Journal of the Egyptian Society of Parasitology, Vol. 53, No.2, August 2023
J. Egypt. Soc. Parasitol. (JESP), 53(2), 2023: 307 – 314
Online: 2090-2549

ASSESSMENT OF METHANOLIC PLANT EXTRACT OF *ALHAGI MAURORUM* IN TREATING *TRICHINELLA SPIRALIS* INFECTIED MICE

By
HAGER F. A. SAAD, MOHAMED FATHY ABOUEL-NOUR HEGAZY AND DINA M. M. EL-SHEWEHY*
Department of Zoology, Faculty of Science, Mansoura University, Mansoura, Dakahlia Governorate, Egypt (*Correspondence: Dinamagdy@mans.edu.eg)

Abstract

Trichinella spiralis is a recurrent parasite that infects people everywhere. This work compared the usage of methanolic extract of *Alhagi maurorum* versus Albendazole in treating trichinellosis in experimental infected mice. Mice (72) were divided in to six groups: CN: Negative control (non-infected-untreated), C-Alb: Mice treated with albendazole but not infected, C-Alhag: Mice treated with methanolic extract of *Alhagi,* but not infected, CI: Positive control (infected, not treated), T-Alb: Mice were infected then treated with Albendazole, T-Alhag: mice were infected then treated with methanolic extract of A. *maurorum*. Mice were infected orally with *T. spiralis* larvae at a dose of 300 larvae/ mouse except control untreated groups. On 7th day post infection half of the groups were sacrificed for intestinal phase and the second half were sacrificed on t 35th day post infection for the muscular phase. Treatment efficacy was determined by parasitological and immunological parameters. The results showed that *A. maurorum* treated mice gave lowest number of *T. spiralis*. The counts of adult in intestine and larval in muscle, and immunologic data showed lowest levels of IFN-γ, TNF-α, TGF-β, IL-4, IL-2 & IL-12 and highest level of IL-10 than infected control mice.

Keywords: Trichinosis, Albendazole, *Alhagi maurorum*, IFN-γ, TNF-α, TGF-β, IL4, IL10.

Introduction

Trichinellosis is a worldwide zoonotic parasite of many mammals, including pigs and man caused by nematodes of genus *Trichinella* reported in Egypt (Morsy *et al*, 2022). Trichinellosis' intestinal phase is significant since it determines the course and results of the illness. The distribution of these larvae in various muscles is related to the public risk posed by their consumption by humans. Because trichinellosis is so prevalent in carnivorous animals, consumers must take special precautions to prevent contracting the disease (Krivokapich *et al*, 2012). By consuming meat from pigs or other animals that have been contaminated with Trichinella larvae, humans can be infected. After consuming contaminated meat, these larvae are expelled from their capsules, entering the intestinal cells at the top and growing into adult worms. They become fertile in two to three weeks. Females give birth to 1500 newborn larvae, which use the lymphatic and blood systems to penetrate and encapsulate in the skeletal muscles (Saad *et al*, 2016). larvae can induce the muscle cell to transform into a new one, called a nurse cell, this ability enables larvae to live for months to years (Despommier, 2009). The available *T. spiralis* therapy is more or less ineffective (Mostafa and Atwa, 2020). Albendazole® (ABZ) and others are the main anthelmintic drugs used to treat trichinellosis (Gottstein *et al*, 2009). But, they only have a moderate effect on encapsulated muscle larvae and a limited bioavailability and a significant risk of resistance (Codina *et al*, 2015). Also, several of these drugs should not be taken by pregnant women or children under the age of three (Yadav and Temjenmongla, 2012), while others are suspected to be carcinogenic (Shalaby *et al*, 2010). Therefore, it is necessary to make attempts to find antitrichinellosis treatments that are safe, effective, and made from plant, which have low toxicity and negligible side effects (Basyoni and El-Sabaa, 2013).

Alhagi maurorum, known as camel-thorn (=Shawkat al-Jamal), is a perennial shrub, of family Fabaceae. It grows up to 2m and blooms in July. The flowers are hermaphrodite, tiny, and brilliant pink to maroon and

hermaphrodite. Local folk medicine has employed *alhagi maurorum* as a therapy for bile duct disorders, glandular tumors, and nasal polyps (Shinwari *et al*, 2006). Because of its diaphoretic, gastroprotective, diuretic, laxative, expectorant, antiseptic, antidiarrheal, and therapeutic properties, it is utilized as a medicinal herb. Haemorrhoids and rheumatism are both treated using oil extracted from leaves, and flowers are applied to cure piles (James, 2011).

Materials and Methods

Animals: Seventy-two Swiss clean laboratory bred Albino male mice, weighing 25-30gm were purchased from Theodor Bilharz Research Institute, Giza. They were provided with regular pellet food and water and were housed in well-ventilated cages with stainless steel lids. Every day, bedding was replaced. Mice were allowed a week to get used to the lab before experimentation.

Parasite: By breaking down muscles of trichinosis infected pigs, infectious larvae were recovered as follows: substance submerged for 12 hours in a digestive solution made up of normal saline 1L, + Hcl acid 0.02L + pepsin 0.02L, which were mixed continuously by a mechanical stirrer at 37°C to separate larvae, suspension was centrifuged at 1,000 rpm for 2 min. after being progressively rinsed in normal saline (Guenther *et al*, 2008). Sediment larvae were then put back into a stable suspension by adding 1.5% gelatins to saltwater. After doing repeated counts by a hemocytometer, each targeted number of larvae requires was an adjusted inoculum. Mice were starved for 12 hours, given light anesthesia before infection, and then were infected with the larvae by a blunt tuberculin syringe directly into the stomach. Each inoculum per mouse was about 300 larvae.

Treatment: 1- Methanolic extract of *Alhagi maurorum* was prepared from the whole plant and given orally as 400mg kg. The freshly harvested plant parts, including leaves, roots, and flowers (2 kg), were collected, and dried before being ground to powder. The dried powder was extracted three times with methanol at room temperature for 21 days, and the crude extracts were then concentrated using a rotary evaporator under decreased pressure. To have the desired fractions, the crude extract was suspended in water and divided with n-hexane, chloroform, ethyl acetate, n-butanol, and soluble residual aqueous fraction (Shinwari *et al*, 2013). 2- Albendazole® (EIPICO): 50mg/kg/day were given orally after being acquired as a 20mg/ml suspension.

Experimental design: Mice (72) were divided in to six group: CN: negative control (neither infected nor untreated), C-Alb: mice not infected, but treated with albendazole, C- *A. maurorum*: mice were not infected, but treated with methanolic extract, CI: positive control (infected, not treated), T-Alb: mice were infected and then treated with Albendazole, T-Alhag: mice were infected and treated with *A. maurorum* methanolic extract. The infected mice were orally infected with 300 larvae/mouse and treatment started one day post infection (Oivanen *et al*, 2002). After seven days post infection (PI) half of the groups were sacrificed for intestinal phase and on the 35th day PI, the second half was sacrificed for muscular phase.

Parasitological examination: Parasite intestinal phase was by a method modified from Benham (Denham, 1965). Worm burden was calculated as total number in intestines in each mouse by counting the adults. To allow worms' travel from the gut to the container, the intestine was dissected out, cleaned, and incubated in 0.01 liter of saline at 37°C for two hours. Fluid was repeatedly washed until being clear then gathered and centrifuged for five minutes at 1,500rpm. After supernatant decanted, sediment was reconstituted in a few drops of saline, and droplet examined by a dissecting microscope to count adults.

Larval extraction and preparation: Muscles from infected mice were digested by immersion in a beaker containing 50ml of water, 0.5ml of Hcl, and 0.5gm of pepsin, 24 hours of mixed incubation at 37^{o}C followed by 15 minutes of electric stirrer agitation. After

sieving the resulting filtrated fluid, remove the supernatant and discard the sediment. By centrifugation for 3 minutes at 1000, discarded the supernatant and applying saline to sediment before repeating this process three times (Wassom *et al*, 1988).

Immunological examination: Mice were killed by cervical dislocation, and blood samples were taken to separate sera by centrifugation at 860G for 20 minutes. ELISA kits (R&D, Minneapolis, MN, USA) were used to assess the quantities of IFN-γ, TNF-α, TGF-β, IL-4, IL-10, IL-2, & IL-12 in according to the manufacturer's instructions.

Ethical consideration: The Ethical Committee, Faculty of Science, Mansoura Univeriity approved the protocol, which went with the Helsinki declaration (2008), Approval Number: Sci-Z-M-2021-53.

Statistical analysis: Data were presented as mean ± SD, by using GraphPad Prism 9 for Windows (GraphPas Software, San Diego, USA). To compare differences the ANOVA was used. It was significant at P <0.05.

Results

When infected controls were compared to treated groups showed a great reduction in intestinal worms. Albendazole recovered ones as compared to the infected control (80.17±3.87), and lowest mean count value was demonstrated (9.5±1.25) with P <0.01 as compared to *A. maurorum* treated one. When compared to positive control, recovered worms from *A. maurorum*-treated mice (37.50±2.14) significantly lower (P< 0.01). On 35th day pi, muscle larvae in treated mice significantly decreased as compared to untreated infected control ones. Larvae recovered from *A. maurorum* treated mice showed count (161.2 ±12.6) as compared to infected control (529.5±17.7, P <0.01), and compared to Albendazole treated mice (P <0.01). When compared to infected control mice, larvae from albendazole-treated mice was significantly reduced (216.8± 13.2, P <0.01).

Immunologically the proinflammatory and anti-inflammatory cytokines showed production of cytokines in sera of control, infected and treated group. There was a significant decrease in IFN-γ, TNF-α, TGF-β, IL-4, IL-2, & IL-12 in *A. maurorum* treated mice compared to infected mice & Albendazole treated ones, with a significant incre-ase in IL-10 cytokine.

Details were given in tables (1 to 9) and figures (1 to16).

Table 1: *T. spiralis* adults and larvae recovered from groups (n=6)

Trichinella spiralis	Variants	CI	T-Alb	T-Hag	p-Value
Adults in intestine	Mean	80.17	9.50	37.50	<0.01*
on 7th day pi	SE±	3.87^{a}	1.25^{b}	2.14^{c}	
Larvae in muscles	Mean	529.5	216.8	161.2	<0.01*
on 35th day pi	SE±	17.7^{a}	13.2^{b}	12.6^{c}	

abc = significant differences; *P≤0.01=significant

Table 2: Concentration of TNF-α in treated mice compared to infected ones in intestinal & muscular phase.

Variants	CN	C-Alb	C-Hag	CI	T-Alb	T-Alhag
Intestinal phase	22.00±0.42^{a}	24.32±0.63^{b}	23.83±0.52^{b}	66.58±1.35^{b}	42.19±1.17$^{a, b}$	31.12±0.36 $^{a, b}$
Muscular phase	26.85±0.50^{a}	33.14±0.33^{b}	28.24±0.50^{b}	56.81±1.02^{b}	36.54±2.67$^{a, b}$	34.67±2.97 ^{a,b}

Table 3: Concentration of IL-4 in treated mice compared to infected ones in intestinal & muscular phase.

Variants	CN	C-Alb	C-Hag	CI	T-Alb	T-Alhag
Intestinal phase	15.67±0.55^{a}	25.26±0.58^{b}	23.23±0.36^{b}	90.24±2.51^{b}	33.65±1.23 ^{a,b}	31.72±0.67$^{a, b}$
Muscular phase	22.36±0.50^{a}	18.64±0.28^{b}	17.56±0.42^{b}	76.36±1.13^{b}	22.74±0.37 $^{a, b}$	21.43±0.31$^{a, b}$

Table 4: Concentration of IL-10 in treated groups compared to infected ones in intestinal & muscular phase.

Variants	CN	C-Alb	C-Hag	CI	T-Alb	T-Alhag
Intestinal phase	106.2±0.20^{a}	124.7±2.09^{b}	116.2±0.26^{b}	43.21±2.05^{b}	84.09±2.26 $^{a, b}$	94.64±1.87 $^{a, b}$
Muscular phase	120.9±0.31^{a}	112.1±0.56^{b}	123.6±0.22^{b}	42.55±0.55^{b}	91.75±0.96 $^{a, b}$	103.3±0.91 $^{a, b}$

Table 5: Concentration of TGF-β in treated groups compared to infected ones in intestinal & muscular phase.

Variants	CN	C-Alb	C-Hag	CI	T-Alb	T-Alhag
Intestinal phase	12.98±0.33^{a}	15.34±0.28^{b}	17.11±0.17^{b}	67.21±1.05^{b}	34.58±0.49 ^{a,b}	26.82±0.40 $^{a, b}$
Muscular phase	14.51±0.66^{a}	20.01±0.19^{b}	17.01±0.21^{b}	57.68±0.49^{b}	34.68±0.33 $^{a, b}$	27.24±0.65 $^{a, b}$

Table 6: Concentration of IFN-γ in treated mice compared to infected ones in intestinal phase.

Variants	CN	C-Alb	C-Hag	CI	T-Alb	T-Alhag
Intestinal phase	30.81±0.24[a]	34.00±0.53[b]	34.14±0.46[b]	103.2±0.91[b]	52.19±0.70[a,b]	42.52±0.58[a,b]
Muscular phase	38.19±0.22[a]	42.45±0.18[b]	40.34±0.16[b]	119.8±0.39[b]	55.33±0.50[a b]	47.81±0.20[a,b]

Table 7: Concentration of IL-12 in treated mice compared to infected ones in intestinal &muscular phase.

Variants	CN	C-Alb	C-Hag	CI	T-Alb	T-Alhag
Intestinal phase	48.51±0.19[a]	50.37±0.26[b]	49.40±0.34[b]	119.7±0.28[b]	66.21±0.79[a,b]	61.04±0.08[a, b]
Muscular phase	43.56±0.28[a]	46.25±0.29[b]	45.57±0.15[b]	126.4±0.155[b]	66.78±0.46[a b]	50.32±0.30[a, b]

Table 8: Concentration of IL-2 in treated mice compared to infected ones in intestinal &muscular phase.

Variants	CN	C-Alb	C-Hag	CI	T-Alb	T-Alhag
Intestinal phase	23.08±0.11[a]	26.00±0.29[b]	24.82±0.37[b]	98.06±0.60[b]	58.03±1.20[a,b]	45.70±2.40[a,b]
Muscular phase	25.89±0.319[a]	27.59±0.314[b]	26.72±0.166[b]	90.73±0.46[b]	51.71±0.47[a,b]	45.03±0.31[a,b]

a = significant difference from control (P <0.05). Compared to infected group, b =significant (P <0.05). Same-letter superscripts= not significant (P > 0.05).

Discussion

Trichinosis, a serious meat-borne zoonosis is characterized by an extremely wide host range and geographical distribution (Pozio, 2021). *T. spiralis* muscle larval (ML) excretion/ secretion (ES) antigen is the most widely used diagnostic antigen of trichinellosis, but preparation of ES antigen requires collecting worms from infected animals, and detection of specific IgG against ML ES antigen may result in a false negative at the early stage of infection (Yue *et al,* 2020). Cytokines and regulatory T cells (Steel and Nutman, 2003), conditional Th2 cells (Díaz and Allen, 2007)altered function of antigen presentation cells or APCs (Venugopal *et al*, 2009). Nevertheless, Bruschi *et al.* (2022) reported that as *Trichinella* infection or molecules interfere with many aspects of the immune system, a word of caution is in order to possible adverse effects of *Trichinella*-based therapies have to be anticipated and thoroughly evaluated to ensure safety for humans. Albendazole and mebendazole are currently the main drugs used to treat trichinosis, albendazole influence good amount of plasma concentration levels and without monitoring but, mebendazole plasma concentration levels differ from one to another requiring individual checking and managing (Yadav and Temjenmongla, 2012). Meanwhile, Pozio (2001) found that it had minor efficacy when used on *T. spiralis* encysted larvae.

In the present study, on the 7th day pi of all treated mice showed significantly less intestinal adults activity than the infected mice.

In the present study, comparison to both control infected and albendazole treated mice, just a small number of worms were detected in mice treated with *A. maurorum* extract. This agreed with Awaad Amani *et al*, (2006), who reported that Th1 immune response was an inflammatory responses. **Also,** Bruschi and Chium- iento (2011) reported that the long-term tri-chinosis in muscles interacted strongly with the host immune system, resulting in muscle inflammation. Koenderman *et al*. (2014) re- ported that the cellular immune response to *T. spiralis* infection was polarized to a Th1 type immune response depending on the sig-nal type provided by dendritic cells (DCs) during the intestinal phase of the infection as an essential bridge between innate and adaptive immunity, DCs play a crucial role in the immune response to parasites.

In the present study, the cytokines decreased in treated *A. maurorum* and Albendazole mice and increase in infected ones. This agreed with Yu *et al*. (2013), who reported that in vitro and in vivo, showed that Th1 cytokines like interleukin (IL)-12 significantly increased during the early stage of *T. spiralis* intestinal infection. Teng *et al*. (2015) reported that naive T cells were encouraged to differentiate into Th1 cells that produce INF by IL-12. Ming *et al*. (2016) found that interferon (INF)-γ, IL-1β and tumor necrosis factor (TNF)-α and that NF-γ (type II interferon) a cytokine that played a significant role in both innate and adaptive immunity. Besides, numerous immune cells, including CD4+ T cells, release NF-γ (type II interferon) in response to some immunological or

inflammatory stimuli, such as infections, specific antigens, or activation of the T cell receptor (Bogdan and Schleicher, 2006). Th1 cell growth and differentiation are accelerated by NF-γ (Pestka *et al*, 2004), activated transcription factors including nuclear factor (NF)-B and increased the production of inducible nitric oxide synthase or iNOS (Mühl and Pfeilschifter, 2003) and also regulated the synthesis of TNF-α as well as other inflammatory cytokines (Neumann *et al*, 1998). Also, it stimulates DCs to express MHC class I & II molecules (Pestka *et al*, 2004).

In Th2 immune response, *T. spiralis* expulsion down-regulatory cytokine IL-10 was extensively expressed by a variety of immune cells, even though it was not cell type specific (Hedrich and Bream, 2010). Thus, to reduce tissue damage from increased immuno-inflammatory reactions and this goes along with this study as the treated groups of *A. maurorum* and Albendazole showed decreased levels of IL-10 as compared with infected mice. Increasing the synthesis of IL-10, a recurring aspect of the host's immune response in helminthiasis, caused immunoregulation (Ilić *et al*, 2021). IL-10 may inhibit the release of IL-12, antigen presentation, DC proliferation, and cell markers. TSL-1 also increased the production of IL-4 and IL-10 from Th2 cells, but lowered the production of INF-γ, polarizing immune response to a high Th2 cellular immunity.

IL-10 is required for a productive gut immune response. This is due to the high vulnerability to the initial *T. spiralis* infection caused by the lack or reduction of IL-10, which results in a notable delay in the expulsion of *T. spiralis* and an increase in the parasite burden (Helmby and Grencis, 2003).

Other cytokines generated and released during the Th2 immune response included the IL-4, IL-5, and IL-13, which promote the production of IgE, which led to the mast cell and eosinophil hyperplasia, trigger acute hypersensitivity reactions, and help to remove *T. spiralis* from host intestine (Rogerio and Anibal, 2012).

Conclusion

The immune response of the Th1 type can be elicited by antigens produced by *T. spiralis*, which can then altered by *T. spiralis* into a Th2 type immune response, which was thought to defend the host by eliminating *T. spiralis*. However, both responses result in the development of intestinal illness, which supports parasitism in the host. Albendazole diminishes the trichinellosis inflammatory response, but with limited pharmacological activity against *T. spiralis* infection.

Alhagi maurorum methanolic extract proved to have potent anti-inflammatory effects.

Conflict of Interest: The authors declared that they neither have conflicts of interest nor received any funds.

References

Awaad, A, Maitland, D, Soliman, G, 2006: Antiulcerogenic activity of *Alhagi maurorum*. Pharm. Biol. 44, 4:292-6.

Basyoni, MM, El-Sabaa, AA, 2013: Therapeutic potential of myrrh and ivermectin against experimental *Trichinella spiralis* infection in mice. Korean J. Parasitol. 51, 3:297-304.

Bogdan, C, Schleicher, U, 2006: Production of interferon-γ by myeloid cells-fact or fancy? Trends Immunol. 27, 6:282-90.

Bruschi, F, Chiumiento, L, 2011: *Trichinella* inflammatory myopathy: host or parasite strategy? Parasit. Vectors. 4, 1:42-8.

Bruschi, F, **Ashour, DS, Othman, AA, 2022:** *Trichinella*-induced immunomodulation: Another tale of helminth success. Food Waterborne Parasitol. May 16;27:e00164. doi:10.1016/j.fawpar.2022.e00164.

Codina, AV, Garcia, A, Leonardi, D, Vasconi, MD, Di Masso, RJ, *et al*, 2015: Efficacy of albendazole: Beta-cyclodextrin citrate in the parenteral stage of *Trichinella spiralis* infection. Int. J. Biol. Macromol. 77:203-6.

Denham, DA, 1965: Studies with methyridine and *Trichinella spiralis*. I. Effect upon the intestinal phase in mice. Exp. Parasitol. 17, 1:10-4.

Despommier, D, 2009: Trichinellida, dioctophymatida and Enoplean parasites. In: Foundations of Parasitology, 8th ed. McGraw-Hill Co, Inc., New York.

Díaz, A, Allen, JE, 2007: Mapping immune response profiles: The emerging scenario from helminth immunology. Eur. J. Immunol. 37, 12:

3319-26.
Gottstein, B, Pozio, E, Nockler, K, 2009: Epidemiology, diagnosis, treatment, and control of trichinellosis. Clin. Microbiol. Rev. 22, 1:127-45.
Guenther, S, Nockler, K, von Nickisch-Rosenegk, M, Landgraf, M, Ewers, C, *et al*, 2008: Detection of *Trichinella spiralis, T. britovi* and *T. pseudospiralis* in muscle tissue with real-time PCR. J. Microbiol. Methods. 75, 2:287-92.
Hedrich, CM, Bream, JH, 2010: Cell type-specific regulation of IL-10 expression in inflammation and disease. Immunol. Res. 47:185-206.
Helmby, H, Grencis, RK, 2003: Contrasting roles for IL-10 in protective immunity to different life cycle stages of intestinal nematode parasites. Eur. J. Immunol. 33, 9:2382-90.
Ilić, N, Kosanović, M, Gruden-Movsesijan, A, Glamočlija, S, Sofronić, L, *et al*, 2021: Harnessing immunomodulatory mechanisms of *Trichinella spiralis* to design novel nanomedical approaches for restoring self-tolerance in auto-immunity. Immunol. Lett. 238:57-67.
James, A, 2011: Alhagi maurorum (Fabaceae). Dr. Duke's phytochemical and ethnobotanical databases.https://phytochem.nal.usda.gov/phytochem/ethnoPlants/show/3405?qlookup=Alhagi+maurorm.
Koenderman, L, Buurman, W, Daha, MR, 2014: The innate immune response. Immunol. Lett. 162, 2 Pt B:95-102.
Krivokapich, SJ, Pozio, E, Gatti, GM, Prous, CL, Ribicich, M, *et al*, 2012: *Trichinella patagoniensis* n. sp. (Nematoda), a new encapsulated species infecting carnivorous mammals in South America. Int. J. Parasitol. 42, 10:903-10.
Ming, L, Peng, RY, Zhang, L, Zhang, CL, Lv, P, *et al*, 2016: Invasion by *Trichinella spiralis* infective larvae affects the levels of inflammatory cytokines in intestinal epithelial cells in vitro. Exp. Parasitol. 170:220-6.
Morsy, T, Sallam, T, Hawam, S, 2022: Trichinosis (trichinellosis) in man and in domestic and wild animals with reference to Egypt: An overview. JESP. 52, 3:431-42.
Mostafa, E, Atwa, H, 2020: Intestinal mastocytosis in *Trichinella spiralis* infection: Immunohistochemical study in murine model. PUJ. 13, 1:52-9.
Mühl, H, Pfeilschifter, J, 2003: Anti-inflammatory properties of pro-inflammatory interferon-γ. Int. Immunopharmacol. 3, 9:1247-55.
Neumann, B, Emmanuilidis, K, Stadler, M, Holzmann, B, 1998: Distinct functions of interferon-gamma for chemokine expression in models of acute lung inflammation. Immunology 95, 4:512-21.
Oivanen, L, Mikkonen, T, Haltia, L, Karhula, H, Saloniemi, H, *et al*, 2002: Persistence of *Trichinella spiralis* in rat carcasses experimentally mixed in different feed. Acta. Vet. Scand. 43, 4: 203-10.
Pestka, S, Krause, CD, Walter, MR, 2004: Interferons, interferon-like cytokines, and their receptors. Immunol. Rev. 202, 1:8-32.
Pozio, E, 2001: New patterns of *Trichinella* infection. Vet. Parasitol. 98, 1-3:133-48.
Pozio, E, 2021: In: *Trichinella and* Trichinellosis. Bruschi F., editor. Academic Press; London.
Rogerio, AP, Anibal, FF, 2012: Role of leukotrienes on protozoan and helminth infections. Mediators Inflamm. 2012:595694.
Saad, AE, Ashour, DS, Abou Rayia, DM, Bedeer, AE, 2016: Carbonic anhydrase enzyme as a potential therapeutic target for experimental trichinellosis. Parasitol Res. 115, 6:2331-9.
Shalaby, MA, Moghazy, FM, Shalaby, HA, Nasr, SM, 2010: Effect of methanolic extract of *Balanites aegyptiaca* fruits on enteral and parenteral stages of *Trichinella spiralis* in rats. Parasitol. Res. 107, 1:17-25.
Shinwari, Z, Watanabe, T, Rehman, M, Youshikawa, T, 2006: A pictorial guide to Medicinal Plants of Pakistan. Kohat: Kohat University of Science and Technology.
Shinwari, ZK, Ahmad, N, Hussain, J, Rehman, NU, 2013: Antimicrobial evaluation and proximate profile of *Nepeta leavigata*, *Nepeta kurramensis* and *Rhynchosia reniformis*. Pak. J. Bot. 45, 1:253-9.
Steel, C, Nutman, TB, 2003: CTLA-4 in filarial infections: implications for a role in diminished T ce-ll reactivity. J. Immunol. 170, 4:1930-8.
Teng, MW, Bowman, EP, McElwee, JJ, Smyth, MJ, Casanova, JL, *et al*, 2015: IL-12 & IL-23 cytokines from discovery to targeted therapies for immune-mediated inflammatory diseases. Nat. Med. 21, 7:719-29.
Venugopal, PG, Nutman, TB, Semnani, RT, 2009: Activation and regulation of toll-like receptors (TLRs) by helminth parasites. Immunol. Res. 43, 1-3:252-63.
Wassom, DL, Dougherty, DA, Dick, T, 1988: *Trichinella spiralis* infections of inbred mice: Immunologically specific responses induced by different *Trichinella* isolates. J. Parasitol. 74, 2: 283-7.

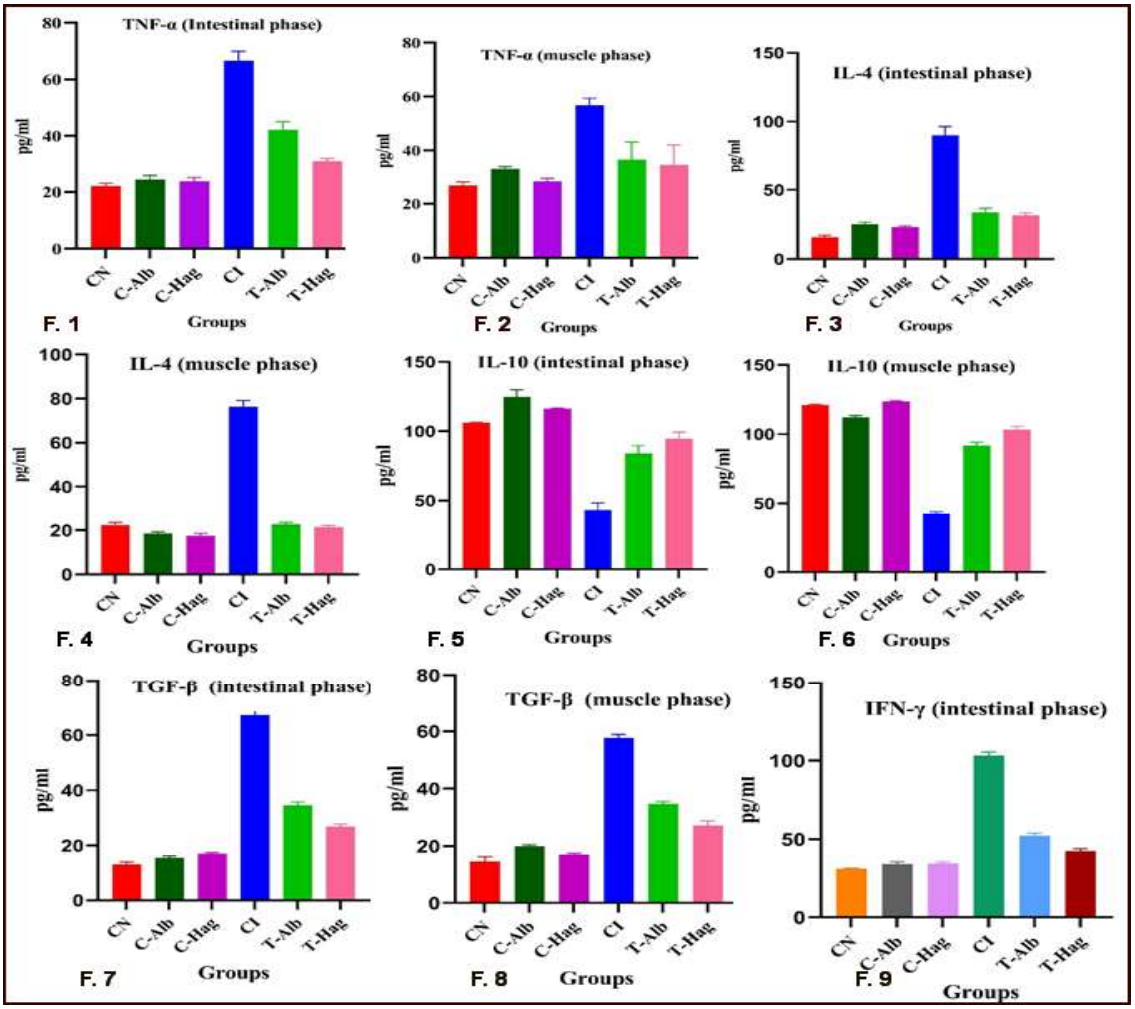
Yadav, AK, Temjenmongla, 2012: Efficacy of *Lasia spinosa* leaf extract in treating mice infected with *Trichinella spiralis*. Parasitol. Res. 110: 493-8.

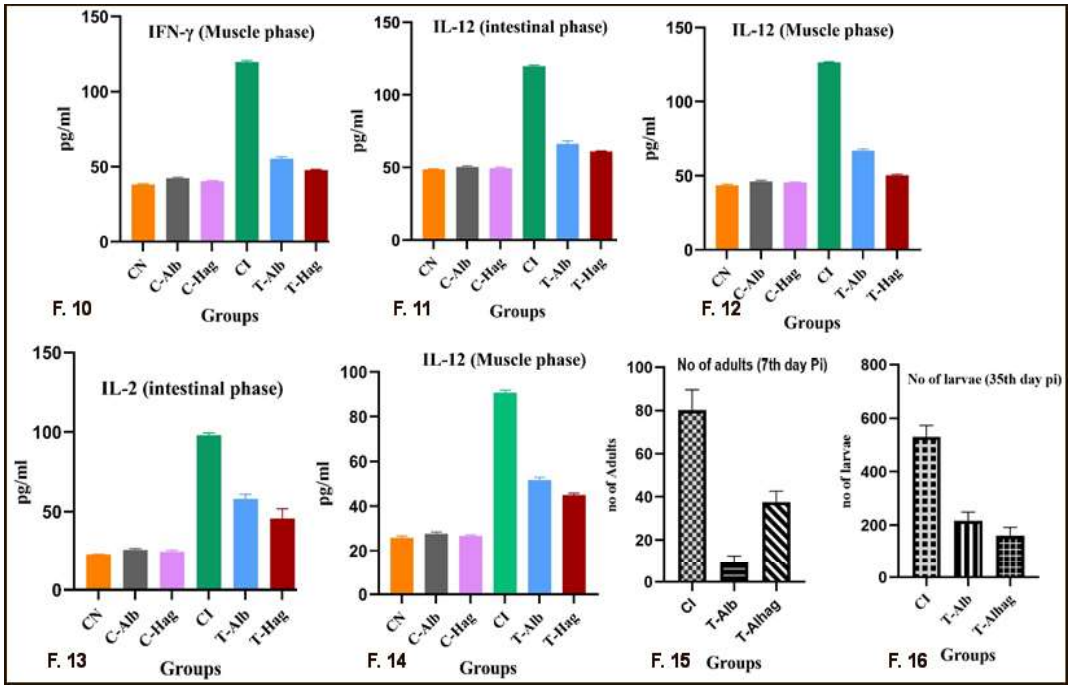
Yu, YR, Deng, MJ, Lu, WW, Jia, MZ, Wu, W, *et al*, 2013: Systemic cytokine profiles and splenic toll-like receptor expression during *Trichinella spiralis* infection. Exp. Parasitol. 134, 1:92-101.

Yue, X, Sun, XY, Liu, F, Hu, CX, Bai, Y, *et al*, 2020: Molecular characterization of a Trichinella spiralis serine proteinase. Vet. Res. Sep 25;51, 1:125. doi: 10.1186/s13567-020-00847-0.

Explanation of figures:

Fig.1: mean levels of TNF-α in intestinal phase in all groups.
Fig.2: mean levels of TNF-α in muscular phase in all groups.
Fig.3: mean levels of IL-4 in Intestinal phase in all groups.
Fig.4: mean levels of IL-4 in muscular phase in all groups.
Fig.5: mean levels of IL-10 in intestinal phase in all groups.
Fig.6: mean levels of IL-10 in muscular phase in all groups.
Fig.7: mean levels of TGF-β in Intestinal phase in all groups.
Fig.8: mean levels of TGF-β in muscular phase in all groups.
Fig.9: mean levels of IFN-γ in Intestinal phase in all groups.
Fig.10: mean levels of IFN-γ in muscular phase in all groups.
Fig.11: mean levels of IL-12 in Intestinal phase in all groups.
Fig.12: mean levels of IL-12 in muscular phase in all groups.
Fig.13: mean levels of IL-2 in Intestinal phase in all groups.
Fig.14: mean levels of IL-2 in muscular phase in all groups.
Fig.15: number of adult worms in infected and treated groups.
Fig.16: number of larvae in infected and treated groups.




IFN-γ (Muscle phase)
pg/ml
150
100
50
0
CN
C-Alb
C-Hag
CI
T-Alb
T-Hag
Groups
F. 10
IL-12 (intestinal phase)
pg/ml
Groups
F. 11
IL-12 (Muscle phase)
pg/ml
Groups
F. 12
IL-2 (intestinal phase)
pg/ml
Groups
F. 13
IL-12 (Muscle phase)
pg/ml
Groups
F. 14
No of adults (7th day Pi)
no of Adults
CI
T-Alb
T-Alhag
Groups
F. 15
No of larvae (35th day pi)
no of larvae
CI
T-Alb
T-Alhag
Groups
F. 16