

## Original Research Article

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## Evaluation of Clinicopathological alterations in mice experimentally infected with *Trichinella spiralis* and the nematocidal effect of tannic acid and albendazole

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### Abstract

*Trichinella spiralis* (*T. spiralis*) is one of the parasitic nematodes of a highly zoonotic importance. It infects various vertebrates, including human. In this study eighty (n =80) Swiss albino mice were experimentally infected by *T. spiralis* to assess the nematocidal activity of tannic acid (TA) and albendazole (ABZ) individually or in combination during the intestinal phase of *T. spiralis*. Mice were divided into 10 equal groups 8 mice for each, (control): non-infected but received saline, (ABZ): non-infected and treated with ABZ 50 mg/kg, (TA1000): non-infected and treated with TA1000 µg/ml, (TA2000): non-infected and treated with TA2000 µg/ml, (ABZ+TA2000): non-infected and treated with ABZ 50 mg/kg + TA2000 µg/ml, (TS): infected non treated, (TS+ABZ) group: infected and treated with ABZ 50mg/kg, (TS+TA1000): infected and treated with TA1000 µg/ml, (TS+TA2000): infected and treated with TA2000 µg/ml and (TS+ABZ+TA2000): infected and treated with ABZ 50mg/kg + TA2000 µg/ml. Mice were sacrificed at 7<sup>th</sup> day post infection. Results demonstrated significant reductions in the adult worm count in TS+TA1000, TS+ABZ, TS+ TA2000 and TS+ABZ+TA2000 with efficacies of 91%, 92%, 92% & 99% respectively. Results were confirmed by histopathological examination of the targeted organs. The degree of necrosis was decreased in treated groups, associated with reduction in the percentage of eosinophils. Elevated concentrations of serum ALT, AST, and urea in the infected group were decreased in treated groups, while albumin and glucose were increased.

It was concluded that the combined albendazole-tannic acid therapy had the highest effect on reducing parasite burden and restoring normal histological architecture.

**Keywords:** *Trichinella spiralis*, Albendazole, Tannic acid, Liver enzymes, Eosinophils.

### Introduction

Trichinellosis is a perilous zoonotic parasitic disease of public health importance (Abd-ELrahman et al., 2020). Worldwide, approximately 11 million individuals and over 150 mammalian species as well as birds and reptiles have been infected by *T. spiralis* (Salama et al., 2021). *Trichinella spiralis* is present in Egypt, and it was diagnosed in man as well as in pigs slaughtered in Cairo abattoirs (Fahmy et al., 2020). Few studies on *T. spiralis* infection in fresh and handled pork in Egypt are accessible. Also, prevalence of *Trichinella spiralis* was reported in 13.3% of rodents collected from and around slaughterhouses in Alexandria (Ismail et al., 2018). While Dyab et al. (2019) reported that prevalence of *Trichinella spiralis*

was 1.08% of slaughtered pigs, in El Bassatine slaughterhouse in Cairo Governorate.

The intestinal phase of trichinellosis is a pivotal stage since it determines the extent and fate of the disease (Abd El-Hamed et al., 2022). Life cycle commences with ingestion of raw or under cooked meat that contain the muscle larvae, where the digestive juice in the stomach dissolves the capsule-like cyst which consequently release of the infective larvae that move to the intestine with subsequent penetration to its columnar epithelium (Diaz et al., 2020). Promptly, the larvae differentiate into adults, then, after mating the adult female worms release large number of newborn larvae that penetrate the intestine (Fadl et al., 2020). Next, the larvae convey through the circulatory system into the skeletal muscle cells

(Othman et al., 2016), where they transform muscle cells into encapsulated nurse cells that remain from months to years (Elmehy et al., 2021).

Albendazole is an oral, broad-spectrum, anthelmintic agent of the benzimidazole class that is commonly used in the treatment of trichinellosis, toxocariasis and other nematode infection in addition to echinococcosis and cysticercosis (Hong, 2018, Verma et al., 2018 and Lin et al., 2020). Albendazole is more effective in early stage of trichinellosis than in late stage (Eid et al., 2020).

Recently, several trials were applied using natural products for prevention and treatment of nematodes (Chaudhari et al., 2019 and Liu et al., 2020). One of these products is tannic acid, which disseminates throughout several natural sources such as grapes, beverages, and others (Youness et al., 2021).

Tannins are a complex group of polyphenolic compounds which are astringent in nature of high molecular weight, and they are majorly categorized into two groups, condensed and hydrolysable tannins, that play an important role in animal nutrition (Lou et al., 2019). They have various therapeutic properties such as an antiparasitic, antiviral, antimicrobial, anti-carcinogenic, anti-inflammatory, antimutagenic and antioxidant (Sharma et al., 2019).

Tannic acid is the simplest hydrolysable tannins (Youness et al., 2021). The importance of tannins as anti-parasitic agent for gastro-intestinal parasites have concentrated in the activity of condensed tannins, while the effect of hydrolysable tannin was rarely studied (Acevedo-Ramírez et al., 2019).

Therefore, the purpose of this study was to investigate the clinicopathological alterations induced due to *T. spiralis* experimental infection in mice and to assess the nematocidal effect of tannic acid and albendazole individually or in combination.

## Materials and Methods

This study was conducted according to the regulation and procedures approved by the ethics committee on animal experimentation of the New Valley University, Faculty of Veterinary Medicine and the guide for the care and use of animals (National Institute of Health Publication NO. 8023, revised 1978).

### Animals

Overall, eighty Swiss albino mice of 30-35 g laboratory-bred parasite free, 6-8 weeks old, were used in the present study. The animals were obtained from Faculty of Veterinary Medicine, Assiut University and maintained in accordance with the institutional and national guidelines. The isolate of *T. spiralis* which were used was originally obtained from infected pork meat from El-Bassatine abattoir in Cairo and it was maintained by routine in vivo passages in BALB/c mice at the Animal House of Assiut University (Assiut, Egypt) under specific pathogen-free conditions. Mice were orally infected with 300 larvae/mouse by gastric gavage.

Where, the larvae were extracted from the carcasses of infected BALB mice after 30 days post infection (dpi) by incubating minced skinned mice with artificial digestive fluid (7.5 g of pepsin + 10 ml of conc. HCl+ 1L of distilled water and stirred to about 10 minute) in conical flask at 37°C overnight. Filtration to larvae by using sieve to remove bones and then washed several times with PBS, and their number/ml was counted under a light microscope X40 according to (Abd-Elrahman et al., 2021).

### Experimental design

This study included ten groups (8 mice each) (control) group: non infected but received saline, (ABZ) group: non infected and treated with albendazole 50 mg/kg, (TA1000) group: non infected and treated with tannic acid 1000 µg/ml, (TA2000) group: non infected and treated with tannic acid 2000 µg/ml, (ABZ+TA2000) group: non infected and treated with albendazole 50mg/kg+ tannic acid 2000 µg/ml, (TS) group: infected untreated, (TS+ABZ) group: infected and treated with albendazole 50 mg/kg and (TS+TA1000) group: infected and treated with tannic acid 1000 µg/ml, (TS+TA2000) group: infected and treated with tannic acid 2000 µg/ml and (TS+ABZ+TA2000) group: infected and treated with albendazole 50mg/kg+ tannic acid 2000 µg/ml. On the 7th dpi, all mice from each group were sacrificed.

### Drugs

Albendazole (Alzental from Epico) 50 mg/kg/day (Attia et al, 2015), the dose taken from the 3<sup>rd</sup> dpi for successive 3 days. Tannic acid powder (CAS No. 1401-55-4, C<sub>76</sub>H<sub>52</sub>O<sub>46</sub>, 98 % purity) was dissolved in drinking tap water and prepared the concentration (1000µg/mL, 2000 µg/mL) and given orally to mice. Tannic acid was given from the first day for six days (Abd-El gaffar et al., 2003 and Omar et al., 2003).

### Isolation and counting of adult *Trichinella spiralis*

The small intestine was removed, washed to remove intestinal content, and was cut into small pieces 1 cm each, with gentle scraping to mucosa to allow migration of adult from mucosa and incubated at 37° C in phosphate buffer saline for 4 h. The fluid was collected in petri dish and counted by stereo microscope (Gamble, 1996).

### Histopathological examination

At the 7<sup>th</sup> dpi, the tissue samples from the TS and treated mice were fixed in 10% formalin for 24 h, washed in water for 12 h, dehydrated in ascending grades of alcohols, cleared in xylene, and embedded in paraffin blocks which were sectioned at 5µm thickness by microtome then stained with hematoxylin and eosin and examined microscopically according to (Attia et al., 2015).

### Hematological and serum biochemical analysis

At the 7<sup>th</sup> dpi, blood samples were collected from retro-orbital vein of each mouse in the studied groups, where two separate blood samples were collected. The first sample was taken in Eppendorf tubes which was mixed with EDTA as anticoagulant for hematological examination for complete blood picture (CBC) investigation using Veterinary Hematology Analyzer (exigo, H400, Sweden). Blood films were made as soon as possible after collection of blood sample, by manual method then stained by Giemsa stain for differential leukocytic count according to (Feldman et al., 2000).

The second sample was taken in Eppendorf tubes to separate the serum for chemical analysis (ALT, AST, total protein, albumin, A/G ratio, glucose, urea, and creatinine). The concentrations of serum biochemical compounds including kinetic method for determination of both alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Young, 2001; Schumann and Klauke, 2003), enzymatic method for the quantitative determination of blood glucose (BG) (glucose oxidase method) (Young, 2001), colorimetric test for the quantitative determination (Biuret method) of total proteins (TP) (Wichselbaum, 1946; Gornall et al., 1949), colorimetric test for the quantitative determination of albumin (bromocresol green method) (Dumas et al., 1971), enzymatic colorimetric test for urea (Fawcett and Scott, 1960) and colorimetric (Jaffé-Reaction) test for kinetic measurement (method without deproteinisation) of creatinine (Bartels and Böhmer, 1971) were quantified using commercial kits (Human, Wiesbaden, Germany) for all except glucose which quantified using commercial kits (Spine React, Spain). Both globulin concentration and albumin/globulin (A/G) ratio were determined using

previously described procedures (Samanta et al., 2016). Concentrations of all these serum biochemical variables were analyzed using spectrophotometric procedures (5010 V5+, semi-automatic photometer, RIELE, Germany) according to the manufacturer's instructions.

### Statistical analysis

The significance of differences between the groups in parasitological examination was calculated using (T) test. p-value of <0.05 was considered statistically significant.

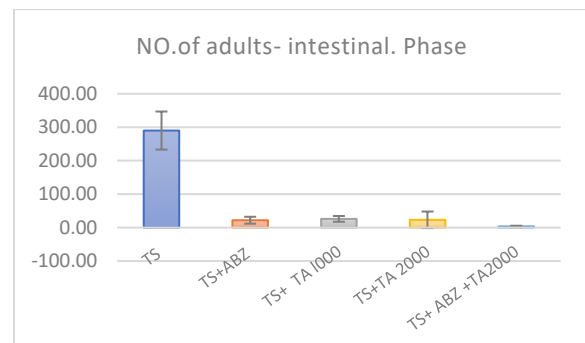
Data for hematological and biochemical variables were analyzed using an analysis of variance (ANOVA) to compare means of different groups utilizing SPSS.23 for the Microsoft Windows operating system. Multiple comparison tests (One Way ANOVA: Post hoc multiple comparison LSD, Duncan) were used to determine whether there were mean differences using SPSS.23. Data are presented as the mean ± SEM. There were differences in mean values among groups when there was a P<0.05).

## Results

### Parasitological results

A significant decrease in the mean number of adult worms was observed in all treated groups compared with TS (p < 0.05). The least count of adult worms was recorded in (TS+ABZ+TA2000) with efficacy of 99% followed by TS+TA2000 and TS+ABZ with efficacy of 92%, then, TS+TA1000 with efficacy of 91%.

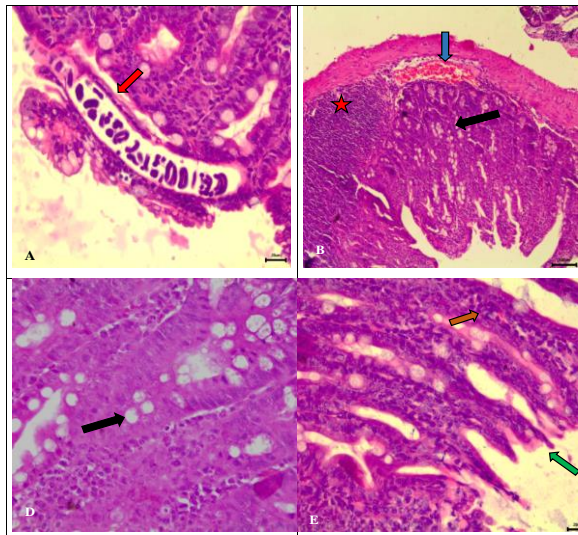
There was insignificant difference in reduction of adult worms between TS+TA1000 and TS+TA2000. In addition, there was a significant decrease in number of adult worms in TS+ABZ+TA2000 as compared to TS+TA1000 and TS+ABZ (chart1).



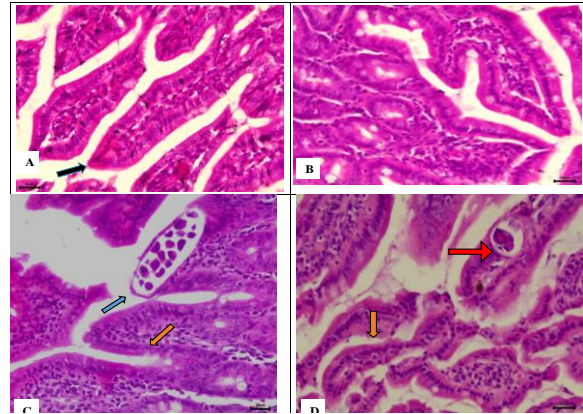
**Chart 1.** Anthelmintic effect of different treatment in intestinal phase

### Histopathological findings

Histopathological examination of the intestinal sections from the TS group showed presence of cross section of adult *T. spiralis* in the lumen of intestine (Fig. 1 A). Also, there was lymphoid follicle hyperplasia (Fig. 1 C). Dense inflammatory cellular infiltrate was observed mainly in the core of the villi and extending into the submucosa. The infiltrate consisted mainly of lymphocytes and plasma cells, with few neutrophils, eosinophils, and fibroblasts (Fig 1E). Also, flattening of the villi (Fig. 1 E) and hyperplasia of the crypts of Lieberkühn was noted evidenced by evident goblet cell hyperplasia (Fig 1 B, D). Regarding the histopathological changes in the intestine of all drug-receiving groups. Marked significant reduction in the number of adults with improvement of other histopathological changes were observed as decreased number of goblet cells (Fig. 2).

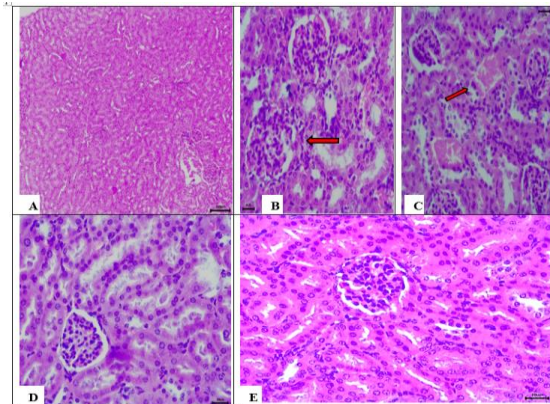


**Fig.1.** Intestine from (TS) group showing presence of adult worms in core of villi (A, red arrow). congestion of blood vessels (B, blue arrow), lymphoid follicle hyperplasia (B, red star), goblet cell hyperplasia (B-D, black arrow), villous atrophy (E, green arrow) and inflammatory cell infiltration in core of villi and extend to submucosa (E, orange arrow).

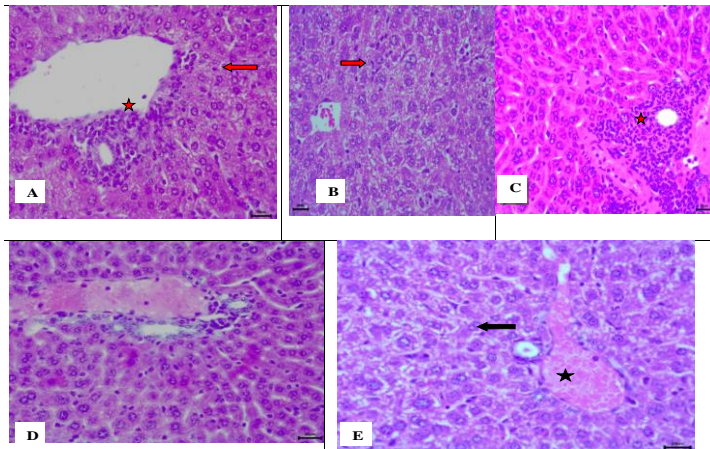


**Fig.2.** Intestine from TS+ABZ group revealing normal villi (A, arrow) with mild inflammatory cell reaction, intestine from TS+TA1000 showing normal villus appearance (B), intestine from TS+TA2000 showing presence of necrosed worm (C, arrow) in the intestinal villi associated with inflammatory cell, intestine from TS+ABZ+TA2000 showing dead parasite (arrow) associated with inflammatory cell infiltration in villi (D, arrow).

The samples of kidney in TS showed congestion in blood vessels (Fig. 3 A), where infected+ treated groups showed normal glomeruli (Fig. 3) expect TS+ABZ (Fig. 3 B) and TS+TA1000 (Fig. 3 C) showed necrobiosis in glomerular tuft and congestion in blood vessels. Where the liver sections revealed vacuolar degeneration and lymphoid cell reaction in portal area in TS group (Fig.4 A) and infected+ treated groups showed mild degeneration in hepatocyte with hydropic degeneration (Fig. 4 B, C, D).



**Fig.3** kidney of group TS showing congestion in blood vessels in kidney (A), kidney from group TS+ABZ showing necrobiosis of glomerular tuft and renal tubular epithelium (B, arrow), kidney from group TS+TA1000 showing congestion in blood vessel (C, arrow) and glomerular tuft, kidney from group TS+TA2000 showing more or less normal glomeruli, some degeneration in renal tubules (D), kidney from TS+ABZ+TA2000 group showing kidney tubules and glomeruli were more or less normal.



**Fig.4** liver of (TS) group showing vacuolar degeneration (arrow) and lymphoid cell reaction in portal area (A, star), liver from group TS+ABZ showing vacuolar degeneration of hepatocyte (B, arrow), liver from group TS+TA1000 showing infiltration of lymphocyte in portal area (C, star), kidney from group TS+TA2000 showing more or less normal glomeruli, some degeneration in renal tubules (D), liver from TS+ABZ+TA2000 group showing hydropic degeneration in hepatocyte (arrow) and congestion (E, star).

**Hematological results:**

Hematological results showed insignificant changes in CBC between all groups before and after treatment except for Hb and PCV which were significantly increased in TS+ABZ+TA2000 in comparison to TS (Table 1).

**Table 1.** Erythrogram Parameters (Mean±S.E.) in Mice Infected by *T. spiralis* and Treatment with TA and ABZ in intestinal phase. Means in the same column not followed by the same letter differ significantly ( $P < 0.05$ ).

Group	RBC ( $\times 10^6/\mu\text{L}$ )	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)
Control	8.74±0.04 ac	13.70±0.20 ad	37.23±1.13 acd	42.53±1.21abc	15.67±0.18abc	36.87±1.21a
ABZ	9.44±0.44 abc	14.15±0.26 acd	39.37±1.73 abcd	42.00±0.78abc	15.80±0.50abc	37.62±0.78a
TA1000	8.96±0.23 abc	14.80±0.72 abcd	39.10±1.93 abcd	43.63±1.57ac	16.50±0.66a	37.83±0.19a
TA2000	9.36±0.32 abc	14.70±0.35 abcd	40.13±0.66 abc	42.93±1.88ac	15.73±0.18abc	36.73±1.35a
ABZ+TA2000	9.73±0.19 ab	16.50±0.29 b	44.05±1.20 b	43.47±0.75ac	16.70±0.25a	38.37±0.13a
TS	9.32±0.49 abc	13.93±1.41 ad	36.97±3.34 cd	39.54±1.54bc	14.89±0.72c	37.71±0.42a
TS+ABZ	9.31±0.25 abc	15.53±0.35 ab	41.17±0.58 abc	44.23±0.75a	16.57±0.20a	37.73±0.39a
TS+TA1000	8.35±0.81 c	12.73±1.37 d	34.30±3.55 d	41.03±0.78abc	15.20±0.26bc	37.13±0.23a
TS+TA2000	9.35±0.15 abc	14.27±0.19 acd	37.67±0.83 abcd	40.50 ±0.59c	15.37±0.12bc	37.97±0.32a
TS+ABZ+TA2000	9.95±0.29 b	16.20±0.60 cb	42.80±1.57 ab	42.97±0.29ac	16.27±0.15ab	37.83±0.29a

Leukogram results revealed insignificant change in TLC in TS as compared with control. Leukocytosis was observed in TS+TA2000 in comparison to TS. Eosinophilia was noticed in TS as compared with control. While TS+ABZ, TS+TA1000, TS+TA2000 and TS+ABZ+TA2000 revealed significant decline in eosinophils in comparison to TS (Table 2). In addition,

lymphopenia was noticed in TS as compared to control. Meanwhile, lymphocytosis was observed in TS+TA2000 in comparison to TS (Table 2). Moreover, neutrophilia was recorded in TS+ABZ, TS+TA1000, TS+TA2000 and TS+ABZ+TA2000 as compared with TS. More than that, monocytosis was noticed in TS in comparison to control. However, TS+ABZ+TA2000 showed significant decrease in monocytes compared with TS (Table 2).

**Table 2.** Leukogram Picture (Mean±S.E.) in Mice Infected by *T. spiralis* and Treatment with TA and ABZ in intestinal phase. Means in the same column not followed by the same letter differ significantly ( $P < 0.05$ ).

Group	TLC $\times 10^3/\mu\text{L}$	Eosinophil $\times 10^3/\mu\text{L}$	Lymphocyte $\times 10^3/\mu\text{L}$	Neutrophil $\times 10^3/\mu\text{L}$	Basophil $\times 10^3/\mu\text{L}$	Monocyte $\times 10^3/\mu\text{L}$
Control	8.00±0.64 acd	0.15±0.03 ad	5.80±0.53 ad	1.54±0.08 ac	0.00±0.00 a	0.46±0.06 ac
ABZ	7.15±0.13 ab	0.17±0.05 ad	3.87±0.21 bc	2.17±0.09 ab	0.00±0.00 a	0.67±0.02 abd
TA1000	9.57±0.38 c	0.22±0.06 acd	5.95±0.58 ad	2.35±0.22 b	0.00±0.00 a	0.86±0.08 bd
TA2000	7.53±0.38 abd	0.08±0.05 a	5.08±0.37 abd	1.87±0.15 ab	0.00±0.00 a	0.50±0.01 ad
ABZ+TA2000	7.00±0.70 ab	0.17±0.06 ad	4.65±0.54 abc	1.78±0.12 ab	0.00±0.00 a	0.40±0.06 c
TS	6.50±0.29 ab	0.89±0.16 b	4.31±0.19 bc	0.76±0.09 c	0.00±0.00 a	0.77±0.16 bd
TS+ABZ	6.83±0.99 ab	0.43±0.11 c	3.75±0.60 c	1.89±0.35 ab	0.00±0.00 a	0.74±0.12 d
TS+TA1000	6.40±0.35 b	0.17±0.03 ad	3.40±0.30 c	2.14±0.20 ab	0.00±0.00 a	0.06±0.09 acd
TS+TA2000	8.89±0.66 dc	0.12±0.04 ad	6.15±0.44 d	1.85±0.62 ab	0.00±0.00 a	0.64±0.04 abcd
TS+ABZ+TA2000	7.00±0.29 ab	0.35±0.12 dc	4.34±0.49 bc	1.75±0.30 ab	0.00±0.00 a	0.50±0.12 ac

**Biochemical Results**

There was significant increase in ALT in TS in comparison to control. While TS+TA2000 showed significant decline in ALT as compared with TS. On the other hand, TS+ABZ+TA2000 showed significant elevation in ALT in comparison to TS. Values of AST was significantly elevated in TS in comparison to control, but TS+ABZ, TS+TA1000, TS+TA2000 showed significant reduction in AST in comparison to TS (Table 3). In addition, hypoglycemia was noticed in TS as compared with control. On the other hand, TS+TA2000 and TS+ABZ+TA2000 showed significant increase in glucose in comparison to TS

(Table 3). Hypoalbuminemia was noticed in TS as compared with control. However, TS+ABZ, TS+TA1000 and TS+ABZ+TA2000 showed significant increase in albumin values as compared with TS. Mice of TS+ABZ, TS+TA1000 and TS+TA2000 showed significant decrease in globulin as compared with TS. In addition, TS+ABZ and TS+TA1000 showed significant decrease in A/G ratio in comparison to TS (Table 3). Moreover, urea was significantly elevated in TS as compared with control, while TS+TA2000 revealed significant decrease in urea in comparison to TS. Also, TS+ABZ showed significant decrease in creatinine as compared to TS (Table 3).

**Table 3.** Some Biochemical Parameters Mean (□S.E.) in Mice Infected by *T. spiralis* and Treatment with TA and ABZ. Means in the same column not followed by the same letter differ significantly ( $P < 0.05$ )

GROUP	ALT U/L	AST U/L	GLUCOSE MG/DL	TP G/DL	ALBUMIN G/DL	GLOBULIN G/DL	A/G RATIO	UREA MG/DL	CREATININE MG/DL
CONTROL	25.33±3.53 a	66.67±2.67 a	121.78±4.09 a	8.31±0.39 ab	3.02±0.01 acd	5.30±0.40 ab	0.58±0.05 ab	32.27±0.98 a	0.42±0.07 A
ABZ	42.00±6.00 bc	115.33±6.96 b	113.77±2.92 ab	7.41±0.29 a	2.88±0.11 abcd	4.52±0.35 a	0.65±0.06 a	36.81±5.27 abc	0.38±0.04 AB
TA1000	32.00±3.06 ab	80.00±5.77 a	103.71±6.36 bd	8.55±0.13 ab	2.82±0.12 abcd	5.73±0.07 ab	0.49±0.02 ab	38.92±2.63 abc	0.24±0.12 AB
TA2000	26.00±4.00 a	78.00±9.24 a	100.77±3.07 bde	8.59±0.40 ab	2.53±0.11 ab	6.07±0.50 ab	0.42±0.05 ab	40.11±3.38 abc	0.38±0.01 AB
ABZ+TA2000	50.00±3.46 cd	145.00±4.04 c	117.89±4.91 ab	7.40±0.44 a	2.82±0.30 abcd	4.58±0.64 a	0.66±0.17 a	36.22±3.32 ab	0.43±0.02 A
TS	41.00±1.73 bc	176.00±1.15 d	78.69±5.87 c	9.27±0.72 b	2.41±0.25 b	6.85±0.93 b	0.37±0.08 b	43.16±5.34 bc	0.42±0.03 A
TS+ABZ	49.67±3.93 cd	117.33±3.53 b	84.44±5.53 ce	8.09±0.11 ab	3.29±0.05 d	4.79±0.16 a	0.69±0.03 a	38.26±0.43 abc	0.20±0.13 B
TS+TA1000	52.67±0.88 cd	123.00±6.35 b	90.92±11.30 cde	7.93±0.47 a	3.14±0.09 cd	4.79±0.56 a	0.68±0.10 a	45.49±1.76 c	0.28±0.06 AB
TS +TA2000	26.00±4.62 a	119.67±2.03 b	105.34±8.24 ad	7.79±0.58 a	2.68±0.19 abc	5.11±0.39 a	0.53±0.01 ab	32.37±0.60 a	0.35±0.00 AB
TS+ABZ+TA2000	56.00±3.46 D	171.00±5.20 D	101.12±3.85 BE	8.35±0.53 AB	3.04±0.26 ACD	5.31±0.78 AB	0.62±0.16 AB	41.29±2.69 ABC	0.34±0.01 AB

**Discussion**

Most drugs that are used for treatment of *Trichinella* infection have low bioavailability and a high degree of resistance (Abd El-Hamed et al., 2022). That’s why the recent research focus on the investigation of naturally originated medications for treatment of trichinellosis (Salama et al., 2021).

The current study revealed significant reduction in the worms count in all *T. spiralis* infected and treated groups compared to TS, where a significant decrease in the counts of adult worms in TS+TA1000, TS+TA2000 and TS+ABZ by 91%, 92% and 92% respectively. In this work, the obtained data were consistent with Attia et al., (2015) who reported high efficacy of albendazole against adult worm, where the number of adults reduced by 94.2%. The co-therapy of albendazole with tannic acid 2000 revealed a good antiparasitic activity against adult worms of *T. spiralis*, where the percentage of the adult reduction was (99%).

Butter et al. (2001) explained that the nematocidal effects of tannin against nematodes were dose dependent, where at high concentrations of quebracho

tannin 2%, all worms died within 2 h and at 1% quebracho tannin, 75% of worms had died in this time, but (0.05%) resulted in some worm mortality. In this study, tannic acid revealed a nematocidal effect on adult worms. Our results were in accordance with (Molan et al., 2002, Waghorn, 2008, Tibe et al., 2013 and Jayanegara et al., 2019). The nematocidal activity of tannic acid could be due to its ability to join protein of the internal mucosa causing autolysis, inducing internal rupture and expulsion of viscera of nematode (Acevedo-Ramírez et al., 2019).

Albendazole inhibited microtubule polymerization from selective binding to beta tubulin monomer of the parasite, with little effect on binding of the host tubulin resulted in impaired uptake of glucose by adult and larvae (Aguayo-Ortiz et al., 2013).

The histopathological investigation is regarded as an important step to understand pathogenesis of the disease (Gamito-Santos et al., 2009). In this study, the histopathological findings of the intestine in TS showed presence of cross section of adult *T. spiralis* in the lumen of intestine. These findings agreed with (Elguindy et al., 2019). The obtained results revealed dense inflammatory cellular infiltrate presented

mainly in the core of the villi and extending into the submucosa. Our result in accordance with (Milcheva et al., 2013). The infiltrate consisted mainly of lymphocytes and plasma cells, with few neutrophils, eosinophils and fibroblasts, these results are in the same aspect with (Hassan et al., 2021). Also, flattening of the villi and hyperplasia of the crypts with goblet cell hyperplasia. These data matches with (Abd El-Hamed et al., 2022).

Examination of TS+ABZ+TA2000 revealed an improvement in the intestinal architecture and a decreased pathological change than found in TS. These changes were manifested by presence of large numbers of dead parasites and presence of mild number of goblet cells but with infiltration of inflammatory cells inside the villi. In addition, the villi were appeared normal as compared with TS.

The results from the present study are in the same line with Salama et al. (2021) who reported an improvement in architecture of intestine in *T. spiralis* infected mice and treated with albendazole and prednisolone. Additionally, Fadl et al. (2020) recorded that the inflammatory cells infiltration and goblet cells were significantly reduced in *T. spiralis* infected mice which treated by albendazole in comparison to infected non treated mice.

In this work, the results indicated there was an increase in Hb and PCV in TS+ABZ+TA2000 in comparison to TS. These results matched with (Jaheed et al., 2019) who mentioned that the goat treated from haemonchiasis with balanites aegyptiaca and albendazole showed significant increase in Hb and PCV at 2 weeks post infection. Moreover, the Hb and PCV values were in higher concentration in lambs treated with condensed tannin, due to the decreased number in the blood feeding adult's nematode in the abomasum and the improved nutrition which allowed the animals to restore blood cells more quickly (Kumar et al., 2020).

Eosinophils were the prominent cell type in the host response to helminth associated with the expulsion of the parasite from the gut (Yasuda and Nakanishi, 2018). The mice of TS showed marked eosinophilia in comparison to control. Similarly, Ribicich et al. (2013) recorded that eosinophilia in cat infected with *T. spiralis* appeared within days 7 and 54 post infection. Additionally, infection with *Trichostrongylus colubriformis* was associated with eosinophilia (Butter et al., 2000). On contrary, eosinophilic count was significantly decreased in TS+ABZ, TS+TA1000, TS+TA2000 and TS+ABZ+TA2000 in comparison to TS, that could be

attributed to reduction of adult burdens in the infected mice. Our results agreed with Musa et al. (2011) who utilized a combined dose of nigella sativa and albendazole in treatment of *Toxocara canis* in mice led to decrease in the level of eosinophils. Also, Movahedi et al. (2017) mentioned that albendazole diminished the eosinophil number during treatment of parasitic meningitis in human caused by the nematode *Angiostrongylus* compared with infected patient. In the same aspect, there was a decrease in the number of eosinophils after albendazole treatment of infected man with soil transmitted helminths (hookworms, *Ascaris lumbricoides* and *Trichuris trichiura*) (de Ruiter et al., 2019). Batzlaff et al. (2014) described that eosinophil count decreased after treatment with albendazole.

In this work, lymphopenia was noticed in TS as compared to control, similarly, Lee and Best (1983) reported that there was a significant decrease in the lymphocytes in infected rats in early stage of trichinosis compared with non-infected rats. On the other hand, lymphocytes increased (returned within control range) in TS+TA2000 in comparison to TS, which might be attributed to the decrease in adults' number plus improvement of histopathological changes in intestine in this group.

Aspartate aminotransferase (AST) catalyzes the transamination of L-aspartate and 2-oxoglutarate to oxaloacetate and glutamate. Aspartate aminotransferase occurs in most cells; however, it is used as indicator in evaluating hepatocellular and muscular injury because of its high activity in these tissues (Kaneko et al., 1997). The cytosolic enzyme alanine aminotransferase (ALT) is nearly specific for hepatocellular injury. The increased serum levels parallel the extent of hepatocellular damage (Kaneko et al., 1997). In this work, the levels of ALT and AST were significantly increased in TS as compared with control. These results were approved histopathologically by vacuolar degeneration in hepatocyte and lymphoid cell reaction in portal area. Our result agreed with Hassan et al. (2021) and Basyoni and Elsabah (2013) who reported that the serum ALT and AST concentrations were in greater concentration in mice of TS than mice of control, due to liver damage by migrating larvae on day 5 and day15 post infection.

Our result showed significant decline in ALT in TS+TA2000 as compared to TS. This reduction could be attributed to tannic acid 2000 µg/ml reduced adverse effect of adult *T. spiralis* and this was approved histologically by the decrease in severity of the lesions in liver of treatment groups in comparison to TS

group. Also, Li et al. (2020) mentioned that tannic acid had a hepatoprotective effect against liver injury induced by arsenic trioxide, because tannic acid activated the Keap1-Nrf2/ARE signaling pathway, led to reduction of oxidative stress, apoptosis, and inflammation.

Serum AST concentration was significantly decreased in TS+ABZ compared with TS. The recorded data matched with Musa et al. (2011) who noticed a significant reduction in AST concentration in serum after treatment with albendazole which reflected the efficacy of albendazole in *Toxocara canis* infections. In the same line, AST levels in TS+TA2000 showed significant decrease in comparison with TS.

In this work hypoglycemia was recorded in TS as compared with control. This result agreed with Castro et al. (1967) and Nishina and Suzuki (2002) who noticed that hypoglycemia was due to malabsorption of glucose due to presence of *T. spiralis* in the intestinal phase. Also, the enteritis which caused by adult, induced depression of absorption rate of glucose led to hypoglycemia (Rodríguez et al., 2009). On the other hand, mice of both TS+TA2000 and TS+ABZ+TA2000 showed significant increase in glucose in comparison to TS. These results were in the same aspect with Jan et al. (2015) who reported that the goat fed the leaf meal which rich in condensed tannin had greater serum glucose concentrations.

In this study hypoalbuminemia was noticed in TS as compared with control. This might be due to migrating larvae which made hepatic and renal damage (Gamble et al., 1997). Similarly, Jaheed (2021) recorded hypoalbuminemia in goats infected with *Haemonchus contortus*. Also, hypoalbuminemia was associated with gastrointestinal disease (Abbas and Humma, 2021). While albumin concentration was increased toward normal in mice of TS+ABZ, TS+TA1000 and TS+ABZ+TA2000 in comparison with TS. Nada et al. (2018) concluded that albumin level was improved toward normal in case of treatment of *T. spiralis* infected mice with albendazole. Moreover, Kumar et al. (2020) proved that albumin was elevated in lambs fed condensed tannin, due to nematocidal activity of condensed tannin through direct effect on the parasite and indirect effect by improvement of protein utilization which were used for repair and immune response.

There were increase in urea levels in mice of TS as compared to control group, this agreed with (Basyoni and Elsabah, 2013), these elevated levels of urea in serum indicated presence of glomerulonephritis

(Soliman et al., 2011). Our histopathological results of mice of TS showed congestion in blood vessels in kidney and glomeruli were swollen with hypercellularity (Reina et al., 2000). On the other hand, urea significantly decreased in TS+TA2000 compared with TS. Where, mice of TS+TA2000 showed apparently normal glomeruli and kidney tubules. There was a reduction in serum urea concentrations in lambs fed sericea lespedeza (legume rich in condensed tannin) compared with control lambs (Acharya et al., 2015). The reduction in serum urea level in goats treated by condensed tannin may be attributed to the decreased rumen protein breakdown and increased essential amino acid absorption (Jan et al., 2015).

## Conclusions

It could be concluded that tannic acid 2000 µg/ml has a successful anti-parasitic effect as albendazole, and consequently restoring the normal tissue architecture of the host. The combined treatment of ABZ and TA2000 has the maximum effect not only on reducing the parasite burden but also on the restoration of normal tissue architecture. Therefore, the combined therapy may be promising alternative treatments for trichinellosis.

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