal and muscle tissue homogenates

Biochemical assays for xanthine oxidase (XO) activity and total anti-oxidant capacity (TAC) were analysed as previously described by Litwack *et al.* (1952) and Koracevic *et al.* (2001), respectively. Additionally, MDA and reduced glutathione (GSH) levels were assayed using commercial kits supplied by Biodiagnostic (Giza, Egypt).

Enzyme-linked immunosorbent assays (ELISAs)

Interleukin 4 (IL-4) and pentraxin 3 (PTX3) levels in intestinal and muscle tissue homogenates were measured using ELISA kits (RayBiotech Inc., Peachtree Corners, Georgia, USA, and Chongqing Biospes Co., Chongqing, China, respectively). All ELISA techniques were performed according to the manufacturer's protocol and read on a microplate reader (Stat Fax®2100, Fisher Bioblock Scientific, Strasbourg, France) at 450 nm with a correction wavelength set at 570 nm.

Histopathological study

Tissue samples from the studied groups were fixed in 10% formalin for 24 h, washed in water for 12 h, dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin blocks that were sectioned at a thickness of 5 µm by a microtome

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and then stained with haematoxylin and eosin. Subjective semi-quantitative histopathological scoring was used to assess the histopathological characteristics of the small intestine and muscle sections (Othman *et al.*, 2016). Tissue section examination and scoring were carried out in a blinded manner.

For small intestinal specimens, histopathological evaluation of the following parameters was performed: the extent of inflammatory cell infiltrates within the core of the intestinal villi and submucosa (+1 = mild reaction; +2 = moderate reaction; and +3 = intense reaction); pathological changes in the intestinal architecture, including epithelial changes and hyperplasia of goblet cells (+1 = mild; +2 = moderate; and +3 = severe); and the mucosal architecture and the degree of villous atrophy (+1 = mild; +2 = moderate; and +3 = severe). For skeletal muscle specimens, the extent of the inflammatory reaction surrounding the capsule was evaluated and scored (+1 = mild reaction; +2 = moderate reaction; and +3 = intense reaction).

To assess the aforementioned histopathological parameters, five histological sections/mouse and ten low-power fields (100×) from each of the examined histological sections were examined, and then the average score was calculated.

Study of vascular endothelial growth factor (VEGF) in skeletal muscles

Muscle sections were deparaffinized and treated with 3% hydrogen peroxide in methanol to block endogenous peroxidase activity. Antigen retrieval was performed. Then, a monoclonal antibody against VEGF (clone: EP1176 y, ready-to-use, GENOVA Diagnostics Company, Asheville, North Carolina, USA) was applied to the sections and incubated for 30 min at room temperature. The antigen–antibody complex was visualized using the biotin–streptavidin–peroxidase method. The colour was developed with diaminobenzidine solution, and the sections were lightly counterstained with haematoxylin. The slides were then dehydrated and mounted.

Analysis of VEGF staining

Cells positive for VEGF immunostaining showed brownish cytoplasmic staining. Immunohistochemical scores (IHSs) were determined by combining the percentage of positively stained cells (quantity score) with the staining intensity score. The scores ranged from 0 to 4 as follows: 0 = no immunostaining; 1 = 1–10% of the cells were positive; 2 = 11–50% of the cells were positive; 3 = 51–80% of the cells were positive; and 4 = ≥81% of the cells were positive. The staining intensity was scored as 0 (negative), 1 (weak), 2 (moderate) or 3 (strong). IHSs were obtained by multiplying the quantity score (0–4) by the staining intensity score (0–3) and ranged from 0 to 12. A score of 9–12 was considered strong immunoreactivity (+3), a score of 5–8 was considered moderate immunoreactivity (+2), a score of 1–4 was considered weak reactivity (+1) and a score of 0 was considered negative (Gou *et al.*, 2011). To evaluate VEGF expression, five histological sections/mouse and ten high-power fields (400×) from each examined section were examined, and then the average score was calculated.

Statistical analysis

The data are presented as the mean ± standard deviation. Analysis of variance was used to compare more than two groups, and the probability of a significant difference between the means of two groups was determined by a post-hoc test (for biochemical indices) and Monte Carlo exact test for chi square (for histopathological and IHSs). Differences were considered significant when $P < 0.05$. Statistical analyses were performed according to conventional procedures using Statistical Package for the Social Sciences (SPSS Inc., Chicago, Illinois, USA) software for Windows version 20.

Results

Number of adult worms in the small intestine and larvae in the muscles

The mean number of adult worms in the small intestines of the early treatment group (23.40 ± 2.41) was significantly lower than that in the positive control group (39.80 ± 3.70) ($P = 0.001$). Likewise, our results revealed a significant reduction in the number of total larvae in the late treatment group (7548.20 ± 840.74) compared to the positive control group (12,635.40 ± 803.61) ($P = 0.001$).

Biochemical findings

Assay of oxidant/anti-oxidant markers

The levels of XO and MDA in small intestinal homogenates and muscle homogenates were significantly increased in the positive control group compared to the negative control group ($P = 0.001$). However, the levels of these markers were significantly decreased in the RSV-treated groups compared to the positive control group ($P = 0.001$). On the other hand, significant decreases in TAC and GSH levels in small intestinal homogenates and muscle homogenates were observed in the positive control group compared to the negative control group ($P = 0.001$). The TAC and GSH levels were significantly enhanced in RSV-treated mice compared to positive control mice (tables 1 and 2).

IL-4 and PTX3 in small intestinal homogenates and muscle homogenates

The levels of IL-4 and PTX3 were compared between all groups, and they were significantly increased in the positive control group compared with the negative control group. Following the administration of RSV to mice, IL-4 and PTX3 levels were significantly reduced compared to those in the positive control group (tables 3 and 4).

Histopathological results

Small intestine findings

Histopathological examination of sections of the small intestine samples from the positive control group showed signs of inflammation in the mucosa and submucosa and the core of the villi. The inflammatory infiltrates principally consisted of eosinophils and neutrophils. Furthermore, there was ulceration and sloughing of the intestinal mucosal epithelium, goblet cell hyperplasia and a decrease in the length of the villi (fig. 1a). Compared to sections from the infected control group, sections from the early treatment group exhibited improvements in histopathological changes and a reduction in inflammatory cell infiltrates (fig. 1b).The histopathological findings in small intestinal sections of all studied groups are shown in Table 5.

Skeletal muscle findings

Histopathological examination of skeletal muscle sections from the positive control group revealed that multiple sections contained encysted *T. spiralis* larvae surrounded by nurse cells and a collagen capsule. Moreover, a massive inflammatory cell infiltrate composed mainly of histiocytes, lymphocytes, plasma cells

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Table 1. Redox parameters in small intestine homogenates ($n = 10$).

	Group	Range	Mean ± SD	F test		
Intestinal xanthine oxidase activity (U/mg protein)	Negative control group	127.33–129.98	129.04 ± 1.08	32.835	***P1***	**0.001***
	Positive control group	219.3–357.6	291.84 ± 63.42		*P2*	0.986
	Early treatment group	127.42–130.98	129.45 ± 1.50		***P3***	**0.001***
Intestinal malondialdehyde level (nmol/g tissue)	Negative control group	1.05–1.92	1.40 ± 0.44	64.958	***P1***	**0.001***
	Positive control group	3.45–4.99	4.25 ± 0.59		*P2*	0.778
	Early treatment group	1.21–1.78	1.49 ± 0.26		***P3***	**0.001***
Reduced glutathione level (mg/g tissue)	Negative control group	5.04–5.98	5.50 ± 0.27	98.561	***P1***	**0.001***
	Positive control group	1.15–1.58	1.24 ± 0.36		*P2*	0.087
	Early treatment group	4.45–5.49	5.06 ± 0.18		***P3***	**0.001***
Intestinal total anti-oxidant capacity (μmol/g tissue)	Negative control group	4.12–4.99	4.70 ± 0.38	103.387	***P1***	**0.001***
	Positive control group	1.02–1.94	1.66 ± 0.37		*P2*	0.999
	Early treatment group	3.98–4.98	4.70 ± 0.41		***P3***	**0.001***

*P*1: negative control group vs positive control group.
*P*2: negative control group vs early treatment group.
*P*3: positive control group vs early treatment group.
n = number of studied mice in each group.
*$P < 0.05$ (significant). SD, standard deviation.

Table 2. Redox parameters in skeletal muscle homogenates ($n = 10$).

	Group	Range	Mean ± SD	F test		
Muscle xanthine oxidase activity (U/mg protein)	Negative control group	169.65–179.59	176.22 ± 4.02	61.784	***P1***	**0.001***
	Positive control group	312.61–423.45	365.66 ± 49.38		*P2*	0.111
	Late treatment group	200.01–213.79	207.62 ± 6.97		***P3***	**0.001***
Muscle malondialdehyde level (nmol/g tissue)	Negative control group	1.69–1.98	1.88 ± 0.12	68.984	***P1***	**0.001***
	Positive control group	3.54–4.29	3.97 ± 0.32		***P2***	**0.020***
	Late treatment group	2.06–2.89	2.38 ± 0.37		***P3***	**0.001***
Reduced glutathione level (mg/g tissue)	Negative control group	3.78–5.57	4.18 ± 0.29	87.951	***P1***	**0.001***
	Positive control group	1.78–2.12	1.47 ± 0.54		*P2*	0.567
	Late treatment group	3.54–4.98	4.09 ± 0.55		***P3***	**0.001***
Muscle total anti-oxidant capacity (μmol/g tissue)	Negative control group	5.32–6.29	5.79 ± 0.40	166.840	***P1***	**0.001***
	Positive control group	1.72–2.52	1.95 ± 0.32		***P2***	**0.001***
	Late treatment group	4.01–4.71	4.31 ± 0.26		***P3***	**0.001***

*P*1: negative control group vs positive control group.
*P*2: negative control group vs late treatment group.
*P*3: positive control group vs late treatment group.
n = number of studied mice in each group.
*$P < 0.05$ (significant). SD, standard deviation.

and eosinophils surrounded the encysted larvae and diffusely infiltrated the affected muscle fibres (fig. 1c). Upon examination of sections from the late treatment group, we found a significant decrease ($P = 0.001$) in the extent of inflammatory cell infiltration and a decrease in the number of muscle larvae compared to those in sections from the positive control group (fig. 1d).The extent of the inflammatory cellular infiltarion in the skeletal muscle sections of the studied groups is shown in Table 6.

Immunohistochemical findings

In the negative control group, no VEGF reaction was detected in muscle sections, whereas a strong (+3) reaction was detected in the positive control group. This positive immunostaining was predominantly obvious in the cytoplasm of nurse cells and the cytoplasm of the surrounding inflammatory cells (fig. 2a). A significant reduction in VEGF expression was observed in the late treatment group compared to the positive control group ($P < 0.05$) (fig. 2b). VEGF expression in the experimental groups is summarized in table 7.

Discussion

Diverse plant-derived polyphenols have been used extensively in various studies in an attempt to find innovative drugs that have inhibitory effects against various pathogens (Lai & Roy, 2004;

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Table 3. Interleukin 4 levels in the different groups ($n = 10$).

	Group	Range	Mean ± SD	F test		
Intestinal IL4 level (pg/mg protein)	Negative control group	118.57–130.08	123.17 ± 4.58	80.994	***P1***	**0.001***
	Positive control group	278.92–357.64	310.33 ± 29.15		*P2*	0.052
	Early treatment group	118.79–200.06	157.30 ± 31.13		***P3***	**0.001***
Muscle IL4 level (pg/mg protein)	Negative control group	138.76–143.21	140.40 ± 1.91	1506.672	***P1***	**0.001***
	Positive control group	387.52–400.23	396.73 ± 5.44		***P4***	**0.001***
	Late treatment group	179.62–210.58	194.80 ± 12.18		***P5***	**0.001***

*P*1: negative control group vs positive control group.
*P*2: negative control group vs early treatment group.
*P*3: positive control group vs early treatment group.
*P*4: negative control group vs late treatment group.
*P*5: positive control group vs late treatment group.
n = number of studied mice in each group.
*$P < 0.05$ (significant). SD, standard deviation.

Table 4. Pentraxin 3 levels in the different groups ($n = 10$).

	Group	Range	Mean ± SD	F test		
Intestinal pentraxin 3 (PTX3) level (pg/mg protein)	Negative control group	199.98–218.06	204.94 ± 7.65	338.674	***P1***	**0.001***
	Positive control group	285.32–304.65	294.58 ± 8.00		***P2***	**0.001***
	Early treatment group	100.51–134.76	116.14 ± 15.16		***P3***	**0.001***
Muscle PTX3 level (pg/mg protein)	Negative control group	150.05–210.23	183.17 ± 25.19	121.139	***P1***	**0.001***
	Positive control group	300.03–310.65	304.34 ± 5.17		***P4***	**0.001***
	Late treatment group	102.54–150.41	123.68 ± 19.70		***P5***	**0.001***

*P*1: negative control group vs positive control group.
*P*2: negative control group vs early treatment group.
*P*3: positive control group vs early treatment group.
*P*4: negative control group vs late treatment group.
*P*5: positive control group vs late treatment group.
n = number of studied mice in each group.
*$P < 0.05$ (significant). SD, standard deviation.

Table 5. Histopathological findings in small intestine sections from the studied groups ($n = 10$).

	Extent of inflammatory cell infiltrates (score)			Mucosal architectural changes and degree of villous atrophy (score)			Epithelial changes and hyperplasia of goblet cells (score)		
Group	+1	+2	+3	+1	+2	+3	+1	+2	+3
Positive control group	0	2	8	0	1	9	0	2	8
Early treatment group	7	3	0	8	2	0	6	4	0
X2	17.334			15.201			14.672		
P-value	0.001*			0.001*			0.001*		

n = number of studied mice in each group.
*$P < 0.05$ (significant).

Taguri *et al*., 2004). One of these compounds is RSV. Many studies have investigated its effectiveness and have shown that RSV has considerable antibacterial, antifungal and antiviral properties (Docherty *et al*., 1999; Chan, 2002; Jung *et al*., 2005). In addition, the inhibitory effects of RSV against protozoans that infect humans, such as *Leishmania major* and *Encephalitozoon cuniculi*, have been demonstrated (Leiro *et al*., 2004; Kedzierski *et al*., 2007). Nevertheless, few studies have examined its anthelmintic activities (Soliman *et al*., 2017).

In the present study, the effect of RSV on experimental trichinellosis was investigated. A significant reduction in the parasite burden in intestinal and muscle tissues was observed in treated mice compared to positive control mice. These results are consistent with the findings of *in vitro* study by Ozkoc *et al*. (2009), which showed that RSV has a significant direct lethal effect on newborn larvae of *T. spiralis* and adult *T. spiralis*. However, Ozkoc *et al*. (2009) showed that a significant lethal effect on muscle larvae was detected only upon exposure to very high

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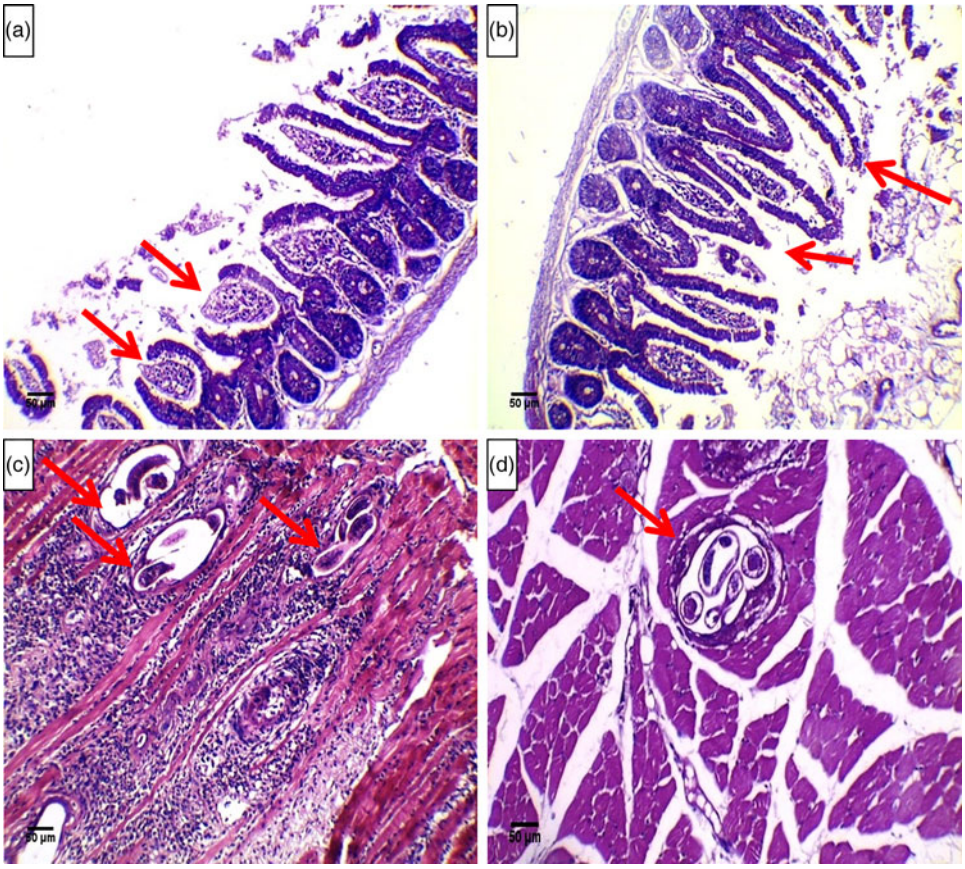

Fig. 1. Histopathological analysis of small intestine sections from (a) the positive control group showing dense inflammatory cellular infiltrates mainly in the core of the villi and extending into the submucosa, hyperplasia of the crypts of Lieberkuhn, a decrease in the villous height to crypt depth ratio and ulceration of the mucosa (arrows) (Haematoxylin and eosin [H&E], 200×) and (b) the early treatment group showing improvements in changes in the small intestines with an evident decrease in inflammatory cell infiltrates (arrows) (H&E, 200×). Histopathological analysis of skeletal muscle sections from (c) the positive control group showing a large number of larvae deposited within the muscle fibres (arrows) surrounded by an intense inflammatory reaction (H&E, 200×) and (d) the late treatment group showing fewer larvae in the muscle (arrows) and a decreased number of inflammatory cell infiltrates (H&E, 200×).

Table 6. The extent of the inflammatory cell infiltrates in skeletal muscle sections from the studied groups ($n = 10$).

	Extent of inflammatory cell infiltrates (score)		
Group	+1	+2	+3
Positive control group	0	1	9
Late treatment group	7	3	0
X2	17.001		
P-value	0.001*		

n = number of studied mice in each group.
*$P < 0.05$ (significant).

concentrations of RSV (440 and 880 µM) for more than 48 h. Researchers have used the ability of RSV to inhibit polyamine metabolism, block calcium channels, induce antimitotic effects by terminating tubulin polymerization and interfere with oxygen consumption by the parasite to explain its antiparasitic effects (Leiro *et al.*, 2004; Kedzierski *et al.*, 2007; Lamas *et al.*, 2009). Additionally, some RSV analogues showed nematocidal effects through the same mechanisms, including the inhibition of tubulin polymerization and oxygen consumption by the parasite (Stadler *et al.*, 1994; Schneider *et al.*, 2003; Chabert *et al.*, 2006). Furthermore, we cannot exclude the possibility that the observed effectiveness of RSV in decreasing muscle larval counts may be attributed to its direct effect on adult worms, which results in a reduction in the number of larvae that reach the muscles.

During the course of *T. spiralis* infection, a large amount of free radicals and ROS are produced both by the parasite itself and by the host during innate and acquired defence reactions against infection (Bruschi *et al.*, 2003; Othman *et al.*, 2016). Anti-oxidants have an important role in metabolism and help protect the host from oxidant-mediated harmful effects (Chiumiento & Bruschi, 2009). Our analysis of oxidative stress biomarkers showed that they were significantly upregulated in the small intestinal and skeletal muscle tissues of the positive control group compared to the negative control group. These results are in agreement with those of several previous studies (Wojtkowiak-Giera *et al.*, 2012; Blum *et al.*, 2013; Kazemzadeh *et al.*, 2014). Oxidative stress was decreased in the RSV-treated groups, as revealed by the increase in TAC and the GSH level and the decrease in XO and MDA levels. These findings are consistent with those of several studies (Jiang *et al.*, 2008; Palsamy & Subramanian, 2008; Das *et al.*, 2010; Toklu *et al.*, 2010; Moridi *et al.*, 2015; Hamza & El-Shenawy, 2017; Soliman *et al.*, 2017; Turkmen *et al.*, 2019) that reported that RSV possesses powerful anti-oxidant properties that help ameliorate oxidative stress.

Infection with *T. spiralis* triggers a very powerful Th2 response, which controls a diversity of responses that are specific to this helminthic infection, including mucosal mastocytosis, goblet cell hyperplasia and intestinal eosinophilic infiltration. There is a broad consensus that Th2 cytokines, including IL-4, IL-5, IL-9 and IL-3, can create a hostile environment for intestinal helminth parasites by producing intense intestinal pathology that may result in the expulsion of these parasites (Urban *et al.*, 1995; Finkleman *et al.*, 1997; Scales *et al.*, 2007). IL-4 plays an important role in the induction of these pathological changes through

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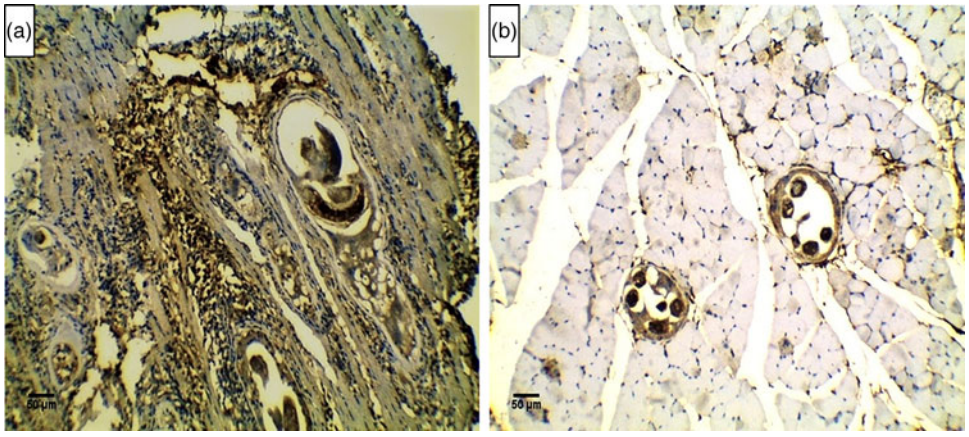

Fig. 2. Immunohistochemical staining of VEGF in skeletal muscle sections from (a) the positive control group showing (+3) VEGF expression within the cytoplasm of the nurse cells and the surrounding inflammatory cells (immunoperoxidase, ×200) and (b) the late treatment group showing weak (+1) cytoplasmic VEGF expression within the nurse cells (immunoperoxidase, 200×).

Table 7. Comparison of VEGF expression in skeletal muscle sections ($n = 10$).

	VEGF immunohistochemistry score					
Group	0	+1	+2	+3	Chi square	*P*-value
Negative control group	10 (100%)	0 (0%)	0 (0%)	0 (0%)	17.334	0.001*
Positive control group	0 (0%)	0 (0%)	1 (10%)	9 (90%)		
Late treatment group	0 (0%)	8 (80%)	2 (20%)	0 (0%)		

n = number of studied mice in each group.
*$P < 0.05$ (significant).

the Stat6 signalling pathway (Arizmendi *et al.*, 2001; Khan *et al.*, 2001). On the other hand, Lawrence *et al.* (1998) showed that in IL-4-deficient mice, the expulsion of *T. spiralis* adult worms can still occur in the absence of severe enteropathy. Furthermore, in the same study, the researchers concluded that intestinal pathological changes are not the main factor that contributes to the expulsion of the parasite. In the present work, there was a significant upregulation in the expression of IL-4 in the small intestinal and muscle homogenates of untreated infected mice compared to negative control mice. Similarly, previous studies by Sofronic-Milosavljevic *et al.* (2013) and Ding *et al.* (2017) reported that IL-4 expression is enhanced in *T. spiralis*-infected animals compared to uninfected animals. According to our results, there was a significant reduction in the expression of IL-4 in the small intestinal and muscle homogenates of RSV-treated infected mice compared to untreated infected mice. These results are in agreement with those of Lee *et al.* (2009), who reported that RSV significantly reduces IL-4 in a mouse model of OVA-induced allergic asthma. We think that a decrease in IL-4 expression may be beneficial in the course of trichinellosis by leading to a decrease in intestinal mastocytosis, which play a major role in the damage and atrophy of the villi during *T. spiralis* infection. Mast cells produce leukotrienes and 5-hydroxytryptamine, which provoke epithelial injury at the tips of villi, a mechanism that may induce villus atrophy (Serna *et al.*, 2006).

The levels of both short and long pentraxins in the blood or tissues rapidly rise under inflammatory conditions and are generally correlated with the severity of the condition that provokes their expression (Norata *et al.*, 2010). The local production of PTX3 (a member of the long pentraxin family) by macrophages, dendritic cells and endothelial cells rapidly occurs upon inflammatory stimulation (Garlanda *et al.*, 2005), and neutrophils liberate PTX3 from cytoplasmic granules at the location of tissue damage or in response to microbial agents (Jaillon *et al.*, 2007). As PTX3 production occurs locally at sites of inflammation, it is believed to be a good self-regulating marker of disease severity (Fazzini *et al.*, 2001). In the present study, PTX3 levels were elevated in the small intestinal and muscle tissues of the positive control group compared to the negative control group. These findings correspond with the results of Savchenko *et al.* (2011), who reported that PTX3 concentrations increase quickly in response to pro-inflammatory signals. However, PTX3 levels are decreased in the treated groups, indicating improvements in tissue injury. These results coincide with those of Erbas *et al.* (2014), who noted a significant reduction in PTX3 levels in RSV-treated diabetic animals compared to untreated diabetic animals.

Due to the noticeable inflammation that occurs in different tissues during the course of *T. spiralis* infection, anti-inflammatory drugs are a basic part of the management protocol of this disease (Shimoni *et al.*, 2007). Interestingly, the current study revealed that RSV ameliorated the severe inflammatory reaction induced by *T. spiralis* infection, as evidenced by the improvements in histopathological changes in the intestines and muscles and the decreased levels of PTX3. Accumulating data have strongly demonstrated the anti-inflammatory activities of RSV in different disease models and different affected organs (Cong *et al.*, 2014; Li *et al.*, 2015; Liu *et al.*, 2016; Wang *et al.*, 2017; Yan *et al.*, 2018). Its inhibitory effects on the expression of inflammatory mediators, including IL-1β, matrix metallopeptidase 13, cyclooxygenases 2, nuclear factor kappa B and many other molecules, have been proposed as mechanisms of its anti-inflammatory actions (Yar *et al.*, 2011; Coutinho *et al.*, 2018).

Angiogenesis is one of the requirements for the maintenance of viability and the proper development of muscle larvae within

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nurse cells. Circulatory rete formation around nurse cells aids in larval nutrition and the efficient disposal of waste products (Ock *et al.*, 2013). During this process, VEGF acts as the chief vascular endothelial stimulating factor (Capó *et al.*, 1998). Hypoxia is the key stimulator of VEGF expression (Sang *et al.*, 2002). In the present study, the RSV-treated group showed weak VEGF expression in skeletal muscles compared with the strong expression in the positive control group. To the best of our knowledge, this is the first study to investigate the effect of resveratrol administration on the expression of VEGF during *T. spiralis* infection. Therefore, we compared our results with those of studies that investigated the effect of resveratrol administration on the expression of VEGF in other disease models. Our results are consistent with those of Liu *et al.* (2012), who reported that RSV exerts anti-osteosarcoma activity through its inhibitory effect on VEGF expression, and those of Yan *et al.* (2018), who concluded that RSV improves cardiovascular function in rats with diabetes-related myocardial infarction via the inhibition of VEGF expression. In the current work, we assume that the reduced expression of VEGF can interrupt the process of angiogenesis, thus depriving developing muscle larvae of necessary nutrients and causing the accumulation of waste products. Altogether, the drug could induce dysontogenesis of muscle larvae, which may be a leading cause of the reduction in larval burden in muscles.

In conclusion, our results collectively show the protective effects of orally administered RSV against several aspects of the pathological consequences of *T. spiralis* infection. To the best of our knowledge, this study demonstrates for the first time that RSV has effects against *T. spiralis* infection *in vivo*. The drug also exhibits notable anti-oxidant and anti-inflammatory activities. Therefore, RSV can be considered a useful adjuvant for the treatment of trichinellosis, and further studies on its usage as an adjuvant in experimental animals and in humans are worth consideration.

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Conflicts of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals. The study protocol was approved by the Laboratory Animal Centre for Research Ethics Committee, Faculty of Medicine, Tanta University (code number 33555/12/19).

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