

Drug resistance and recent therapeutic measures in controlling of fascioliasis

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Abstract: Fascioliasis is a widely distributed disease affecting herbivorous animals. As a result of drug resistance a mixture of two antifasciola drugs (Triclabendazole and Superivomec) was used in trial to overcome this drug resistance. Twenty eight newly weaned white Boskat rabbit aging 1.5 month were divided into 7 groups, six of them were experimentally infected with metacercaria of *Fasciola gigantica* and one kept as -ve control group. Faecal egg count during the clinical course of the disease, counting the worm and its morphological studies and lesion score after postmortem examination were the parameters used to evaluate the effect of different drug mixtures. It had been concluded that the mixture of triclabendazole and superivomec was the mixture of choice.

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Key words: Fascioliasis, Metacercaria, Triclabendazole, Rabbit and Superivomec.

1. Introduction:

Fascioliasis or liver flukes is a disease affecting herbivorous animal and caused by *Fasciola hepatica* and *Fasciola gigantica*. It has a worldwide distribution in a large variety of grass-grazing animals as sheep, goats, cattle, buffaloes, horses and rabbits. In Egypt, donkeys and camels as well, are hosts for *Fasciola gigantica*. Fascioliasis may occasionally affect man (Haseeb et al., 2002; Sanad and Al-Megrin, 2005).

Fascioliasis gives rise to important economic losses such as great expenses with anthelmintics, liver condemnation, production loss due to mortality, lower production of meat, milk and wool; reduced weight gain, and impaired fertility (Parr and Gray, 2000; Marques and Scroferneker, 2003).

Two clinical stages are recognized in fascioliasis. An acute stage coincides with the larval migration and worm maturation in the hepatic tissue, and a chronic stage coincides with the persistence of *Fasciola* worms in the bile ducts (Haseeb et al., 2002).

Fascioliasis is controlled by a combination of anthelmintic therapy and management measures. These methods are costly and may lead not only to anthelmintic resistance, but also to undesirable residues in food or the environment (Pérez et al., 2002). Consequently, the control of fascioliasis is one of important aims in our work.

These studies were designed to select an effective treatment against different stages of *Fasciola gigantica* were including immature worm, adult worm, and eggs using mixture of drugs to avoid development of drug resistance.

2. Materials and methods

I. Materials

Experimental animals:

A total number of 28 newly weaned white Boskat rabbit aging 1.5 months (obtained from faculty of agriculture farm, Assiut University) were divided into 7 groups (4 rabbits each).

Group (1): (Control +ve group): Rabbits were infected with 30 metacercaria/ rabbit orally by stomach tube. Rabbit were slaughtered 11 weeks post infection (PI).

Group (2): Flubendazole treated group: Rabbits were infected with 30 metacercaria/ rabbit orally and treated orally with flubendazole at 6 weeks (PI) (100 mg/kg b.wt).

Group (3): Flubendazole and superivomec treated group: Rabbits were infected with 30 metacercaria/ rabbit and treated orally with flubendazole (50 mg/kg b.wt.) plus superivomec (ivermectin 100 µg and clorsulon 1 mg/ kg b.wt).

Group (4): Superivomec treated group: Rabbits were infected with 30 metacercaria/ rabbit orally and treated S/C with superivomec at 6 weeks (PI) (ivermectin 200 µg and clorsulon 2 mg /kg b.wt).

Group (5): Triclabendazole treated group: Rabbits were infected with 30 metacercaria/ rabbit orally and treated orally with triclabendazole (10 mg/kg b.wt).

Group (6): Triclabendazole and superivomec treated group: Rabbits were infected with 30 metacercaria/ rabbit orally and treated orally with triclabendazole (5 mg/kg b.wt) plus superivomec (ivermectin 100 µg and clorsulon 1 mg per kg b.wt).

Group (7): (Control -ve group): Rabbits were slaughtered 11 weeks (PI).

II. Methods

A) Parasitological examination:

Faecal samples:

A piece of faecal sample was dispersed and thoroughly mixed with about 10 parts by volume of saline solution. Examination of faecal samples was carried out according to Melvin and Brooke, (1982)

Fluke samples:

Samples of flukes were taken to study changes in morphology by carmine stain and scanning electron microscopy.

Methods used to study the effect of the drug on the worm:

- Egg count at different periods of infection during the experiment.
- The number of worms were counted and measuring the length of the flukes.
- The morphology of adult flukes was studied by carmine stain and Scanning Electron Microscope (S.E.M).

B) Pathological examination:

Tissue samples: Samples from liver were taken for histopathological examination.

Gross pathology: Rabbits were dissected for the presence/absence of any gross lesions in the liver.

Histopathological examination: Routine review histopathological picture were carried on formalin fixed paraffin embedded sections from the liver and stained by H & E (Bancroft et al., 1994).

C) Statistical analysis:

The data were statistically analyzed using general linear model (G. L. M) procedure of SAS (1996). The significance differences between treatment means were tested by Duncan multiple range test (Steel and Torrie, 1982).

3. Results

The clinical signs were observed 4 weeks post infection with *Fasciola gigantica*. The signs in the form of dullness, rough hair coat. At 9 weeks (PI), loss of hair coat, anorexia, paleness of the mucous membranes and emaciation were observed

especially in control +ve group and flubendazole treated group when compared with normal rabbits (Figs. 1A,B).

Faecal examination revealed the presence of eggs of *Fasciola gigantica* as early as 40 days (PI) in all infected groups. The eggs appeared oval, operculated, delicate light yellow in color (Fig. 1C).

Statistical analysis of the egg counts from 7 to 11 weeks (PI) revealed significant increase in eggs in group 1 and 2 when compared with other groups. In group 3 and 4, highly significant decrease of egg count was observed when compared with group 1 and 2. Statistical investigation of egg count in the group of rabbits treated with triclabendazole either alone or in combination with superivomec revealed highly significant decrease in the egg count during the 7th week (PI) when compared with group 1. Complete cessation of the eggs was observed in both groups during the 8th weeks (PI) (Table 1).

Gross examination of the liver in group 1,2 and 3 showed enlargement, congestion and multiple necrotic foci on the liver surface. In addition, multiple migratory tracts were observed. Perihepatitis was observed. Normalization of the liver with no migratory tracts and liver flukes were seen in group 4, 5 and 6.

Histopathological examination of the liver in group 1 and 2 showed congestion, thrombosis, vasculitis and perivascularitis of hepatic vasculatures. Bile duct proliferation, hyperplasia, cholangitis and pericholangitis were observed. The bile duct changes in group 2 were more severe than group 1. The histopathological changes of the hepatic cells were ranged from degenerative changes (vacuolar degeneration and glycogen infiltration), necrosis (coagulative and lytic) and hepatitis either focal or diffuse (Figs. 1D-G).

The histopathology of the liver in group 3 showed the same pathological changes as group 2 but less severe. The histopathological changes of liver in group 4 were quite similar to those observed in the previous groups. While triclabendazole treated group revealed normal appearance of the different hepatic structures except a minor changes expressed by congestion and thrombosis of some hepatic vasculatures. The bile ducts showed bile duct proliferation and hyperplasia, cholangitis and pericholangitis. The hepatic parenchyma showed focal granular degeneration. In superivomec + triclabendazole treated group, most of the hepatic tissues appeared more or less normal except minor pathological alterations.

As shown in Table (2) the lesion scores of histopathological results of group 1 revealed significant increase in the bile duct lesions, portal lesions, migratory tracts and focal liver lesions in

group 1 when compared with other groups except group 2. In group 3, the lesion score demonstrated that the bile duct lesion and portal lesion were significantly decreased when compared with group 1 and 2. In group 4, minimal lesion scores were observed. In group 5, the lesion scores revealed absence of migratory tracts and diffuse liver lesion. The lesion scores of bile duct and portal lesions were significantly decreased. In group 6, lesion scores obtained after treatment with the mixture revealed significant decrease in all parameter when compared with other groups.

Careful counting of the liver flukes and statistical analysis revealed significant increased of flukes numbers in group 1 when compared with other groups (Table. 3). *Fasciola gigantica* stained with carmine revealed maturity of the worm while in flubendazole treated group it was decreased than control +ve group. The flukes stained with carmine showed that the mixture of flubendazole and superivomec had a prominent effect on the maturation of the fluke especially those of the sexual organs. Significant decrease in the number and the length of flukes in superivomec treated group when

compared with other groups was seen. Also, it has a prominent effect on maturation of sexual organs and to lesser extend on the digestive organs (Table. 3) & (Figs.2A-D).

Scanning electron microscopy of the liver flukes in control +ve group revealed rough tegumental surface of the adult *Fasciola gigantica*. The surface covered with posterior directed spines and transverse folds. The anterior ventral portion showed oral and ventral suckers and gonopore between them (Fig. 3). In flubendazole treated group minor changes in the form of shrunken oral sucker and dilated ventral sucker. The spines surrounded the oral sucker and mid-lateral aspect of the ventral surface of the flukes showed shortening of the scale-like spines. The ventral surface of the flukes showed large area of sloughed tegument which filled with debris while the dorsal surface showed disruption in the form of furrowing of the tegumental surface (Fig. 4). Scanning electron microscopy to the liver flukes of flubendazole + superivomec treated group showed more prominent changes especially in the anterior half of the fluke (Figs. 5).

Table (1): Mean values of numbers of eggs at 7weeks, 8 weeks, 9 weeks, 10 weeks and 11 weeks post infection in different groups.

	7 wks.	8 wks.	9 wks.	10 wks.	11 wks.	Average
Control + ve group	14.00±1.75 ^a	20.57±1.91 ^a	53.71±6.44 ^a	101.43±5.24 ^a	194.14±15.48 ^a	76.77±11.86 ^a
Flubendazole	13.71±1.25 ^a	17.57±3.25 ^{ab}	42.71±4.97 ^a	61.86±5.26 ^b	111.14±9.05 ^b	49.40±6.50 ^a
Flu + Super	8.29±0.75 ^b	10.57±2.44 ^b	22.00±2.90 ^b	10.67±2.23 ^c	17.00±2.65 ^c	13.79±1.32 ^b
Superivomec	8.14±1.10 ^b	13.00±1.09 ^{ab}	28.86±3.18 ^b	8.00±1.14 ^c	-	15.00±1.96 ^b
Triclabendazole	5.00±1.33 ^b	-	-	-	-	5.00±1.33 ^b
Super + Tric	5.57±1.07 ^b	-	-	-	-	5.57±1.07 ^b

^{a, b and c} means in the same column differ at (p<0.05)

Table (2): Mean values of lesion scores in different groups.

	Control + ve	Flubendazole	Flub + Super	Superivomec	Triclabenda-zole	Sup + Tric
Bile duct lesions	11.80±1.03 ^b	13.80±0.92 ^a	9.00±0.77 ^c	7.60±0.58 ^c	4.90±0.48 ^d	2.10 ±0.31 ^e
Portal lesions	11.90±1.05 ^a	11.90±1.03 ^a	9.50±0.79 ^b	7.40±0.45 ^{bc}	6.30±0.93 ^c	2.40±0.45 ^d
Migratory tracts	6.70±1.00 ^a	5.10±0.85 ^{ab}	4.00±0.71 ^{ab}	3.00±0.55 ^b	-	-
Focal liver lesions	9.33 ±2.19 ^a	4.67± 0.50 ^{bc}	3.78±1.10 ^{bc}	7.50±1.43 ^{ab}	2.00±0.00 ^c	1.75±0.25 ^c
Diffuse liver lesions	6.00±1.08 ^a	5.70±1.07 ^a	4.25±0.98 ^{ab}	1.33±0.33 ^c	-	1.33±0.33 ^c

^{a, b, c and d} means in the same column differ at (p<0.05)

Table (3): Mean values of liver flukes numbers in different groups.

Groups	Numbers of liver flukes
Control +ve group	13.90±1.38 ^a
Flubendazole	8.60±1.53 ^b
Flub + Superivomec	5.60±0.93 ^{bc}
Superivomec	-
Triclabendazole	-
Super + Tricla	-

Table (4): Mean values of the length of liver flukes in different groups.

Groups	Length of liver flukes
Control +ve group	4.35±0.26 ^a
Flubendazole	3.73±0.16 ^b
Flub + Superivomec	2.33±0.18 ^{bc}
Superivomec	-

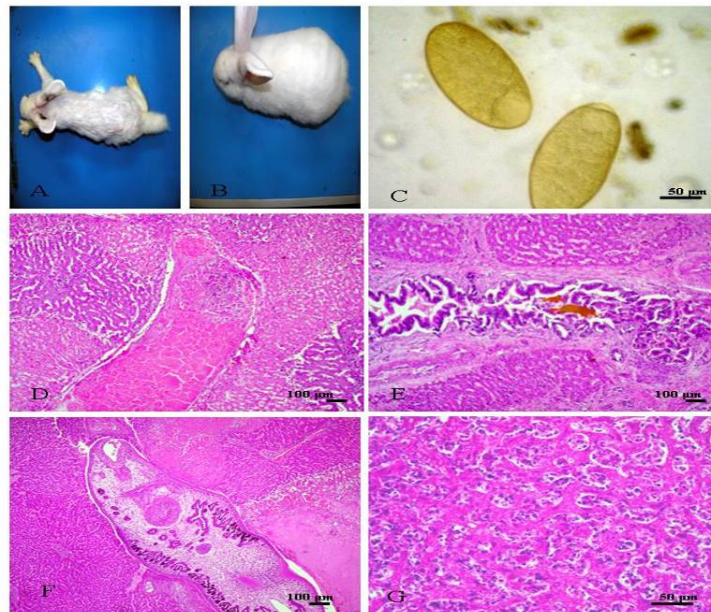
**Fig. 1**

Fig. (1): A. Rabbit showing emaciation, loss of hair coat. Control +ve group. B. Normal rabbit. C. Eggs of *Fasciola gigantica*. Faecal smear. X 40. D. Mixed thrombus in a large blood vessels. E. Cholangitis of bile duct with bile thrombi (arrow). F. Liver showing migratory tract containing mature fluke. G. Liver showing hepatitis demonstrated by necrosed hepatocytes and impaction of sinusoids with dead eosinophils. Control +ve group. H&E.

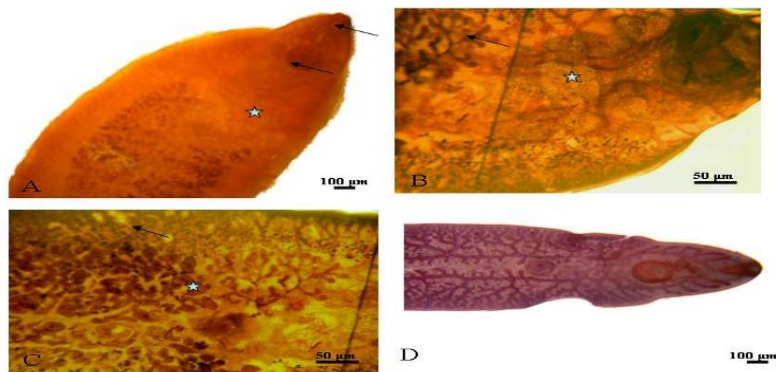
**Fig. 2**

Fig. (2): A. Anterior portion of *Fasciola gigantica* showing oral and ventral suckers (arrows) well developed uterus (star). B. Anterior portion of *Fasciola gigantica* showing well developed uterus (star) and well developed intestinal ceca (arrow). C. Posterior portion of *Fasciola gigantica* showing well developed testis (star) and vitelline glands (arrow). Control +ve group. Carmine stain X10 D. Anterior portion of *Fasciola gigantica* showing immature sexual organs and stunted growth. . Carmine stain X10.

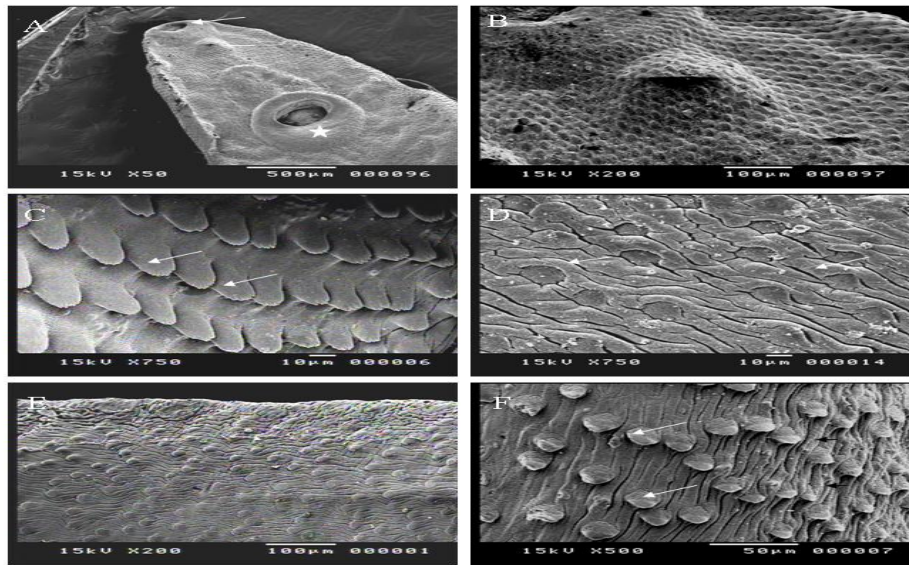
**Fig. 3**

Fig. (3): Scanning electron micrograph (SEMs) of *Fasciola gigantica*. Control +ve group.

A. Oral (arrow), ventral (star) suckers and gonopore (line) of the liver fluke. X 50.

B. The oral sucker and gonopore surrounding with spines. X 200

C. The spines posteriorly directed with serrated margin (arrows). X 750.

D. The posterior ventral surface showing transverse folds (arrows) and grooves in between with less and depressed spines. X 750.

E. The dorsal surface showing tegument with spines. X 200.

F. Higher power showing less number of spines (arrows) and transverse folds (arrowheads) X 500.

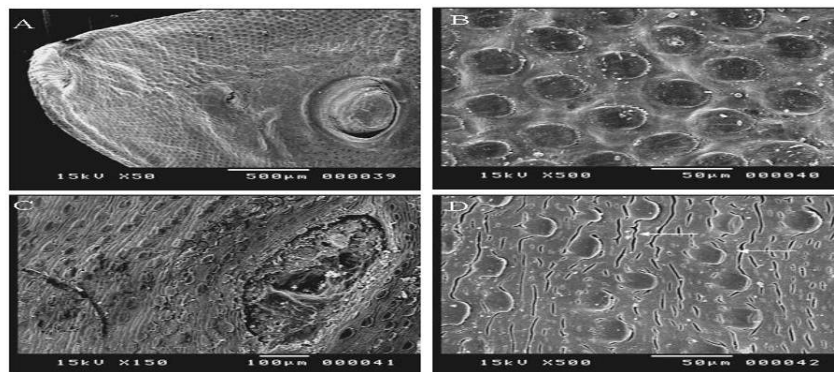
**Fig. 4**

Fig. (4): Scanning electron micrograph (SEMs) of *Fasciola gigantica*. Flubendazole treated group. A.

Anterior portion showing shrunken oral sucker. X 50. B. The spines of the anterior portion showing

shortening and compression. X 500. C. Ventral surface posterior to the ventral sucker showing large

area of tegumental sloughing. X 150. D. Dorsal surface showing furrowing of the tegument (arrows). X

500.

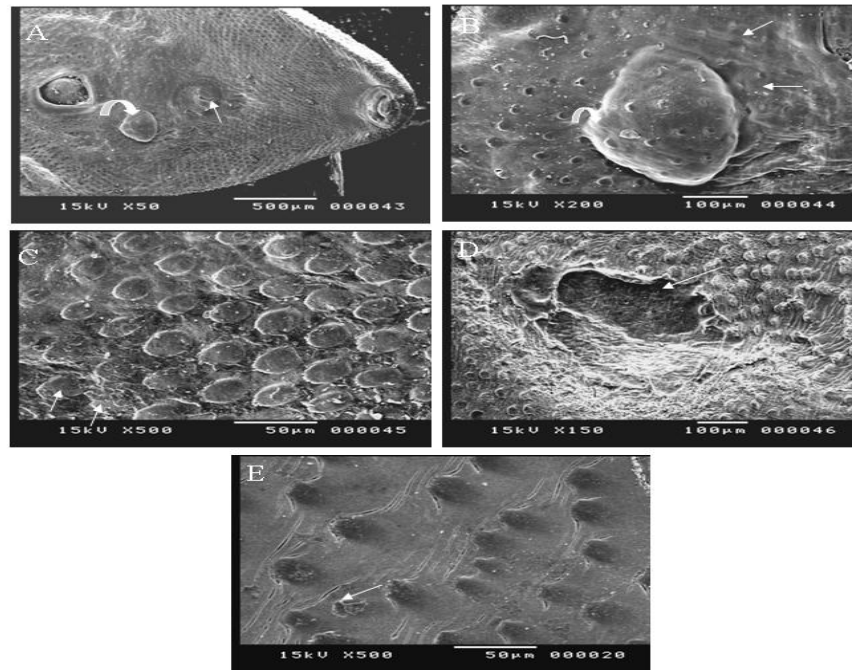
**Fig. 5**

Fig. (5): Scanning electron micrograph (SEMs) of *Fasciola gigantica*. Flubendazole + superivomec treated group. A. Anterior portion showing sloughing to the gonopore between oral and ventral sucker (arrow) and swelling (curved arrow). X 50. B. Higher power showing large swelling (curved arrow) and swelling of adjacent area (arrows) with no spines around it. X 500. C. The spines surrounded the oral sucker and mid-lateral aspect showing destruction (arrows). X 500. D. The middle portion of the ventral surface showing sloughing of the tegument (arrow). X 150. E. Dorsal surface showing furrowing of the tegument with destructed spines (arrow). X 500.

4. Discussion:

Fasciola hepatica remains one of the single most important helminth parasites of many countries in the world. Its tropical counterpart is *Fasciola gigantica*. There are effective strategies for the control of fascioliasis, based largely on drug (fasciolicide) use but allied to epidemiological data (Boray, 1997 and Malone, 1997). While most of the experimental work has been conducted with *Fasciola hepatica*, the problems associated with fascioliasis and its control is similar in large parts of the world where only *Fasciola gigantica* is present. All drugs effective against one of the species are equally effective against the other (Boray, 1986).

In the present study, the histopathological changes in the form of vascular changes, bile duct lesions, portal lesions, migratory tracts and hepatocellular changes were reported by many authors (Farha, 1993; Yoshida et al., 1996; Pérez et al., 1999 and Adedokun and Fagbemi, 2001).

The eggs of *Fasciola gigantica* were appeared in the faeces at 40 days post infection as oval, operculated and delicate yellow in color. This finding was in agreement with the studies of many authors (Haseeb et al., 2002; Lotfy and Hillyer, 2003). Statistical analysis of the egg counts from 7 to 11 weeks (PI) revealed significant increase in eggs in control +ve group when compared with other groups.

The egg counts increased gradually from 7 to 11 weeks. These results are in harmony with the studies of Sherif et al., (2001) who reported that the egg counts increased gradually from 10 weeks to 16 weeks (PI). Also, Sewell, (1966) mentioned that egg counts of *Fasciola* increased gradually up to a peak 108 days (PI).

In the present work, counting of the liver flukes and statistical analysis revealed significant increase of fluke's numbers in control +ve group (13.90 ± 1.38) when compared with other groups. These results were obtained by Schillhorn Van Veen et al., (1980) and Adedokun and Fagbemi, (2001) who reported that the pathological findings were related to the number of the flukes recovered at post mortem.

Fasciola gigantica stained with carmine revealed maturity of the worm with eggs in the uterus and well developed intestinal ceca and testes at 9 and 11 weeks post infection. Similarly, Olaechea et al., (1991) found that there was individual variation in numbers of flukes per animal and the mean percentage of mature worm with eggs in uterus at 10 weeks post infection. Kolodziejczyk et al., (2006) said that the juvenile form of the flukes in the liver tissue and mature forms in the bile ducts of rats were seen at 7 weeks (PI).

Scanning electron microscopy to the liver flukes in control +ve group revealed rough tegumental surface of the adult *Fasciola gigantica*. The surface covered with posterior directed spines and transverse folds. These results obtained by many investigators (Meany et al., 2006 and Mahmoud and Hegazi, 2007). This can be explained by the increasing efficacy of absorption and exchange of materials by the tegument, such features are also observed in other trematodes (Jinxin and Yixun, 1981). The anterior and middle regions tend to have more developed spines or folds than the posterior region. These results were agreed with the studies of Mahmoud and Hegazi, (2007) who suggested different capabilities of absorption in various regions of tegument. The presence of numerous spines, covering the body surface may facilitate movement of *Fasciola* in the bile ducts of the liver. In the flubendazole treated group, minor changes of the flukes in the form of shrunken oral sucker and dilated ventral sucker, shortening of the scale-like spines and sloughed tegument which filled with debris. These findings coincide with those described by Omran et al., (2006) who studied the effect of flubendazole on *Schistosoma mansoni*.

In superivomec treated group, there was significant decrease in the number and the length of flukes when compared with other groups. Also, it has a prominent effect on maturation of sexual organs

and to lesser extend on the digestive organs. These results were in agreement with the findings of Vera-Montenegro et al., (2003) who reported that clorsulon causing stunted growth of the fluke and egg production was markedly decreased and the findings of Fetterer et al., (1985) and Sundlof et al., (1991) who reported that clorsulon was highly effective in reducing worm burden.

It had been concluded that experimental fascioliasis in rabbits resulted in severe deleterious effect on the liver tissue. The flubendazole was not effective against fascioliasis. The uses of triclabendazole in the treatment of fascioliasis eliminate the gross lesions and liver fluke and minimize the histopathological alterations. The results produced by the mixture of triclabendazole and superivomec may be due to its effectiveness in overcoming the eggs as early as one week of treatment and its effectiveness against the fluke. So we advise using this mixture in the treatment of *Fasciola gigantica* in the field (mixture of choice).

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