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TRICHINELLA SPIRALIS INFECTION PROTECTS AGAINST OVALBUMIN-INDUCED ALLERGIC BRONCHIAL ASTHMA IN A MURINE MODEL

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Abstract

Allergic bronchial asthma is a long-lasting illness of the respiratory system characterized by chronic inflammation of the respiratory airways with abnormal Th2-type immune responses to specific allergens. The accumulation of scientific evidence supports the beneficial effects of some parasitic infections in animal models of allergic diseases. Thus, the aim of this work was to discover the influence of infection with *T. spiralis* on ovalbumin-induced acute allergic airway inflammation in mice and to investigate whether this effect is correlated to the infection dose or not. Therefore, the severity of respiratory airways inflammation, the leukocyte counts in the blood and BALF, immunohistochemistry of $FOXP3^{+}CD4^{+}$T cells and the levels of IL-6, IL-13, and IL-10 were assessed. In this experiment, 120 male laboratory bred mice were randomly divided into six groups: induced asthma, saline control, low dose preventive, high dose preventive, low dose therapeutic and high dose therapeutic groups. *T. spiralis* infection attenuated the intensity of pulmonary inflammation, decreased numbers of eosinophils in BALF and blood, reduced levels of IL-6 and IL-13 and elevated levels of IL-10 with a significant upregulation of $FOXP3^{+}CD4^{+}$T cells expression compared to the uninfected induced asthma group. The preventive groups showed the best results with no significant difference between effects of the low and the high doses. In conclusion, *T. spiralis* infection reduced Th2 type infammation and augmented regulatory immune response in ovalbumin-induced acute allergic airway inflammation with better preventive than therapeutic effects. Further investigations are needed to determine the minimal dose of infection that gives the best influence.

***Key words*:** *Trichinella spiralis*; Helminthic therapy; Ovalbumin; Acute allergic airway inflammation; IL-6; IL-13; IL-10; $FOXP3^{+}$ $CD4^{+}$T cells

Introduction

Allergic bronchial asthma is a long-lasting illness of the respiratory system characterized by extensive, reversible, chronic inflammation of the respiratory airways with acute attacks of broncho-obstruction (Peters and Fritz, 2011). At least 10 % of the world's population suffered from allergic diseases (Theofilopoulos, *et al*, 2017) and about 300 million people globally are suffering from bronchial asthma (Loukas *et al*, 2016). Besides, the incidence of bronchial asthma was increasing in the last 40 years particularly in the more developed countries (Bahadori *et al*, 2009; Ananden *et al,* 2010). The inflammatory reaction in allergic asthma was initiated by abnormal T helper 2 (Th2)-type immune responses to specific allergens leading to production of large amounts of cytokines including interleukin (IL-4), IL-5, IL-9, IL-13 & IL-14. Also, there was recruitment of inflammatory cells predominantly eosinophils, mast cells, basophils & Th2 lymphocytes. Also, there were higher levels of allergen specific IgE (Di Lorenzo *et al*, 2009). Th2-type immune response was a common feature of allergic conditions, eosinophilic syndromes, and parasitosis (Fahy, 2015). Th2 lymphocytes were the main promoter and regulatory T-cells (Treg) the main suppressor of numerous characteristics of allergic inflammatory reaction in asthmatic patients (Lambrecht and Hammad, 2013).

The immune response to helminth infections was characterized by the release of certain regulatory cytokines that help in the reduction of the resulting inflammation and facilitate establishment of the chronic infection of helminth in the host. The effects may indirectly help the host by protecting him

from autoimmune or atopic diseases (Cheng *et al*, 2018). The accumulation of scientific evidence supports the beneficial effects of some parasitic infections in animal models of allergic disorders (Chenery *et al*, 2016; Obieglo *et al*, 2018) and autoimmune diseases (El-Malky *et al*, 2011). The hopeful results from clinical studies supported the suppressor effect of some parasitic infections on these diseases (Fleming *et al*, 2011; Summers *et al*, 2005). Various studies in animal models of allergic asthma revealed that some parasites had the capacity to reduce the pathology of asthma by production of regulatory T cells and anti-inflammatory cytokines (Buck *et al*, 2014; Ebner *et al*, 2014; McSorley *et al*, 2014; Finlay *et al*, 2017). That's why the role of helminths and Helminthic therapy attract the attention of many authors worldwide (Zwiernik *et al*, 2019).

Trichinella spiralis (*T. spiralis*) is a distinctive luminal and tissue-dwelling parasitic helminth. It has the ability to manipulate the host immune response by shifting from Th1 to Th2/Treg response (Beiting *et al*, 2007; Guo *et al*, 2016). Several studies reported that *T. spiralis* infection, or the worm products had the capability to repress a set of autoimmune diseases in experimental animals including inflammatory bowel diseases (Yang *et al*, 2014), experimental autoimmune encephalomyelitis (Gruden-Movsesijan *et al*, 2010), collagen induced arthritis (Eissa *et al*, 2016; Cheng *et al*, 2018), and type 1 diabetes (Saunders *et al*, 2007). Also, it could significantly alleviate allergic disorders (Park *et al*, 2011).

Little studies investigated the influence of *T. spiralis* infection on bronchial asthma. This work aimed to discover the possible influence of *T. spiralis* infection on ovalbumin-induced acute allergic airway inflammation in mice and to investigate whether it correlated to the infection dosage or not.

Materials and Methods

Parasite and Animals: *T. spiralis* strain (Istituto Superiore di Sanità code: ISS6158), maintained at Medical Parasitology Department, Tanta Faculty of Medicine, was used in per oral infection. 120 parasite-free male Swiss Albino mice (6-8 weeks old & weighed 25-30gm supplied from Theodore Bilharz Research Institute (Giza) were used in this study.

Ethics statement: Animals were housed in appropriate cages. The care and use of the animals was according to the national and institutional guidelines. All the experiments were done in the Faculty of Medicine, Tanta University. The research ethics committee, Faculty of Medicine, Tanta University, approved the study and its approval code was (33530/11/19).

Induction of *T. spiralis* infection: Three previously infected mice were sacrificed, skinned and eviscerated. After being grinded in meat grinder, the ground mice were put in an artificial gastric juice laboratory prepared in formed 1% pepsin (weight/vol.) and 1% concentrated HCL (volume/vol.) in 200ml. warm tap water per mouse, and incubated at 37˚C for 2 hours with persistent agitation by electric stirrer. The resultant fluid was filtered and suspended in a urine flask for about 30 minutes to allow larval sedimentation. The supernatant fluid was removed and the concentration of larvae in sediment was determined and adjusted to be 100 larvae/0.25 ml. or 200 larvae/0.25 ml. according to the required infection dose of every mouse and given orally (Dunn and Wright, 1985).

Induction and confirmation of acute allergic airway inflammation: Sensitization of the mice was done as follows; they received two intraperitoneal injections of 100µg of Ovalbumin (OVA) (Sigma-Aldrich, USA) in 200 µl of sterile normal saline adsorbed to 1 mg of Aluminum hydroxide (alum) (Sigma, Aldrich, USA) as an adjuvant on days 0 and 7 followed by administration of two doses composed of 50µg of OVA in a total volume of 50µl of sterile normal saline per dose on days 14 &16 by intranasal route (Aranzamend *et al*, 2012). The establishment confirmation of the acute allergic airway inflamma-

tion model was settled by histopathological examination of the mice lung tissues as well as by the manifestations development including scratching of the head and nose, frequent sneezing followed OVA inhalation, development of abdominal muscle twitching and weight loss.

Experimental animals were randomly divided into six groups of 20 mice each: G1 (Induced asthma): was sensitized only. G2 (Saline control): received sterile normal saline by intra-peritoneal injection on day's 0 & 7, & 50µl sterile normal saline intranasal instillation on days 14 & 16. G3 (Low dose preventive): infected with 100 *T. spiralis* muscle larvae/mouse and their sensitization started on the 28th day post infection (P.I.). G4 (High dose preventive): infected with 200 *T. spiralis* muscle larvae/mouse and the sensitization started on the 28th day P.I. G5 (Low dose therapeutic): infected with 100 *T. spiralis* muscle larvae/mouse and the sensitization started on the 10th day P.I. G6 (High dose therapeutic): infected with 200 *T. spiralis* muscle larvae/mouse and the sensitization started on the 10th day P.I.

Mice were sacrificed on the 18th day of sensitization using the approved methods of euthanasia (Close *et al*, 1997) and were submitted to the following procedures:

Histopathological study of lung tissues: The right lung of each mouse was inflated with 0.5mL paraformaldehyde, 10% in phosphate buffer saline (PBS) (0.4m NaCl & 10mM NaPO4) prior to removal. They were fixed in 10% phosphate-buffered formalin, dehydrated, embedded in paraffin, sectioned at 3µm thickness, and then stained with hematoxylin and eosin (H&E). Lung sections were sliced and mounted onto clean glass slides. Three thin cut sections per sample were done. Slides were examined by a blinded pathologist using a light microscope to determine the degree of histopathological changes. The pathological lesions in form of (peribronchiolar inflammation, perivascular inflammation, hypertrophy and hyperplasia of goblet cells) were blindly scored using a semi-quantitative scoring system ranging from absent (no inflammatory cells), mild (a circle of inflammatory cells of 1 cell layer depth), moderate (a circle of inflammatory cells of 2-4 cells depth) to marked inflammation (a circle of inflammatory cells of more than 4 cells depth) (Trujillo-Vargas, 2005). The histopathological parameters were evaluated in 5 low-power fields (×10) per section, and the average score was determined.

Immunohistochemical study to assess the FOXP3^{+}CD4^{+}T cells expression in lung tissues: Paraffin-embedded sections were submitted to deparaffinization in xylene, washed in PBS (pH 7.4), rehydration in a graded series of ethanol solutions, and microwave heating for 15 min in citric buffer (pH 6.0) for antigen retrieval. Next, endogenous peroxidase activity was blocked by addition of 3% hydrogen peroxide and incubation for 10 min. Blocking with normal serum (Invitrogen, Carlsbad, CA) was done followed 30 min later by addition of the primary Goat Anti-FOXP3 (Mouse) Antibody (Catalog No #OAEB00854, Aviva Systems Biology, USA) and incubation overnight at 4°C. Slides washing with PBS was repeated three times, each for 5 min. After that, the biotin-labeled secondary antibody and the streptavidinbiotin complex were added, each for 1hr incubation at room temperature. Slides were dipped in PBS, followed by immersion for 10min in 3, 3′-diaminobenzidine-tetrahydrochlorhydrate (DAB) (Merck, Darmstadt, Germany) solution (0.4mg/mL with 0.003% hydrogen peroxide). Microscopic monitoring of reaction was done and terminated by adding distilled water. The counter-staining with hematoxylin, dehydration and coverslipping of slides were done. Immunoreactivity of FOXP3^{+}CD4^{+}T cells appeared as brown staining of variable degrees of intensity in cytoplasm and/or nucleus of cells. For negative control, normal mouse serum was used instead of the primary antibody and human normal tonsil tissue was used as a positive control.

For the assessment of FOXP3^{+}CD4^{+}T cells

expression in the lung tissues, examination of 10 high-power fields (×400) in each slide by light microscope was done, and the average immunohistochemical score (IHS) was determined. First staining intensity score: (0 = negative, 1 = weak, 2 = moderate, & 3 = strong) and the quantity score (percentage of stained cells) (0 = no immunostaining; 1 = 1-10% of cells are positive; 2 = 11-50% are positive; 3 = 51-80% are positive; and 4 = ≥ 81% of positive) cells were determined. The IHS ranged from 0 to 12 and calculated by means of multiplication of staining intensity score by the quantity score. Negative, weak, moderate or strong immunoractivity corresponded to 0, (1-4), (5-8), and (9-12) IHS scores respectively (Gou *et al*, 2011).

Examination of blood samples and Bronchoalveolar Lavage fluid (BALF): Collection of 20µl blood sample from the bleeding surface at distal part of each mouse tail was done using a pipette containing EDTA anticoagulant. Besides, to collect BALF, ligation of the main stem bronchus of the left lung was done, followed by exposure and cannulation of the trachea through a minor slit inferior to its proximal end using a 1 ml syringe and 23-gauge needle sheathed with polyethylene tubing. Bronchoalveolar washing was done by infusing 1-ml aliquots of sterile saline, followed by gentle massage of the thorax and careful aspiration of the fluid. This procedure was done three times. The entire collected specimen was assembled in a centrifuge tube and rapidly, within 1 hr, centrifugation of 1 ml of the fluid at 400×g, 4°C for 10min. was done. After elimination of the supernatant, sediment was resuspended in 200µl saline and used for cell counting. Leukocytes in blood and BALF were quantified using a hemocytometer to determine total leukocyte counts followed by performance of differential leukocyte counts on Giemsa and Wright's stained slides. Two hundred cells were counted per slide.

ELISA measurement of IL-6, IL-13 & IL-10 in lung tissue homogenates: A part weighed 100 mg of each lung was washed with PBS, dissected, homogenized in 1ml of PBS and stored overnight at -20°C. The homogenates were submitted two times to freezing and thawing to breakdown the cell membranes followed by centrifugation for 20min. at 12,000×g, 2-8°C. The supernatant fluid was collected and kept at -80°C. Centrifugation of the samples was done again after thawing before the examination using ELISA kits for quantitative detection of mouse IL-6 (Catalog No #M6000B, R&D Systems Inc, Minneapolis, MN, USA), IL-13 (Catalog No #88-7137-22, eBioscience, San Diego, CA, USA) & IL-10 (Catalog No #OKBB00194, Aviva Systems Biology, USA) following the manufacturer's protocol.

Statistical analysis: Data were expressed as mean ± standard deviation, analyzed for significance ($P < 0.05$) by statistical analysis of variance (ANOVA) and the Tukey test for multiple comparisons to determine statistical differences between more than two groups. Student's *t*-test was used for comparison between two groups. Monte Carlo exact test for chi-square was utilized for analysis of histopathological and immunohistochemical scores. Analyses were done by software for windows, SPSS version 20.

Results

Histopathological and immunohistochemical results: To determine whether *T. spiralis* infection could prevent and/or reduce features of lung pathology associated with allergic airway inflammation in an OVA-alum acute allergic airway inflammation mouse model, existence of related histopathological parameters including peribronchiolar, perivascular inflammation and hypertrophy and hyperplasia of goblet cells were examined in H&E stained lung sections. Typically inflammatory cell infiltration of lungs, which was characteristic to asthma acute phase, was exacerbated in induced asthma group with marked peribronchial and perivascular inflammatory cell infiltration and adjacent blood vessels congestion (Fig. 1A). In contrast, saline control ones did not show any signs of inflammation or cell infiltration (Fig. 1B). Sem-

iquantitative analysis of pathological features in low & high dose preventive group showed significantly reduced peribronchiolar & perivascular inflammatory cell infiltration of lungs with mild score (Fig. 1C, & D). Decreased inflammation was in low & high dose therapeutic groups as compared to induce asthma group with moderate inflammatory cell infiltration (Tab. 1; Fig. 1E, & F).

Immunohistochemical score of $FOXP3^+$ $CD4^+$T cells expression in lung tissues showed upregulation in *T. spiralis* infected mice in comparison with induced asthma group. It was strong to moderate in low & high dose preventive mice (Fig. 2C, & D) and weak to moderate in low & high dose therapeutic groups (Figs. 2E, & F), but weak to negative in induced asthma group (Tab. 2; Fig. 2A).

Leukocyte counts in blood & BALF: Blood samples and the BALF of induced asthma group presented elevated levels of total leukocyte, eosinophil counts in comparison with the saline control group. Similarly, counts of neutrophils, monocytes and lymphocytes in the blood of induced asthma group were significantly higher than those of the saline control group. Mice in low and high dose preventive groups had significantly decreased cell counts relative to cell counts of induced asthma group. Reduction in cell counts in preventive groups was significantly higher than the therapeutic groups, without significant difference between low dose & high dose preventive groups. Also, difference was non-significant between low dose & high dose therapeutic groups except for blood eosinophil counts were significantly lower in low dose therapeutic group compared to high dose therapeutic one (Tabs. 3 & 4).

Assay of cytokines levels in lung tissue homogenates: Trying to find out the underlying mechanisms of the possible preventive or therapeutic effects of *T. spiralis* infection on acute allergic airway inflammation, this study investigated some key cytokines in the pathogenesis of both allergic asthma and *T. spiralis* infection including Th1 proinflammatory cytokine (IL-6), Th2 cytokine (IL-13) and regulatory cytokine (IL-10) in lung tissue homogenates. IL-6 & IL-13 levels were significantly elevated in induced asthma group compared with saline control. But, there was a significant decrease in both cytokines levels in all *T. spiralis* infected ones. This reduction was significantly higher in the preventive groups relative to the therapeutic groups. In contrast, a significant upregulation in IL-10 levels was observed in all the infected groups compared with the induced asthma group. The low dose preventive group showed the lowest levels of the proinflammatory cytokines IL-6 and IL-13 and the highest level of the regulatory cytokine IL-10 in comparison with the other *T. spiralis* infected groups (Tab. 5).

Table 1: Semiquantitative analysis of histopathological changes in the lung tissues of groups (n=10)

Examined mice	Peribronchiolar inflammation				Perivascular inflammation				Hypertrophy and hyperplasia of goblet cells			
	Absent	Mild	Moderate	Marked	Absent	Mild	Moderate	Marked	Absent	Mild	Moderate	Marked
G1	0	0	1	9	0	0	2	8	0	0	2	8
G2	10	0	0	0	10	0	0	0	10	0	0	0
G3	0	8	2	0	1	7	1	1	2	6	2	0
G4	0	7	3	0	0	7	2	1	2	5	3	0
G5	0	2	7	1	0	3	5	2	0	2	8	0
G6	0	1	8	1	0	3	4	3	0	1	9	0
X^2	108.286				82.917				103.073			
P value	0.001*				0.001*				0.001*			

* $P < 0.05$, Chi-square (X2) test of significance compared proportions between qualitative parameters

Table 2: Immunohistochemical score of $FOXP3^+CD4^+$T cells expression in lung tissues of groups (n=10)

Number of examined mice	$CD4^+$ $FOXP3^+$ T cells Immunohistochemical Score			
	Negative	Weak	Moderate	Strong
G1	7	3	0	0
G2	10	0	0	0
G3	0	0	2	8
G4	0	0	3	7
G5	0	1	9	0
G6	0	2	8	0
X2	58.001			
P value	0.001*			

Table 3: Total and differential leukocyte counts in blood samples of groups (n=10)

(cells /microliter)	G1	G2	G3	G4	G5	G6
Total leukocyte count (Mean ± SD)	17790 ± 1112.65	3790 ± 617.62	12790 ± 733.48	13470 ± 875.79	14762 ± 524.61	14820 ± 488.11
F test	120.985					
P1		0.001*	0.001*	0.001*	0.001*	0.001*
P2			0.001*	0.001*	0.001*	0.001*
P3				0.280	0.004*	0.003*
P4					0.046*	0.038*
P5						0.926
Eosinophils	8760 ± 827.16	736 ± 177.29	3732 ± 861.84	4218 ± 401.15	6968 ± 536.58	7974 ± 582.39
F test	123.679					
P1		0.001*	0.001*	0.001*	0.001*	0.053
P2			0.001*	0.001*	0.001*	0.001*
P3				0.221	0.001*	0.001*
P4					0.001*	0.001*
P5						0.016*
Monocytes	2724 ± 452.36	62 ± 24.88	530 ± 111.36	602 ± 77.91	2574 ± 477.89	2602 ± 440.87
F test	71.277					
P1		0.001*	0.001*	0.001*	0.477	0.562
P2			0.034*	0.016*	0.001*	0.001*
P3				0.732	0.001*	0.001*
P4					0.001*	0.001*
P5						0.894
Segmented neutro-phils	4140 ± 288.10	1530 ± 269.81	2260 ± 207.36	2320 ± 189.21	3140 ± 151.66	3304 ± 161.96
F test	90.862					
P1		0.001*	0.001*	0.001*	0.001*	0.001*
P2			0.001*	0.001*	0.001*	0.001*
P3				0.667	0.001*	0.001*
P4					0.001*	0.001*
P5						0.245
Lymphocytes	3846 ± 55.50	1276 ± 193.98	2306 ± 148.93	2392 ± 98.34	2680 ± 216.68	2792 ± 116.92
F test	155.998					
P1		0.001*	0.001*	0.001*	0.001*	0.001*
P2			0.001*	0.001*	0.001*	0.001*
P3				0.370	0.001*	0.001*
P4					0.005*	0.001*
P5						0.246

* *P* <0.05. *P1*: Comparison with G1, *P2*: Comparison with G2, *P3*: Comparison with G3, *P4*: Comparison with G4 & *P5*: Comparison with G5

Table 4: Total leukocyte count and eosinophils count in bronchoalveolar lavage fluid (n=10)

(cells / microliter)	G1	G2	G3	G4	G5	G6
Total leukocyte count (Mean ± SD)	284 ± 14.82	29 ± 6.44	59.8 ± 6.98	64 ± 6.89	107.2 ± 4.97	113 ± 4.90
F test	613.280					
P1		0.001*	0.001*	0.001*	0.001*	0.001*
P2			0.001*	0.001*	0.001*	0.001*
P3				0.427	0.001*	0.001*
P4					0.001*	0.001*
P5						0.276
Eosinophils	67 ± 11.90	1.4 ± 0.39	10.6 ± 1.14	11.2 ± 1.30	24.4 ± 3.58	26 ± 3.39
F test	95.428					
P1		0.001*	0.001*	0.001*	0.001*	0.001*
P2			0.012*	0.008*	0.001*	0.001*
P3				0.860	0.001*	0.001*
P4					0.001*	0.001*
P5						0.639

* *P* <0.05. *P1*: Comparison with G1, *P2*: Comparison with G2, *P3*: Comparison with G3, *P4*: Comparison with G4 & *P5*: Comparison with G5

Table 5: Cytokine levels in lung tissue homogenates (n=10)

	G1	G2	G3	G4	G5	G6
IL – 6 (pg/mg protein) (Mean ± SD)	153.4 ± 4.04	101.6 ± 4.62	105.8 ± 3.11	110.6 ± 3.91	136.8 ± 2.77	138.6 ± 1.52
F test	186.166					
P1		0.001*	0.001*	0.001*	0.001*	0.001*
P2			0.068	0.001*	0.001*	0.001*
P3				0.039*	0.001*	0.001*
P4					0.001*	0.001*
P5						0.421
IL – 13 (pg/mg protein)	606.2 ± 76.59	108.8 ± 7.36	174.6 ± 29.31	185.2 ± 25.20	355.8 ± 54.06	368 ± 56.28
F test	74.173					
P1		0.001*	0.001*	0.001*	0.001*	0.001*
P2			0.038*	0.018*	0.001*	0.001*
P3				0.727	0.001*	0.001*
P4					0.001*	0.001*
P5						0.688
IL – 10 (pg/mg protein)	162.6 ± 7.27	114.4 ± 5.37	588.2 ± 9.63	567 ± 17.84	268.8 ± 14.75	242 ± 26.32
F test	891.879					
P1		0.001*	0.001*	0.001*	0.001*	0.001*
P2			0.001*	0.001*	0.001*	0.001*
P3				0.037*	0.001*	0.001*
P4					0.001*	0.001*
P5						0.011*

* *P* <0.05. *P1*: Comparison with G1, *P2*: Comparison with G2, *P3*: Comparison with G3, *P4*: Comparison with G4 & *P5*: Comparison with G5

Discussion

The accumulating data about the substantial role of helminths in modulation of the host immune response has encouraged investigation into the medical applications of helminths (Dunne and Cooke, 2005; Zwiernik *et al*, 2019). *T. spiralis* infection modulates the host immune response by different me-chanisms including down regulation of Th1 and Th17-associated cytokines, upgrading of Th2-associated cytokines, promotion of Treg cell-associated cytokines, and the stimulation of various regulatory cells (Sofronic-Milosavljevic *et al*, 2015). These immune-modulating effects of *T. spiralis* were explo-red in the treatment of numerous immunological disorders. For example, experimental autoimmune encephalomyelitis, inflammatory bowel disease and collagen-induced arthritis (Gruden-Movsesijan *et al*, 2010; Yang *et al*, 2014; Cheng *et al*, 2018).

The role of helminths in damping allergic diseases was widely investigated (Mangan *et al*, 2006; Smits *et al*, 2007; Dittrich *et al*, 2008). Here in, this study looked at the effects of *T. spiralis* infection in alleviation of the pathological features of ovalbumin induced mouse model of acute allergic airway inflammation. Furthermore, it aimed to determine whether this modulation could be affected by reducing the infection dosage or not. The marked peribronchitis and perivasculitis, the upregulation in the total leukocyte counts and eosinophil counts in the blood and BALF together with the elevated Th2-cytokine IL-13 in the lung tissue homogenates of the induced asthma group indicate that the induction of allergic airway inflammation model was efficient. These findings agreed with Marinho *et al*. (2016) who described matching results.

The histopathological score of lung tissue inflammation in *T. spiralis* infected groups was significantly reduced than non-infected induced asthma group. Similar reduction in the inflammatory reaction was also reported in models of arthritis and colitis treated with *T. spiralis* infection (Cheng *et al*, 2018; Ashour *et al*, 2014) signified a direct effect of *T. spiralis* infection in reduction of the inflammatory response. Also, the results of cell counts in the blood and BALF were supportive to histopathological findings as there was significant reductions in the total leukocyte counts, eosinophil counts, neutrophil counts in preventive groups compared to induced asthma group. In this context, it was well established that the eosinophils are

the chief cells in the response to allergens. Additionally, they are the key inflammatory cells implicated in the pathophysiology of asthma whether allergic and nonallergic (Lambrecht and Hammad, 2014; Brusselle *et al*, 2013). They release proinflammatory cytokines that aggravate the associated allergen induced inflammation (Erpenbeck *et al*, 2003; Shamri *et al*, 2010). That's why; the reduction in the intensity of pulmonary inflammation could be attributed to the reduced eosinophils recruitment. Besides, the reduction in neutrophils influx in the lung tissues following *T. spiralis* infection could be explained by the ability of the parasite to secrete a 45 kDa glycoptotein capable of hindering neutrophils migrations (Bruschi *et al*, 2000).

$CD4^+CD25^+$regulatory T cells represented around 5-10% of $CD4^+$T cells in healthy mice. Their regulatory function necessitates the expression of the forkhead-winged helix transcription factor gene (FOXP3) (Fontenot *et al*, 2003; Khattri *et al*, 2003). They contributed in the downregulation of Th1 mediated inflammation and Th2-mediated disorders (Tang and Bluestone, 2008). A growing body of evidence supports the existence of alterations or impairments in either the count or the function of Treg cells in allergic individuals relative to healthy controls (Ling *et al*, 2004; Shi *et al*, 2004). During *T. spiralis* infection, there is an increase in the counts and the activity of these populations of cells. Moreover, they have an active role in the immune response throughout the muscular phase of trichinellosis (Beiting *et al*, 2007). In this work, immunohistochemical staining of the lung tissue sections showed the absence or the presence of a very few number of $FOXP3^+CD4^+$T cells within inflammatory cell infiltrate in induced asthma group. By contrast, the expression was strong to moderate in the preventive groups and weak to moderate in the therapeutic groups. These results coincide with the in vitro results of Aranzamendi *et al*. (2012) which showed a significant effect of *T. spiralis* extracts in increasing the expansion of functional Treg cells. Furthermore, Eissa *et al*. (2016) detected similar effects of adminstration of *T. spiralis* antigen in the treatment of induced arthritis and Ashour *et al*. (2014) reported same effects of *T. spiralis* infection on $FOXP3^+CD4^+$T cells expression in induced colitis model.

In the present study, the detected changes in the cytokines profile in the lung tissue homogenates included upregulation of regulatory arm of the immune response and inhibition of both Th1 and Th2 immune responses in the infected groups. These findings are confirmative to the findings of other studies that have shown similar effects of *T. spiralis* infection on the immune response (Beiting *et al*, 2007; Cho *et al*, 2012; Ilic *et al*, 2012). Other investigators showed that IL-10 has an inhibitory effect on the production of proinflammatory cytokines including both Th1 and Th2 related cytokines. Besides, it interferes with eosinophils activation (Hawrylowicz and O'Garra, 2005). This could explain the decrease in the levels of IL-6 and IL-13 in lung tissue homogenates and the reduction in eosinophil counts in the BALF in the infected groups. Additionally, this suggests a correlation between the reduction of the intensity of inflammation in the lungs and the augmentation in the levels of the anti-inflammatory cytokine IL-10. These results go hand in hand with those of the expression of $FOXP3^+CD4^+$Tcells within the lung tissues as they proved to have a major role in the production of IL-10 (Asseman *et al*, 1999; Annacker *et al*, 2001; Suri-Payer and Cantor, 2001; Klein *et al*, 2003).

Of interest, this study examined the effect of two different doses of *T. spiralis* infection 100 or 200 muscle larvae/ mouse 28 days or 10 days before induction of asthma. The results showed a protective effect of either dose at both time points on induced asthma. However, the best results were those of the preventive groups with non-significant difference between the effect of low and high doses of infection. This agreed with Aranza-

mendi *et al*. (2012) who concluded that the degree of the protection intensified with the progression of the course of infection reaching the best effects in the chronic muscle phase. The time needed for the muscle larval stage of *T. spiralis* to develop and induce proliferation of Treg cells was the main factor to determine the protective effect that help in the relief of allergic airway inflammation (Fabre *et al*, 2009).

Conclusion

The pulmonary inflammatory response in an ovalbumin-induced acute allergic airway inflammation murine model was significantly alleviated by associated infection with *T. spiralis*. Activation of $FOXP3^{+}CD4^{+}$T cells and the release of the anti-inflammatory cytokine IL-10 were potential mechanisms included in this effect. These protective effects were not dependent on the infection dose but affected by the period of the preexistence of *T. spiralis* infection before onset of ovalbumin sensitization. Studies to determine the smallest dose of infection that produces the best effects and to understand the mechanistic role of $FOXP3^{+}CD4^{+}$T cells in controling allergic airway inflammation are ongoing and will be published in due time.

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Legends of figures

Fig 1. Representative photomicrographs of histopathological findings in lung sections, A- induced asthma group showed marked peribronchial and perivascular inflammatory cell infiltration mainly eosinophils, thickening of bronchial walls and congested adjacent small blood vessels (yellow arrows), B- saline control group showed normal brochial architecture, clear alveoli with intact lumen and no inflammatory cell infiltration, C-low dose preventive group showed scattered areas of mild peribronchial and perivascular inflammatory cell infiltration (yellow arrows) , D- high dose preventive group showing mild peribronchial and perivascular inflammatory cell infiltration, nearly similar to low dose preventive group, E- low dose therapeutic group showed moderate peribronchial and perivascular inflammatory cell infiltration, &F- high dose therapeutic group showed moderate peribronchial and perivascular inflammatory cell infiltration (H&E ×100)

Fig 2: Representative photograph of immunohistochemical stained lung sections showing $FOXP3^{+}CD4^{+}$T cells expression in inflammatory cell infiltrate (yellow arrows). A- induced asthma group showed weak expression, B- saline control group showed negative expression, C- low dose preventive group showed strong expression, D- high dose preventive group showed strong expression, E- low dose therapeutic group showed moderate expression, &F- high dose therapeutic group showed moderate expression (Immunoperoxidase ×400)

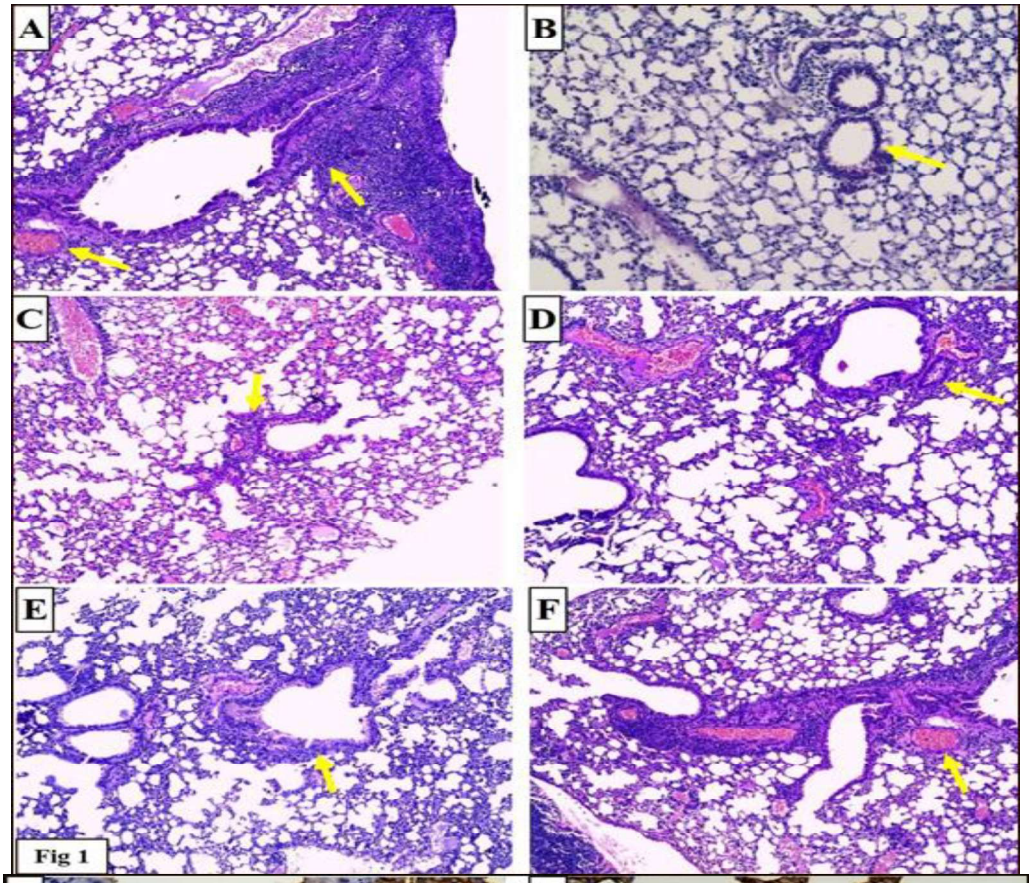

Fig 1

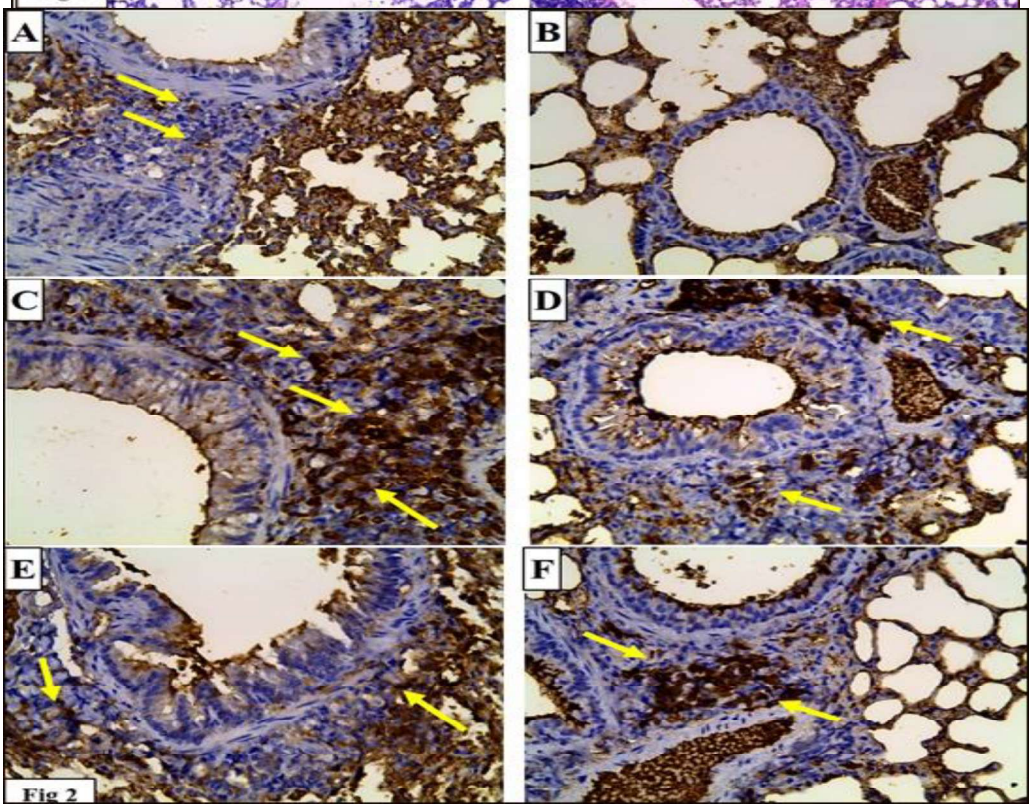

Fig 2