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EFFECT OF PRAZIQUANTEL ON ADULT WORM AND EGGS OF *HYMENOLEPIS NANA* IN EXPERIMENTALLY INFECTED MICE

(With 3 Plates)

By

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تأثير عقار البرازيكوانتيل على دودة الهيمينوليبس نانا وبويضاتها في فئران التجارب

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تعد الهيمينوليبس نانا من أكثر الديدان الشريطية انتشارا في مصر. وشملت هذه الدراسة مدى انتشار الدودة القزمية في المرضى المترددين على مستشفى ابنوب المركزي. كما شملت الدراسة استخدام عقار البرازيكوانتيل في علاج الفئران البيضاء المعدية بدودة الهيمينوليبس نانا من الانسان. وكانت الجرعة المستخدمة هي ١٠٠ملجم/كيلو. و تمت مناقشة تأثير العقار على كل من الديدان البالغة والبويضات. ومن هذه الدراسة نستخلص أن عقار البرازيكونتل هو العلاج الأمثل للديدان الشريطية في كل من الانسان وحيوانات التجارب.

SUMMARY

Hymenolepis nana is the most common tapeworm in Egypt. The present work was concerned with estimation of the prevalence of *Hymenolepis nana* in patients attending Abnoub Central Hospital. It was also concerned with experimental infection of mice with human *Hymenolepis nana* eggs. Praziquantel was applied to treat the infected mice, it was administered in a single oral dose of 100-mg/kg-body weight. The effect of the drug on adult worms and eggs was discussed. It can be concluded that Praziquantel remains the drug of choice for treatment of *Hymenolepis nana* in both man and experimental animals.

Key words: *Praziquantel, Hymenolepis nana, experimental infection*

INTRODUCTION

Parasitic diseases are still one of the major medical problems in Egypt where they predispose to impairment of physical and mental development particularly in children (Sabbour and Farid, 1978).

Hymenolepis nana is the most common tapeworm in Egypt. El-Nazer (1983) reported its incidence to be (5.1 %) as multiple infection. Ismail *et al.* (1988) found the infection rate (10 %) and Morsy *et al.* (1991) estimated (10.1 %) infection rate.

Treatment of helminthic parasites has two main aims, namely to prevent or reduce further tissue damage in infected individuals and to reduce egg excretion and thus transmission in the community is prevented (Sleigh *et al.*, 1986; Cuesta *et al.*, 1992 and Ettoum *et al.*, 1993).

Praziquantel is undoubtedly the most important advance in antihelminthic chemotherapy of recent decades (Younis and Khalil, 1998).

The present work was conducted to induce experimental infection in laboratory mice with human *Hymenolepis nana* eggs and to study the effect of the currently used drug (Praziquantel) on adult worms and eggs of *Hymenolepis nana* in the experimentally infected mice.

MATERIAL and METHODS

The present work was concerned with *Hymenolepis nana* infecting patients attending Abnoub Central Hospital. It was also concerned with application of Praziquantel in treatment of experimentally infected mice with human *Hymenolepis nana eggs.*

Diagnosis of human *Hymenolepis nana* infection:

Stool samples were collected from 500 patients in clean plastic containers with covers and examined by both direct smears and concentration methods by floatation, Ritchie (1948) and formol ether sedimentation techniques, Garcia (2001).

Highly positive stool samples for *Hymenolepis nana* eggs were chosen for experimental infection of albino mice as follows:

- The specimen was diluted to homogenous suspension in normal saline and strained.
- Repeated centrifugation were done using normal saline till the supernatant became clear.

-The supernatant was discarded and the number of eggs per ml. was calculated using a Haemocytometer and adjusted to be 100 eggs per ml.

Experimental *Hymenolepis nana* infection to mice :

15 clean laboratory albino mice with an average weight 22 grams were used in this study. The mice were maintained on a well-balanced diet. Stool of all mice was examined every three days for 2 weeks to exclude *Hymenolepis nana* natural infections.

The mice were divided into the following groups:

1- Control group:
2- normal non-infected mice (control negative).
3- mice infected with 2 ml.of the egg suspension (control positive). After the 7th.day the stool was examined daily for *Hymenolepis nana* eggs.

Test group:

10 mice were infected each with 2 ml. of the egg suspension, administered directly into the esophagus by means of a tuberculin syringe fitted to a polythene tube.

After the 7th.day the stool was examined daily for *Hymenolepis nana* eggs.

Praziquantel administration:

Infected mice of the test group were weighed and given Praziquantel in an oral dose of 100-mg/kg-body weight (Gonnert and Andrews, 1977). One mouse was sacrificed after 1, 2 and 24 hours post-treatment. The intestine was cut longitudinally and examined for the presence of adult *Hymenolepis nana* and the stool was examined for eggs.

Staining of adult *Hymenolepis nana* with acetic acid alum carmine:

The small intestine of the treated positive mice and the positive control mice were cut longitudinally and examined for the presence of adult worms. They were collected in two petri dishes fixed in Formaline 10%. The worms were left in the fixative for 24 hours then stained with acetic acid alum carmine according to Faust *et al.* (1976).

RESULTS

The stool examination of human cases revealed that *Hymenolepis nana* was common in children. The total percentage of infection was (2.7 %), the highest prevalence was in age group 2-7 years old (4 %).

The efficacy of Praziquantel against *H. nana* in experimentally infected mice:

1- Effect on eggs:

Stool samples examined for detection of eggs of *H. nana* after one and 2 hours of treatment showed the eggs with disfigured shell outline. The embryophore was ill-defined in some eggs and lost in others, (Pl. I Fig. B) in comparison to the control egg (Pl. I Fig. A). After 24h the eggs completely disappeared.

2- Effect on adult fresh worms:

- After one hour of treatment with Praziquantel, adult worms of *H. nana* were detected in the large intestine of mice; they were immobilized and completely separated.
- After 2 hours of treatment, fragments of adult worms were detected in the faecal matter in the rectum of mice.
- After 24 hours of treatment no worms were detected in the intestine.

3- Effect on adult stained worms:

Examination of stained worms revealed that the scolex is intact, the tegument showed maceration at the neck and immature segments, (Pl. II Fig. A, B). Both the mature and gravid segments showed loss of muscle tone and the segments became more flattened with ill-defined inter- segmental lines, in comparison to normal segments, (Pl. II Fig. C,D) & (Pl. III Fig. A,D). Eggs were irregularly distributed in the gravid segments, (Pl. III Fig. A,B).

DISCUSSION

The study of parasites causing human diseases is important not only because these infections are associated with high morbidity and mortality but also because most of them are preventable when people are armed with the necessary health knowledge (Sun, 1988).

The present investigation revealed that the total prevalence of *Hymenolepis nana* infection was (2.7 %), the highest prevalence was in age group 2-7 years old (4 %).

Previous results obtained from different Egyptian localities, showed that the rate of infection with *Hymenolepis nana* was fluctuating as reported by El-Naggar *et al.* (1978), Sidky *et al.* (1987), Mohammed *et al.* (1988) and Morsy *et al.* (1991). This fluctuation of infection depended on age, social condition and health habits.

Praziquantel is a broad-spectrum antihelminthic drug. It had been approved to be effective against a wide range of trematodes and cestodes especially schistosomiasis and *H. nana* (Pearson & Current, 1983).

Praziquantel is rapidly absorbed after oral administration and the enteral absorption is nearly complete, maximum serum concentration is attained in 1-2 hours. (Mandour *et al.*, 1990 and El-Guinady *et al.*, 1994). It is well tolerated and accepted by the patients (Hardman *et al.*, 1996).

Regarding the efficacy of Praziquantel therapy on the human strain of *H. nana* in experimentally infected mice, it was found that treatment with Praziquantel in a single dose of 100 mg / kg lead to absence of eggs excreted in stool of mice. The intestine was found free of adult worms (100 % cure rate). This was in agreement with the efficacy of the drug in human cases with equivalent dose 25 mg / kg (El-Guinady *et al.*, 1994; Metwally *et al.*, 1995 and Kabil *et al.*, 1996).

It was observed in this study that the onset of the effect of Praziquantel in mice was rapid and the parasites were immobilized and excreted within a few hours with faeces, this was in agreement with Thomas *et al.* (1982). Moreover the scolex was completely separated from mucosa of the intestine. The neck region and immature segments showed maceration of the tegument, while mature segments showed loss of muscle tone. The gravid segments became more flattened and the eggs were irregularly distributed in the segments.

Becker *et al.* (1981) observed that vacuolization of the tegument was exclusively confined to the neck region of the tapeworms and proglottids of the middle or posterior regions of the worms never showed destruction.

The main targets for Praziquantel action were the *Schistosoma* adult worm`s tegument and musculature (Wingard *et al.*, 1991). Schistosomes exposed to Praziquantel develop an almost instantaneous contraction of the muscles followed by spastic paralysis and rapid vacuolization of the syncytial tegument, (Davis *et al.*, 1979).

The effects of Praziquantel could be attributed to alteration in intracellular Ca^{2+} homeostasis at one or more site in the worm. Increased intracellular Ca^{2+} was the cause of cytoskeletal disruption and membrane blebbing in a wide variety of cell types, (Day *et al.*, 1992). Blair *et al.* (1992) noted that the tegument as well as the sarcolemma seemed to contain Praziquantel sensitive sites. These sensitive Ca^{2+} influx sites in the tegument would explain why the tegument is damaged so easily by Praziquantel (Redman *et al.*, 1996).

From the present study, it can be concluded that Praziquantel remains the drug of choice for treatment of *Hymenolepis nana* in both man and experimental animals, the reason being high therapeutic efficacy, excellent patient tolerance and few and relatively minor side effects.

REFERENCES

Becker B.; Mehlhorn H.; Andrews P. and Thomas H. (1981): Ultrastructural investigations on the effect of Praziquantel on the tegument of five species of cestodes. Z. Parasitenk., 70 : 120 – 138. .

Blair K.L.; Bennett J.L. and Pax R.A. (1992): Praziquantel: Physiological *H. nana.* Parasitology., 104: 59-66.

Cuesta R.A.; Kaw Y.T. and Duwaji M.S. (1992): S. mekongi infection in a leiomyosarcoma of the small bowel: Case report. Hum. Pathol., 23 (4): 471-473.

Davis A.; Biles J.E. and Ulrich A.M. (1979): Initial experiences with Praziquantel in the treatment of human infection due to *S. haematobium. Bull. WHO., 57(5): 773-779.*

Day T.A.; Bennett J.L. and Pax R.A. (1992): Praziquantel: The enigmatic antiparasitic. Parasitology Today. 8 (10): 342 - 344

El-Guinady M.A.; El-Touny, M.A.; Abdel-Bary S.A.; Abdel-Fatah S.A. and Metwally A. (1994): Clinical and pharmacokinetic study of praziquantel in Egyptian schistosomiasis patients with and without liver cell failure Am. J. Trop. Med. Hyg., 51 (6) : 809-818.

El-Naggar B.A.; El-Tork S.A. and Morsy T.A. (1978): Parasitic infections among preschool and school aged children. J.Egypt. Pub & Hth Assoc., 53 (5-6): 374-376.

El-Nazer M.M.A. (1983): Parasites of children in Assiut province. M.Sc. thesis (Parasitology), Faculty of Medicine, Assiut University.

Ettoum I.A.; Saad A.M.; Ismail B.M.; Ali M.M.; Suliman S.; Bennett J.L. and Homeida M.A. (1993): Efficacy of treatment in patients with advanced hepatic fibrosis. Am. J. Trop. Med. Hyg., 48 : (1) : 77-81.

Faust E.C.; Russel P.F. and Jung R.C. (1976): Craig & Faust's clinical Parasitology, Lea & Fabiger, Philadelphia, 8th ed.

Garcia, L.S. (2001): Diagnostic medical Parasitology, 4th ed. ASM Press, Washington. pp. 746

Gonnert R. and Andrews P. (1977): Praziquantel a new broad-spectrum antischistosomal agent. Z Parasitenk., 52 : 129.

Hardman J.G.; Limbird L.E.; Molinoff P.B.; Ruddon R.W and Gilman A.G. (1996): Goodman and Gilman's the pharmacological basis of therapeutics. 9ed.. International Edition, McGraw-Hill.

Ismail M.M.; Bruce J.I.; Ross Mussen S.L.; Attia M. and Salama M. (1988): Schistosomiasis and other helminthic infections in kafr Soliman Village, Sharkia Governorate, Egypt. J. Egypt. Soc. Parasitol., 18, 1 : 42-46.

Kabil S.M., Abdel-Fattah M.S., Nawar M.A. and Atta M.M. (1996): Evaluation of different doses of Praziquantel in the cure of intestinal schistosomiasis. In Egyptian Society of Tropical Medicine, Infectious and Parasitic Diseases (ESTIP), 3rd Annual Congress., P. 94 .

Mandour E.M.; Mohammed A.; Turabi E.L.; Hamid Homeida M.A. and Manoun (1990): Pharmacokinetics of Praziquantel in healthy volunteers and patients with schistosomiasis. Trans . Roy. Soc. Trop. Med. Hyg ., 84 : 389-393.

Metwally A.; Bennett J.L.; Botros S.; Ebeid F. and Attar G.D.M. (1995): Impact of drug dosage and brand bioavailability and efficacy of Praziquantel. Pharmocol. Res., 13: 53-59.

Mohamed N.H.; Fawzy A.F.A.; Sarwat M.A. and Farrage A.M.K. (1988): Parasitic diseases in El-Korean, Sharkia Governorate with evaluation of some methods used in stool examination. J. Egypt. Soc. Parasitol., 18, 1 : 305-311.

Morsy T.A.; Farrag. A.H.K.; Sabry A.A.; Salama M.H.I. and Arafa Magdi A.S. (1991): Ecto and Endo parasites in two primary schools in Qualyob City, Egypt., J. E.gypt. Soc. Parasitol. 21 (2): 391- 403.

Pearson. R.P. and Current, R.L. (1983): Praziquantel; A major advance in antihelmintic therapy. Ann. Intern. Med., 99: 195.

Redman C.A.; Robertson. A. and Roger, A.M. (1996): Praziquantel: An urgent and exiting challenge. Parasitology Today., 12 : 14-20.

Ritchie L.S. (1948): Ether sedimentation technique for routine stool examinations. Bull U.S. Army Medical importance.

Sabbour M.S. and Farid N. (1978): Textbook of infectious diseases, Dar El-Maaraf, Cairo, Egypt.

Sidky H.A.; Abdel Ghani S.M.; Hassan A.T.; Mostafa A.M.; Hassan R.R. and Abdel Hamid, S. (1987): Study on different helminthic infection among Al-Azhar University Students. J. Egypt. Soc. Parasitol., 17: 161-168.

Sleigh, A.G.; Hoff R.; Mott K.E.; Maguire J.H. and Da france Silves J.T. (1986): Mansoni schistosomiasis in Brazil: 11 years evaluation of successful disease control with oxamniquine. Lancet., 635-637.

Sun, T. (1988): Colour Atlas and Text book of Diagnostic Parasitology , Igako-Shoin, New York, Tokyo, 317 pp.

Thomas, H.; Andrews. P. and Mehlhorn. H. (1982): New results on the effect of Praziquantel in experimental cysticercosis. Am. J. Trop. Med. Hyg., 31: 803-10.

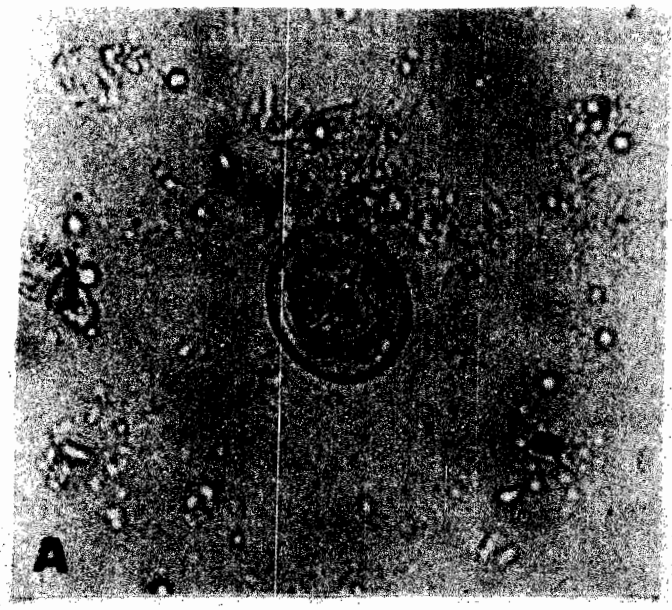
Wingard L.B.; Brody T.M.; Larner J. and Schwart Z.A. (1991): Human pharmacology-molecular to clinical, Wolfe publishing Limited-International Student Edition, London and Philadelphia, pp. 733-760.

Younis T.A. and Khalil H.H. (1998): Chemotherapy of schistosomiasis present and past J. Egypt Soc. Parasitol., 28 (1): 293-299.

Plate I:

Fig. A - Control egg of *H. nana.* X.66

Fig. B - Eggs of *H. nana* post- treatment. X.66



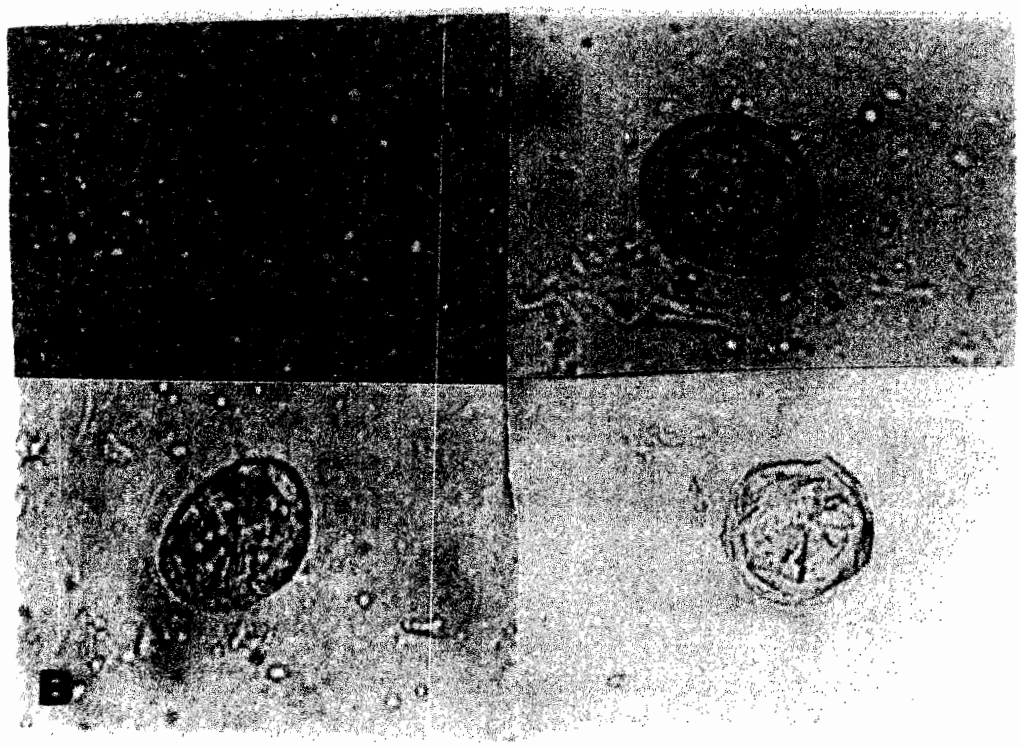


Plate II:

Fig. A - Scolex of *H. nana* post- treatment X.13.2

Fig. B - Immature segments of *H. nana* post- treatment. X. 13.2

Fig. C&D - Mature segments of *H. nana* control and post-treatment.X16.5

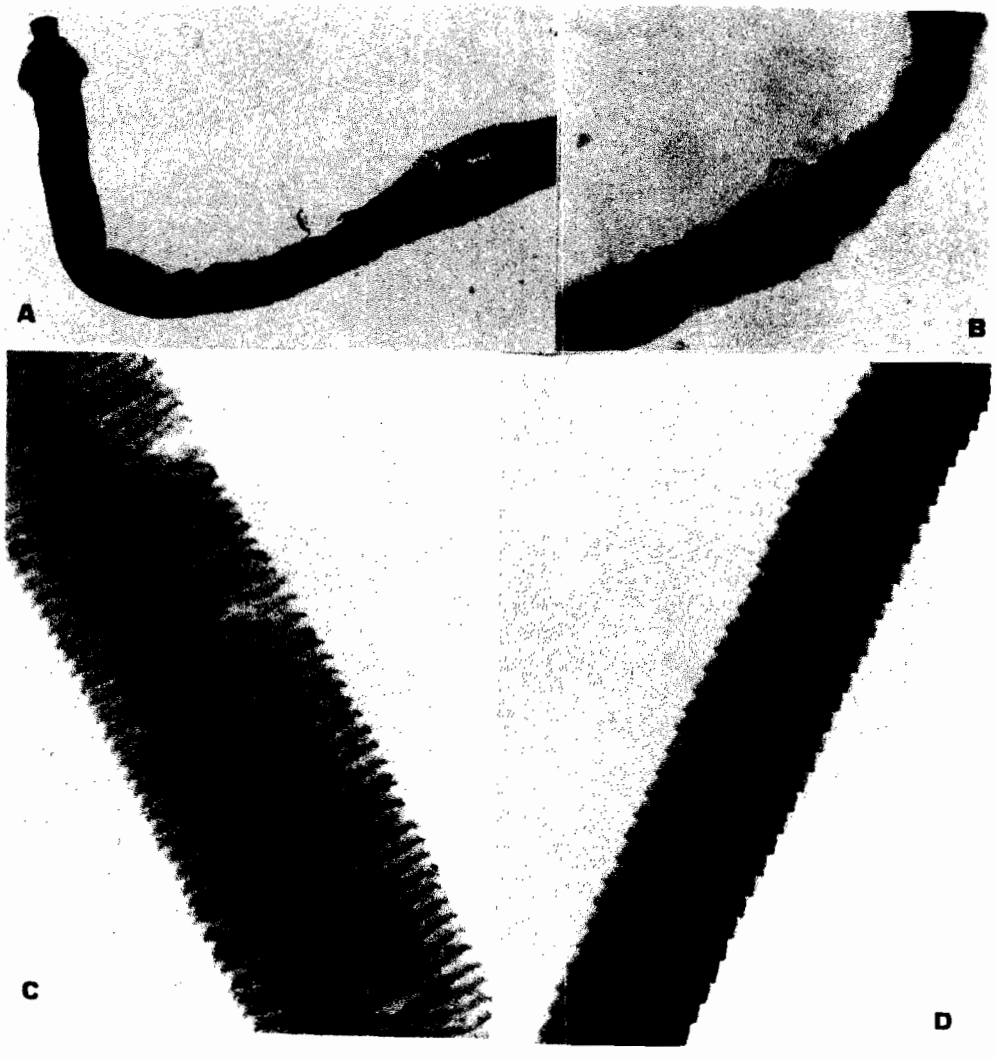


Plate III:

Fig. A&B- Gravid segments of *H. nana* control.X.16.5

Fig. C&D- Gravid segments of *H. nana* post- treatment X 16.5