ACTA ABP BIOCHIMICA POLONICA

Vol. 59, No 4/2012
575–580
on-line at: www.actabp.pl

Regular paper

Some heterocyclic thione derivatives exhibit anticoccidial activity by inhibiting glycosidases

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Coccidiosis is one of the most common parasitic diseases affecting many species of domestic animals. This disease has a major economic significance and the search for new compounds having anticoccidial activity is of great importance. In this article, different levels of protection from coccidian infection by *Eimeria stiedae* were developed in rabbits by treatment with compounds incorporating the skeleton of thiourea. These compounds include 4,5-diphenylimidazole-2-thione (1), 4,5-Diphenyl-1,2,4-triazole-3-thiol (2) and 5-(2-Hydroxyphenyl)-4-phenyl-1,2,4-triazole-3-thiol (3) compared to the anticoccidial drug toltrazuril as a reference compound. Compounds 1-3 inhibit coccidiosis-induced activity of α-glucosidase. The protection from coccidial infection by compound 1 was higher than that shown for compounds 2 and 3. These data suggest that diazole and triazole thione derivatives have a mimetic effect for anticoccidial drugs through their inhibition of glycosidases.

Key words: Coccocidiosis, anticoccidial compounds, imidazolethione, triazolethiol, toltrazuril, inhibitor of glycosidase

Received: 12 February, 2012; revised: 10 June, 2012; accepted: 22 August, 2012; available on-line: 16 October, 2012

INTRODUCTION

Coccidiosis is one of the most common and problematic parasitic diseases in all classes of domestic animals caused by several species of coccidia (Tolebi and Mulcahy, 1995). It is a ubiquitous protozoan infection of animals seriously impairing their growth and food utilization (Abdel-Megeed *et al*., 2005) and causes significant mortality in domestic rabbits (Hauptman *et al*., 2001). Hepatic coccidiosis (*Eimeria stiedae*) is one of the most pathogenic coccidian protozoans in domestic rabbits causing severe coccidiosis and increased mortality (Hauptman *et al*., 2001). Consequently the search for new compounds having anticoccidial activity is of prime significance for scientific and economic values. There are many well-known anticoccidial agents such as toltrazuril which is a triazinetrione derivative. It acts on all intracellular development stages of the infection (Mathis *et al*., 2004) and is used for the treatment of *Eimeria stiedae* infection (Cam *et al*., 2008). Since coccidiosis occurs in the liver of the host, it is interesting to study the effect of drugs used for treating the illness on some hepatic enzymes. Glycosidases are important enzymes in processing cellular as well as extracellular carbohydrates (Zhao *et al*., 2010) and the activity is affected by coccidiosis (Major and Ruff, 1978; Kudweis *et al*., 1991; Adams *et al*., 1996).

Glycosidases (EC 3.2.1.-) are enzymes hydrolyzing *O*- and *S*-glycosyl residues involved in the biosynthesis of oligosaccharide chains and N-linked glycoproteins in the endoplasmic reticulum (Helenius and Aebi, 2001; Long *et al*., 2006; Yusa *et al*., 2006). In addition, inhibition of these glycosidases has various effects (Nadanaka *et al*., 2004; Goffard *et al*., 2005; Torrelles *et al*., 2006). Recently, we have reported the glycosidase inhibition activity of 4,5-diphenylimidazole-2-thione (compound **1**), 4,5-diphenyl-1,2,4-triazole-3-thiol (compound **2**), and 5-(*o*-hydroxyphenyl)-4-phenyl-1,2,4-triazole-3-thiol (compound **3**) (Balba *et al*., 2011). The objective of the present study is to evaluate the anticoccidial activity of these compounds compared to toltrazuril through the inhibition of coccidiosis-induced elevation of α-glucosidase and α-amylase, taking into consideration the presence of thiourea moiety in their cyclic structure (Fig. 1). The ultimate goal is to look for some new effective anticoccidial compounds.

MATERIALS AND METHODS

Materials. The enzymatic colorimetric assay kit for glucose (phenol, 4-amino-antipyrine, glucose oxidase and peroxidase) was purchased from Boehringer (Mannheim, Germany). Bovine serum albumin (BSA), soluble starch and Foline-Ciocalteau reagent were purchased from Sigma (St. Louis, Mo., USA). Toltrazuril was a kind gift from the Department of Poultry and Fish Diseases, Edfina Faculty of Veterinary Medicine, Alexandria University. Other reagents were of analytical grade. Compounds **1**, **2** and **3** were prepared according to the methods described by El Ashry *et al.* (2008 & 2010).

Animals. Male Spanish V. line rabbits were obtained from the animal house of the Faculty of Agriculture, Alexandria University. The animals were one month old with an approximate body weight of 1000 g. They were housed individually in metal cages and kept healthy under conventional conditions of temperature, humidity and 12 h photoperiod. Animal work was conducted in

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Abbreviations: Compound **1**, 4,5-diphenylimidazole-2-thione; compound **2**, 4,5-Diphenyl-1,2,4-triazole-3-thiol, compound; **3**, 5-(2-Hydroxyphenyl)-4-phenyl-1,2,4-triazole-3-thiol; PI, post infection; NINM, non-infected non-medicated; INM, infected non-medicated; NIM1, non-infected medicated with compound **1**; NIM2, non-infected medicated with compound **2**; NIM3, non-infected medicated with compound **3**; IM1, infected medicated with compound **1**; IM2, infected medicated with compound **2**; IM3, infected medicated with compound **3**; NIMR, non-infected medicated with toltrazuril; IMR, infected medicated with toltrazuril.

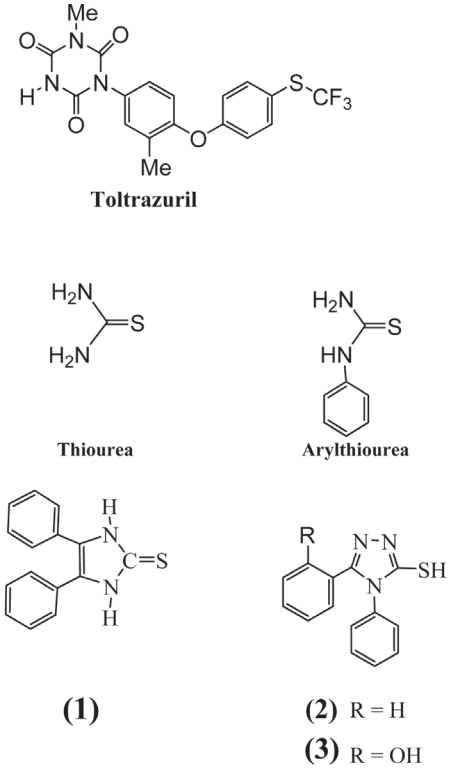

Figure 1. Structures of toltrazuril and the investigated thiourea analogues.

accordance with the institution protocol and the national legal requirements.

Infection and medication of animals. Isolation and sporulation of *E. stiedae* oocysts: *E. stiedae* was isolated from infective field cases. The isolated oocysts of *E. stiedae* strain were developed to sporulated oocysts (infective stage), where the optimal conditions for sporulation are 38°C and a relative humidity of 76–80%. Complete sporulation was taking place after 72 hours.

Propagation of pure *E. stiedae* strain. Baby rabbits infected with 50000 sporulated oocysts showed symptoms of liver coccidiosis after 15 days post infection (PI). The symptoms were observed as loss of appetite, emaciation, diarrhea and distended abdomen. An enlarged greenish pale liver with whitish yellow necrotic nodules of various sizes on the surface was noticed (data not shown) and ascites also occurred. Fresh and pure oocysts of *E. stiedae* strain were reisolated from the gall bladder 30 days PI and then were sporulated. The obtained fresh and sporulated oocysts were used for experimental infection of rabbits.

Experimental observation. The rabbits were divided into ten groups (five for each) as non-infected non-medicated (NINM, group 1), infected non-medicated (INM, group 2), non-infected medicated with compound **1** (NIM1, group 3), non-infected medicated with compound **2** (NIM2, group 4), non-infected medicated with compound **3** (NIM3, group 5), infected medicated with compound **1** (IM1, group 6), infected medicated with compound **2** (IM2, group 7), infected medicated with compound **3** (IM3, group 8), non-infected medicated with toltrazuril (NIMR, group 9) and infected medicated with toltrazuril (IMR, group 10). The infected groups 2, 6, 7, 8 and 10 were infected by 50000 fresh, sporulated *E. stiedae* oocysts. The inoculation of the oocysts was carried out orally as a single dose using a wide–mouthed 1.0 ml plastic pipette. At the 5th day PI, the animals were treated with a specific amount of each compound (10% of LD_{50}) divided in three doses given once daily. Groups 3 and 6 were treated with 20.4 mg of compound **1** per rabbit (three doses of 6.8 mg). Groups 4 and 7 were treated with 10.7 mg of compound **2** (3.6 mg per dose). Groups 5 and 8 were treated with 15.5 mg of compound **3** (5.16 mg per dose). Groups 9 and 10 were treated with 2.5% toltrazuril by adding the drug solution to the drinking water at the rate of 3 ml/L for treatment period of 8 h per day during two consecutive days as reported previously (Mathis *et al*., 2004).

After different periods PI, up to 30 days, (starting 24 h post treatment with the third dose of the compounds **1**, **2** or **3**), three rabbits from each group were sacrificed and their livers were collected, excised and subjected to assays. In the positive control group (group 2), signs of disease were observed on the 15th day PI. These were loss of appetite, emaciation, diarrhea and distended abdomen. The signs of disease were observed later (20th day PI) in the IM1, IM2 and IM3 groups. They were of considerably variable intensity, from light in IM1 group to moderate in the IM2 and IM3 groups. In the IMR group and in the non-infected groups, neither clinical symptoms nor side effects were observed throughout the observation period (30th day PI).

Enzymes assay. The assay of α–glucosidase activity in the serum and liver homogenate is based on its effect towards maltose as a substrate (Balbaa *et al*., 2002, Dahlquist, 1970) and the determination of the liberated glucose with the glucose oxidase method as described previously (Tinder, 1969). Briefly, the assay mixture contained 28 mM maltose and 50 mM maleate at pH 4.5. The reaction was run for 60 min at 37°C in a final volume of 1.1 ml with an appropriate amount of the en-

Table 1. Oocysts output by infected rabbits.

Groups/ days PI	Mean oocyst output x 10^3/g feces ± S.E.*							
	16	18	20	22	24	26	28	30
2: INM	28.4 ± 0.406	92.0 ± 0.108	156.0 ± 0.807	238.1 ± 0.377	318.5 ± 0.513	398.6 ± 0.664 0.0721	364.5 ± 0.377	375.0 ± 0.513
6: IM 1	18.2 ± 0.409	43.4 ± 0.669	88.1 ± 0.378	142.5 ± 0.806	205.1 ± 0.604	192.3 ± 0.116	156.0 ± 0.886	163.1 ± 0.166
7: IM 2	20.7 ± 0.203	56.0 ± 0.403	102.0 ±0.813	164.3 ± 0.197	222.3 ± 0.392	258.1 ± 0.803	218.4 ± 0.15	238.0 ± 0.206
8: IM 3	22.5 ± 0.204	74.0 ± 0.102	128.0 ± 0.148	180.1 ± 0.132	238.0 ± 0.146	266.0 ± 0.102	296.0 ± 0.176	250.4 ± 0.148
10: IMR	Non	Non	Non	Non	Non	Non	Non	Non

*The values are the means of five rabbits ± S.E.

Table 2. Serum α-glucosidase in all experimental groups of rabbits.

Groups/days PI	Specific activity of serum α-glucosidase (μM/min/mg protein) ± S.E.*				
	1	8	15	22	30
1: NINM	21.19 ± 0.19	21.99 ± 0.14	22.19 ± 0. 04	21.8 ± 0.16	22.14± 0. 07
2: INM	20.38 ± 0.10	29.30 ± 0.11[a]	36.48 ± 0.11[a]	43.55 ± 0.07[a]	38.48± 0.01[a]
3: NIM 1	19.8 ± 0.20	15.67 ± 0.34[b]	19.62 ± 0.08[b]	20.65 ± 0.04[b]	21.30±0.11[b]
4: NIM2	20.94 ±0.15	17.03 ± 0.06[b]	19.88 ±0.08[b]	20.46 ± 0.04[b]	21.76±0.01[b]
5: NIM 3	20.63 ±0.16	16.25 ± 0.07[b]	18.70 ± 0.10[b]	19.45 ± 0.06[b]	21.12±0.02[b]
6: IM 1	20.78 ± 0.16	23.92 ± 0.14[c]	25.28 ± 0.03[c]	28.17 ± 0.07[c]	26.14±0.03[c]
7: IM 2	20.98 ± 0.17	24.53 ± 0.13[c]	27.57 ± 0.08[c]	30.25 ± 0.03[c]	27.69± 0.01[c]
8 : IM 3	20.50 ± 0.13	26.54± 0.13[c]	30.56 ± 0.04[b]	32.27 ± 0.05[c]	29.49±0.02[c]
9: NIMR	20.45 ± 0.15	20.92±0.06	21.43 ± 0.13	20.75±0.06	21.01 ± 0.03
10: IMR	20.68 ± 0.22	21.63±0.13	23.16 ± 0.03	22.70±0.04	21.57± 0.07

*The values are the means of five rabbits ± S.E. Means in the same column with different superscripts are significantly different ($P < 0.05$).

zyme source and the produced red color was measured at 500 nm. The assay of α-amylase in serum as well as in liver homogenate of rabbit was determined according to the method described previously (Bernfeld, 1995; Fischer and Stein, 1961). The reaction was carried out by incubating 5.0 ml of 0.02 M sodium phosphate buffer, pH 6.9, 2.0 ml of buffered soluble starch and 1.0 ml of sodium chloride in a water bath at 37°C for 5 min. A volume of 0.5 ml of diluted enzyme source was then added to the sample tube while 0.5 ml of phosphate buffer was added to the blank one. The assay mixture was incubated for 30 min at 37°C followed by the addition of 0.5 ml of dinitrosalicylic acid reagent to stop the reaction. After heating in a boiling water bath for 5 min, 0.5 ml of sodium hydroxide was added, heated for further 5 min and cooled under tap water. Finally, the amount of maltose liberated was measured at 540 nm against blank. Maltose concentration was calculated from a standard curve. All assays were run in triplicate and the average was calculated.

Protein determination. The protein content of serum and tissue samples was determined as reported previously (Tsuyosh & James, 1978) for calculating the values of the specific activity of the studied enzymes.

RESULTS

Mortality of rabbits

Throughout the experimental observation period, no death occurred in the non-infected groups (NINM, NIM1, NIM2, NIM3 and NIMR) or in the IMR group (infected reference group). This result agrees with that reported previously (Pommier *et al*., 2003). On the other hand, in the INM group (positive control group), four rabbits died, one each on the 20th, 23rd, 25th and 28th day of PI. Moreover, in the IM1 group one rabbit died on the 26th day PI. In IM2 group one rabbit died on the 24th and a second one on the 29th day PI. In group 8 (IM3), one rabbit died on day 23rd day of PI and second rabbit on the 28th day. Thus, the mortality was 80, 20, 40, 40 and 0 percentage in the INM, IM1, IM2, IM3 and IMR groups, respectively. These data show that compound **1** significantly reduced the mortality caused by *E. stiedae* as compared with the positive control group.

Parasitological observation

We noted a nearly complete absence of oocysts in the non-infected groups (NINM, NIM1, NIM2, NIM3 and NIMR). In the positive control (INM), the peak production of was at the 26th day PI, and then decreased (Table 1). The mean of oocysts abundance per gram feces was 28.4 ± 0.406 on the 16th day PI increased to 398.6 ± 0.664 at the 26th day PI and then decreased to 364.5 ± 0.377 on the 28th day PI in agreement with previous studies by others (Jenkins *et al*., 1993). In the IM1 group, the peak period of oocyst output reached maximum on the 24th day PI and then decreased. The peak periods of oocysts output of the IM2 and IM3 groups reached to maximum in the 26th and 28th days of PI, respectively, then decreased (Table 1). Thus, compounds **1**, **2** and **3** caused a significant decrease ($P = 0.001$) of the oocyst output as compared to positive control but did not prevent it. No oocysts were found in the fecal samples from the IMR group throughout the experiment.

Serum and hepatic α-glucosidase and α-amylase

Serum level of α-glucosidase

As shown in Table 2, at the first day PI the specific activity of serum α-glucosidase was nearly similar in all experimental groups with non-significant change ($P = 0.1676$). At the 8th day PI, the positive control group displayed a significant elevation of the specific activity of serum α-glucosidase, from 20.38 ± 0.10 to 29.30 ± 0.11 ($P = 0.0019$). The specific activity of serum α–glucosidase of the INM group was elevated by 33% compared to the NINM group (negative control group). In addition, the protein content was significantly reduced ($P = 0.0042$). The specific activity of serum α-glucosidase in the NIM1, NIM2, and NIM3 groups was significantly reduced compared to the NINM group ($P = 0.0001$) by

Table 3. Serum α-amylase in all experimental groups of rabbits.

Groups/days PI	Specific activity of serum α-glucosidase (μM/min/mg protein) ± S.E.*				
	1	8	15	22	30
1: NINM	11.16±0.03	11.14 ± 0.28 [a]	10.96 ± 0.03 [a]	11.17 ± 0.05 [a]	11.24 ± 03[a]
2: INM	10.92±0.03	12.07 ± 0.36 [a]	12.65 ± 0.04[a]	13.48 ± 0.15[a]	13.79 ± 0.05[a]
3: NIM 1	10.68±0.02	7.64 ± 0.02 [b]	9.51 ± 0.07	10.19 ± 0.322	10.85 ± 0.07
4: NIM2	10.47±0.11	8.14 ± 0.04 [b]	9.80 ± 0.10	10.61 ± 0. 08	10.43 ± 0.01
5: NIM 3	10.66±0.03	8.96 ± 0.07 [b]	9.60 ± 0.03	10.11 ± 0.01	10.23 ± 0.08
6: IM 1	11.01±0.12	10.64 ± 0.03 [a]	10.72 ± 0.04 [a]	11.26 ± 0.03 [a]	11.87 ± 0.03 [a]
7: IM 2	11.20±0.31	10.72 ± 0.04 [a]	10.88 ± 0.15 [a]	11.49 ± 0.09 [a]	11.86 ± 0.03 [a]
8: IM 3	11.02±0.14	11.82 ± 0.04 [a]	10.85 ± 0.07 [a]	11.52 ± 0.03 [a]	12.16 ± 0.01 [a]
9: NIMR	11.08±0.02	11.07 ± 0.03 [a]	9.99 ± 0.05[a]	10.43 ± 0.07 [a]	10.66 ± 0.03 [a]
10: IMR	11.03±0.32	11.94 ± 0.03 [a]	10.89 ± 0.13 [a]	11.21 ± 0.04 [a]	10.37 ± 0.02 [a]

*The values are the means of five rabbits ± S.E. Means in the same column with different superscripts are significantly different ($P < 0.05$).

a value of 30, 24, and 26%, respectively. Groups IM1, IM2 and IM3 showed a slight elevation in the specific activity of serum α-glucosidase with a slight reduction of the protein content.

At the 15th day PI, the specific activity of serum α-glucosidase in the INM group was continuously elevated by 64% compared to the negative control group (P = 0.0001). Also, the protein content was significantly reduced. The specific activity of serum α-glucosidase in the IM1, IM2 and IM3 groups was elevated (P = 0.0005) by 14, 23 and 38%, respectively. On the other hand, the specific activity of serum α-glucosidase in the non-infected groups (groups 3, 5, 7 and 9) and the IMR group was not significantly changed (P = 0.1791). At the 22nd day PI, the specific activity of serum α-glucosidase in the INM group was highly elevated by a value of 99% compared to the negative control group (P = 0.0001). In the IM1, IM2 and IM3 groups, it was elevated by a 29, 38 and 47%, respectively (P = 0.0001). The specific activity of serum α-glucosidase in groups 3, 5, 7, 9 and 10 showed a non-significant difference from the negative control (P = 0.2729).

At the 30th day PI, the elevation of the specific activity of serum α-glucosidase in the INM, IM1, IM2 and IM3 groups compared to the negative control group was less than that observed at the 22nd day PI: 74, 18, 25 and 33%, respectively (Table 2). On the other hand, the specific activity of serum α-glucosidase in groups 3, 5, 7, 9 and 10 showed a non-significant change (P = 0.2002). This implies that the specific activity of serum α-glucosidase in the non-infected treated groups (NIM1, NIM2 and NIM3) is inhibited by compounds **1**, **2** and **3**. Also, these compounds lowered the elevation of the specific activity of serum α-glucosidase resulting from the hepatic coccidiosis in the infected treated groups (IM1, IM2 and IM3). The inhibition of coccidiosis-induced serum α-glucosidase in the IM1, IM2 and IM3 did not bring it to the normal level. The specific activity of the enzyme from the NIMR and IMR groups showed a non-significant change throughout the observation period ($P > 0.05$). The protein content in the serum of these two groups was slightly reduced.

Serum level of α-amylase

The specific activity of serum α-amylase from rabbits in all experimental groups was determined weekly throughout the observation period. As shown in table 3, the specific activity of serum α-amylase in the INM group was slightly elevated throughout the observation period by 8, 16, 21 and 19% at 8th, 15th, 22nd and 30th days PI, respectively, comparing to NINM as a negative control. In the IM1, IM2, and IM3 groups it displayed a non-significant change ($P = 0.460$) throughout the observation period, indicating that compounds **1**, **2** and **3** prevented effectively the slight increase of the activity induced by the infection. In the NIM1, NIM2 and NIM3 groups, the specific activity of the serum α-amylase was reduced at 8th day PI by 31, 27 and 20%, respectively. In groups IMR and NIMR, it was non-significantly changed ($P = 0.2002$) throughout the observation period.

Table 4. Hepatic α-glucosidase and α-amylase in different experimental groups of rabbits at 8th day PI.

Groups		Specific activity (μM/min/mg protein) ± S.E.*	
Number	Name	α-Glucosidase	α-Amylase
1	NINM	14.53 ± 0.638	1.113 ± 0.107
2	INM	17.41 ± 0.208[b]	1.194 ± 0.227[c]
3	NIM1	9.27 ± 0.254[a]	0.855 ± 0.226[a]
4	NIM2	10.15 ± 0.178[a]	0.808 ± 0.208[a]
5	NIM3	10.18 ± 0.146[a]	0.891 ± 0.216[a]
6	IM1	13.00 ± 0.432[c]	1.061 ± 0.192[c]
7	IM2	14.19 ± 0.323[c]	1.094 ± 0.147[c]
8	IM3	14.08 ± 0.248[c]	1.088 ± 0.266[c]
9	NIMR	12.95 ± 0.178[c]	1.023 ± 0.254[c]
10	IMR	13.73 ± 0.216[c]	1.114 ± 0.204[c]

*The values are the means of five rabbits ± S.E. Means in the same column with different superscripts are significantly different ($P < 0.05$).

Table 5. Hepatic α-glucosidase and α-amylase in different experimental groups of rabbits at 30th day PI.

Groups		Specific activity (μM/min/mg protein) ± S.E.*	
Number	Name	α-Glucosidase	α-Amylase
1	NINM	15.02 ± 0.308	1.116 ± 0.107
2	INM	29.13 ± 0.392[a]	1.294 ± 0.227[b]
3	NIM1	14.74 ± 0.334[b]	1.025 ± 0.226
4	NIM2	13.45 ± 0.409[b]	1.108 ± 0.108
5	NIM3	14.94 ± 0.324[b]	1.021 ± 0.216
6	IM1	19.48 ± 0.248[c]	1.145 ± 0.192[b]
7	IM2	21.14 ± 0.394[c]	1.197± 0.141[b]
8	IM3	22.37 ± 0.154[c]	1.218 ± 0.266[b]
9	NIMR	13.27 ± 0.0148[b]	1.023 ± 0.254
10	IMR	14.73 ± 0.294[b]	1.114 ± 0.204[b]

*The values are the means of five rabbits ± S.E. Means in the same column with different superscripts are significantly different ($P < 0.05$).

Hepatic α-glucosidase and α-amylase

At the 8th day PI (24 h post medication) and at 30th day PI (at the end of the observation period), the specific activity of α-glucosidase and α-amylase was determined in liver homogenateI (Tables 4 and 5). At the 8th day PI, the specific activities of hepatic α-glucosidase and α-amylase were elevated in the INM group by a value of 20 and 7%, respectively compared to the negative control group. In the IM1, IM2, IM3, IMR and NIMR groups, they showed a non-significant change ($P > 0.05$) compared to the NINM group. The specific activity of hepatic α-glucosidase was reduced by 36, 30 and 27%, in NIM1, NIM2 and NIM3 groups respectively (significant change compared to INM group, $P < 0.05$). Also, the specific activity of hepatic α-amylase was significantly reduced in the NIM1, NIM2 and NIM3 groups compared to INM group, $P < 0.05$ (Table 4).

At the 30th day PI, the specific activity of hepatic α-glucosidase in the INM, IM1, IM2 and IM3 groups was significantly elevated compared to the NINM group ($P < 0.05$) while for the hepatic α-amylase the elevation was non-significant ($P < 0.05$). The specific activities of hepatic α-glucosidase and α-amylase in the NIM1, NIM2, NIM3, NIMR and IMR groups displayed a non-significant changes ($P < 0.05$) compared to the NINM group (Table 5). So, both serum and hepatic α-amylases were slightly affected by hepatic coccidiosis. The target compounds **1**, **2** and **3** reduced the coccidiosis-induced elevation of serum and hepatic α-glucosidases and α-amylases to a level near that of the non-infected groups.

DISCUSSION

The ultimate goal of the current study was to establish effective anticoccidial compounds. The efficacy of the tested compounds has been confirmed by many criteria such as weight gain, oocyst counts, parasitological, biochemical, and histopathological findings. The biochemical studies involved serum and hepatic α-glucosidase and α-amylase. The compounds **1**, **2** and **3** had structural patterns similar to thioureas and arylthioureas. Similar compounds which have been used as anticoccidial drugs (Sabrina & Shoaa, 2001).

The toxic effect of the chemical groups or substituents in compounds **1**, **2** and **3** should be taken into consideration**.** Recently, we reported that LD_{50} values for compounds **1, 2** and **3** of 0.204, 0.107 and 0.155 mg/g body weight, respectively (Balba *et al*., 2011) and the persistence of those compounds in rabbits was approximately 120 h.

The presented results show that a field strain of *Eimeria stiedae* was successfully isolated from the gall bladder of infected field cases and sporulated in a similar manner to that described previously (Baghdadi & Al-Mathal, 2010). The field strain of *E. stiedae* was reisolated from experimental animals at 28th day PI. This reisolated strain was confirmed by microscopical examination and its oocysts were formed to be ellipsoidal (data not shown). This is consistent with the results reported by others (Pakes & Gerrity, 1994). It was reported that stored sporulated oocysts of *E. stiedae* were viable and infective for up to 18 months at 4°C (Drouet-Viard *et al*., 1997). In the present study, baby rabbits successfully infected by sporulated oocysts were treated with compounds **1**, **2** or **3** as anticoccidial factors and toltrazuril as a reference. The data for the positive control group, which developed a typical *E. stiedae* infection, are in agreement with that described previously (Singla *et al*., 2000).

The infected rabbits treated with the tested compounds were compared with those treated with the reference drug. The mortality decreased from 80% in the positive control group to 20, 40 and 40% in the groups treated with compounds **1**, **2** and **3,** respectively, compared to 0% for toltrazuril (reference drug). In addition, there was a decrease in oocyst output from 100% in positive control group to 11–54% in the groups treated by the tested compounds with respect to zero (no oocysts) for toltrazuril. Furthermore, the postmortem examination and histopathological pictures for the groups treated by the tested compounds were clear and the degree of infection was decreased by 36–73%. Therefore, the prevention of lesion in groups treated by these compounds was significant, especially for compound **1.**

Toltrazuril induced complete protection against *E. stiedae* infection in rabbits (Morton-Smith, 1997; Haberkorn & Mundt, 1999). The histopathological examination showed all stages of Eimeria protozoan except schizonts in treated animals with compounds **1**, **2** or **3** (not shown). This means these compounds prevent the formation of schizonts, probably through their inhibitory effect on the liver lysosomal α-glucosidase, which is involved in wall formation of schizonts or by other mechanisms which may be clarified by further. The results confirmed that toltrazuril induced complete protection against *E. stiedae* infection as discussed elsewhere (Morton-Smith, 1997; Haberkorn & Mundt, 1999), while compounds **1**, **2** and **3** caused only a partial protection.

In the present study, we report an increase in the activity of hepatic α-glucosidase and α-amylase in INM as compared to NINM rabbits. Also, we compare the activity of enzymes in IM1, IM2 and IM3 to NINM to detect the reduction of coccidiosis-induced activity. Moreover, the enzyme activities in IM1, IM2 and IM3 are also compared to NIM1, NIN2, NIN3 to confirm the inhibitory effect of the tested compounds. The significant induction of the specific activity of α-glucosidase in coccidiosis was significantly reduced in the groups treated with compounds **1**, **2** and **3** but not fully to normal values**.** This reduction indicates that α-glucosidase was significantly inhibited by compounds **1**, **2** and **3** which lead to decrease the lesion and lowered the side effect of hepat-

ic coccidiosis. On the other hand, the specific activity of serum α-amylase was slightly elevated in the INM group as explained in "Results". That non-significant elevation in hepatic coccidiosis was reduced by compounds **1**, **2** or **3** nearly to the normal level.

In general, the treatment with compound **1** in three successive oral doses on three consecutive days alleviated the clinical symptoms and decreased the mortality from 80% in the positive control group to 20%. Also, it brought about a significant decrease in oocyst output nine-fold and reduced the elevation of relative specific activity of serum α-glucosidase from 99% in positive control group to 29%. In addition, it decreases the lesion formation by 73%. Taken together, compound **1** offers a protection against hepatic coccidiosis in rabbits, whereas compound **2** and **3** were less effective. However, many synthetic compounds of different structure have been reported to show an anticoccidial activity against different species of *Eimeria* (Biftu *et al*., 2005; Yusa *et al*., 2006; Wang *et al*., 2009; Zhao *et al*., 2010).

In conclusion, diaryl derivatives of imidazole-thione and 1,2,4-triazole-thiol which incorporate the thiourea skeleton in their cyclic structure lower the coccidiosis-induced activity of α-glucosidase and offer variable degree of protection against that disease Among them, compound **1** seems to be a promising drug for the treatment of coccidiosis by *Eimeria stiedae*.

Acknowledgements

The authors thank the technical staff of Departments of Biochemistry at the Faculty of Science and the Edfina Faculty of Veterinary Medicine, Alexandria University for their assistance.

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