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Original Research

Evaluate therapeutic efficacy of triclabendazole and mirazid in Guinea Pigs infected with *Fasciola gigantica*

Mohamed E. El-Boshy,[1,2] Husien S. Husien[2]. Osama A. Abdulla[3], Fatma M. Abdelhamid[4], Engy F. Risha[4]

ABSTRACT

Aim and Background: Fascioliasis is the most important parasitic diseases that affect the human and animal all over the world. Triclabendazole (TCBZ), and Mirazid is the most common trematodicidal drug used in Egypt The present study, aimed to evaluate the therapeutic efficacy of the TCBZ and Mirazid in Guinea pigs experimentally infected with *Fasciola gigantica* **Methods:** Thirty two, Guinea pig (1-2 month old) was allocated into four equal groups to study the efficacy of treatment of facioliasis with triclabendazole (TCBZ) and mirazid. Group (Gp.1) was the control, GPs (2 -4) were orally inoculated with 20 *Fasciola gigantica* metacercariae (FGM) for each as a single dose by using stomach tube. Gps (3&4) were treated orally, with TCBZ, as a single dose (36 mg/ Kg Bw) and mirazid 200 mg/ Kg Bw for six successive days respectively. The drugs were administered in the 8th week post-infection to evaluate their efficacy against adult stages of *Fasciola gigantica*. Blood samples were collected at the end of the 1st and 2nd week post treatment for hematological and serum biochemical examination. **Results:** TCBZ treated groups (GP 3) showed macrocytic hypochromic anemia, which disappeared at the end of the 2nd week post treatment. Heterophilia, esinophilia and lymphopenia were encountered in non treated group (GP 2) and mirazid treated group (GP 4). The liver transaminase (ALT, AST), gamma glytamyl transferase (GGT) and alkaline phosphatase (ALP) as well as total bilirubin, urea and creatinine, were elevated while serum albumin was decreased in GP (2) and returned to the normal value in GP (3) and GP (4) after two weeks post-treatment. **Conclusion:** It could be concluded that; the triclabendazole is more effective than mirazid in the treatment of *Fasciola gigantica* infection. Further research should be done for more precise knowledge about the efficacy of mirazid as a fasciolicidal drug.

KEY WORDS: Triclabendazole, Mirazid, Hematology, Hepatorenal Markers, Guinea Pigs, *Fasciola gigantica*

[1]Dept. of Laboratory Medicine, Faculty of Applied Medical Sciences, Umm al Qura University, Makkah, Saudi Arabia
[2]Dept. of Pathology, Fac. Vet. Med., Mansoura University
[3]Dept. of Clinical Pathology, Fac. Vet. Med., Suez Canal University
[4]Dept. of Clinical Pathology, Fac. Vet. Med., Mansoura University

Address for correspondence: Mohamed El-Boshy, Dept. of Clinical Pathology, Fac. Vet. Med., Mansoura University, dr_elboshy@yahoo.com

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INTRODUCTION

Distomiasis is among the most important parasitic diseases that affect the domesticated and wild animal besides man all over the world. The disease causes significant economic losses due to the high financial losses through the great expenses on therapy, and to liver condemnation. Moreover the production is hindered due to the mortalities and the lower production of milk, meat and wool in addition to the decrease body gain and impaired fertility [1].

Triclabendazole (TCBZ), benzimidazole antithelmintic drug, unlike other broad spectrum benzimidazole drugs it shows only marginal activity against liver fluke, its efficacy appears to be restricted to both adult and juvenile flukes of *F .hepatica*, *F. gigantica* and *F. magna.* TCBZ, produces a gradual suppression in the activity of the flukes unlike other benzimidazole which makes prolonged stimulation before the movement is finally declined [2].

Mirazid is a new herbal antithelmintic drug formed from myrrh extract that is derived from *Commiphora molmol (C. molmol)* tree, family burseraceae. It's one of the oldest known medicinal plants used by ancient Egyptians for medical purposes as well as in mummification. Recently it had been licensed for medical use in Egypt as a trematodicidal drug with high efficacy and safety. As it is well tolerated with a wide margin of safety for the liver, kidneys, hemopoietic system and chromosomes. It is non-mutagenic besides it can be repeated for a long period [3].

This work aimed to compare the efficacy of TCBZ and mirazid, in the treatment of the *Fasciola gigantica* in Guinea pigs.

METHODS

Experimental Animals

Thirty two Guinea pigs, 1-2 month old of both sexes were

obtained from Helwan farm of Laboratory Animals (Ministry of Public Health).The animals were kept in galvanized zinc plate cages under strict hygienic conditions. The animals were ensured freedom from parasitic infection, through a regular parasitological examination. The daily requirement of ascorbic acid (50 mg/liter of drinking water) was supplied throughout the experiment according to [4].

The Metacercariae

Fasciola gigantic a metacercariae were obtained from the Parasitology Department of Theodor Bilharz, Research Insitiute, Imbaba, Egypt and was examined for the viability. The infective dose for each animal was prepared by counting metacercariae on the polyethylene strips.

Drugs

Mirazid: It was obtained as a soft gelatin capsule containing an oleo-resin purified *Commiphora molmol* extract as 300 mg (Pharco Pharmaceuticals, Alexandria, Egypt). The active ingredients of the different fractions extract of *Commiphora myrrha* are sesquiterpenes, diterpenes, diterpenic acids, sandaracopimaric acid and abietic acid [5]

Triclabendazole: Fasinex (10%) a white color suspension prepared for oral uses (Novartis.Co. Switzerland).

Experimental Design

The experiment was conducted on 32 Guinea pigs, 1-2 month age, after acclimatization for 15 days in cages; the animals were divided into four equal groups. GP (1) was the control. Each animal in GPs (2-4) was orally inoculated with 20 FGM as a single dose by using stomach tube. GP (2) was experimental control, infected and non-treated. GP (3) was orally treated with TCBZ at a dose 36 mg/ Kg Bw and GP (4) was orally treated with mirazid (200 mg/ Kg Bw) for six successive days in the early morning on an empty stomach, according to the dose carried out by Soliman et al [6] and adjusted based on the animal body weight [7]. The drugs were administered at the end of the 8^{th} week post infection to evaluate their efficacy against adult stages of *Fasciola gigantica*. Eight Guinea pigs were randomly picked up from each group 1st and 2nd week post treatment. Blood samples were individually collected by heart-puncture for hematological examination (RBC count, hemoglobin concentration, PCV value, total WBC count and differential leukocyte count) according to [8-10] respectively. Serum chemistry (ALT, AST, ALP, total bilirubin, total protein, albumin, urea and creatinine were assayed spectrophotometrically (5010, Photometer, BM Co. Germany) using commercial test kits according to the enclosed pamphlet (Spine React Spanish Co.), while the gamma glutamyltransferase (GGT) was determined by using ready made kits provided by Emapole, (Poland). Fecal samples were collected at the end of the 8th week post-infection and examined for fluke eggs by the sedimentation method according to [11].

Statistical Analysis

The data among treatment groups were analyzed by one way analysis of variance (ANOVA) with post-hoc LSD multiple comparison test using State View software, [12] for windows (Version 4.01. Abacaus Institute. Berkeley, California) to find out the significant differences among treatment groups when $P < 0.05$.

RESULTS

The erythrogram revealed macrocytic hypochromic in infected non-treated group as well as the infected and treated groups at on week post treatment when compared with the control group, while those parameters returned to the normal two weeks post treatment (Table 1-2). Regarding to the leukogram in the investigated groups, there was heterophilia , eosinophilia and lymphopenia in both infected non-treated and mirazid treated groups as compared with the control group, while the leukogram return to normal at 2 weeks post treated with TCBZ (Table 3-4).

Table 1. Studeis on Erythrogram Parameters (Mean ± S.E), One Week Post Treatment in Guinea pigs Eperimental infected with, *Fasciola gigantica*.

Group	RBC 10^6/L	Hb g/dL	PCV %	MCV fl	MCH pg	MCHC %
GP. 1	5.45^b ± 0.42	15.54^b ± 1.12	40.51^b ±1.25	74.32^b ±3.51	28.58 ±1.23	37.65^a ±1.35
GP. 2	4.25^a ±0.35	11.01^a ±0.81	36.9^a ±0.75	86.8^a ±4.12	25.9 ±1.12	30.1^b ±2.01
GP. 3	4.31^a ±0.32	11.19^a ±0.78	36.7^a ±0.80	85.2^a ±4.01	26.1 ±1.18	30.2^b ±1.42
GP. 4	4.71^b ±0.45	12.86^b ±1.32	38.7^b ±1.47	82.26^b ±3.58	27.3 ±1.42	33.43^b ±1.85

The same Column not followed by the same letter differs significantly ($P<0.05$)

Table 2. Studeis on Leukogram Parameters (Mean ± S.E), One Week Post Treatment in Guinea pigs Eperimental infected with, *Fasciola gigantica*.

Group	TLC 10^3 / µL	Hetrophil 10^3 / µL	Esinophil 10^3 /µL	Basophil 10^3 / µL	Lymphocyte 10^3 / µL	Moncyte. 10^3 / µL
GP. 1	9.30 [a] ± 0.57	3.02[b] ±0.21	0.56[b] ± 0.21	0.04 [a] ± 0.02	4.97 [a] ± 0.54	0.71[a] ±0.11
GP. 2	10.65[a] ±0.42	4.77[a] ±0.43	2.03[a] ±0.51	0.08[a] 0.08	3.19[b] ±0.51	0.69[a] ±0.09
GP. 3	9.45[a] ±0.78	3.28[b] ±0.11	0.59[b] ±0.27	0.06 [a] ±0.06	4.77[a] ±0.54	0.75[a] ±0.15
GP. 4	10.39[a] ±1.01	4.65[a] ±0.39	2.01[a] ±0.21	0.05 [a] ±0.05	3.05[b] ±0.59	0.63[a] ±0.13

The same Column not followed by the same letter differs significantly (P<0.05)

Table 3. Studeis on Erythrogram Parameters (Mean ± S.E), Two Week Post Treatment in Guinea pigs Eperimental infected with, *Fasciola gigantica*.

Group	RBC 10^6/µL	Hb g/dL	PCV %	MCV Fl	MCH Pg	MCHC %
GP. 1	5.41 [a] ± 0.47	14.75 [a] ± 1.03	39.2 [a] ±1.32	72.5 [a] ±3.32	27.3 [a] ±1.16	37.91 [a] ±1.68
GP. 2	4.76 [a] ±0.39	12.92 [a] ±1.09	37.1 [a] ±0.98	77.9 [a a] ±4.85	27.2 [a] ±0.91	34.91 [a] ±1.59
GP. 3	4.71 [a] ±0.40	12.38 [a] ±0.81	37.3 [a] ±0.79	79.19 [a] ±5.03	26.3 [a] ±1.05	33.12 [a] ±1.81
GP. 4	4.95 [a] ±0.41	13.17 [a] ±1.12	38.9 [a] ±1.47	78.6 [a] ±4.15	26.6 [a] ±1.28	34.41 [a] ±1.75

The same Column not followed by the same letter differ significantly (P<0.05)

Table 4. Studeis on Leukogram Parameters (Mean ± S.E), Two Week Post Treatment in Guinea pigs Eperimental infected with, Fasciola gigantica.

Group	TLC 10^3 / µL	Hetrophil 10^3 / µL	Esinophil 10^3 /µL	Basophil 10^3 / µL	Lymphocyte 10^3 / µL	Moncyte 10^3 / µL
GP. 1	9.26 [a] ± 0.51	2.91[b] ±0.49	0.51[c] ± 0.19	0.00 0.00	5.20[a] ± 0.43	0.64[a] ±0.12
GP. 2	9.72[a] ±0.59	3.69 [a] ±0.42	1.95[a] ±0.35	0.12 0.08	4.29[b] ±0.51	0.67[a] ±0.08
GP. 3	9.79[a] ±0.53	3.28[b] ±0.41	0.61[c] ±0.27	0.08 ±0.08	5.13[a] ±0.49	0.69[a] ±0.13
GP. 4	10.01[a] ±0.61	3.99 [a] ±0.36	0.88[b] ±0.17	0.00	4.48[b] ±0.57	0.75[a] ±0.10

The same Column not followed by the same letter differs significantly (P<0.0)

The disorders of liver functions, documented in our study of elevated liver enzymes, total bilirubin and decreased serum albumin in infected non-treated group as compared with the control group, while the liver functions were normal in TCBZ treated group 2 week post treatment (Table 5-6). The liver enzymes, ALT, AST, ALP and GGT were significantly increased in mirazid treated group at 2 weeks post-treatment when compared with the control and significantly decreased as compared with the infected group.

Regarding to renal function tests, the creatinine and urea were significantly elevated in infected non-treated group when compared with the control group. In the treated groups (TCBZ & mirazid) the renal function tests were non significant difference when compared with control group as displayed in the Table (5-6)

Table 5. Some Serum Biochemical Profiles (Mean ± S.E), One Week Post Treatment and Two Month P.I with *Fasciola gigantica*.

Groups	ALT U/L	AST U/L	GGT U/L	ALP U/L	T. Bili. mg/dl	T. P. gm/dl	Alb. gm/dl	Urea mg/dl	Creat. mg/d
GP.1	15.6^{c} ±1.41	24.2^{c} ±2.32	16.25^{b} ±1.19	14.32^{c} ±1.31	0.44^{b} ±0.07	5.56^{b} ±0.35	3.05^{b} ±0.28	45.59 ±4.42	0.65^{b} ±0.05
GP.2	34.9^{a} ±1.85	49.29^{a} ±4.3	28.93^{a} ±1.45	31.23^{a} ±1.51	0.89^{a} ±0.08	4.52^{a} ±0.31	2.12^{a} ±0.25	54.2 ±5.62	0.86^{a} ±0.07
GP.3	24.5^{b} ±1.19	32.5^{b} ±3.01	23.61^{a} ±1.49	16.2^{c} ±1.32	0.54^{b} ±0.07	4.69^{b} ±0.39	2.24^{a} ±0.27	50.78 ±5.44	0.81^{ab} ±0.09
GP.4	26.2^{b} ±1.12	30.3^{b} ±2.99	23.45^{a} ±1.51	25.9^{b} ±1.25	0.51^{b} ±0.06	4.81^{b} ±0.41	2.20^{a} ±0.26	49.35 ±6.01	0.74ab ±0.06

Table 6. Some Serum Biochemical Profiles (Mean ± S.E), Two Week Post Treatment and Two Month P.I with *Fasciola gigantica*.

Group	ALT U/L	AST U/L	GGT U/L	ALP U/L	T.Bili. mg/dl	T. P. gm/dl	Alb. gm/dl	Urea mg/dl	Creat. mg/d
GP.1	13.9^{b} ±1.21	23.8^{b} ±2.14	17.19 ±1.23	15.04c ±1.29	0.49b ±0.06	5.62^{a} ±0.39	3.18^{b} ±0.27	42.32b ±4.01	0.61b ±0.06
GP.2	32.6^{a} ±1.70	51.9^{a} ±4.7	29.82^{a} ±1.34	34.95a ±1.42	0.91a ±0.09	4.75^{a} ±0.42	2.24^{a} ±0.29	59.18a ±5.14	0.94^{a} ±0.08
GP.3	16.4^{c} ±1.26	26.2^{c} ±2.15	18.21^{c} ±1.38	16.83c ±1.27	0.52b ±0.06	5.35^{a} ±0.37	2.83^{ab} ±0.24	41.85b ±4.06	0.73^{b} ±0.08
GP.4	22.7^{b} ±1.02	34.1^{b} ±2.35	22.15^{b} ±1.32	25.75b ±1.19	0.50b ±0.05	5.41^{a} ±0.42	2.92^{ab} ±0.27	42.49b ±3.95	0.70^{b} ±0.07

T.Bili; Total Bilirubin, T.P, Total Protein, Alb, Albumin, Creat, Creatinine

The same column not followed by the same letter differs significantly ($P<0.05$)

DISCUSSION

Facioliasis is diseases caused by a trematode that invades the hepatic parenchyma and bile ducts of ruminants and other mammals, causing significant economic losses such as mortalities, low production of meat, milk and wool as well as reduced weight gain and impaired fertility [13].

The erythrogram result showed regenerative macrocytic hypochromic anemia in the infected non-treated groups. Such regenerative anemia could be the result of traumatic lesions induced during the migration of the juvenile flukes in the hepatic parenchyma, causing hepatobilary hemorrhage till establishing themselves in the bile ducts [14]. Moreover the anemia may be a result of the hepatotoxic effect of the worm metabolites (proline) which induces hemolysis and impaired erythropoiesis [15]. The anemia was a common feature during the late stage of infection, which partially agrees with Waweru et al [16] who reported that the common cause of anemia in two breeds of sheep infected with *Fasciola gigantica* at late stage was the heamatophagic nature of the parasite in the bile duct. Also agree with Yadav et al., [14] who found that the anemia started to occur from the 7th week PI in bovine calves and from the 6th week PI in buffalo calves.

The triclabendazole is a benzimidazole anthelmintic which was reported to have a specific flukicidal effect against the mature and immature stages of Fasciola hepatica and *Fasciola gigantica* in the small and large domesticated ruminants [17]. The infected group and treated with TCBZ showed anemia 2 month PI and for one week post treatment, then disappeared after two weeks of treatment as the drug eliminated most of the late stages of the immature and mature flukes. Consequently, there was a blood loss through the hepatobilary hemorrhage [18]. Moreover, the TCBZThis reported that the produced a significant increase in RBC at the 3rd week post treatment of sheep infected with fascioliasis [19].

Heterophilia, lymphopenia and insignificant change in the monocytes were observed in the Guinea pigs of infected non-treated group one week post treatment. These changes could be attributed to stress condition caused by early migration of the flukes in the hepatic parenchyma and the associated hemorrhage. The leukocytosis has been reported in different species of fascioliasis sheep [20,21]. Also the decrease in the lymphocytic response may be due to decreased production of IL-2 produced by the splenocytes of the infected rats as it is essential for the lymphocytes development and growth [22]. Meanwhile, our result are not in accordance with Bashandy et al [21] and Venguest et al [23] who found lymphocytosis and monocytosis in sheep and fallow deer infected with *Fasciola gigantica* and Fasciola hepatica respectively.

Eosinophils, provide a defense against the larvae of parasitic worms and unicellular organisms [24]. The eosinophil granules contain a substance called the major basic protein (MBP) which is toxic to many parasitic larvae [25]. The eosinophils have surface receptors for the antibody immunoglobulin E (IgE), these receptors are believed to be of importance in fighting the parasitic infection [26]. Eosinophilia that continued till the end of the experiment was the most predominant leukogram change in infected non-treated group (GP2). Similarly, to our resort, the eosinophilia in the current work could be attributed to the increased IL-5 from Th2 cytokines that contribute to the growth and differentiation of the eosinophils [22,27]. Eosinophilia was seen in rats and mice that were injected with Fasciola hepatica antigen as a result of increased eosinophilic precursors in the bone marrow [28].

The leukogram of infected and treated with TCBZ (GP, 3) showed eosinophilia, returned rapidly to the normal level after treatment. TCBZ eliminated most of the immature and mature flukes, reducing the hematological alterations to take place in fascioliasis [18]. This is indicated that the drug has high efficacy against immature and mature flukes. Similarly, several authors documented the efficacy of TCBZ in the treatment of fascioliasis [18,29,30].

The eosinophilia remained even after the 2nd week post treatment in the mirazid treatment group (GP, 4). This indicates that the mirazid is not highly effective against the *Fasciola gigantica*. This agrees with Fatem and Soheir [31] who reported that there is a considerable doubt about the efficacy of this drug in the treatment of schistosoma species. On the other hand, the fasciolicidal effect of mirazid in the patient endemic area with fasioliasis approved by significant decreasing eosinophilia after treatment with mirazid [3,32].

The liver transaminase enzymes (ALT &AST) are used to evaluate the liver damage during experimental infection of *Fasciola gigantica* in Guinea pigs. The ALT and AST were significantly increased in the infected group till the end of the experiment [23]. Such elevation is an indicator for the hepatocyte degeneration and fibrosis besides blockage of the bile ducts by migration of the juvenile flukes during the first stage of infection [33]. Similar results were reported by many authors who studied fascioliasis in different species. [23, 34, 35]. The GGT and ALP levels were significant increased in infected non-treated group (GP, 2). As the GGT and ALP are located in the bile duct epithelium, so its elevation is considered as a biomarker of a cholestatic effect and hyperplastic cholangitis [36]. Our results are in agreement with several authors, reported biliary hyperplasia in different animal species suffering from fascioliasis [23,33,36].

The liver enzymes ALT, AST, ALP and GGT were returned to the normal level in TCBZ group, while were significantly increased in mirazid treated group when compared with control. This means that the mirazid is less effective against the fascioliasis compared with TCBZ.. This result agrees with Fatem and Sohaire [31], who doubted the efficacy of mirazid in the treatment of schistosomiasis in hamsters. Moreover, the GGT and ALP returned to the normal level in patients of fascioliasis 3 month post treatment [37]. Regarding to the TCBZ treatment, our results are in accordance with [19, 20], who reported that the alteration in the liver enzyme which returned to the normal level four weeks post-treatment (PT) with TCBZ. Such results disagreed with [38], who found that the GGT enzyme remained elevated 24 days PT in sheep. This difference may be attributed to the difference in species.

The bilirubin level in infected non-treated group (GP, 2) revealed that the total bilirubin was significantly increased till the end of the experiment. The hyperbilirubinemia occurred as a result of an increased production rate of the bilirubin either from increase catabolism of cytochrom P450 which represents a high percentage of the total heme synthesized in the liver [39] and or from an increased erythrocyte degradation due to hematophagia by the flukes .Our results agree with [40], who studied the effect of experimental fascioliasis on the bilirubin metabolism in rats.The total bilirubin, were insignificantly changed in the treated groups (GPs, 3&4) as drug eliminated most of the immature and mature flukes. This gets along with our results about the inefficacy of mirazid against the immature stages when compared with the adult ones.

The total protein and albumin were decreased in the infected group compared with control. The hypoproteinemia in the present work, could be the result of anorexia and inefficient food utilization that accompanied the fluke infection, or from the hepatic damage caused by the migration of the flukes. The hypoalbuminemia could be due to the inhibition of its synthesis or the increased loss or breakdown of albumin [41]. Our results agree with [38, 35], who studied fascioliasis in naturally infected sheep and cattle respectively. The alteration in the serum protein returned rapidly to the normal level after treatment with TCBZ and mirazid.

The elevation of urea and creatinine levels in infected none treated (GP.2) could be as a result of renal injury caused by deposition of granular and pesudolinear IGg fasciola antigen in the mesangial region of the glomeruli, leading to membranoproliferative and mesangioproliferative glomerulonephritis in bovine fascioliasis[1,36]. The urea and creatinine were insignificantly changed two weeks after treatment with TCBZ and mirazid. Ahamed et al [37] reported that the urea and creatinine levels in patient with fascioliasis were within the normal range before and after treatment with mirazid.

CONCLUSION

It could be concluded that; the triclabendazole is more effective than mirazid in the treatment of *Fasciola gigantica* infestation. Further research should be done for more precise knowledge about the efficacy of mirazid as antifasciolicidal drug.

CONFLICT OF INTEREST

There is no conflict of interest of authors to declare

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