ANTHELMINTHIC EFFECT OF TAMOXIFEN ON EXPERIMENTALLY HYMENOLEPIS NANA INFECTED MICE

By

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Abstract

   Hymenolepis nana is one of the commonest parasites worldwide especially in children. Praziquantel was the most effective drug in hymenolepiasis treatment, but with the risk of resistance development. There was a need for search for drug(s) to overcome this problem. Tamoxifen (an estrogen-receptor modulator), is available, low cost with potential anti-parasitic efficacy. This study assessed the therapeutic effect of tamoxifen drug on mice experimentally infected mice with *H. nana* at different developmental stages. Clean laboratory bred mice infected with *H. nana* eggs were divided into 4 groups according to different schedule of tamoxifen administration (20mg/kg body weight) at the parasite different developmental stages and control one (infected non treated). At the experimental mice were sacrificed and parasitological evaluated for worm burden count, their weight, egg count and viability. The results did not show statistically significant effect on worm burden as compared to control one, with the exception of mice treated two days post-infection that showed significant increase in worm burden. There was significant decrease in worm weight, egg count and viability as compared to control ones, but without difference between different treated groups.

Keywords: Mice, *Hymenolepis nana*, Tamoxifen[®], Parasitological evaluation.

Introduction

   Hymenolepis nana or the dwarf tapeworm is a worldwide prevalent zoonotic parasitic disease. It is one of the commonest cestode infections among rodents and man (Sadaf *et al,* 2013). It is widespread parasite of children owing to the lack of fecal sanitation together with bad feeding practice (Mirdha and Samantray, 2002). The three drugs used for the hymenolepiasis treatment were praziquantel, nitazoxanide, and niclosamide, with praziquantel (PZQ) the drug of choice for *H nana* (Juan *et al,* 2002). Indication of the development of PZQ resistance by other parasites appeared over recent decades (Beshay, 2017). Additionally, PZQ tablets are large, taste bitter, without readily available pediatric formulation (Cioli *et al*, 2014). Tamoxifen is the most used selective estrogen-receptor modulator to prevent and treat estrogen receptor positive breast cancer (Bogush *et al,* 2018), as well as antibacterial, antiviral and anti-parasitosis against echinococcosis and leishmaniasis (Bogush *et al,* 2012). Tamoxifen is cheap, safety and worldwide available (WHO, 2019).

   The present study aimed to evaluate Tamoxifen[®] activity on mice experimentally infected with *Hymenolepis nana* and its different life cycle stages.

Materials and methods

   The study was conducted at the experimental animal house of Department of Parasitology, Benha Faculty of Medicine, from October 2018 to January 2019.

   Parasite: *Hymenolepis nana* eggs were obtained fresh from stool samples of infected patient and inoculated into rats to have a laboratory strain.

   Fresh stool samples were collected in dry clean containers from patients attended Pediatric Department, Benha University's Hospitals. Stool samples were examined microscopically to insure *H. nana* eggs (Garcia and Bruckner, 1997) and the egg viability was confirmed by using Chausov's methodlogy (Moazeni and Rakhshandehroo, 2011). The dead eggs acquired a yellow color within 20 minutes, the oncospheres turned orange and magenta, but living eggs was yellow in color and spherical or subspherical oncosphere in closed in a thick membrane; embry-

ophore, and armed with six hooks: same as hexacanth embryo (Ridley, 2011). The collected eggs were then administered orally by using a tuberculin syringe to experiment with mice groups. Examination of stool of infected mice to confirm infection was done 2weeks post-infection by direct smear method (Ferretti *et al*, 1980). Dilution in normal saline and sieving for each sample was done. The collected fluid was then left to concentrate by sedimentation at room temperature for 1 to 3hrs or very carefully centrifuged (5 to 7 minutes at a rate of 500revolutions/ minute). Mice were sacrificed three weeks post-infection, by ether inhalation in a closed container. Each mouse was dissected and small intestine was removed and put in a Petri-dish with normal physiological saline and left for half an hour to release the worms from the intestinal mucous. Intestine was then slit opened, the worms were released and the gravid segments of adult worms were recovered and homogenized to get the eggs.

Experimental animals: One hundred laboratory-bred male Swiss albino mice, 6-8 weeks old, weighed about 20-25gm each were used in both *H. nana* maintenance and in experimental study in clean metallic cages. They were allowed bread mixed with barely, crushed corn and beans in a ratio 3:2:1 & water was provided in hanging bottles. Cages were kept in the animal house under daily observations

Tamoxifen® was obtained from Egyptian International Pharmaceutical Industries Co. (EPICO) each tablet contained 40mg. The tablets were dissolved in sunflower oil (Sigma-Aldrich) and given orally by tuberculin syringe at a dose of 20mg/kg body weight (Nicolao *et al,* 2014).

Infection design: Parasite-free mice received orally a dose of about 200 *Hymenolepis nana* eggs/mouse, using a syringe with curved esophageal needle specifically to infect mice. Infected mice were divided into 5 groups: G1: 20-infected mice orally treated with tamoxifen (20mg/kg orally) at 2nd day post-

infection for 3 successive days to evaluate the drug effect upon the cysticercoids. G2: 20-infected mice treated orally with tamoxifen (20mg/kg) at 6^{th}day post-infection for 3 successive days to evaluate the drug action upon juvenile form. G3: 20-infected mice treated orally with tamoxifen (20mg/kg) at 10th day post-infection for 3 successive days to evaluate the drug action on immature worms, not yet initiated oviposition. G4: 20-infected mice treated orally with tamoxifen (20mg/kg) at 14th day post-infection for 3 successive days to evaluate the drug action on mature worm laying eggs. G5: 20-infected, non-treated (infected control) to evaluate the parasite normal development in comparison with the drug treated mice.

Drug efficacy on total worm burden: Mice were sacrificed 20 days post-infection and the small intestines were removed, each one in a Petri-dish containing normal saline and left for sometimes then small intestines were dissected and the petri-dishes were examined to recover the free worm mucosa. Worm' affected was identified based on the morphology of scolex, mature segments, ova in gravid segments and each worm weight in mg.

Egg counting: Worms were separately crushed and the average eggs number was microscopically counted.

Egg viability: A vital staining method was used to confirm viability (Chausov, 1964).

Statistical analysis; Data were collected, tabulated and analyzed using the statistical package SPSS version 12. Data were given as mean and standard deviation (SD) for quantitative variables and percent for qualitative variables. Fisher exact test was used to compare between groups and P values <0.05 were considered significant.

Results

The present study did not show significant reduction in worm burden in infected mice treated with tamoxifen as compared to control ones (P >0.05) with exception of mice started treatment two days post-infection. This group showed an increase in worm burden as compared to control one (Tab.1). Also,

comparing the results of groups treated with different schedules showed no significant difference (P>0.05) in worm burden except mice treated after two days post-infection, where the worm burden was significantly higher than in other groups (P<0.05). There was a highly significant reduction in worm weight (P<0.001) in infected mice treated with tamoxifen when compared with control ones (Tab. 2). By comparing the results of mice treated with different schedules did not show significant difference (P>0.05) in worm's weight. There was a highly significant reduction in $H.$ $nana$ egg count (P <0.001) in infected mice treated with tamoxifen when compared with the control ones (Tab. 3). By comparing results in mice treated with different schedules did not show significant difference (P>0.05) in egg count, buta highly significant reduction in percentage of viable eggs (P <0.001) in infected mice treated with tamoxifen when compared with the control ones (Tab. 4). Also, comparing the results in mice treated with the different drug schedules did not show significant difference (P>0.05) in viable eggs percentage.

Table 1: Tamoxifen efficacy on $H .nana$ worm burden in mice intestine

Mice group	Mice number	Worm burden per mice		Pairwise group significance				
				P value against other groups in order				
		Mean	SD	G 1	G2	G3	G4	G5
G1	20	2.83	2.29	-	<0.05*	<0.05 *	<0.05 *	<0.05 *
G2	20	1.75	1.0	<0.05 *	-	>0.05	>0.05	>0.05
G3	20	1.76	1.0	<0.05 *	>0.05	-	>0.05	>0.05
G4	20	1.74	1.0	<0.05 *	>0.05	>0.05	-	>0.05
G5	20	1.85	1.0	<0.05 *	>0.05	>0.05	>0.05	-

*Significant difference

Table 2: Tamoxifen efficacy on weight of $H. nana$ worm

Mice group	Mice number	Worm weight (mg)		Pairwise group significance				
				P against other groups in order				
		Mean	SD	G 1	G2	G3	G4	G5
G1	20	6.8	2.73	-	>0.05	>0.05	>0.05	<0.001**
G2	20	6.9	2.53	>0.05	-	>0.05	>0.05	<0.001**
G3	20	6.73	2.53	>0.05	>0.05	-	>0.05	<0.001**
G4	20	7.47	2.94	>0.05	>0.05	>0.05	-	<0.001**
G5	20	11.0	3.08	<0.001**	<0.001**	<0.001**	<0.001**	-

**Highly significant difference

Table 3: Tamoxifen efficacy on $H .nana$ egg count

Mice group	Mice number	Egg count per worm		Pairwise group significance				
				P against other groups in order				
		Mean	SD	G 1	G2	G3	G4	G5
G1	20	8245.71	2414.7	-	>0.05	>0.05	>0.05	<0.001**
G2	20	7760.97	2105.34	>0.05	-	>0.05	>0.05	<0.001**
G3	20	8506.12	2219.27	>0.05	>0.05	-	>0.05	<0.001**
G4	20	8686.84	2286.05	>0.05	>0.05	>0.05	-	<0.001**
G5	20	12129.82	2358.42	<0.001**	<0.001**	<0.001**	<0.001**	-

**highly significant difference

Table 4: Tamoxifen efficacy on $H .nana$ eggs viability

Mice group	Mice number	Viable egg percentage		Pairwise group significance				
				P against other groups in order				
		Mean	SD	G 1	G2	G3	G4	G5
G1	20	36.47	8.76	-	>0.05	>0.05	>0.05	<0.001**
G2	20	36.85	13.52	>0.05	-	>0.05	>0.05	<0.001**
G3	20	34.72	7.16	>0.05	>0.05	-	>0.05	<0.001**
G4	20	33.64	10.74	>0.05	>0.05	>0.05	-	<0.001**
G5	20	71.47	12.93	<0.001**	<0.001**	<0.001**	<0.001**	-

**Highly significant difference

Discussion

Hymenolepis nana is the commonest worm infected man worldwide (Smyth, 1994). In fection can persist for years by auto infection (Cho *et al,* 2009). Praziquantel is effective against *H. nana*. But, frequent drug reg-

imens were necessary to organize spreading infections especially in institutional and familial settings. Besides, the incomplete understanding of its mechanism of action delayed efforts to combat its resistance (Murray and Baron 2007; Doenhoff *et al*, 2008).

In the present study, tamoxifen on the worm burden showed no significant effect. Besides, there was unexplained significant increase in mice treated at two days post infection. The present results showed that tamoxifen did not affect the adult worm. This disagreed with Escobedo *et al.* (2013) who found that tamoxifen caused a strong cysticidal and antitaeniasic effect on *Taenia solium*. The present results also disagreed with Miguel *et al*. (2009) and Eissa *et al.* (2011) who reported that tamoxifen had good efficacy on *L. braziliensis and L. chagasi* infected animals, with marked reduction of parasite burden at lesion site or liver and spleen by about 99% & 95% to 98% respectively. The present study showed a highly significant reduction in *H. nana* weight in infected mice treated with tamoxifen as compared to control ones. This find agreed with Nicolao *et al*. (2014) who evaluated the tamoxifen effect against larval stages of *Echinococcus granulosus* in-vitro and in-vivo that showed reduction in cyst weight. In the present study, tamoxifen affected egg count and viability with significant reduction in both. This result coincided with the theory that tamoxifen is estrogen receptor modulator affecting parasitic fecundity more than the adult itself. More studies are needed to study drug effect and mechanism of action.

Conclusion

To the best of knowledge, none has evaluated tamoxifen® efficacy on *Hymenolepis nana*. Data showed that tamoxifen affected *H. nana* by reduction its egg count and viability, but without effect on the worm burden.

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Explanation of figure

Fig.1-A*: H. nana* egg obtained from crushed worms, **B:** *H. nana* egg in unstained stool smear (x10)**.C:** Chausov methodology showed viable *H.nana* egg with yellow onchosphere against pinkish background (x10).**D**: Dead *H. nana* egg : by using chausov methodology showed onchosphere with magenta color (x10)