The impact of anthelmintic therapeutics on serological and tissues apoptotic changes induced by experimental trichinosis

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Abstract Trichinosis is a sharable parasitic disease caused by *Trichinella* spp., the disease occurred on eating inappropriate cooked pork infected by the parasite encysted larvae. This study aimed to evaluate experimentally the impact of treatment by thiabendazole, praziquantel (PZQ) and prednisone on *T. spiralis* induced parasitological, serological and apoptotic changes. Forty albino rats were infected orally each by ± 1000 larvae, divided into four groups each of 10 rats, group (A) infected control, group (B) thiabendazole tested, group (C) PZQ tested and group (D) prednisone tested. On the seventh and 40th days post-infection, all groups were evaluated parasitologically by the number of the intestinal worms and the muscular encysted larvae, while IFN- γ and TNF- α were estimated by ELISA, histopathological and histochemical assessment of the tissue changes during both phases were performed by different stains. In conclusion, thiabendazole was a potent and curable drug, it showed nearly 100% efficacy on intestinal worms, highly significant variations in cytokines levels during both the intestinal and muscular phases, while it induced moderate effects on encysted muscular larvae number, In addition it ameliorated myocytes apoptotic changes induced by trichinosis.

Keywords *Trichinella spiralis* · Thiabendazole · Praziquantel · Prednisone · IFN- γ · TNF- α · Apoptotic changes

Introduction

Trichinosis is a zoonotic parasitic disease caused by *Trichinella* spp. It is affecting wide range of countries in the world (Pozio 2007), trichinosis is a health problem because it affects human patients globally (Gottstein et al. 2009), the disease occurred by eating inappropriate cooked pork infected by the encysted larvae of *Trichinella* spp.; for which there is no absolute successful treatment, despite great control efforts (Ashour and Elbakary 2011; García et al. 2013).

There are other species can infect humans; unlike *T. spiralis*, *Trichinella pseudospiralis* does not undergo encystment in skeletal muscle but leads to larval migration and clinical symptoms (Gottstein et al. 2009).

Trichinella spiralis is characterized by completing its life cycle in a single host, it remained intracellular, in the small intestine the female worms produce larvae which migrate to settle and survive inside skeletal muscles cells, and this induces myocytes transformation to nurse cells that support the life of the larva for months to years (Fabre et al. 2009; Despommier 2009), the associated defense and sensitivity responses occurred by muscles infection by larvae presented by myalgia of various muscle groups (Ashour and Elbakary 2011).

Matsuo et al. (2000) and Boonmars et al. (2004b) reported that apoptosis of muscle cell resulted by *Trichinella* infection was mediated by cell conversion to nurse cell. Degenerative changes of muscle cell and its conversion to nurse cell gave up myopathy changes; this is investigated by mononuclear cell infiltrations which appear by histopathology examination (Boonmars et al. 2005).

The death receptor and mitochondrial mechanisms are the most important for illustration apoptosis (Gupta 2003). In the mitochondrial pathway, the mitochondrial

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cytochrome c is released and combined with apoptotic protease activating factor 1 (Apaf 1) resulting in stimulation of caspases (Bratton et al. 2001). The combination between them and the death receptors provide stimulation to initiate apoptosis (Dempsey et al. 2003; Wajant et al. 2003). Boonmars et al. (2004) reported that mitochondria-pathway is responsible for the *T. spiralis*-infected muscle cells apoptosis.

The conversion of muscles cells to nurse cells is mediated by some genes related to apoptosis that expressed during cyst formation. In trichinosis, the basophilic and eosinophilic cytoplasm shows pro-apoptosis factors but eosinophilic cytoplasm shows anti-apoptosis factors that remain alive for a longer time (Boonmars et al. 2004).

Trichinella spiralis-infected muscle cells showed different features of basophilic cytoplasm unique for apoptosis (Matsuo et al. 2000), it is proved by showing the genes related to mitochondria-pathway in the formation of cyst in muscles cells infected by *T. spiralis* (Boonmars et al. 2004).

Harris et al. (2000) found that the production of some cytokines like IL-4 and IFN- γ regulates the immune responses to infectious pathogens that mediate transformation of CD4+ T cells to TH1 and TH2 cells. Therefore, *Trichinella spiralis* in both phases may survive by different ways related to the dominant lymphocytes.

IFN- γ shared in immune response against newborn larvae, but has no effect on the adult worms' expulsion from intestine (Helmby and Grencis 2003). Also Dvorožnáková et al. (2011) reported that the increased level of IFN- γ proved its role in muscle phase immune response.

Wu et al. (2005a) referred the increasing levels of endogenous TNF- α in myocytes infected by *T. spiralis* to incorporation in both apoptosis and anti-apoptosis pathway, which represents mechanism of the nurse cell formation.

The aim of the treatment in the early phase of infection is to decrease larval muscle infection; but if infection has already occurred the aim is to decrease muscle pathology that causes the clinical complains (Campbell et al. 1994).

Mebendazole, albendazole, thiabendazole, which kill adult worms, are most effective therapies early in the illness; albendazole may have the advantage of being better tolerated. Short courses of systemic corticosteroid therapy ameliorate clinical symptoms and are administered in severe cases (Gottstein et al. 2009), however praziquantel (PZQ) is potent also against a wide range of cestode, trematode and nematode infections in man and animals (Andrews et al. 1983). So we tried to test it against trichinosis.

This study aims to evaluate experimentally the impact of treatment by thiabendazole, PZQ and prednisone on trichinosis-induced apoptotic changes which are one of the

main causes of trichinosis clinical manifestations during the muscular phase, as they are translated to myocytes DNA changes. Parasitological, serological, histopathological and histochemical studies were performed to assess this impact and changes by using different techniques and stains.

Materials and methods

Animals and parasite

Forty male albino rats (7–9-weeks old) weighing 80–100 g apparently healthy and free of parasites were obtained from Faculty animal house, where rats were bred till the end of the study. All rats were inoculated by oral route with ± 1000 larvae per rat. The infective larvae of *T. spiralis* were taken from muscles of infected pork, obtained from pig slaughter house in Elbasatin, Cairo.

Infection of animals

Infective larvae were prepared from infected pork muscles by mixing the muscles in digestive fluid for 12 h (20% concentrated HCl and 20% pepsin). The mixture was centrifuged (at 1000 rpm for 2 min) and washed (0.9% NaCl) repeatedly to obtain the larvae (Guenther et al. 2008). The infective larvae were mixed with 1.5% gelatin and saline to become stable and ready for infection, rats were prevented from food 12 h before infection, then were given infective larvae through oral route using a tuberculin syringe with blunt nozzle.

Study design

Classification of infected rats into 4 groups, 10 rats each; A, B, C, and D. Group A infected untreated (infected controls), Group B received thiabendazole, Group C received PZQ and Group D received prednisone.

Drugs form and dose

PZQ (Distocide; EPICO, Egypt) in the form of an aqueous suspension in 2% Cremophore EL solution, at a dose of 25 mg/100 g rat.

Thiabendazole (Mintezol; Pharco Pharm, Egypt) at a dose of 270 mg/100 g rat.

Prednisone (Cortisone; Pharco Pharm, Egypt) at a dose of 0.072 mg/100 g rat.

All drugs were given by oral route using a tuberculin syringe.

On drug administration, each group was divided into two halves; the first half received drugs on day 3 post-

infection (PI) and the second half received drugs on day 30 PI till time of scarification.

Animals which received the drugs on day 3 PI were sacrificed on day 7 PI to assess the impact and changes on the intestinal phase, while animals which received the drugs on day 30 PI were sacrificed on day 40 PI to assess the effects on the muscle phase. Control infected group which received no drugs, was subdivided also into 2 halves, and sacrificed on the same times.

Assessment

Parasitological methods

Intestinal adult worms counts (Denham 1965) The intestine was opened, washed, then left in saline for 2 h to allow worms to exit, washing was carried out several times till the fluid becomes clear. Then, the collected fluid centrifuged at 1500 rpm for 5 min to obtain the sediment containing the worms for counting using dissecting microscope.

Muscle larvae counting The diaphragm was dissected, digested and centrifuged to sediment the larvae, then the larvae per each rat diaphragm were counted (Guenther et al. 2008).

Assessment the effects of drug

Drugs effects were evaluated by counting the mean number of living worms and larvae per rat, so to then calculate the efficacy according to; $\text{Efficacy (\%)} = (A - B/A) \times 100$, where A = counted worms or larvae from control animals and B = counted worms or larvae from tested animals (Hosking et al. 1996).

Serological examination

Blood samples were collected from the corner of the rats eyes (Hoff and Rlagt 2000), sera were separated and stored at -20°C until the determination of serum TNF- α and IFN- γ levels by ELISA (R&D systems, DuoSet ELISA, USA) according to manufacturer's instructions using polyclonal antibody and monoclonal antibody specific for TNF- α , IFN- γ and measured at 450 nm optical density (OD). Concentrations were determined by available standard curves.

Histopathological examinations of the different tissues reactions

After sacrificing animals in each group on the 7th and 40th day PI, tissues (jejunum and muscle) samples were taken from different groups, fixed in 10% formol-saline, dehy-

drated in ascending grades of alcohols, cleared in xylene, paraffin blocks were prepared, sectioned by microtome at 4 μm thicknesses and stained by Hematoxylin and Eosin (H and E), then microscopically examined by different magnifications (100 $\times$ and 400 $\times$) to study: the shape and size of villi, intestinal crypts and cellular infiltration, as well as the presence of goblet cells in jejunum specimens, while the cellular and tissue reactions surrounding the encysted larvae in muscle specimens were investigated (Carleton et al. 1967).

Histochemical examinations of the muscular tissues

Feulgen stain was used to investigate the apoptotic changes which depend on nuclear DNA acid hydrolysis, stained tissues were fixed with avoidance of strong acids usage to avoid excess de-staining, so normally appeared bright red coloration of nuclear DNA, against green coloration of the background. Apoptosis was classified according to changes of nuclear DNA coloration as: light red, faint red, less bright red and bright red staining of nuclear DNA indicating severe, moderate, mild apoptosis and normal nuclei respectively (Chieco and Derenzini 1999).

Statistical analysis

SPSS 19.0 soft-ware package were used and presented as Mean $\pm$ SD. ANOVA test was used for significant differences between groups.

Ethical consideration

The experimental animals were reared at the animal house, Faculty of Medicine, Zagazig University, Egypt. The Scientific Research Ethical Committee of the Faculty approved the study.

Results

Parasitological and serological result

Table 1 showed *T. spiralis* living worms numbers per rat (Mean $\pm$ SD) and the impact of the tested drugs on the third day PI, thiabendazole was found to be the most effective drug that gave nearly 100% efficacy, eradicated mostly all *T. spiralis* worms, with highly significant difference when compared with group (A), while the mean worm count in group (C and D) (188.8 and 196) with efficacy (18 and 14%) respectively, with insignificant difference with group (A). Concerning the drugs' effects on the encysted larvae in muscles (diaphragms) assessed on day 40 PI (Table 2), there were significant difference

Table 1 Effects of drugs on *T. spiralis* intestinal adult worms when administered on day 3 PI and sacrificed on day 7 PI

Group	Range	Mean $\pm$ SD	Efficacy (%)
Infected untreated group (A)	245–223	230.8 $\pm$ 6.6 a	–
Thiabendazole tested group (B)	0	0 b	100
PZQ tested group (C)	190–185	188.8 $\pm$ 1.5 c	18
Prednisone tested group (D)	200–186	196 $\pm$ 4 c	14
F	39.2		
P	< 0.001**		

Groups with different letters = significantly different, *P* value = probability, F = ANOVA test

**Highly significantly difference

Table 2 Effects of drugs on *T. spiralis* larvae in muscles when administered on day 35 PI and sacrificed on day 40 PI

	Range	Mean $\pm$ SD	Efficacy (%)
Infected untreated group (A)	5653–5102	5336 $\pm$ 177 a	–
Thiabendazole tested group (B)	2411–2050	2260 $\pm$ 286 b	58
PZQ tested group (C)	4935–4599	4790 $\pm$ 114 c	10
Prednisone tested group (D)	4925–4536	4511 $\pm$ 303 c	15
F	92.5		
P	< 0.001**		

Groups with different letters = significantly different, *P* value = probability, F = ANOVA test

**Highly significantly difference

between thiabendazole tested group (B) with other groups (58% reduction). Table 3 showed serum IFN- γ and TNF- α levels on 7th day and 40th day PI, where there were increase of IFN- γ and decrease of TNF- α levels in most of tested groups, group (B) gave the highest variation in both

cytokines on both phases with highly significant difference when compared with other groups.

Histopathological examination (Figs. 1, 2)

Infection with *T. spiralis* induced severe pathological changes in the intestine where worms were noticed in the lumen with hyperplasia of lymphoid tissue, villus atrophy and crypt hyperplasia, excess eosinophilic infiltration with increased number of goblet cells in the control infected group (A). In thiabendazole-tested group (B), there were no adult worms or larvae, folded papillae, few ulcers in the mucosa with acute inflammatory cell in the mucosa. The PZQ-tested group (C) adult worms were seen in between and inside the villi with edematous mucosa, mild degeneration of the worms was noticed. In prednisone-tested group (D), worms were seen in-between the flattened villi, penetrating the mucosa, inflammatory reactions were not obvious, and some worms were piercing deeply the intestinal wall. The pathological changes in the diaphragm of the control infected group (A) showed severe parasitic infection, many larvae were noticed encysted, inflammatory tissue reaction infiltrating the cysts walls, the muscles

Table 3 Serum IFN- γ and TNF- α level on day 7 and day 40 PI

	Day 7 PI		Day 40 PI	
	IFN- γ (pg/ml). Mean $\pm$ SD	TNF- α (pg/ml). Mean $\pm$ SD	IFN- γ (pg/ml) Mean $\pm$ SD	TNF- α (pg/ml) Mean $\pm$ SD
Infected untreated group (A)	142.7 $\pm$ 37.82 a	380.1 $\pm$ 18.4 a	192.4 $\pm$ 56.6 a	404.1 $\pm$ 45.46 a
Thiabendazole tested group (B)	630.3 $\pm$ 7.82 b*	97.4 $\pm$ 45.32 b*	743.5 $\pm$ 43.4 b*	104.5 $\pm$ 32.23 b*
PZQ tested group (C)	184.7 $\pm$ 13.71 a	291.5 $\pm$ 6.14 a	239.6 $\pm$ 45.6 a	319.43 $\pm$ 27.6 a
Prednisone tested group (D)	179.6 $\pm$ 2.24 d	129.5 $\pm$ 43.35 d	212.3 $\pm$ 46.7 d	219.43 $\pm$ 47.23 d
F	432	845	653	263
P	< 0.001	< 0.05	< 0.001	< 0.05

Groups with different letters = significantly different

*Significantly difference of group (B) with other groups

Fig. 1 Intestinal sections (H and E stained) showed worms (black arrows) in the lumen with hyperplasia of lymphoid tissue, villus atrophy (red arrow) and crypt hyperplasia, and excess eosinophils infiltration with increased number of goblet cells in infected untreated group (a). In thiabendazole tested group (b), there were no adult worms or larvae with folded papillae and few ulcers in the mucosa (black arrows) with acute inflammatory cell in the mucosa. In PZQ tested group (c), adult worms (black arrows) were seen in between

and inside the villi with mild edematous mucosa, villus atrophy (red arrows) and degeneration of the worms. In prednisone tested group (d), worms (black arrows) seen in between villi and penetrating mucosa with flattened villi (red arrow), no inflammatory reaction were seen with adult worms present embedded deeper inside intestinal wall. e refers to cut section of normal rat intestine showing normal villi. (Magnification $\times 100$) (color figure online)

fibers showed mild cloudy swelling. Thiabendazole-tested group (B) showed disintegration of encysted larvae within incomplete cyst wall, it was surrounded by tissue reactions.

In PZQ-tested group (C), more than one larva were noticed inside the cyst, chronic inflammatory tissue reaction infiltrated the thick cyst wall, with fragmentation and necrosis

Fig. 2 *Trichinella spiralis* infected rat diaphragm muscle sections (H and E stained) at 40th day PI. Infected untreated group (**a**) showed multiple depositions of *T. spiralis* encysted larval (black arrows) with mononuclear cell infiltrates surrounding the capsule (red arrows). Thiabendazole tested group (**b**) showed disintegration of encysted larvae (black arrow) within incomplete cyst wall surrounded by mild tissue reactions (red arrow). In PZQ tested group (**c**), larvae appeared

inside the cyst (black arrow) with chronic inflammatory tissue reactions (red arrow) infiltrating the thick cyst wall with fragmentation and necrosis of the muscles fibers. Moderate infections with encysted disintegrated larvae (black arrow) inside thick cyst wall surrounded by granulomatous reaction (red arrow) were seen in prednisone tested group (**d**). **e** refers to cut section of normal rat muscle. (Magnification $\times 100$) (color figure online)

of the muscles fibers. Moderate infection by encysted larvae, they were inside thick cysts wall, and surrounded by granulomatous reaction, this was noticed in prednisone-tested group (D).

Histochemical results (Fig. 3)

By Feulgen staining of group (A), degenerative changes of the muscular tissue were shown with very faint red coloration of the myocytes nuclear DNA (severe apoptosis). Group (B) showed normal circular shape of nuclei and less bright red coloration of the myocytes nuclear DNA (mild apoptosis), both groups (C and D) showed pale red coloration of DNA in muscles cells nuclei (moderate apoptosis).

Discussion

The present study assessed the impact of some drugs (thiabendazole, PZQ and prednisone) on the apoptotic changes induced by *T. spiralis* infection in albino rats, as the rat is a suitable and selective host for experimental trichinosis (Mikkonen et al. 2005). The transformation of the skeletal muscle cell to a nurse cell in case of trichinosis, is a unique phenomenon, the accommodation of this parasite with the host involving some apoptotic changes that accelerate this process (Matsuo et al. 2000; Boonmars et al. 2005; Wu et al. 2005a, b, 2008).

Parasitological study in the present work showed that there were almost complete eradication of adults and larvae in the group tested by thiabendazole, it showed the highest efficacy (100%) with significant difference with other drugs on the intestinal phases, it is in agreement with Brown et al. (1961) who attributed the therapeutic effect of thiabendazole to its inhibitory effects on the infective larvae, interference with larvae development to adult worms, also abrupt destruction of larvae due to suppression of their metabolic function (Campbell and Cuckler 1964). While Pozio et al. (2009) reported that the early administration of chemo-therapy gave effective cure of trichinosis and its pathological changes. In relation to muscular phase, the moderate therapeutic effect of thiabendazole (58% efficacy) may be explained, by Jarczewade et al. (1974) that its damaging action on encysted larvae is facilitated by the entry of antibodies into the cyst to disintegrate it, while the immunosuppression effect of the parasite leads to suppression of humoral immunity with few released antibodies, so the effect of thiabendazole on encysted larvae is moderate.

The parasitological effects of PZQ on the worm count were mild with insignificant difference with the control infected group (A) with minute efficacy in treating

encysted larvae in muscles, so it has slight therapeutic action on trichinosis on both phases, this was supported by Schenone (1980), who accused the resistant cuticle of the parasite to PZQ entry.

In relation, prednisone has also mild therapeutic action on trichinosis, but anti-inflammatory action was seen in the intestinal and muscular phases, but there were deep penetration of the parasite in the intestinal wall (Dupouy-Camet et al. 2002), regarding the parasitological impact, there was insignificant difference compared to the control. Also Dupouy-Camet et al. (2002) added that glucocorticosteroids can interrupt the expulsion of *Trichinella* from the intestine, so must be used in combination with anthelmintic drugs. In addition in the infected muscle tissues, the parasite burden was higher than dexamethasone tested mice (Piekarska et al. 2010).

Modification of the characters and levels of pro- and anti-inflammatory cytokines by the parasites explained that these cytokines incorporated and regulated the inflammatory response (Else and Finkelman 1998). Cytokines profile (IFN- γ , TNF- α) shown in Table 3, revealed increase of IFN- γ and decrease of TNF- α in all treated groups, but thiabendazole-tested group (B) gave the highest variation in both cytokines on both periods with highly significant difference compared to other groups. In the control infected group (A), TNF- α level was at high level on the 7th and 40th days as it increased as proinflammatory cytokines, this is partially in agreement with Wu et al. (2005a, b) who demonstrated that induction of TNF- α in *T. spiralis* infected muscle cells, it is resulting in induction of apoptosis with the conversion of muscle cells to nurse cells.

In relation to thiabendazole-tested group (B), it showed the most increasing level of IFN- γ on both phases, mostly due to its protective effect against the parasites, this is in agreement with Helmby and Grencis (2003) that IFN- γ included in protection against newborn larvae, the increasing production of IFN- γ correlated with its participation in the immune response on both phases (Dvorová et al. 2011). Also Lawrence et al. (1998) reported that mice deficient in IFN- γ receptor develop enteropathy, while mice deficient in TNF- α receptor develop less enteropathy. Moreover, IL-10 had an antagonistic action with IFN- γ and the balance between the two gave the protection against the parasite different stages (Helmby and Grencis 2003). In IL-10-deficient mice when infected with *Trichinella spiralis*, IFN- γ was markedly increased while Th2 responses were maintained causing death of the parasite with mixed memory response (Beiting et al. 2007).

Also Lee and Ko (2006) reported that the parasite preferred the Th2 response for remaining alive in tissues during the muscle phase of *T. spiralis*, as Th1 response mediated the destructive granulomatous response, so the

Fig. 3 *Trichinella spiralis* infected rat diaphragm muscle sections (Feulgen stained) showing: infected untreated group (**a**) very faint red coloration (arrows) of the nucleus (severe apoptosis), thiabendazole tested group (**b**) normal circular shape of nuclei and less bright red coloration of

the nuclei (mild apoptosis), both PZQ and prednisone tested groups (**c**, **d** respectively): pale red coloration (arrows) of the nuclei of muscles cells (moderate apoptosis). **e** refers to cut section of normal rat muscle stained with Feulgen stain. (Magnification $\times 1000$) (color figure online)

selection of Th2 response by the parasite, enabled the growing larvae to resist rejection at an early stage.

Considering the results of PZQ-tested group (C), the levels of IFN- γ and TNF- α were $(184.7 \pm 13.71,$

$291.5 \pm 6.14)$ on 7th day PI and $(239.6 \pm 45.6,$ $319.43 \pm 27.6)$ on 40th day PI, this had insignificant difference compared with the control group, so PZQ has no therapeutic activity on the worms as reported by Andrews

et al. (1983) who added that the uptake of PZQ by the nematode *Heterakis spumosa* was very slow, due to the impermeability of the cuticle to the drug. However, Schenone (1980) reported that there was therapeutic effect of PZQ on certain nematodes as *Enterobius vermicularis* and *Trichuris trichiura*.

Concerning prednisone-tested group (D), the levels of IFN- γ and TNF- α (179.6 ± 2.24 , 129.5 ± 43.35) on 7th day PI and (212.3 ± 46.7 , 219.43 ± 47.23) on 40th day PI, revealed the decreasing of both cytokines that may be due to the immunosuppression action of prednisone, it decreases all immune responses particularly the cellular with decrease the secretion of cytokines, this is explained by Machnicka et al. (1994) and Kisiel and Kaszuba (2011) who reported that the treatment for immediate hypersensitivity accompanied trichinosis by glucocorticoids showed inhibition of the activity of immune cells (including lymphocytes), this is leading to decrease in cytokine synthesis. On the other hand, Frydas et al. (2002) suggested that mimosine (anti-inflammatory compound) has a big role on inhibiting TNF- α and IL-6 and a small role on inhibiting IL-4, IFN- γ was inhibited only up to the 21st day. However some findings may be explained by the predominance of the immunosuppressive effect of trichinosis on the therapeutic role of the anthelmintic drugs as thiabendazole.

In the present study, histopathological examination revealed that thiabendazole-tested group (B) showed no adult worms or larvae with folded papillae, few ulcers in the mucosa, acute inflammatory cell in the mucosa of the intestine, in addition to disintegration of encysted larvae within incomplete cyst wall, they were surrounded by mild tissue reactions in the diaphragm (Figs. 1, 2). This is in agreement with Leung and Barriaga (1966) and El-Ridi et al. (1990) who said that thiabendazole was very effective in eradication of all intestinal forms of the parasites.

Regarding PZQ-tested group (C), adult worms were seen in between and inside the villi, mild edematous mucosa, some degeneration of the worms inside the intestine, more than one larvae were noticed inside the cyst, chronic inflammatory tissue reaction infiltrated the thick cyst wall, in addition to fragmentation and necrosis of the muscles fibers (Figs. 1, 2), these findings may be attributed to the shortage of PZQ that lacks the nematocidal properties and has no apparent effect on *T. spiralis* during both phases of the disease.

In prednisone-tested group (D), worms were seen in-between the villi and penetrating mucosa with flattened villi, no inflammatory reaction was seen, adult worms were noticed also embedded inside intestinal wall, moderate infection with encysted larvae inside thick cyst wall, they were surrounded by less granulomatous reaction as seen in the diaphragm muscle (Figs. 1, 2), these findings are explained by Castro et al. (1980) that cortisone therapy

during the intestinal phase had suppressor effect on granuloma formation by the anti-inflammatory and immunosuppression effect. Also Piekarska et al. (2010) reported that larvae in the infected muscle tissue were higher than the dexamethasone treated control mice.

Apoptosis is the programmed cell death, a process that balances the cell proliferation (Osborne 1996). Parasites can induce apoptosis by two ways; directly by active mediators or indirectly by inflammatory mediators (Lundy et al. 2001; Tato et al. 2004). Many studies have reported apoptosis induced by *Trichinella* infected muscle cells (Matsuo et al. 2000; Boonmars et al. 2004), by induction modifications and reprogramming of host to accommodate it, this is mostly in accordance with this study at the histochemical level (Fig. 3). Infected control group (A) showed degenerative changes presented by the light red stain of the nuclear DNA, with marked apoptosis indicating the adverse effects of the parasite on myocytes.

Thiabendazole-tested group (B) showed normal circular shape of nuclei, less than bright red color of the DNA of the nuclei i.e. mild apoptosis, although thiabendazole had moderate therapeutic effect on the encysted larvae during muscular phase, it ameliorated early most apoptotic changes.

Concerning groups (C and D), they showed pale red coloration of the nuclear DNA in muscle cells; indicating moderate apoptosis, Wu et al. (2005a, b) declared that apoptosis occurred in the muscle cell tissue not the inflammatory cells, this is in agreement with the present study, especially the prednisone-tested group (D), the tested drug acted as anti-inflammatory with decreasing inflammatory cells; but the apoptosis was moderate in the muscles cells. These findings are in contrary to Boonmars et al. (2008) who reported that apoptosis changes occurred for inflammatory cells and epithelial bile duct cells nuclei after praziquantel treatment of hamster opisthorchiasis. So, *Trichinella* infection induced apoptosis in infected muscles cells, thiabendazole was the most effective drug that alleviated apoptotic changes induced by trichinosis, in addition to its curative impact during the intestinal phase, highly significant positive variations in cytokines levels (IFN- γ and TNF- α) on both phases of trichinosis, while it induced moderate effects on encysted muscular larvae.

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