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**COMPARITIVE STUDIES ON THE EFFECT
OF BITHIONOL, PRAZIQUANTEL AND
TRICLABENDAZOLE IN RABBIT'S FASCIOLIASIS
1. PARASITOLOGICAL STUDIES**

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ABSTRACT

Rabbits were infected with 30 metacercariae each. They were then divided into four batches each of two groups. One batch served as controls and the others were treated each with a drug. The first group from each batch was treated when worms were still immature, the other group was treated after maturity. The effect of the drugs was monitored by: (1) Stool examination for eggs and studying the percent of egg reduction. (2) Worm recovery at autopsy and calculating the percent of the drug efficacy. (3) Culturing some eggs and following their embryonation and hatching. Results pointed out that Triclabendazole was the best fasciolicide followed by Bithionol. Praziquantel had a negligible effect.

INTRODUCTION

Fascioliasis caused by the liver flukes, *Fasciola gigantica* or *Fasciola hepatica* is the cause of considerable loss in sheep and cattle production all over the world. It is becoming frequently diagnosed in the human host particularly among Egyptians. Several drugs have been proposed for the treatment of human fascioliasis mainly, Emetine hydrochloride (Ragab and Farag, 1978), Chloroquine (Hardman et al., 1970), Bithionol (Farag et al 1988), Praziquantel (Wahn and Mehalhorn, 1984), and Triclabendazole

(Wessely et al., 1987). Bithionol has a wide spread use in the treatment of various parasites in different hosts. It is effective against cestodes and trematodes particularly in paragonimiasis, in variable doses and schedules of drug administration. (Xiaosu et al., 1982, Maki & Yanagisawa, 1985). Praziquantel is considered the drug of choice in almost all trematode and cestode infections; as to its fasciolicidal activity, reports were contradictory (Andrews et al., 1983). The benzimidazole compound, Triclabendazole was reported to be the fasciolicidal drug of choice, equally affective against both mature and immature stages of the parasite (Boray et al., 1986, Rapic et al., 1988).

The present work aims at comparing the effect of the three drugs: Bithionol, Praziquantel and Triclabendazole on the viability of *Fasciola* worms (immature and mature), on oviposition and development of eggs.

MATERIAL AND METHODS

The present study was accomplished on forty healthy male rabbits aged one month. They were divided into the following groups: **Group Ia:** Five infected control rabbits sacrificed on the 65th day simultaneously with rabbits of groups (II,III & IV) a (immature stage of worms). (In the rabbits worms mature and begin oviposition on day 70-80) (El-Sayad, 1992). **Group Ib:** Five infected control rabbits sacrificed on the 85th day simultaneously with rabbits of groups (II,III & IV) b. **Group IIa,b:** Ten infected rabbits given Bithionol, 100 mg/kg body weight, orally, every other day, for five doses (Abou Basha et al., 1982). In group (IIa) administration of the drug started on the 55th day after infection i.e. before maturation of worms, and in group (IIb) administration of the drug started on the 75th day. i.e. after worm maturation. **Group IIIa,b:** Ten infected rabbits given Praziquantel, 300 mg/kg body weight, every day for five doses (Pearson and Guerrant, 1983). In group (IIIa) administration of the drug began on the 60th day of infection. In group (IIIb) drug administration began on the 80th day after infection. **Group IVa,b:** Ten infected rabbits given Triclabendazole, 45 mg/kg body weight daily for two consecutive days (Taira et al., 1983). In group (IVa) drug administration began on the 64th day after infection while in group (IVb) drug administration began on the 83rd day after infection.

Naturally infected *Lymnaea cailliaudi* were crushed and the released cercariae were allowed to encyst on *Trifolium alexandrina* leaves (Kendall, 1967). Rabbits one month old were starved for 24 hours, they then fed on the leaves of *T. alexandrina*. All rabbits were infected on the same day, with 30

Fasciola metacercariae each. (El-Sayad, 1992).

Bithionol was used as Bitin (a product of Tanabe Seiyaku Co., Ltd Japan, 200 mg tablets). Praziquantel (Bayer, 600 mg tablets) and Triclabendazole as Fasinex (Ciba-Geigy AG, 10% suspension). The tablets were homogeneously suspended in arabic gum. The suspensions were administered orally using a stomach tube (Kammerer et al., 1976).

Rabbits were sacrificed on the 65th day and 85th day post infection. The liver of every animal was removed and washed. The main bile duct and its branches were opened and any flukes present were extracted, washed, counted and examined for colour and tegumental integrity (Craig et al., 1986). Viability of flukes was determined on the basis of movement, immobile worms were tested by pricking with a sharp needle (Craig et al., 1984). Percentage efficacy of the drug was expressed as the percentage reduction in the number of worms after treatment according to the following formula:

$$\% \text{ efficacy} = \frac{\text{Average number of worms in control group} - \text{Average number of worms in treated group}}{\text{Average number of worms in control group}} \times 100$$

Effect of drugs on oviposition: Starting, on the day of the drug administration, the stools of the infected control group and the groups treated after worm maturation were examined daily until rabbits sacrifice. Stool examination was carried out after simple sedimentation and eggs were counted after the Kato-Katz method (Kremer & Molet, 1975). The percentage reduction in the number of eggs after treatments was calculated according to the following equation (Besvir et al., 1986).

$$\% \text{ reduction in number of eggs} = \frac{\text{Average number of eggs before treatment} - \text{Average number of eggs at end of treatment}}{\text{Average number of eggs before treatment}} \times 100$$

Effects of drugs on embryonation and hatching of eggs: The effect of the drugs on the development of fluke eggs was determined using a modification of the procedure of Coles and Briscoe (1978). Depending on findings of the above studies this assay was designed as follows: in case of Bithionol and Triclabendazole, two rabbits were infected and given reduced doses of drug, Praziquantel was administered to one rabbit, in the usual dose. The contents of the large intestine of sacrificed rabbits (infected and infected treated) were

allowed to sediment in water. The sediment was washed several times with tap water. Batches of approximately 30 eggs were picked up by a pipette under the dissecting microscope and placed in 50 ml tap water in clean beakers. Beakers were incubated in the dark at room temperature for 12 days. The development of miracidia, their activity, viability and the possibility of hatching were studied microscopically (Allam, 1992).

RESULTS

In case of the control group (Ia) the rabbits had a swollen abdomen which on opening contained a large amount of partly clotted blood. The enlarged liver was covered with sheets of fibrin and contained haemorrhagic areas. Numerous immature flukes (varying in length from 0.4-1.1 cm with a mean of 0.99 cm) were found adhering to the surface of the liver and free in the peritoneal cavity. Similar observations were found in the treated infected groups (IIa & IIIa) but with less pathology and fewer worms. In the Triclabendazole treated group (IVa) the liver was fibrosed and the bile ducts were dilated and thickened. It was (table,1) observed that the percent reduction of worms in group IIa was 60%, it was 46.6% in group IIIa; in group IVa the highest percentage reduction (93.3%) was observed. All worms collected after Bithionol and Triclabendazole were whitish in colour and sluggish in movement. No worm (table, 2) were detected in the Bithionol treated group II_b (efficacy 100%). In the Praziquantel treated group, all rabbits harboured worms but the number of worms was reduced by 20% as compared to controls. Only one rabbit harboured two worms in the group treated with Triclabendazole. The worms were whitish, with sluggish movement and their tegument showed necrotic areas. The percent efficacy of drug was 95%. In the control group (table, 3) the average number of eggs increased gradually until sacrificed. In group II_b, two rabbits stopped egg-excretion after the 3rd dose of bithionol, the other rabbits stopped egg excretion after the 4th dose. In group III_b, the faecal egg counts were gradually reduced until sacrifice. The percentage reduction was 27.7%. In group IV_b, Triclabendazole reduced the faecal egg count to zero in one rabbit after the 1st dose, and in all the others after the 2nd dose.

All eggs obtained after treatment (Triclabendazole and Bithionol) were disorted and broken (Figs. 1,2). After cultivation eggs from the control and praziquantel groups developed mature miracidia that hatched starting on day 12. Eggs of the treated groups developed through morula and blastula stages only.

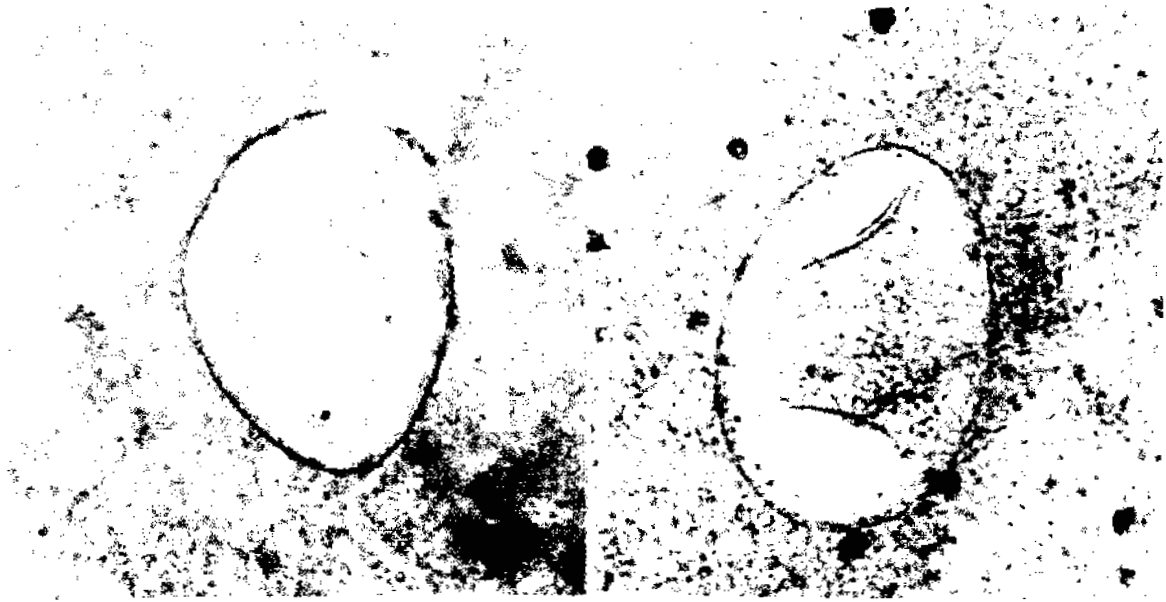


Fig. (1): Distorted and broken eggs after exposure to Triclabendazole and Bithionol. X 100.

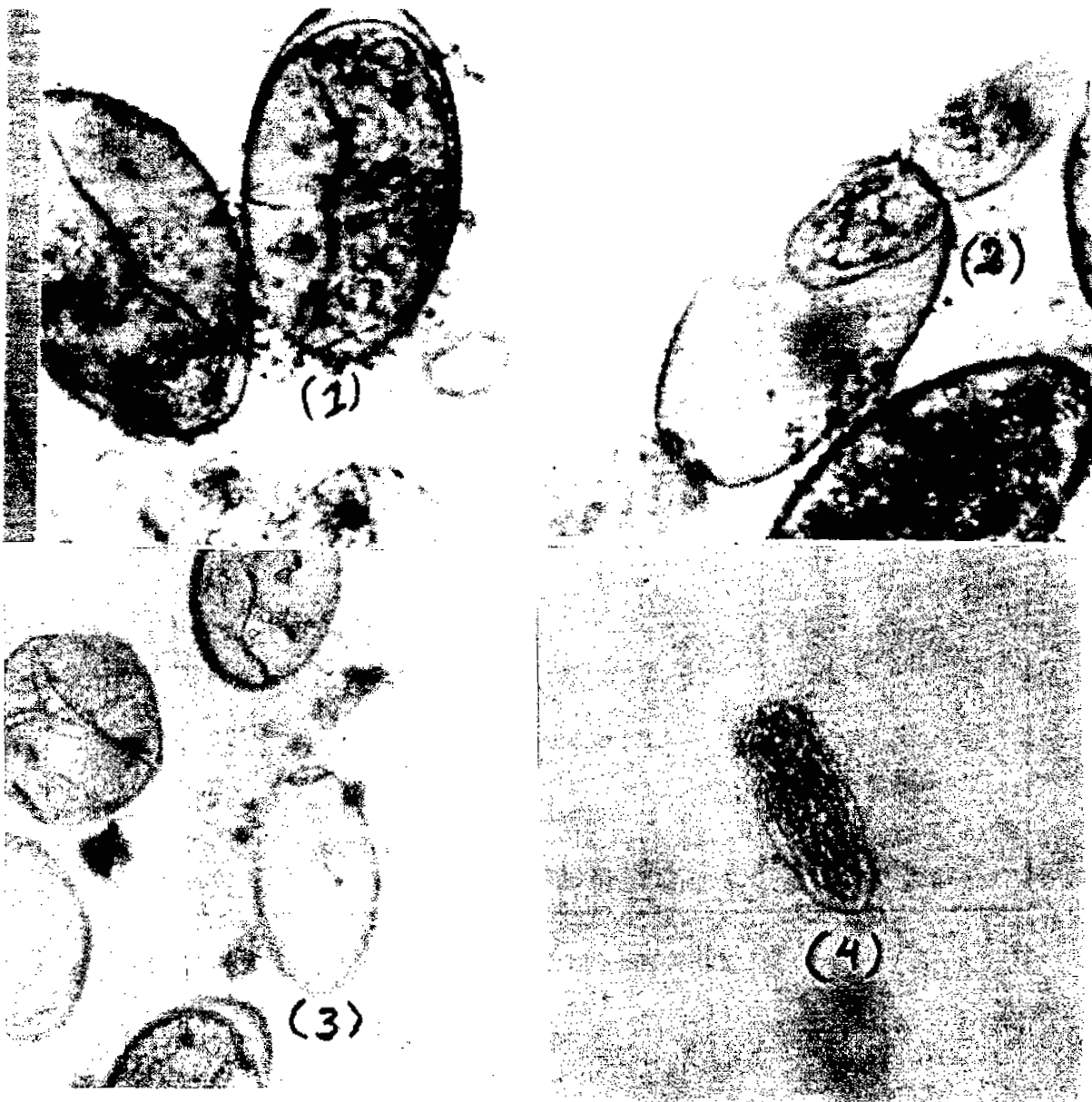


Table 1: Efficacy of the drugs on immature* *Fasciola* worms in rabbits.

Group	Immature worms	% efficacy
Group Ia (control)	range : 12-18 mean : 15	-
Group IIa (Bithionol)	range : 3 - 8 mean : 6	60
Group IIIa (Praziquantel)	range : 5 - 11 mean : 8	46.6
Group IVa (Triclabendazol)	range : 0 - 2 mean : 1	93.3

* Rabbits were sacrificed after 65 days from infection.

Table 2: Efficacy of the drugs on mature worms* in rabbits.

Group	mature worms	% efficacy
Group Ib (control)	range : 7 - 12 mean : 10	-
Group IIb (Bithionol)	range : 0 - 0 mean : 0	100
Group IIIb (Praziquantel)	range : 4 - 12 mean : 8	20
Group IVb (Triclabendazol)	range : 0 - 2 mean : 0.5	95

* Rabbits were sacrificed after 85 days from infection.

Table 3: Quantitative coprologic examination of control rabbits and rabbits treated with the three drugs.

Group **	Number of eggs per gram of faeces in successive stool examinations						% reduction •
	0*	1 st	2 nd	3 ^{er}	4 th	5 th	
Group Ib (control)							
range	1-139	1-153	6-160	9-170	10-179	11-180	
means	56	60	63	69	72	70	
Group IIb (Bithionol)							
range	1-150	1-110	1-90	0-60	0	0	100
means	60	44	32	13	0	0	
Group IIIb (Praziquantel)							
range	1-30	1-100	1-113	1-120	1-115	3-110	27.7
means	72	61	58	60	63	53	
Group IVb (Triclabendazol)							
range	1-133	0-35	0	0	0	0	100
means	58	23	0	0	0	0	

** : Every group comprised five rabbits.

* : Day : before treatment.

• : Percent redction was calculated relative to the count of the same group on day zero (0).

DISCUSSION

The most important criteria of a good anthelmintic drug for the treatment of fascioliasis are high efficacy against both the immature and adult flukes, safety to the target animal and to the user, absence of persistent chemical residues in the host tissues and ease of application at an economic price. The present work evaluated the efficacy of Bithionol, Praziquantel and Triclabendazole, as fasciolicidal drugs. Considering the immature worms, it was observed that Triclabendazole was highly effective, Bithionol killed a good proportion of flukes, while Praziquantel was less effective. These results which confirm the high efficacy of Triclabendazole against immature flukes, might ensure successful treatment of acute fascioliasis. Excellent results have been achieved with the same drug in treatment of acute fascioliasis in sheep, cattle, goats and other experimental animals with 90-100% efficiency (Wolff et al., 1983, Stevenson et al., 1986, Bogan et al., 1988). Bithionol followed Triclabendazole on its effect on immature worms. Bithionol was first used for fascioliasis by Yoshida et al. (1962). In 1966, Dawes described the interference of Bithionol with reproductive function of *Fasciola hepatica* experimentally in rats and he reported that it killed all immature worms in the liver. The present results of the poor action of Praziquantel on the immature worms agree with many reports (Knobloch et al., 1985 & Farid et al., 1986). However, Praziquantel was reported successful in treatment of acute *Fasciola* in humans by Schiappacasse et al. (1985).

The surviving immature worms after treatment with Triclabendazole and Bithionol might develop to adult flukes. However, the whitish colour of the worms and their sluggish movement together with the severely injured tegument might be considered as signs of irreversible damage. This tegumental injury suggests that uptake of the drugs (Triclabendazole and Bithionol) was facilitated by transtegumentary absorption. Fair-Weather et al. (1984 and James et al. (1987) reported that a 24 hrs. exposure of *Fasciola hepatica* worms to Triclabendazole *in vitro*, resulted in strong inhibition of the parasites motility. As to the efficacy on the mature worms, no worms were detected in the Bithionol treated group. Similar findings were reported by Abou Basha et al. (1983) and Jenkins et al. (1987). Only 5% of worms survived in the group treated with Triclabendazole. They were sluggish in movement and their tegument was necrosed. These results agree with those of Stevenson et al. (1983) in sheep. Craig and Huey (1984) reported that

Triclabendazole affected the tegument of *Fasciola hepatica*. The majority of worms were alive in the group given Praziquantel. They were normal in colour and movement. In spite of the presence of few alive worms after Triclabendazole, eggs were not recovered after the second day of the treatment denoting a direct effect of the drug on the process of oviposition. The cessation of oviposition after Triclabendazole and Bithionol agrees with the results reported by Stansfield et al. (1987) in sheep and cattle. Although immature and mature worms were expected to co-exist in their animals, eggs disappeared after one week of treatment in heavy infections. Stoppage of egg excretion after the first dose in two rabbits means that a single dose of Triclabendazole was sufficient to destroy all the liver flukes in them. These findings agree with the results of Lecuyer et al. (1985) in cattle. As to Praziquantel, Schiappacasse et al. (1985) reported that its use in chronic human infection, did not stop the excretion of eggs.

Eggs from the control and Praziquantel groups developed active mobile and hatchable miracidia in 12 days. All the eggs collected after treatment with a single dose of Triclabendazole or three doses of Bithionol were distorted and broken in comparison with control eggs. They developed to the morula stage only. These observations agree with those of Wessely et al. (1988), Abou Basha et al. (1983), Coles and Briscoe (1978). Fetterer (1986) found that Triclabendazole *in vitro* was effective in preventing embryonation of fluke eggs.

In conclusion, Triclabendazole is considered the most effective fasciolicidal drug affecting both immature and mature worms. Bithionol affected essentially the adult worms. Both drugs influenced worm fecundity, and interfered with eggs development. Praziquantel has a negligible effect in treatment of fascioliasis.

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