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The Apoptotic Effect of PTX on Inflammatory Cells in the Liver of Mice Infected by Schistosoma mansoni: An Ultrastructural Study

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ABSTRACT

Pentoxifylline (PTX) is known for its suppressor effect in various inflammatory lesions. It has been recently suggested that PTX may play a role in downregulating the migration and participation of inflammatory cells in the granuloumatous lesions in murine models of schistosomiasis. This could help to alleviate the injurious effect exerted by inflammation on the host tissue. Accordingly, PTX was administered in Schistosoma mansoni-infected experimental mice (at 7 weeks & 15 weeks stages) and its effect on inflammatory cells was investigated by transmission electronmicroscopic ultrastructural study of the migrating inflammatory cells in the intravascular compartment and those residing in the extravascular granulomatous lesion. The inflammatory cells in the PTX-treated groups showed obvious apoptotic morphological changes (chromatin condensation and margination, shrinkage of the nucleus, widening of the perinuclear space, cytoplasmic condensation, intact organelles and cytoplasmic membrane) during both early and late stages of the disease. These results denote that the induction of apoptosis of inflammatory cells by PTX could be regarded as one mechanism whereby this drug produces its antiinflammatory action. Thus the administration of PTX could be a beneficial therapeutic approach in ameliorating the noxious effect produced by the full blown inflammatory process.

Key Words: *Pentoxifylline - Schistosomiasis - Inflammatory cells - Hepatic granulomatous lesions - Ultrastructure - Apoptosis.*

INTRODUCTION

Pentoxifylline (PTX), a methyl xanthine derivative, has recently been shown to have an antiinflammatory effect by interfering with migration and adherence of immune cells **[1-5]**.

Many studies have addressed the mechanisms underlying such effect and suggested the inhibition of production of the proinflammatory cytokine tumor necrosis factor -alpha (TNF-α) and the subsequent suppression of ICAM-1 (intercellular adhesion molecule-1) expression on the recruited cells **[5-11]**.

Many therapeutic trials using PTX have been conducted to evaluate its antiinflammatory potentiality in various inflammatory diseases **[12-15]**.

Studies have been performed to investigate effects of PTX on hepatic periovular granuloma, an inflammatory injurious lesion responsible for morbidity and mortality in schistosomiasis, which is usually superceded by mobilization and accumulation of inflammatory cells in the hepatic tissue. In one study using an experimental model **[16]**, PTX was found to produce significant reduction of the expression of the adhesion molecule LFA-1 (lymphocyte function-associated molecule-1) on leucocytes infiltrating the liver in both intra- and extarvascular compartments during the vigorous stage of granuloma. This indicated an antiinflammatory effect of PTX through inhibiting LFA-1- mediated adherence and subsequent migration of inflammatory cells forming the granulomatous lesion.

Another evidence for PTX antiinflammatory potency was presented by Reis et al. **[17]** who showed decrease in the intragranulomatous eosinophil accumulation due to interference with cellular recruitment and/or differentiation.

In the present work, a detailed ultrastructural study of the migrating inflammatory cells was conducted in order to demonstrate the possible PTX -induced alterations in the integrity and activity of the inflammatory cells. This would,

in turn, throw more light on the therapeutic efficacy of PTX in ameliorating the murine Schistosoma mansoni granulomatous lesion during the acute vigorous and chronic stages of the infection.

MATERIAL AND METHODS

Animals:

Twelve white albino mice were infected with Schistosoma mansoni by SC injection of 60 cercariae/ mouse. They were then equally divided into 4 groups:I. The first group was sacrificed at 7 weeks post infection, II. The second group was sacrificed 15 weeks after infection, III. The third group was treated with pentoxifylline (Sigma) 3 weeks before sacrifice and then sacrificed at 7 weeks postinfection, IV. The fourth group was treated with PTX for 3 weeks using the same dose level as in group III before sacrifice and then sacrificed at 15 weeks postinfection.

The therapeutic dose was calculated first by transforming the human dose of trintal, the commercial name for PTX (400 mg three times daily), into a murine dose (3.12 mg three times daily) using special tables devised by Paget and Barnes **[18]**. The murine dose was further multiplied by 1.5 (4.68 mg daily) in order to ensure higher PTX effect. The treatment was continued for three weeks until the date of sacrifice.

Transmission Electronmicroscopy:

Liver samples (1mmX1mm in size) were prepared for transmission electronmicroscopy according to Clement et al. **[19]** as follows:

1- Fixation in 2.5% glutaraldehyde (Merk) in cacodylate buffer [0.2M, pH=7.4] (Electronmicroscopy Sciences) for 2 hours at 4ºC.
2- Post fixation in 1% OsO4 (Electronmicroscopy Sciences) in cacodylate buffer [0.3M, pH=7.4] for 1 hour at 4ºC.
3- Dehydration in ascending grades of ethanol (30%, 50%, 70%, 90% and 100%).
4- Substitution in a mixture of epoxy resin (Electromicroscopy Sciences) and absolute ethanol.
5- Inclusion in three washes of epoxy resin at room temperature twelve hourly.
6- Embedding in epoxy resin capsules.
7- Polymerization of the resin at 60ºC for three days.
8- Ultrathin sectioning of capsules and examination of sections under the electronmicroscope, Joel 1200 EX II.

RESULTS

In the 7 weeks S.mansoni-infected untreated animals:

By EM, the inflammatory cells are seen both intravascular and intragranulomatous (Fig. 1). Most granuloma cells and those present in the lumen of blood vessels are composed of lymphocytes, plasma cells, monocytes, some neutrophils and very few eosinophils (Fig. 2).

In the intravascular compartment, the cells are abundant and some appear adherent to the endothelial cells lining the vessel. Most of the cells exhibit normal morphology with intact nuclei displaying normal size, shape and chromatin pattern. Also the cytoplasm density is normal and contains normal intact well-defined granules and other cellular organelles such as mitochondria and endoplasmic reticulum. Slight collagen deposition is encountered (Fig. 1).

In the 7 week S.mansoni-infected PTX- treated mice:

The same types of inflammatory cells are present in the treated and untreated groups of the corresponding stage and they are still represented inside the lumen of blood vessels and in the granulomata (Fig. 3). Also the relative number of cells to fibrous tissue is not affected by treatment. However, EM revealed morphological alterations of many cells with appearance of apoptotic and degenerative elements as evidenced by intranuclear chromatin condensation and margination, shrinkage of the nucleus, widening of the perinuclear space as well as condensation, shrinkage and budding of the cytoplasm (Figs. 3,4).

In the 15 weeks S.mansoni-infected untreated animals:

As the disease passes from the 7 to 15 weeks stages, the inflammatory cells start to disappear from the lumen of the blood vessels but continue to be present in the granulomata, though reduced in number relative to the amount of collagen deposits. The differential counts start to divert from the pattern mentioned during the 7-weeks stage towards predominance of eosinophils and fibroblasts (Figs. 5,6). Also some morphological changes are observed in some cells reflecting

a mild degree of degeneration (Fig. 7). This is evidenced by partial disintegration of cytoplasm and rupture of cytoplasmic membrane.

In the 15 weeks S.mansoni-infected PTX treated mice:

The inflammatory cells have disappeared from the intravascular compartments as in the15 weeks untreated group (Fig. 8). Cells also continue to appear in the same relative proportions in the granuloma as in the untreated 15 weeks stage. Furthermore, deposits and fibroblasts are increased (Fig. 9). A shift towards the increase of eosinophils is also encountered. Morphological apoptotic alterations have been observed (Fig. 10).

DISCUSSION

The inflammatory reaction incited in the liver of Schistosoma mansoni-infected mice is a double-edged weapon exerting both a beneficial role in combating the parasite and a noxious effect on the host tissue. The antiinflammatory agent PTX was thus used in this study in a therapeutic attempt to alleviate such injurious inflammatory reaction.

The present work has introduced a morphological analysis of inflammatory cell types in the murine hepatic granuloma revealing the characteristic chronological events that occur during the progression of the granuloma from the seventh week to the fifteenth week post-infection in the infected untreated group of animals. The 7 week granuloma was composed mainly of lymphocytes and plasma cells followed, in the 15 weeks, by their retreat and replacement by eosinophils, fibroblasts and collagen. Also abundant inflammatory cells that appeared in the intravascular compartment during the 7 weeks stage were significantly decreased during the 15 weeks. These findings agree with previous ultrastructural studies of Schistosoma mansoni granulomatous reactions **[20,21]**.

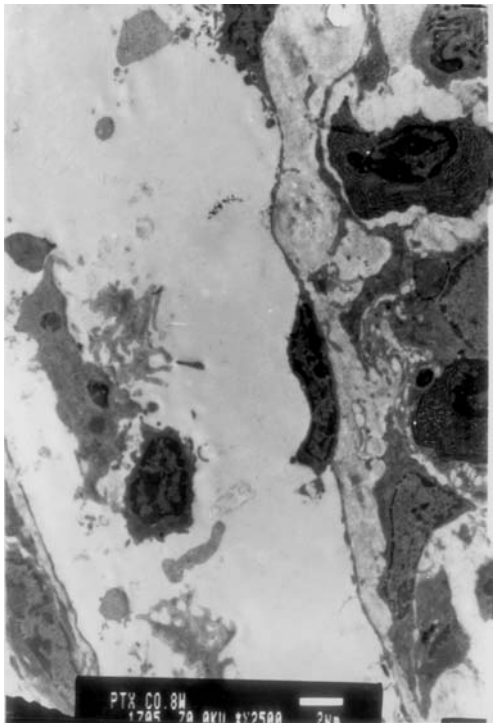

Fig. (1): TEM photo of a 7 wks S. mansoni- infected mouse showing a longitudinal section of a blood vessel in the liver lined by well-defined endothelial cells (EC). The inflammatory cells seen within the vessel are a lymphocyte (LY) and an activated neutrophil (NE) displaying many villous projections. Many inflammatory cells are seen recruited extravascularly in close contact to the vessel. X 2500.

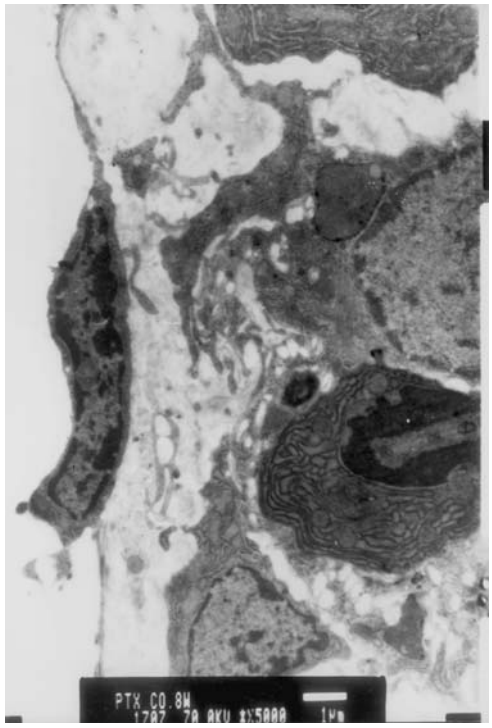

Fig. (2): A granulomatous inflammatory reaction in proximity to the same blood vessel shown in the previous photo. The granuloma is composed mainly of plasma cells (PC) exhibiting the specific onion-like appearance of endoplasmic reticulum in the cytoplasm. X5000.

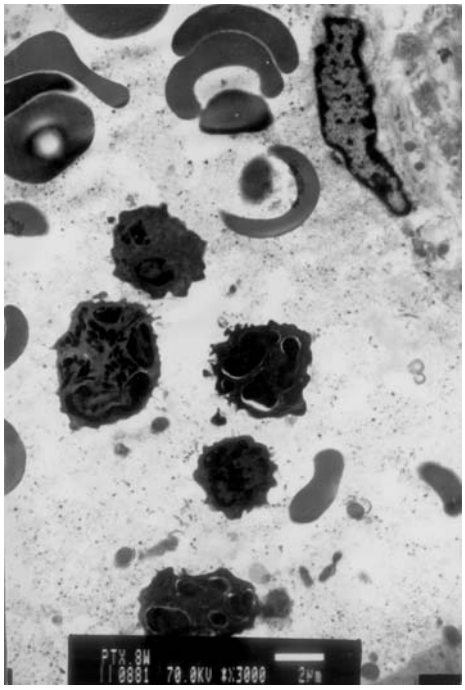

Fig. (3): A micrograph of a liver section of a 7wks PTX-treated S. mansoni- infected mouse showing a collection of apoptotic inflammatory cells in the lumen of a blood vessel. The cells show condensation and pyknosis of their nuclei with widening of perinuclear space as well as condensation and shrinkage of the cytoplasm. X3000.

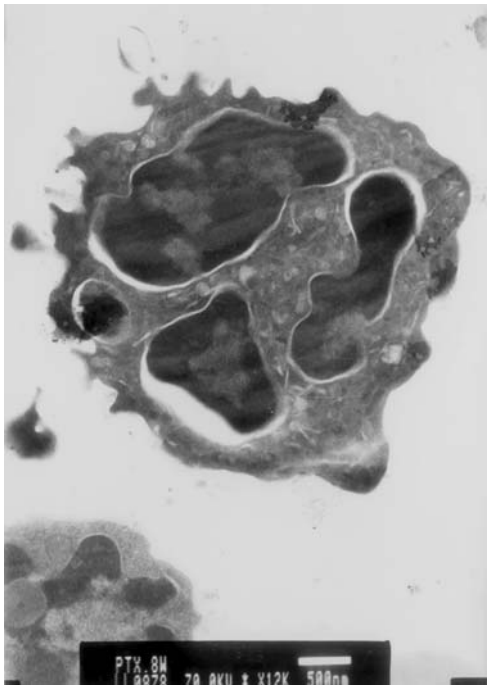

Fig. (4): An apoptotic neutrophil (in a 7wks p.i. PTX-treated mouse) with shrunken and condensed nuclear lobes surrounded by widened perinuclear spaces. The cytoplasm appears condensed but studded by intact granules. X12,000.

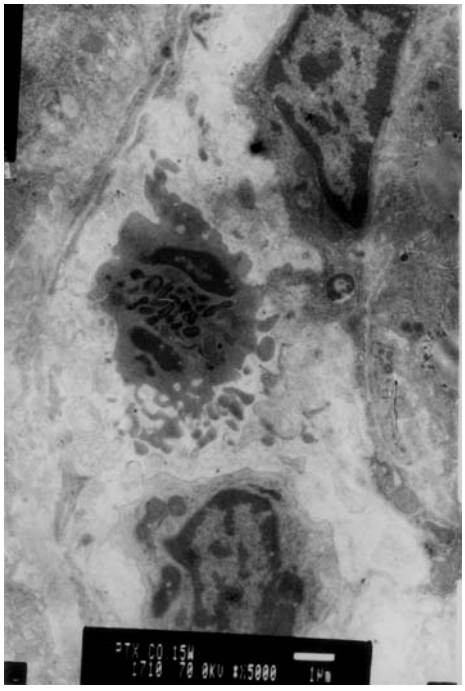

Fig. (5): A TEM photograph in the liver of a 15 wks S. mansoni-infected mouse revealing a blood vessel lined by an endothelial cell (EC) which is seen throwing some projections to reach villi of an eosinophil lying in the lumen of the vessel. A lymphocyte (LY) is also encountered inside the lumen. X5000.

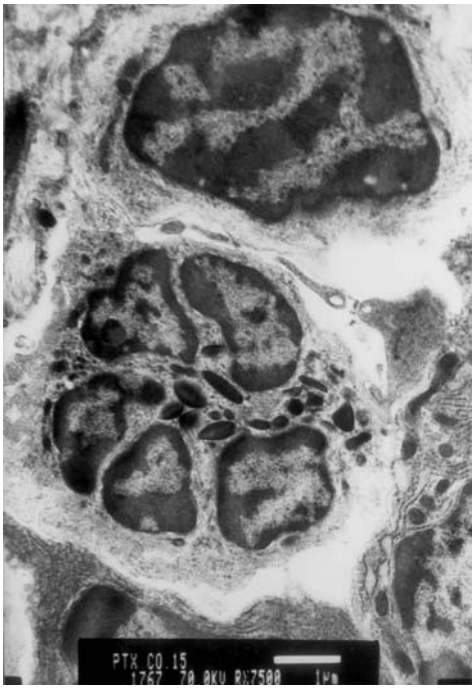

Fig. (6): A cross section in a 15wk hepatic granuloma of a S. mansoni-infected mouse showing an eosinophil (EO) and a lymphocyte (LY) with normal morphology. X7500.

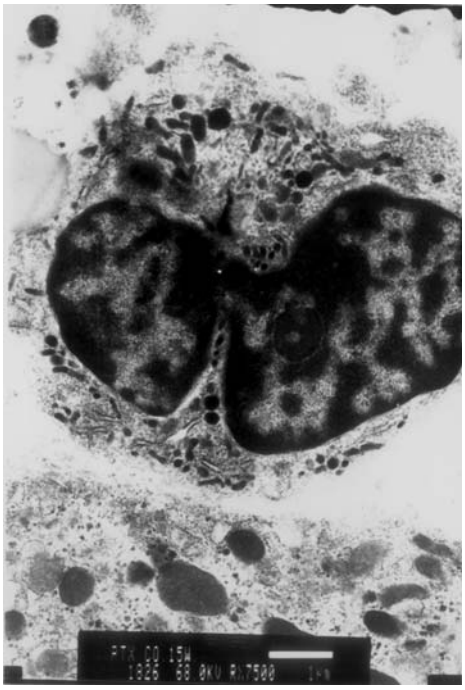

Fig. (7): A photograph of a 15 wk granuloma showing a degenerating eosinophil with disappearance of its plasma membrane and partial disintegration of the cytoplasm. X7500.

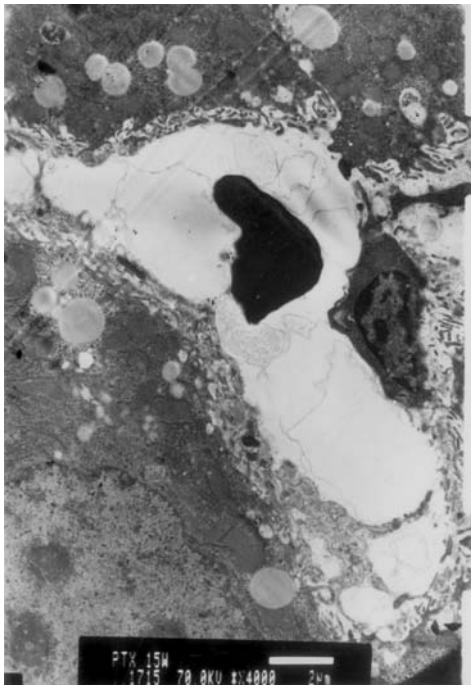

Fig. (8): A blood vessel in the liver of a mouse infected with S. mansoni (15 wks p.i.) and treated with PTX. The vessel is empty from inflammatory cells. Only mature red cells can be seen. X4000.

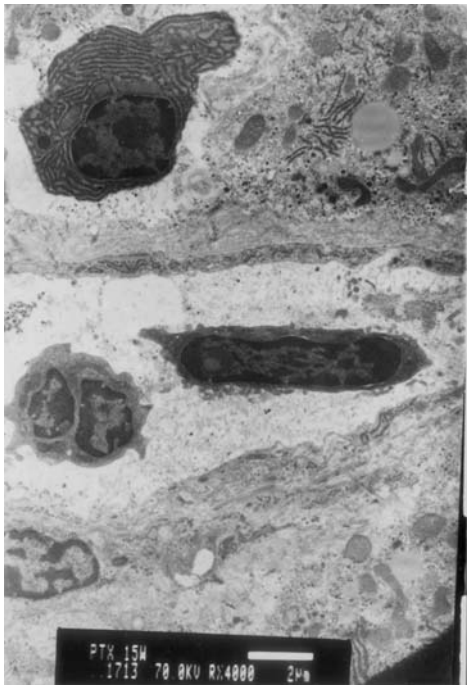

Fig. (9): TEM micrograph of a liver section from a PTX-treated mouse after 15wks infection with S. mansoni. A blood vessel is revealed with a lymphocyte (LY) and a fibroblast (FB) in its lumen. An extravascular plasma cell (PC) is seen nearby the blood vessel wall. X4000.

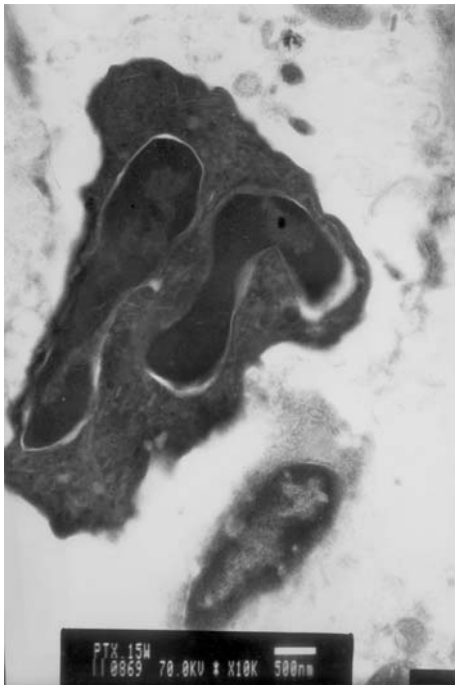

Fig. (10): A neutrophil showing apoptotic features with chromatin condensation and shrinkage of the nucleus leaving a space surrounding it. The cytoplasm also appears condensed and shrunken yet the granules are intact. X10,000.

Ultrastructurally, most cell types were of normal morphology in the 7th week granuloma except for appearance of pronounced cytoplasmic projections. The latter might subserve either adherence of inflammatory cells to endothelial lining for their extravasation into the hepatic tissue or contact of inflammatory cells to each other for cellular interactions. In the 15th week stage, some degenerative changes were observed in a small population of granulomatous cells explaining the demolishment of cellular elements in the downregulated granuloma.

In the PTX- treated group, the 7th week stage showed apoptotic morphological features in both intravascular and granulomatous inflammatory cells (Figs. 3,4) while disintegration and degeneration of the cytoplasm were also observed in other cells. During the 15 weeks stage, PTX rendered the apoptotic changes more pronounced than in the untreated group. The apoptotic effect of PTX probably presents a mechanism for alleviating inflammation in Schistosoma mansoni infection.

Another mechanism of PTX antiinflammatory effect was shown by Ghanem et al. **[16]** to be through the reduction of the expression of the adhesion molecule LFA-1 on the intravascular and granulomatous inflammatory cells, thus hindering LFA-1 mediated adherence and migration of inflammatory cells. Reis et al. **[17]** also showed a decrease in the intragranulomatous eosinophil accumulation by PTX. However, they related this effect to the possible immunosuppressive properties of PTX being capable of inhibiting proliferation of mononuclear cells and lymphocytes induced by T- and B- cell mitogens **[22]** reducing indirectly the number of eosinophils which are strongly dependent on T-cells cytokines such as IL-3, IL-5 and GM-CSF **[23]**.

Therefore the above mechanisms suggestesd by various studies could together explain the potentiated antiinflammatory action of PTX in hepatic schistosomiasis.

On the other hand, the direct morphological evidence of apoptotic effect of PTX on inflammatory cells, as encountered in the present study, needs further dissection of the underlying mechanisms through the analysis of apoptotic pathways in the in vitro and/or in vivo granuloma.

It is also important in future studies to standardize the dose and the stage of administration of the drug in order to obtain an appropriate antiinflammatory therapeutic effect without possible interference with the defensive mechanisms of the body.

REFERENCES

1- Bruynzeel I, Vander Raaij LM, Willemze R, et al. Pentoxifylline inhibits human T-cell adhesion to dermal endothelial cells. Arch. Dermatol. Res. 1997, 289: 189.

2- Gonzalez-Amaro R, Portales-Perez D, Barandal L, et al. Pentoxifylline inhibits adhesion and activation of human T-lymphocytes. J. Immunol. 161: 65, 1998.

3- Krakauer T. Pentoxifylline inhibits ICAM-1 expression and chemokine production induced by proinflammatory cytokines in human pulmonary epithelial cells. Immunopharmacology. 2000, 46: 253.

4- Neuner P, Kloser G, Pourmojib M, et al. Pentoxifylline in vivo and in vitro downregulates the expression of the intercellular adhesion molecule-1 in monocytes. Immunology. 1997, 90: 435.

5- Rieneck K, Diamant M, Haahr PM, et.al. In vitro immunomodulatory effects of pentoxifylline. Immunol. Lett. 1993, 37: 131.

6- Balazs C, Kiss H and Farid NR. Inhibitory effect of pentoxifylline on HLA-DR expression and glycosaminoglycan synthesis by retrobulbar fibroblasts. Horm. Metab. Res. 1998, 30: 496.

7- Farkas G, Marton J, Mandi Y, et al. Complex treatment of infected necrotizing pancreatitis. Orv. Hetil. 1998, 139: 2235.

8- Hohenberger P, Latz E, Kettel C, et al. Pentoxifylline attenuates the systemic inflammatory response induced during isolated limb perfusion with recombinant human tumor necrosis factor-alpha and melphalan. Ann. Surg. Oncol. 2003, 10: 562.

9- Mandi Y, Farkas G and Oesovszky I. Effects of pentoxifylline and Pentaglobino on TNF and IL-6 production in septic patients. Acta Microbiol. Immunol. Hung. 1995, 42: 301.

10- Naveau S. Acute alcoholic hepatitis: treatments. Presse Med. 2001, 9: 1024.

11- Roujeau JC. Treatment of severe drug eruptions. J. Dermatol. 1999, 26: 718.

12- Gomez-Cambronero L, Camps B, de-la-Asuncion JG, et al. Pentoxifylline ameliorates cerulein-induced pancreatitis in rats; role of glutathione and nituric oxide. J. Pharmacol. Exp. Ther. 2000, 293: 670.

13- Park WS, Chang YS and Lee M. The efficacy of pentoxifylline as anti- inflammatory agent in experimental Escherichia coli meningitis in the newborn piglet. Biol. Neonate. 2000, 77: 236.

14- Shimizu N, Watanabe T, Arakawa T, et al. Pentoxifylline accelerates gastric ulcer healing in rats: roles of tumor necrosis factor alpha and neutrophils during the early phase ulcer healing. Digestion. 2000, 61: 157.

15- Silva JC, Rocha MF, Lima AA, et al. Effects of pentoxifylline and nabumetone on the serum levels of IL-1 beta and TNF-α in rats with adjuvant arthritis. Inflamm. Res. 2000, 49: 14.

16- Ghanem LY, Lotfi AA, Salah M, et al. Immunoelectronmicroscopic, immunohistochemical and immunocytochemical study of the effect of pentoxifylline and anti-transforming growth factor -ß on two adhesion molecules in murine schistosomiasis. Egypt. J. Lab. Med. 2001, 13: 1.

17- Reis LF, Ventura TG, Souza SO, et al. Quantitative and Qualitative interferences of pentoxifylline on hepatic Schistosoma mansoni granulomas: effects on extracellular matrix and eosinophil population. Mem. Inst. Oswaldo Cruz, 2001, 96: 107.

18- Paget and Barnes. Evaluation of drug activities. Vol.I Academic Press. 1964.

19- Clement B, Rissel M, Peyrol S, et al. A procedure from light and electronmicroscopic intracellular immunolocalization of collagen and fibronectin in rat liver. J. Histochem. Cytochem. 33: 407, 1986.

20- Epstein WL, Fukuyama K, Danno K, et al. Granulomatous inflammation in normal and athymic mice infected with Schistosoma mansoni: An ultrastructural study. J. Path. 1979, 27: 207.

21- Ghanem LY. A study of the ultrastructure of eosinophils in mice infected with Schistosomiasis. M.Sc. thesis presented to the Faculty of Medicine, Cairo University. 1985.

22- Rosenthal LA, Taub DD, Moors MA, et al. Methylxanthine-induced inhibition of the antigen and superantigen-specific activation of T and B lymphocytes. Immunopharmacology. 1992, 24: 203.

23- Rott O, Cash E and Fleischer B. Phosphodiesterase inhibitor pentoxifylline, a selective suppressor of T helper type 1-but not type 2-associated lymphokine production, prevents induction of experimental autoimmune encephalomyelitis in lewis rats. Eur. J. Immunol. 1993, 23: 1745.