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Toxicological And Parasitological Studies Of The Secnidazole Drug (Compared With Amprolium Sulphate) As A Trial For Using It In Treatment Of *E.Stiedae* Infection In Rabbits

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ABSTRACT

Eighty apparently healthy and coccidia-free New Zealand white male rabbits (average weight of 774.69gm) were used. They were divided into eight equal groups in separate cages. The 1st group served as the normal (negative) control rabbits.The 2nd group was orally administered 50mg secnidazole /kg.B.wt., once a day, for 6 consecutive days. The 3rd group was orally administered 50mg amprolium sulphate 20% /kg.B.wt., once a day, for 6 consecutive days. The 4th group was administered the combination of the above two drugs (with the same doses and duration as in the 2nd and 3rd groups). Each rabbit of the 5th, 6th, 7th and 8th groups was orally inoculated with infective dose of 5×10^4 sporulated oocysts of *Eimeria stiedae* (*E.Stiedae*). The 5th group served as positive infected non-treated control. The 6th group was treated with secnidazole. The 7th group was treated with amprolium sulphate 20%, and the 8th group was treated with the combination of both drugs (the doses and duration of drugs as in the non infected groups). Haematological, serum biochemical, parasitological and histopathological investigations were carried out at 15 days post *E.Stiedae* infection (7th day of drug treatments) and 25 days post infection (10th day post stopping of drug administration). Rabbits were weighed twice at 15 and 25 days post infection. Treatment of rabbits with the combination of secnidazole and amprolium 20% elicited slight toxic effects than amprolium alone especially in the non-infected rabbits, but was the best anticoccidial activity, followed by treatment with amprolium 20% alone which induced a moderate toxicosis and a moderate anticoccidial activity. The secnidazole treatment showed the least toxicity; but induced a significant anticoccidial activity (inspite of its least anticoccidial activity compared with the other two systems of treatment). For achieving a better anticoccidial activity and least toxicity, we suggest more trials for reducing doses and/or duration of treatment with a combination of amprolium and secnidazole. Trials for field application of secnidazole as anticoccidial drug are suggested for more evaluation.

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INTRODUCTION

The isolation of the antibiotic Azomycin (2-nitroimidazole) from the streptomycete, which showed trichomonocidal activity led to the chemical synthesis and biological testing of many other nitroimidazoles *(1)*.

From the biologically active Nitroheterocyclic compounds are the 2-nitroimidazoles and the 5-nitroimidazoles, and from the biologically active 5-nitroimidazoles are : metronidazole (flagyl), Tinidazole, ornidazole, benzoylmetronidazole, nimorazole and secnidazole (flagentyl) were used against *Entamoeba histolytica, Trichomonas vaginalis* and *Giardia muris* in rodent models *(2)*.

The mode of action of nitroimidazoles (which are electron acceptors) on the protozoa and anaerobic bacteria is due to their reduction of nitroreductase enzyme of the microorganisms giving a reduced cytotoxic product, which in turn reacts with DNA, and consequently the DNA synthesis of the microorganisms was inhibited causing their death *(3)*.

The role of 5-nitroimidazole (metronidazole) for controlling coccidiosis in animals and poultry was tested by several authors. The highly satisfactory control of coccidiosis outbreaks in rabbits by metronidazole through reduction of hepatic and intestinal lesions and clinical signs by the oral dose level of 40mg/kg.B.wt. for 3 days, in parallel with subcutaneous injection of 20mg/kg.B.wt. of 1% of its solution. No significant toxic signs were observed*(4)*. The metronidazole may be used for the control of rabbit coccidiosis*(5)*. In Egypt, ***Shakshouk et al.*** *(6)* compared the anticoccidial activity of metronidazole (by oral dose of 25mg/L drinking water), sulfaquinoxaline (oral dose of 25mg/L water) and Lasalocid (75ppm in ration) in groups of Balady chickens. The results indicated that all the three drugs improved the body weight gain, feed

conversion and reduced the faecal oocyst output. The mortality rate was reduced to 5%, 10% and 2.5% in cases of metronidazole, sulfaquinoxalene and lasalocid treatments respectively.

Secnidazole is hydroxy-l-2-propyl-1-methyl-2-nitro-5-imidazole and a long acting 5-nitroimidazole. It is twice as active as metronidazole in the treatment of *Trichomonas vaginalis* and *Giardia muris* in mice. Its toxicity is low (oral LD_{50} = 2.4g/kg.B.wt.). The drug persists in the blood longer than does the metronidazole, it is also twice as active as metronadazole for the treatment of the intestinal and liver amoebiasis *(7)*.

Hepatic coccidiosis caused by *Eimeria stiedae* in rabbits, is known to modify the morphological and physiological properties of liver resulting in structural and functional alterations similar to those appearing in human hepatic amoebiasis*(8)*. The development of resistance to the anticoccidial drugs is the major problem in poultry and rabbit industries. Anticoccidial drug-resistance occurs when the Eimeria parasite can multiply and/or survive in the presence of a therapeutic concentration of the anticoccidial drug which normally destroy the parasite or prevents its multiplication*(9)*.

The objective of the current study was to elucidate the anticoccidial activity of secnidazole in treatment and control of rabbit coccidiosis, as well as to reduce resistance towards anticoccidial drugs.

MATERIALS AND METHODS

A-Animals :

Eighty apparently healthy and coccidia-free New Zealand white male rabbits (average body weight of 774.69 ± 2.2 grams) were used. The rabbits were put under observation with daily faecal examination for two weeks for excluding any animal with *E.stiedae* or any illness. The rabbits were divided into 8 equal groups which were kept in separat wire-floored metal cages and given pelleted feed and previousely boiled then cooled drinking water.

B-Coccidia parasite :

The oocysts of *E.Stiedae* were collected from the gallbladder and liver lesions of naturally infected rabbits with hepatic coccidiosis. The collected oocysts were suspended in 2.5% potassium dichromate solution and incubated at 28°C for 4 days (the period for maximum sporulation), then the oocysts were washed 2-3 times with distilled water by centrifugation for 10 minutes at 1500 rpm. The Eimeria oocysts were propagated in Eimeria-free rabbits (4-5 weeks old) to obtain sufficient numbers of coccidia-oocysts. The oocysts were preserved in 2.5% pot.dichromate solution and then microscopically counted by haemocytometer to determine the number of sporulated oocysts in 1ml of the solution culture, which was then stored at 4-8°C until used. Faecal and biliary oocysts of *E.Stiedae* were counted by McMaster technique.

C-Chemicals and Equipments :

-Potassium dichromate (for oocysts preservation) (El-Nasr Co).
-Haymes and Turkey's solution (for haematological study) (El-Nasr Co.)
-Formalin, sodium chloride, xylol, haematoxylin and. eosin, soft and hard paraffins, and Canada balsam (for histopathological technique) (El-Nasr Co.).
-Biochemical kits: ALT enzyme, total bilirubin, albumin, total protein, globulin, total lipids and creatinine determinations (El-Nasr Chem. Co.).
- Spectrophotometer.
-Haemocytometer (for parasitological and haematological counting).
- Light microscope.
-McMaster Slide (for counting the shedded oocysts in bile & faeces).

D- Drugs :

-Secnidazole tablets (Flagentyl) (El-Nile Co.) (one tablet contains 500mg secnidazole).
-Amprolium sulphate 20% powder (ADWIA Co.).

E- Methods :

Groups : Eighty rabbits were divided into 8 equal groups in well separated cages.

Inoculation of the *E.Stiedae* sporulated oocysts : Each rabbit of the last four groups (5th, 6th, 7th, and 8th, groups) was orally inoculated with 5 x 10^4 sporulated oocyst of *E.Stiedae* by a stomach tube*(10)*.

Drug treatments :

1-The rabbits of the 1st group were non-infected, non-drug treated and served as a negative (normal) control.

2-The rabbits of the 2nd group were orally administered 50mg secnidazole/ kg.B.wt., once a day, for 6 consecutive days.
3-The rabbits of the 3rd group were orally administered 50mg amprolium sulphate 20% /kg.B.wt., once a day, for 6 consecutive days.
4-The rabbits of the 4th group were orally administered the combination of secnidazole and amprolium sulphate 20% (the same dose and duration as in the 2nd, and 3rd groups).
5-The rabbits of the 5th group were *E.stiedae* infected (as previously mentioned) and non-drug treated, and served as infected (positive) control.
6-The rabbits of the 6th group were *E.stiedae* infected and treated with secnidazole (as in the 2nd group).
7-The rabbits of the 7th group were *E.stiedae* infected and treated with amprolium sulphate 20% (as in the 3rd group).
8-The rabbits of the 8th group were *E.stiedae* infected and treated with the drug combination of both secnidazole and amprolium sulphate 20% (as in the 4th group).

Sampling and methods of investigations:

a-Blood samples were collected (with heparin anticoagulant) from half the number of rabbits of the eight groups after sacrificing them on the 7th day of drug treatment (15th day post Eimeria infection) and another blood samples from the rest of rabbits on the 10th, day from stopping of the treatment (on the 25th day post Eimeria infection) for red and white blood corpuscle counts*(11)*.
b-Serum samples were prepared (on the 15th and 25th day post infection) for determination of the following serum biochemical constituents: Alanine aminotransferase (ALT) enzyme activity *(12)*, total bilirubin *(13)*, total protein *(14)*, albumin *(15)*, globulins (the difference between total protein and albumin), total lipid *(16)* and the serum creatinine*(17)*.
c-Bile samples, from the gallbladders of the sacrificed rabbits of all groups, were collected on the 15th and 25th day post *E.Stiedae* infection and the oocysts were counted by the McMaster technique in 1ml bile*(10)*.
d-The faecal samples were daily collected from the rabbits of all groups for 10 days between 15-25 days post infection, and the oocysts were counted in 1gm of faecal matter by the McMaster technique*(18)*.
e-The liver gross lesion scores were graded from 0-4 according to*(19)* in the sacrificed rabbits of all groups on days 15 and 25 post Eimeria infection.
f-Fresh liver tissue specimens of the sacrificed rabbits on the 15th and 25th day post infection were fixed in 10% neutral formalin saline for the histopathological technique *(20)* and the histopathological changes were microscopically examined for different histopathological lesions.
g-The body weights of rabbits of all groups were recorded on the 15th and 25th day post infection.

The statistical analysis : The obtained data were statistically analysed using F-test through the analysis of variance (ANOVA)*(21)* for the body weight, biochemical and haematological analysis. The analysis of Randomized Complete Block Design (RCBD) by the Duncan Multiple Range-Test (Multiple F-test) was used for the statistical analysis of parasitological data *(22)*.

RESULTS

A-Clinical signs

The *E.Stiedae* infected rabbits showed a gradual decrease of the appetite in the first week, marked emaciation in the 3rd week, semisolid faecal droppings in some cases and distension of the abdomen by the end of the experimental period. One rabbit from the positive control (infected, non-treated) died in the 3rd week post infection.

B-Post mortem findings

The slaughtered rabbits of coccidia infected rabbits showed gastro- intestinal distension with gases, visible and sometimes protruded liver nodules which decreased by treatment with amprolium and/or secnidazole.

C-Effects of drug treatment and/or *E.Stiedae* infection on the body weight :

At both periods (15 or 25 days post Eimeria infection), all the drug treated groups of rabbits showed a significant decrease in the body weights than that of the normal (negative) control. The group of rabbits infected with Eimeria without treatment (positive control) showed a significant decrease in the body weight (lower body

weight) than that of all the other groups of rabbits, but the groups of rabbits administered secnidazole, amprolium or the combination of both showed a significant improvement in their body weights than that of the positive control rabbits (table, 1).

D-Effects of drug treatments and/or *E.Stiedae* infection on some haematological parameters

1-Red Blood Corpuscles (RBCs) count

On the 7th day of treatment (15 days of *E.Stiedae* infection), the RBCs count was significantly decreased in all groups than normal (non-infected) control (except by the combined drug treatment of Eimeria infected rabbits which revealed nearly normal RBCs count). On the 10th day post last drug treatment (25 days post infection) the secnidazole or its combination with amprolium induced only a significant decrease of RBCs compared with that of the normal control, but the coccidia infected groups showed nearly normal RBCs count (tables, 2 and 3).

2-White Blood Corpuscles (WBCs) Count

The combined drug treatment (secnidazole and amprolium) induced a significant increase of WBCs count in coccidia infected and non-infected rabbits compared with the normal control rabbits (on the 7th day of treatment), in contrast, the WBCs counts decreased significantly by treatments of both coccidia infected and non-infected rabbits (when each drug was given alone) compared with the normal control. On the 10th day post last treatment (25 day post infection), the WBCs count was significantly decreased in all groups compared with that of the normal control rabbits (except by secnidazole or the combination of both drug treatment in coccidia infected rabbits, which returned back to nearly the normal count of WBCs) (tables, 2 and 3).

E-Effects of treatments and/or *E.Stiedae* infection on some serum biochemical constituents

1-Alanineaminotransferase (ALT) enzyme activity

The ALT enzyme activity was significantly increased by the combined treatment of both secnidazole and amprolium in the non-infected rabbits and in all the coccidia infected groups compared with that of the normal (non-infected) control on the 7th day of drug treatment and on the 10th day post last treatment (25 days post infection). No significant change of ALT activity between secnidazole or amprolium treatments of the non-infected rabbits was recorded (tables, 4 and 5).

2-Total Bilirubin

The total bilirubin was significantly increased in all groups compared with the normal control except in cases of : (a) secnidazole treatment of the non-infected rabbits (at both periods), (b) secnidazole or amprolium treatments of coccidia infected rabbits (on the 7th day of treatments). The treatments of coccidia infected rabbits significantly reduced the total bilirubin compared with that of the positive control (coccidia infected) rabbits (in the 1st period) (tables, 4 and 5).

3-Serum Creatinine

The serum creatinine was significantly increased in all groups when compared with the negative (normal) control rabbits in both periods, except by (a) the secnidazole treated non-infected rabbits (in both periods, (b) the amprolium treated rabbits on the 10th day post last treatment,. (c) rabbits with combinted treatment on the 7th day showed a nearly normal creatinine concentration (tables, 4 and 5).

4-Total Lipids

The total lipids were significantly increased in all groups of rabbits when compared with that of the negative (normal) control group (in both periods) except by (a) the secnidazole treated rabbits (in both periods), (b) the positive (coccidia infected) control, (c) the combination of both drugs on the 7th day of treatment, where the total lipids were nearly normal. At both periods, no significant change in the total lipid concentration between the coccidia infected groups (tables, 4 and 5).

5-Albumin

The albumin concentration was non-significantly changed in all groups (in both periods) (tables, 4 and 5).

6-Globulins

The globulin contents were significantly increased in all groups of rabbits when compared with that of the normal control (in both periods), except by (a) secnidazole treatments, (b) treatment with the combination

of the two drugs (on the 7th day of treatments), (c) non-infected rabbits which were treated with amprolium alone or drug combination (on the 10th day post last treatment), where the globulin concentration was nearly normal (tables, 4 and 5).

7-Total proteins

On the 7th day of treatment, the total proteins were significantly increased in all groups than the normal control except in the cases of : (a) the non – infected rabbits treated with secnidazole, (b) treatment with combination of both drugs, where the total proteins were nearly normal.

On the 10th day post last drug treatments (25 days post infection), the total proteins showed irregular pattern, where : (a) it was significantly increased in coccidia infected rabbits treated with the combination of both drugs, (b) it was significantly decreased in the serum of amprolium treatment of the non-infected rabbits, (c) it appeared nearly normal in the other five groups, (d) no significant change of the total protein was noticed among the four coccidia infected groups (tables, 4 and 5).

F-Parasitological study

1-Oocyst output in faeces

All coccidia infected (treated and non-treated) rabbits began to excrete oocysts of *E.Stiedae* in their faeces from the 15th day post infection (PI). The daily oocyst output per gram of faeces increased progressively until the 23rd day PI (the peak of oocyst production), then began to decrease from the 24th day PI. The decrease of the shedded oocyst in 1gm of faeces was associated with the combined treatment by both secnidazole and amprolium (lower oocysts output), followed by amprolium treatment, then secnidazole. However all the three systems of drug treatments showed a significant decrease in oocyst output/gm of faeces than that of the infected – non treated (positive) control rabbits (starting from the 19th day PI). In most cases, there were no significant changes of faecal oocyst output between amprolium treatment and secnidazole treatment (table, 6).

2-Oocyst output in the bile on the 15th and 25th day PI

There was a statistically significant decrease of oocyst output in the bile of all the treated rabbits against *E.Stiedae* infection than that of the infected (positive) control rabbits. All the three systems of treatments showed a significant decrease of the oocyst output per 1ml of bile, but the most lower oocyst output was shown by the combined drug treatment, followed by the amprolium then by the secnidazole treatment (table, 7).

3-Liver lesion scores

On the 15th or 25th day PI, there was a significant decrease in the liver lesion score in all *E.Stiedae* infected and treated rabbits than only the infected (non treated) positive control. There was no significant change in the liver lesion score between the amprolium treated group and the combined treatment of both drugs on the 25th day PI (table, 8).

G-Histopathological findings: The histopathological changes are recorded in table (9).

Table (1): The body weights (in grams) of rabbits at different periods after secnidazole and/or amprolium sulphate treatments, with or without *E.Stiedae* infection (Means ± SE).

Days PI / Groups (gr.)	Initial body weights	Body weights on the 15th day post *E.stiedae* infection	Body weights on the 25th day post *E.stiedae* infection
-ve (Normal) control gr.(1)	777.33 ± 7.53 A	1355.67 ± 21.40 A	1540.83 ± 15.21 A
Secnidazole treated gr.(2)	772.17 ± 9.76 A	1125 ± 37.49 A	1317.50 ±10.20 BDE
Amprolium treated gr.(3)	780.50 ±9.12 A	1139.67 ±14.61 B	1310.67 ±50.54 B
Secnidazole and amprolium treated gr.(4)	786.67 ± 6.39 A	1159.50 ± 49.31 C	1313.00 ±14.78 BD
E.Stiedae infected (+ve control) gr. (5)	773.33 ± 9.02 A	1036.33 ± 25.05 C	1140.00 ±25.48 C
E.Stiedae infected and secnidazole treated gr.(6)	771.50 ± 8.23 A	1102.00 ± 26.82 CD	1306.00 ± 7.72 D
E.Stiedae infected and amprolium treated gr.(7)	767.50 ± 4.67 A	1158.00 ± 11.85 D	1382.33 ±15.18 EF
E.Stiedae infected and secnidazole and amprolium treated gr.(8)	770.67 ± 4.62 A	1233.00 ± 14.43 E	1441.67 ± 14.67 F
F-test	NS	*	*
LSD (at P ≤ 0.05)	---	68.969	67.191

N.B. : (1) N.S. = Non Significant change. (2) LSD = Least significant difference between means (at P ≤ 0.05) (3) The different capital litters in columns denote the significant difference between means (at P ≤ 0.05) and vise versa. (4) * = significant changes among means (at P ≤ 0.05)

Table (2): Some haematological changes of rabbits with secnidazole and/or amprolium sulphate treatments, with and without *E.stiedae* infection (on the 7th day of drug treatment or 15th day PI) (Means ± SE).

Groups (gr.)	RBCs ($X10^6$)	WBCs ($X10^3$)
-ve (Normal) control gr.(1)	5.415 ± 0.217 AD	3.925 ± 0.097 AD
Secnidazole treated gr.(2)	4.440 ± 0.136 B	2.567 ± 0.406 BF
Amprolium treated gr.(3)	3.942 ± 0.115 C	4.975 ± 0.242 C
Secnidazole and amprolium treated gr.(4)	3.200 ±0.390 C	4.025±0.107 AD
E.Stiedae infected (+ve control) gr. (5)	4.776 ±0.243 DB	3.600 ± 0.466 DE
E.Stiedae infected and secnidazole treated gr.(6)	4.412± 0.241 B	2.850 ± 0.116 BE
E.Stiedae infected and amprolium treated gr.(7)	3.800 ± 0.107 C	1.975 ± 0.090 F
E.Stiedae infected and secnidazole and amprolium treated gr. (8)	5.580 ± 0.316 A	5.002 ± 0.198 C
F-test	*	*
LSD (at P ≤ 0.05)	0.798	0.841

N.B. : (1) * = significant change (at P ≤ 0.05) (2) LSD = Least significant differenc (at P ≤ 0.05). (3) The different capital litters in columns denote presence of significant changes among means (at P ≤ 0.05) and vice versa

Table (3): Some haematological changes of rabbits with Secnidazole and/or amprolium sulphate treatments and with or without *E.stiedae* infection (on the 10th days post drug treatment cessation or 25th day PI) (Means ± SE).

Groups (gr.)	RBCs ($X10^6$)	WBCs ($X10^3$)
-ve (Normal) control gr.(1)	4.615 ± 0.137 ADE	4.250 ± 0.378 A
Secnidazole treated gr.(2)	3.692 ± 0.343 BC	1.550 ± 0.128 B
Amprolium treated (+ve control) gr. (3)	4.265 ± 0.180 ABE	2.075 ± 0.141 B
Secnidazole and amprolium treated gr.(4)	3.060 ± 0.162 C	1.975± 0.101 B
E.Stiedae infected gr.(5)	5.005 ± 0.151 DE	2.150± 0.227 B
E.Stiedae infected and secnidazole treated gr.(6)	4.000 ± 0.078 AB	4.875±0.295 A
E.Stiedae infected and amprolium treated gr. (7)	4.683 ± 0.322 E	1.225±0.049 B
E.Stiedae infected and secnidazole and amprolium treated gr. (8)	4.565 ± 0.202 ADE	4.200±0.187 A
F-test	*	*
LSD (at P ≤ 0.05)	0.646	1.654

N.B. : (1) * = significant change (at P ≤ 0.05) (2) LSD = Least significant difference (at P < 0.05). (3) The different litters in columns denote presence of significant changes among means (at P ≤ 0.05), and vice versa

Table (4): Some serum biochemical constituents of rabbits with Secnidazole and/or amprolium sulphate treatment with and without *E.stiedae* infection (on the 7th day of treatments on the 15th day PI) (Means ± SE).

Groups(gr.)	ALT-activity (U/L)	Total bilirubin (mg/dl)	Serum creatinine (mg/dl)	Total lipid (mg/dl)	Albumin (g/dl)	Globulins (g/dl)	Total proteins (g/dl)
-ve (Normal) control gr.(1)	20.44A ± 0.854	0.227A ± 0.063	0.835A ± 0.116	9.450AE ± 0.129	2.691A ± 0.368	2.793A ± 0.316	5.484A ± 0.144
Secnidazole treated gr.(2)	22.611AB ± 0.981	0.356AB ± 0.102	0.970A ± 0.081	7.98A ± 0.216	2.545A ± 0.261	2.725A ± 0.215	5.270A ± 0.123
Amprolium treated gr.(3)	23.611AB ± 0.900	0.691BD ± 0.095	1.240BCD ± 0.158	14.99BC ± 1.533	2.532A ± 0.141	4.198B ± 0.253	6.730BC ± 0.490
Secnidazole and amprolium treated gr.(4)	25.28BCE ± 1.731	0.788D ± 0.143	1.160C ± 0.148	18.69C ± 2.156	2.546A ± 0.111	3.332C ± 0.354	5.878A ± 0.208
E.Stiedae infected (+ve control) gr.(5)	28.475CE ± 1.499	0.511C ± 0.062	1.200C ± 0.145	10.32AD ± 0.300	2.634A ± 0.252	4.244D ± 0.274	6.878C ± 0.309
E.Stiedae infecte and secnidazole treated gr.(6)	32.625 DE ± 2.035	0.173A ± 0.028	1.330 D ± 0.113	11.93BDE ± 0.974	2.487A ± 0.315	4.246D ± 0.322	6.733C ± 0.185
E.Stiedae infected and amprolium treated gr. (7)	33.15 D ± 1.195	0.178A ± 0.028	1.340D ± 0.129	13.25BD ± 1.123	2.572A ± 0.283	3.951D ± 0.137	6.523C ± 0.416
E.Stiedae infected and secnidazole and amprolium treated gr. (8)	28.834 E ± 1.747	0.994 D ± 0.206	0.820A ± 0.026	10.495AD ± 1.028	2.546A ± 0.236	3.026A ± 0.185	5.572A ± 0.273
F-test	*	*	*	*	NS	*	*
LSD (at P ≤ 0.05)	3.961	0.400	0.167	3,727	--	0.370	0.835

N.B. : (1) * = significant change at P ≤ 0.05 (2) LSD = Least significant difference (at P < 0.05). (3) NS = Non Significant change. (4) The different capital litters in columns denote presence of significant changes among means (at P ≤ 0.05), and vice versa.

Table(5): Some serum biochemical constituents of rabbits with secnidazole and/or amprolium sulphate treatments with and without *E.stiedae* infections (on the 10th day post treatment cessation or 25th day PI) (Means ± SE).

Groups (gr.)	ALT-enzyme activity (U/L)	Total Bilirubin (mg/dl)	Serum Creatinine (mg/dl)	Total Lipid (mg/dl)	Albumin (g/dl)	Globulins (g/dl)	Total proteins (g/dl)
-ve (Normal) control gr.(1)	12.32A ± 0.632	0.229A ± 0.072	1.06A ± 0.080	9.86A ± 0.482	2.723A ± 0.238	2.454ACD ± 0.306	5.177AB ± 0.290
Secnidazole treated gr.(2)	13.588AB ± 0.712	0.375A ± 0.036	1.18AC ± 0.098	10.14A ± 0.628	2.465A ± 0.322	2.826BEF G ± 0.211	5.291AB D ± 0.236
Amprolium treated gr.(3)	15.98B ± 1.02	0.684BC D ± 0.127	1.32BDE ± 0.127	13.46B ± 0.551	2.322A ±0.211	2.197C ± 0.273	4.519C ± 0.3654
Secnidazole and amprolium treated gr.(4)	16.358B ± 1.143	0.770C ± 0.164	1.14A ± 0.113	13.92B ± 0.401	2.399A ± 0.261	3.02EG ± 0.086	4.987A ± 0.273
E.Stiedae infected (+ve control) gr.(5)	19.40C ± 1.166	0.960DE ± 0.084	1.27CD ± 0.129	14.64C ± 0.285	2.467A ± 0.342	2.588BDF ± 0.086	5.487BD ± 0.127
E.Stiedae infected and secnidazole treated gr.(6)	30.16D ± 1.649	0.840E ± 0.097	1.33DE ± 0.113	14.88C ± 0.261	2.764A ± 0.424	2.603ABH ± 0.236	5.367BD ± 0.224
E.Stiedae infected and amprolium treated gr. (7)	20.16C ± 1.649	0.699C ± 0.126	1.28CBD ± 0.097	14.36C ± 0.43	2.43A ± 0.215	2.88FGH ± 0.326	5.31ABD ± 0.357
E.Stiedae infected and secnidazole and amprolium treated gr. (8)	26.167E ± 1.60	0.691CD ± 0.203	1.42E ± 0.127	14.77C ± 0.362	2.557A ± 0.354	2.956G ± 0.357	5.153D ± 0.319
F-test	*	*	*	*	NS	*	*
LSD (at P: ≤ 0.05)	2.982	0.151	0.121	0.590	--	0.322	0.333

N.B. : (1) * = significant change at P ≤ 0.05 (2) LSD = Least significant difference (at P < 0.05). (3) NS = Non. Significant change. (4) The different litters in columns denote presence of significant changes among means (at P ≤ 0.05) and vice versa.

Table (6): Daily faecal oocyst output in 1gm faeces (X 10^3) from the rabbits of different groups infected with 5 X 10^4 oocysts of *E.Stiedae* after treatment with secnidazole and/or amprolium sulphate 20% for 6 consecutive days (Means ± SE).

Groups(gr.)	15th day (X 10^3)	16th day (X 10^3)	17th day (X 10^3)	18th day (X 10^3)	19th day (X 10^3)	20th day (X 10^3)	21st day (X 10^3)	22nd day (X 10^3)	23rd day (X 10^3)	24th day (X 10^3)
-ve (Normal) control gr.(1)	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa
Secnidazole treated gr.(2)	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa
Amprolium treated gr.(3)	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa
Secnidazole and amprolium treated gr.(4)	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00 Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa	0.00 ± 0.00Aa
E.Stiedae infected (+ve control) gr. (5)	44.75 ± 1.67 Aa	76.5 ± 2.75 Aa	85 ± 1.75Aabc	109 ± 3.95 Bbc	138 ± 1.77Bc	250.75 ± 4.68Bde	284.5 ± 2.25Bde	380.25 ± 3.25Beg	564 ± 3.62Bf	392.25± 3.65Bg
E.Stiedae infected and secnidazole treated gr.(6)	37.25 ± 0.96 Aa	66.25 ± 2.22 Aa	74 ± 1.46 Aa	85.5 ± 2.56 Bab	105.75 ± 2.30Cb	166.5 ± 2.56Cc	191 ± 1.62Cc	221.25 ± 3.09Cc	364 ± 2.09Cd	229 ± 0.94Cc
E.Stiedae infected and amprolium treated gr.(7)	27 ± 1.37 Aa	41.75± 1.52 Aa	43 ± 1.54 Aa	53.5 ± 0.75ABab	68.5± 1.35Cab	108.25 ± 2.16Db	164.75± 2.22Cc	185.75 ± 2.79Cc	305.75 ± 20.2Dd	201.5 ± 3.75Cc
E.Stiedae infected and secnidazole and amprolium treated gr.(8)	22.00 ± 1.37 Aa	31.5 ± 1.35 Aab	36.00 ± 1.54 Aab	42.25 ± 1.29ABab	54.5 ± 1.6Cabc	80.00 ± 2.5Dbc	106± 2.26Dc	126.75 ± 1.29Dc	183.5 ± 2.56Ed	134 ± 0.79Ddc

N.B. :

1- LSD of (period x treatment) interaction = 54.885 (at $P \leq 0.05$)

2- The different capital litters in columns denote presence of significant changes among means of groups at the same period (at $P \leq 0.05$), and vise versa.

3- The different small litters in rows denote presence of significant changes among means of the same group at different periods (at $P \leq 0.05$), and vise versa.

Table (7): The oocyst outputs in 1ml of bile (X 10^4) of the rabbits of the different groups with secnidazole and/or Amprolium 20% treatments for 6 days (on the 15th and 25th day post *E.Stiedae* infection) (Means ± SE).

Days PI / Groups (gr.)	15th day (X 10^4)	25th day (X 10^4)
-ve(Normal) control gr.(1)	0.00± 0.00 Aa	0.00± 0.00 Aa
Secnidazole treated gr.(2)	0.00± 0.00 Aa	0.00± 0.00 Aa
Amprolium treated gr.(3)	0.00± 0.00 Aa	0.00± 0.00 Aa
Secnidazole and amprolium treated gr.(4)	0.00± 0.00 Aa	0.00± 0.00 Aa
E.Stiedae infected (+ve control) gr.(5)	409 ± 13.005 Ba	332.5±11.924Bb
E.Stiedae infected and secnidazole treated gr.(6)	316.5± 5.154 Ca	207.5±7.395 Cb
E.Stiedae infected and amprolium treated gr. (7)	194 ± 4.323 Da	150 ± 7.906 Db
E.Stiedae infected and secnidazole and amprolium treated gr. (8)	96.75± 2.408 Ea	107.5 ± 7.395 Ea

N.B. : (1) LSD of period x treatment interaction (at $P \leq 0.05$) = 19.650 (2) Different capital litters in columns denote significant variation between treatments (at $P \leq 0.05$) and vise versa, (3) different small litters in rows denote significant variation among periods (at $P \leq 0.05$) and vise versa.

Table (8): Liver lesion scores of rabbits of different groups treated with secnidazole and/or Amprolium 20% treatments for 6 days (on the 15th and 25th day post *E.Stiedae* infection) (Means ± SE).

Days PI / Groups (gr.)	15th day (X 10^4)	25th day (X 10^4)
-ve(Normal) control gr.(1)	0.00+ 0.00 Aa	0.00± 0.00 Aa
Secnidazole treated gr.(2)	0.00± 0.00 Aa	0.00± 0.00 Aa
Amprolium treated gr.(3)	0.00± 0.00 Aa	0.00± 0.00 Aa
Secnidazole and amprolium treated gr.(4)	0.00± 0.00 Aa	0.00± 0.00 Aa
E.Stiedae infected (+ve control) gr. (5)	3.5 ± 0.08 Ba	3.75 ± 0.14 Ba
E.Stiedae infected and secnidazole treated gr.(6)	2.92 ± 0.14 Ca	2.82 ± 0.0 7 Ca
E.Stiedae infected and amprolium treated gr. (7)	2.58 ± 0.14 Ca	2.50 ± 0.19 Da
E.Stiedae infected and secnidazole and amprolium treated gr. (8)	2.00 ± 0.12 Ea	2.42 ± 0.07 Db

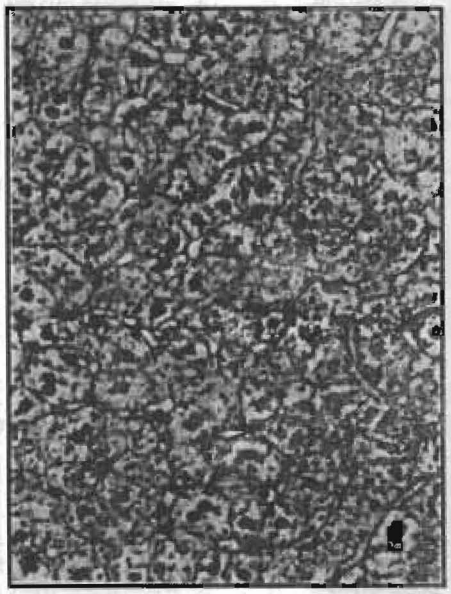
N.B.: (1) LSD of (period x treatment) interaction (at $P \leq 0.05$) = 0.293 (2) Different capital litters in columns denote significant variation between treatments (at $P \leq 0.05$) and vise versa, (3) different small litters in rows denote significant variation among periods (at $P \leq 0.05$) and vise versa.

Table (9): The histopathological findings of the Secnidazole and/or Amprolium sulphate treatment of the non-infected and coccidia infected rabbits on the 7th day of drug treatment (15 days PI) and 10th day of drug treatment cessation (25 days PI).

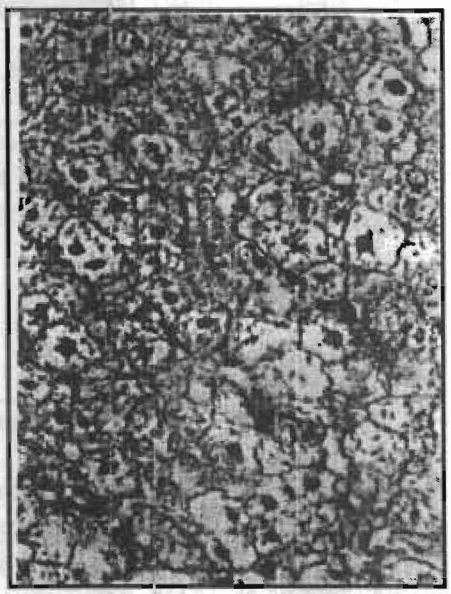
Groups (gr.)	Lesions of liver on the 7th day of treatment(15th day PI)	Lesions of liver on the 10th post treatment cessation (25th day PI)
Normal (negative) control gr.(1)	Normal histological structures	Normal histological structure
Secnidazole treated gr. (2)	1) Vacuolar degeneration of some hepatocytes 2) Congestion of blood vessels (Fig.1-A)	1) Slight to non-detectable degenerative changes (Fig.1-B)
Amprolium sulphate treated gr. (3)	1) Hydropic (vacuolar) degeneration. 2) Focal necrotic areas. 3) Congestion of blood vessels (Fig.1-C)	1) Hydropic degeneration 2) Focal necrotic areas
Secnidazole and amprolium treated gr. (4)	1) Diffuse hydropic (vacuolar) degeneration 2) Focal and diffuse necrotic areas. 3) Haemorrhages in the liver tissue 4) Congestion of blood vessels (Fig. 1-D)	The same features of liver lesions on the 7th day but with less severity
Coccidia infected non-treated (+v control) gr. (5)	- Numerous developmental stages of *E.Stiedae* in the epithelial lining of the bile ductules (Fig.2-A)	1) Severe (malignant) cystic formation 2) Hyperplasia of bile ductules which were completely occluded with developmental stages and oocysts in their lumens. 3) Hepatic cells replaced by cystic formation of bile ductules and connective tissue (Fig.3-A)
Coccidia infected and secnidazole treated gr. (6)	1) Moderate proliferation of bile ductules 2) slight degenerative changes of hepatocytes (Fig.2-B)	- Few cystic formation of bile ductules (Fig.3-B)
Coccidia infected and amprolium treated gr. (7)	- Mild proliferation of bile ductules (Fig.2C)	- Few cystic formation of bile ductules (Fig.3-C)
Coccidia infected and secnidazole and amprolium treated gr. (8)	- Mild proliferation of bile ductules (Fig.2-D)	1) Slight destruction of epithelial lining of bile ductules. 2) few developmental stages and oocysts 3) Mild cystic formation of bile ductules (Fig.3-D).

A- Secnidazole (15-days PI) (gr.2) X 400

B- Secnidazole (25 days PI)(gr.2) X 400

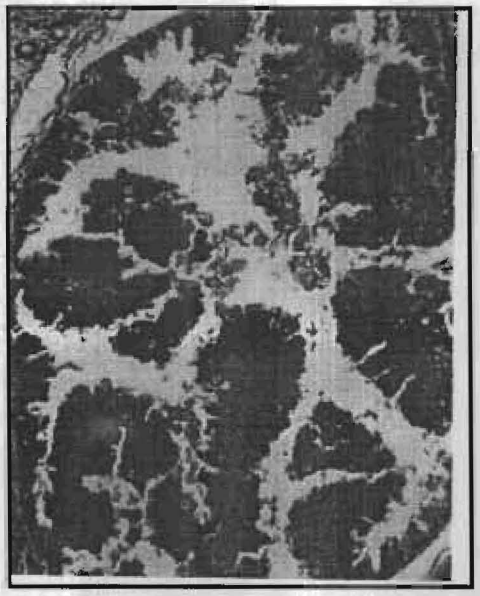


C-Amprolium (15 days PI)(gr.3) X 400



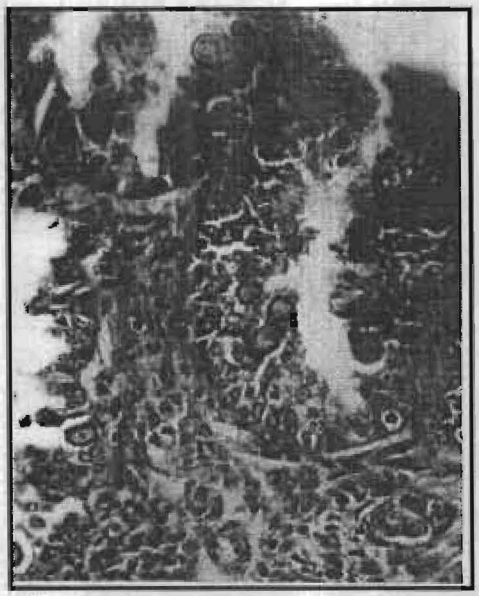
D- Secnidazole +Amprolium (15 days PI)(gr.4) X 400

Fig.(1): Liver sections of rabbits orally administered with 50mg/k.gB.wt. of secnidazole and/or amprolium sulphate 20% (once a day, for 6 days) as follow : A-(gr. 2) non-infected rabbit at 7th day post secnidazole treatment [15 days post infection (PI)] showing vacuolation and slight degenerative changes of some hepatocytes. B- (gr.2) non-infected rabbit after 10 days of secnidazole treatment cessation (25 days PI), showing focal degenerative changes of some hepatocytes with congestion. C- (gr. 3) non-infected rabbit on the 7th day of amprolium treatment showing vacuolar degeneration and focal necrosis of hepatocytes. D- (gr. 4) non-infected rabbit at 7th day of combined treatment showing clear vacuolation and degenerative changes of hepatocytes with focal necrotic areas. (H & E , X 400).

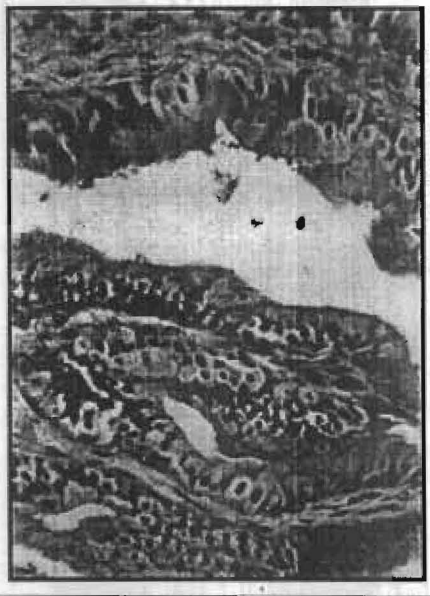


A-Coccidia infected (+ve control), 15 days PI (gr. 5)X 200.

B- Coccidia infected + secnidazole treated (15 days PI) (gr.6) X 400

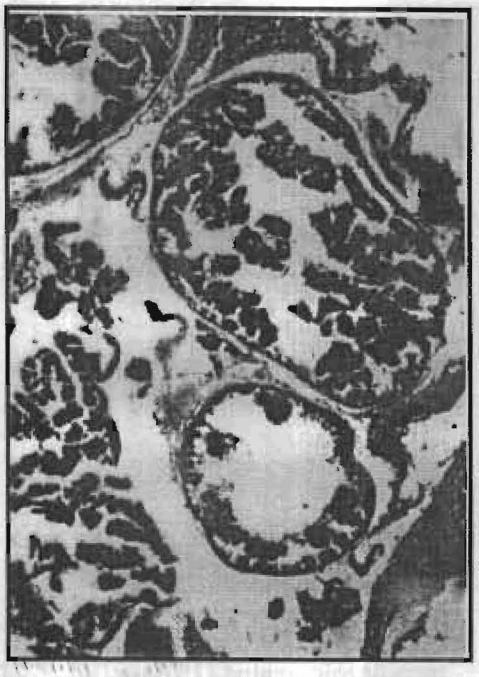


C-Coccidia infected + amprolium treated (15 days PI) (gr. 7) X 400

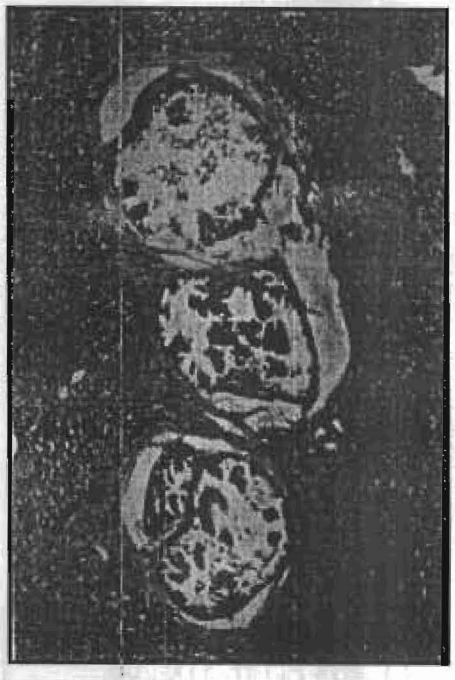


D- Coccidia infected (15 days PI) +(secnidazole and amprolium) treated (gr.8) X 400

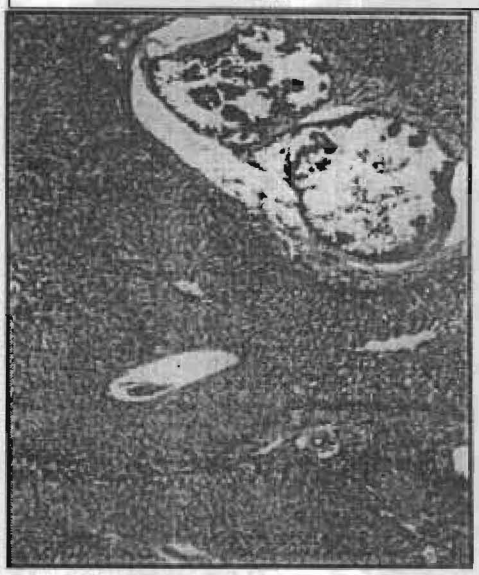
Fig.(2): Liver sections of coccidia infected rabbits orally administered with 50mg/kg.b.wt. of secnidazole and/or amprolium, once a day, for 6 days, as follow : A- (gr. 5) positive (infected, non-treated) control rabbits on the 15th day post infection, showing numerous developmental stages of *E.stiedae* (shizonts, gametes and oocysts) in the epithelial lining of the severly destructed bile ductules (H & E , 200). B- (gr. 6) coccidia infected rabbit with secnidazole treatment (15 days pI) showing different developmental stages of *E.stiedae* in the slightly destructed epithelial lining of bile ductules. C- (gr. 7) coccidia infected rabbit treated with amprolium (15 days PI) showing different developmental stages of *E.Stiedae* in the moderately destructed epithleial lining of bile ductules. D- (gr. 8) coccidia infected rabbit treated with combined drugs (15 days PI) showing few number of developmental stages of *E.Stiedae* without destruction of the epithelial linining of the bile ductules. (H & E, X 400) .



A- Coccidia infected
(+ve control) 25 days PI (gr. 5)
X 40



B-Coccidia infected +
secnidazole treated (25 days PI)(gr. 6)
X 40



C- Coccidia infected
+ amprolium treated (25 days PI) (gr.7)
X 40

A- Coccidia infected (25 days PI)
+(secnidazole and amprolium treated (gr.8)
X 40

Fig. (3) : Liver sections of coccidia infected rabbits, orally administered with 50mg/kg.B.wt. of secnidazole and/or amprolium, once a day, for 6 days, as follow : A- (gr. 5) positive control rabbit (infected, non-treated) 25 days PI showing great numbers of cystic formation of bile ductules causing pressure atrophy of the surrounding hepatic parenchyma. B- (gr. 6) coccidia infected rabbit treated with secnidazole at 25 days PI (10 days post stopping of treatment) showing few cystic formation of bile ductules surrounded by fibrous connective tissue. C- (gr. 7) coccidia infected rabbit previousely treated with amprolium (at 25 days PI) showing few number of cystic formation of bile ductules surrounded by fibrous tissue. D- (gr. 8) coccidia infected rabbit treated with combined drugs (25 days PI) showing very few cystic formation of bile ductules surrounded by connective tissue. (H & E, X 40).

DISCUSSION

The hepatic coccidiosis of rabbits, caused by the sporulated oocysts of *E.stiedae*, remains one of the dangerous and common disease of Egyptian rabbits causing economic losses in rabbit production. The resistance of coccidia against many anticoccidial drugs causes an important problem in controlling the disease. The metronidazole (as one of 5-nitroimidazoles) has been proven to be effective in controlling coccidiosis ***(4-6).*** So, we tried to use secnidazole (as a new and long acting 5-nitroimidazole) in the present work.

Concerning the safety evaluation of the drug treatments, the haematological study showed that during the first studied period (7th day of treatment or 15 days after *E.Stiedae* infection), all the treatments induced a significant decrease of the RBCs counts compared with that of the normal rabbits, except the combination of treatments restored the normal values of the RBCs. During the second period (10th day post stopping drug treatment or 25 days post infection), the RBCs counts regained their normal levels in all the coccidia infected groups of rabbits and with amprolium treatment than the normal control. The reduced erythropoiesis was attributed to reduced erythropoietin following a chronic renal diseases ***(23).*** The biochemical study revaled that the serum creatinine concentration was significantly increased in all groups than that of the normal control (except by secnidazole, in both periods) indicating the presence of a certain degree of renal dysfunction by amprolium sulphate treatment.

The WBCs counts showed a significant decrease in all the treated rabbits (infected or non-infected) in both periods, except in case of the combination of the two drugs which nearly normalized the leukocytic counts. A mild leukopenia could also be induced by one of the 5-nitroimidazole compounds (the metronidazole) in goats ***(24).*** Also, a transient and reversible leukopenia during metronidazole therapy was detected ***(25).***

The current biochemical analysis indicated that the ALT enzyme activity was significantly increased in all the treated groups compared with that of the normal control rabbits except by secnidazole treatment (in both periods), and by amprolium 20% (in the first period), where the ALT enzyme could be normalized. The same pattern of ALT changes could be detected also for bilirubin concentration in response to either secnidazole and/or amprolium treatments. The ALT enzyme level was usually elevated in cases of acute hepatic necrosis and the extrahepatic obstruction ***(26).*** The increased concentrations of total bilirubin is an indicative of hepatic dysfunction***(27).*** The present histopathological studies showed the presence of substantial liver damage which caused by the treatments, from slight degenerative (vacuolar) changes by secnidazole treatment to clear vacuolar (hydropic) degeneration and focal necrotic areas by amprolium to clear hydropic degeneration with focal and diffuse necrotic areas (in both periods) by the combination treatment of secnidazole and amprolium. So the treatment with such drug combination could induce a higher hepatotoxic effects (especially in the non-infected rabbits) compared with the amprolium (of moderate hepatotoxicity) then secnidazole (with slight to undetectable hepatotoxicity) as recorded for metronidazole ***(24)***.

Mostly a significant increase of the total lipids were induced by amprolium or the combination of the two drugs in the present work; but the treatment with secnidazole for 6 days did not elevate the total lipid above the normal level (at both periods). Such hyperlipidemia could be attributed to the interference of the drugs with the lipid metabolism ***(28)***.

The total globulins were significantly increased, in most groups compared with the control. Secnidazole treatment could normalize globulin in non-infected rabbits (in the first period) and in the infected rabbits (in the 2nd period). Amprolium or the combination of both drugs normalized the globulin in the 2nd period. The globulins are composed of alpha, beta and gamma globulins ***(29).*** Most alpha and beta globulins and albumin are synthesized in the liver, whereas the gamma globulins are synthesized by the mature B-lymphocytes (plasma cells) in the lymphatic organs ***(23)***. The serum albumin concentration was not significantly affected by all treatments and the liver of all rabbits was not severely impaired ***(30)***. As the albumin concentration was not significantly changed, the changes in globulins were the major factor in the total protein changes.

Based on the current toxicological study, it could be concluded that the secnidazole treatments of rabbits showed the least toxic effects among the three treatments, as reported by ***Mandal et al.*** in goats ***(24)***. The authors recorded no significant biochemical changes by metronidazole.

The current study revealed a significant reduction of the oocyst output in either the bile or faeces and a significant reduction of the liver lesion scores in rabbits treated with 50 mg secnidazole/kg.B.wt. for 6 days, compared with that of coccidia infected (positive control) rabbits. Similar results were recorded by using an oral dose of 40mg/kg.B.wt. metronidazole for three days in rabbit without showing toxic effects ***(4)***. Similar significant anticoccidial activity was recorded in the current study by amprolium 20% (50mg/kg.B.wt.) and also with the combination of both secnidazole and amprolium. Such anticoccidial activity was represented by reduced oocyst counts and liver lesion scores compared with the positive control. The amprolium sulphate 20% treatment showed more significant reduction of the above parameters than did the secnidazole treatment in the present study. Similar results were obtained by metronidazole (5-nitroimidazole compound) with a less anticoccidial activity than that showed by amprolium sulphate in goats ***(31)*** and in chickens ***(32)***.

The current study revealed that the oocyst output per 1ml of bile in all the coccidia infected rabbits (treated and non-treated) on the 25th day post infection showed significant increase than that on the 15th day post infection. This may be attributed to the presence of hyperplasia of the bile ductules that led to a partial or complete occlusion of such ductules resulting in cessation of bile outflow to the duodenum, leading to accumulation of more oocysts in the bile than in faeces. Moreover, an increased total bilirubin concentration was obtained in the coccidia infected rabbits on the 25th day PI than on the 15th day PI in most cases. These results coincide with those for metronidazole ***(33)***.

The treated and non-treated coccidia infected rabbits revealed a gradual increase of the liver lesion scores by time, till the 25th day PI, and this result was similar to that reported by ***Pellerdy (34)***.

The liver lesion scores of the infected rabbits on the 25th day PI were mild, as a result of treatment with the combination of the drugs. It became moderate by either secnidazole treatment or amprolium treatment separately, but the lesion scores became severe in the liver of the positive control (infected, non-treated), indicating a powerful anticoccidial activity for the drug combination than that produced by each drug alone. This explains the significant improvement in the body weight of the rabbits given the combined treatment in the present study than those given any single drug. The albumin concentration was not significantly changed in all groups, consequently the hepato-and nephrotoxicities were not severe. The damaging effects of the coccidia infection predominated on that due to the toxicity of the drugs, leading to a lower number of cystic formation of bile ductules found with the combined drug treatment (compared to any of the two drugs alone).

It could be concluded that the highest efficacy of the anticoccidial activity was for the combination of the two drugs, followed by amprolium and ending with secnidazole. On the other hand, the toxicosis side effects was more for the combination of the two drugs than amprolium than secnidazole.

Further studies on such drug combination (secnidazole and amprolium) by reducing their doses (to be less than 50mg/kg.B.wt) and/or duration of treatment (to be less than 6 days) should be tried to obtain good anticoccidial activity without toxicosis side effects.

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REFERENCES

1- Cosar, C. and Julou, L. (1959): Activity de L' (Hydroxy-2-ethyl)-1- methyl-2-nitro-5 imidazole (8823R.P.) Vice-avisdes infections experimentales a trichomones vaginalis. Ann. Inst. Pasteur, Paris,(96): 235.

2-Rossignol, J.F.; Maisonneuve, H. and Cho, Y.M. (1984): Nitroimidazoles in the treatment of trichomoniasis, giardiasis and amoebiasis. International Journal of Clinical Pharmacology, Therapy and Toxicology, 22(2): 63-72.

3-Sisson, P.R. and Ingham, H.R. (1985): Action of metronidazole on facultative anaerobes. J. Infect. Dis., (151): 569.

4-Reshetnyak, U.Z.; Bartenev, U.S. and Rubanov, A.A. (1970): Trichopol (metronidazole), an effective drug against coccidiosis in Rabbits: Veterinaria, (3): 75.

5-Jones, L.M.; Booth, N.H. and McDonald, L.E. (1977): Veterinary Pharmacology and Therapeutics (4th, ed), the Iowa State University Press.

6-Shakshouk, A.G.; Gomaz, H.E. and Ashmawi, I.E. (1995): The anticoccidial efficacy of metronidazole in comparison with sulfaquinoxaline and lasalocid in control of coccidiosis in baladay chickens. J.Egypt. Vet. Med. Assoc., 55 No. (1,2): 517-527.

7-Benazet, F. and Guillaume, L. (1976): Amoebicidal and trichomonacidal activities of secnidazole in the laboratory. Bulletin de la Societe de Pathologie Exotique, 69 (4): 309-319.

8-Fernandez, E.; Roman, I.D.; Cava, F.; Galan, A.L.; Esteller, A.; Munoz, M.E. and Jimenez, R. (1996): Acid–base disturbances in the rabbit during experimental hepatic parasitism. Parasitology Research, 82 (6): 524-528.

9-Chapman, H.D. (1997): Biochemical, genetic and applied aspects of drug resistance in Eimeria parasites of the fowl. Avian Pathology (26): 221-244.

10-Peeters, J.E.; Geeroms, R.; Antonine, O.; Mammerickx, M. and Halen, P. (1981): Efficacy of naracin against hepatic and intestinal coccidiosis in rabbits. Parasitology, 83 : 293-301.

11-Shalm, O.W. (1975): Veterinary Haematology, 3rd Ed.

12-Reitman, S. and Frankel, S. (1957): Determination of SGOT and SGPT. J. Clin. Path. (28): 56.

13-Jendrassiki, G.P. (1938): Vere in fachte photometriche metheden zur bestimmung de blutbilirubins. Biochemical, 2 (297): 81.

14-Doumas, B.T.; Watson, W.A. and Bigs, H.G. (1971): Determination of serum total protein. Clin.Chem.Acta,31(1): 87.

15-Rojkin, M.L.; Olguin de Mariani, M.C.; Drappo, G.A. and Sosa, C.F. (1974): Fraccionamiento proteico per determinacion directa de albumina. Bioq. Clin.VIII (4): 241.

16-Schmit, J.M. (1964): Determination of serum total lipids.Conc. Thesis, Lyon.

17-Henry, R.J. (1974): Clinical Chemistry, Principle and Technics 2nd ed., Harper and Row, PP: 525.

18-Abd El-Rahman, M.S.; Hilali, M.A. and Selim, M.K. (1982): Handbook of diagnostic techniques of parasites of veterinary medicine. Parasitology Department, Fac. of Vet. Med., Cairo Univ., PP. 34-36.

19-Johnson, J. and Reid, W.M. (1970): Anticoccidial drugs : Lesion scoring technique in battery and floor pen experiments with chickens. Exp. Parasitol. (28): 30-36.

20-Lillie, R.D. and Fulmer, H.N.(1976): Histopathological Technique and Practical Histochemistry, McGrow Hill Book Company.

21-Snedecor, G.W. and Cochran, W.G. (1969): Statistical Methods, 6th, ed. Iowa State University, Press Ames, IOWA.

22-Duncon, D.B. (1955): Multiple Range and Multiple F-test., Biometrics (11): 1-42.

23-Duncan, J.R. and Prasse, K.W. (1986): Veterinary Clinical Medicine. Clinical Pathology. 2nd ed. Iowa State University Press, Ames IA.

24-Mandal, T.K.; Yadava, K.P. and Tirkey, K. (1987): Effect of metronidazole on the haematological and biochemical parameters in goats. Indian Journal of Animal Sciences, 57 (7): 706-708.

25-Sanders, C.V.; Hanna, B.J. and Lewis, A.C. (1979): Metronidazole in the treatment of anaerobic infections. Amer. Rev. Resp. Dis. (120): 337.

26-Zimmerman, H.J. (1984): Function and integrity of the liver. In Clinical Diagnosis and Management by Laboratory Methods. Edited by Henry, J.H. and Sounders, W.B. Phildelphia.

27-Kachman, J.F. and Moss, D.W. (1976): Enzymes : In : Fundamentals of Clinical Chemistry. Sounders, W.B., Phildelphia.

28-Stroev, E.A. (1986): Biochemistry, translated from Russian, B.V. Cand. Sc. (Chem.), Mir Publishers, Moscow.

29-McPherson, R.A. (1984): Specific protein in : Clinical Diagnosis and Management by

Laboratory methods. Edited by Henry, J.B.; Saunders, W.B., Philadelphia.

30-Vorhaus, L.J. and Kark, R.M. (1953): Serum cholinesterase in health and disease. Amer. J. Med. (14): 707.

31-Sabu, L. and Pillai, K.M. (1996): Amprosol, sulmet, coxysol, A.K. and Metrogyl in treatment of caprine coccidiosis. Journal of Veterinary and Animal Sciences, 27(2): 141-144.

32-Saif, A.S.A.B. (2003): Some pharmacological studies on antiparasitic drug. M.D. Thesis (pharmacology), Fac. Vet. Med., Kafr-El-Sheikh, Tanta University.

33-Bothaina, A., Badawy; Tanios, N.I. and Assia, M.El-Sawy (1999): Parasitological, biochemical and histopathological studies on hepatic coccidiosis in rabbits. Egypt. J. Com. Path. & Clinic. Path.Vol. (12): 149-159.

34-Pellerdy, L.P. (1974): Coccidia and Coccidiosis, Text book, 2nd ed., Akademiai, Kiado, Budapest.

الملخص العربى

دراسات سمية وطفيلية على عقار السيكنيدازول (مقارنة مع سلفات الأمبروليوم) كمحاولة لإستخدامه فى علاج عدوى الأيميريا ستيدى فى الأرانب

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استخدمت فى هذه الدراسة ثمانون من ذكور الأرانب النيوزيلاندى البيضاء السليمة والخالية من الكوكسيديا (بمتوسط وزن ٧٧٤,٧ جرام) حيث قسمت الأرانب إلى ثمانية مجموعات متساوية فى أقفاص منعزلة كالتالى : المجموعة الأولى من الأرانب استخدمت كضابط سلبى للتجربة، والمجموعة الثانية تم تجريعها بالفم عقار السيكنيدازول بجرعة ٥٠مجم/ كجم من وزن الجسم مرة فى اليوم ولمدة ٦ أيام متتالية، والمجموعة الثالثة تم تجريعها بالفم بعقار سلفات الأمبروليوم (٢٠%) بجرعة ٥٠مجم/كجم وزن، مرة فى اليوم ولمدة ٦ أيام متتالية، والمجموعة الرابعة تم تجريعها بالفم كلا من العقارين معا (بنفس الجرعة والمدة كما فى المجموعتين الثانية والثالثة)، وتم تجريع كل أرنب من أرانب المجموعات الخامسة والسادسة والسابعة والثامنة بجرعة معدية (٥×١٠^{٤}) من حويصلات الأيميريا ستيدى المتجرثمة (قبل العلاج الدوائى بثمانية أيام) حيث تم إعطاء الأرانب المعالجات الآتية : المجموعة الخامسة هى مجموعة ضابطة موجبة (مصابة بالكوكسيديا وغير معالجة) والمجموعة السادسة أرانب مصابة بالكوكسيديا وتم إعطاءها عقار السيكنيدازول (بنفس الجرعة والطريقة المتبعة فى المجموعة الثانية) والمجموعة السابعة فهى أرانب مصابة بالكوكسيديا وتم إعطاءها عقار سلفات الأمبروليوم (كما فى المجموعة الثالثة) والمجموعة الثامنة فهى أرانب مصابة بالكوكسيديا وتم إعطاءها كل من العقارين معا (كما بالمجموعة الرابعة)٠ وتم عمل الفحوص الدموية والبيوكيميائية والطفيلية والهستوباثولوجية فى اليوم الخامس عشر من عدوى الأيميريا ستيدى (اليوم السابع من العلاج بالعقاقير) وفى اليوم الخامس والعشرون من عدوى الأيميريا (اليوم العاشر من توقف العلاج الدوائى). وقد تم عد حويصلات الأيميريا ستيدى يوميا ولمدة عشرة أيام ما بين ١٥-٢٤يوم، كما تم وزن الأرانب فى أيام ١٥، ٢٥ من عدوى الكوكسيديا٠ وبناء على النتائج المتاحة، فقد أمكن إستنتاج أن العلاج المزدوج بكلا العقارين معا أحدث سمية أكثر بقليل من العلاج بالأمبروليوم (خاصة فى الأرانب الغير مصابة بالمرض) ولكن هذا العلاج المزدوج كان أكثر تأثيرا فى علاج الكوكسيديا عن أى من العقارين بمفرده، ثم يتبعه العلاج بالأمبروليوم فهو أقل سمية من العلاج المزدوج وأحدث تأثيرا أقل فى علاج الكوكسيديا من العلاج المزدوج أيضا، ثم العلاج بالسيكنيدازول الأقل سمية من العلاجين السابقين والأقل تأثيرا فى علاج الكوكسيديا بالمقارنة بالعلاج المزدوج أو العلاج بالأمبروليوم (بالرغم من احتفاظه بالتأثير المعنوى المعالج لعدوى الكوكسيديا)٠ ولهذا يجب تقليل جرعة ومدة العلاج المزدوج بالعقارين للحصول على أفضل تأثير فى علاج كوكسيديا الأرانب بدون إحداث تأثير سمى معنوى وكذلك يجب تجريب العلاج الحقلى الاقتصادى بعقار السيكنيدازول كمضاد للكوكسيديا لتقييم الدواء من الأوجه الأخرى المختلفة٠