1 **Title**

2 Evaluation of the inhibitory effect of N-acetyl-L-cysteine on *Babesia* and *Theileria* parasites

3 **Running title**

4 In vitro effect of N-acetyl-L-cysteine on *Babesia* parasites

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22

23 **Competing interests**

24 The authors declare that they have no competing interests.

25 **ABSTRACT**

26 N-acetyl-L-cysteine is known to has antibacterial, antiviral, antimalarial, and antioxidant
27 activities. Therefore, the in vitro inhibitory effect of this hit was evaluated in the present study on
28 the growth of *Babesia* and *Theileria* parasites. The in vitro growth of *Babesia bovis*, *Babesia*
29 *bigemina*, *Babesia divergens*, *Theileria equi*, and *Babesia caballi* that were tested was
30 significantly inhibited ($P < 0.05$) by micromolar concentrations of N-acetyl-L-cysteine. The
31 inhibitory effect of N-acetyl-L-cysteine was synergistically potentiated when used in
32 combination with diminazene aceturate on *B. bovis* and *B. caballi* cultures. These results indicate
33 that N-acetyl-L-cysteine might be used as drug for the treatment of babesiosis, especially when
34 used in combination with diminazene aceturate.

35

36 **Keywords:** *Babesia*; *Theileria*; N-acetyl-L-cysteine; In vitro

37 **1. Introduction**

38 Babesiosis is a tick-transmitted disease, causes great economic losses in the bovine and
39 equine industries worldwide. *B. bovis* and *B. bigemina* are the main etiological agents of bovine
40 babesiosis, which has considerable impact on cattle health and productivity (Uilenberg et al.,
41 2006). Moreover, the significance of *Babesia divergens* is almost certainly underestimated for
42 livestock industry in Europe, with the possibility of human infection (Zintl et al., 2003). Equine
43 piroplasmosis caused by *T. equi* and *B. caballi*, is considered one of the most important
44 protozoan diseases affecting horses, mules, and donkeys (El-Sayed et al., 2015). Fever,
45 hemolytic anemia, and hemoglobinuria are the principal clinical manifestations of such infection
46 (Homer et al., 2000; Uilenberg et al., 2006). To date, the newly developed antibabesial drugs,
47 such as epoxomicin, ciprofloxacin, thiostrepton, rifampicin (AbouLaila et al., 2010, 2012),
48 pyronaridine tetraphosphate, luteolin, nimbolide, gedunin, and enoxacin (Rizk et al., 2015, 2016)
49 aren't available for use in the veterinary market. Moreover, some currently available antibabesial
50 drugs in the veterinary field either have toxic side effects, as imidocarb dipropionate, or have
51 developed a resistance to *Babesia* parasites from prolonged use, as in the case of diminazene
52 aceturate (Mosqueda et al., 2012). Therefore, developing new antibabesial drugs with low toxic
53 effects on animal and with no resistance from the parasite is urgently needed. To address this
54 concern, the antimalarial effect of N-acetyl-L-cysteine (NAC) has been reported (Quadros
55 Gomes et al., 2015). Additionally, many studies have validated the usage of N-acetyl-L-cysteine
56 in the treatment of respiratory diseases (Grandjean et al., 2000; Stey et al., 2000; Poole and Black,
57 2001) and acute renal diseases (Tepel et al., 2000). Also, this drug has antioxidant (Quadros

58 Gomes et al., 2015), antiviral (Geiler et al., 2010), and antibacterial effects (Buijtels and Petit,
59 2005). However, its potential as an antibabesial drug has not been examined. Therefore, the
60 antibabesial effect of N-acetyl-L-cysteine was evaluated in the current study against the in vitro
61 growth of bovine *Babesia* and equine *Babesia* and *Theileria* parasites.

62

63 **2. Materials and methods**

64 *2.1. Chemical reagents*

65 SYBR Green I (SGI) nucleic acid stain (Lonza, USA; 10,000x) was stored at -20°C and
66 thawed before use. A lysis buffer consisting of Tris (130 mM; pH 7.5), EDTA (10 mM), saponin
67 (0.016%; W/V), and TritonX-100 (1.6%; V/V) was prepared in advance and stored at 4°C.
68 Diminazene aceturate (Novartis, Japan) was used as a positive control drug. N-acetyl-L-
69 cysteine (from Sigma-Aldrich, Japan) were prepared as 100 mM stock solutions and stored at -
70 30° until use.

71

72 *2.2. In vitro cultivation of Babesia parasites*

73 A Texas strain of *B. bovis* (Hines et al., 1992), an Argentina strain of *B. bigemina* (Hotzel
74 et al., 1997), a German bovine strain of *B. divergens* (Lengauer et al., 2006), a US Department
75 of Agriculture (USDA) strain of *B. caballi* (Avarzed et al., 1997), and *T. equi* (Bork et al., 2004)
76 were cultivated in purified bovine or equine red blood cells (RBCs) using a microaerophilic
77 stationary-phase culture system (Igarashi et al., 1998). Briefly, Medium 199 was used for *B.*
78 *bovis*, *B. bigemina*, and *T. equi*, whereas RPMI 1640 medium was used for *B. divergens* and *B.*

79 *caballi* (both from Sigma-Aldrich, Japan). Media were supplemented with 40% normal bovine
80 serum (for bovine *Babesia* isolates) or 40% normal horse serum (for equine *Babesia* and
81 *Theileria* isolates), 60 U/ml penicillin G, 60 μg/ml streptomycin, and 0.15 μg/ml amphotericin B
82 (all three drugs from Sigma-Aldrich). Additionally, 13.6 μg of hypoxanthine (ICN Biomedicals,
83 Inc., USA) per ml was added to *T. equi* culture as a vital supplement. Cultures of parasitized
84 RBCs (pRBCs) were incubated at 37°C in an atmosphere of 5% CO_2, 5% O_2, and 90% N_2.
85
86 *2.3. In vitro Babesia fluorescence assay*
87 The in vitro *Babesia* fluorescence assay (*B*FA) was performed as previously mentioned
88 by Rizk et al. (2015 and 2016). *B. bovis*, *B. bigemina*, *B. divergens*, *T. equi*, and *B. caballi*
89 pRBCs were diluted with non-parasitized bovine or equine RBCs to start the assay at 1%
90 parasitemia. M199 medium was used for the culture of *B. bovis*, *B. bigemina*, and *T. equi*
91 parasites; RPMI 1640 medium was used for *B. divergens* and *B. caballi*. The specific medium for
92 each parasite was used alone or mixed with the indicated concentrations: 10, 5, 1, 0.5, and 0.25
93 µM for diminazene aceturate or 400, 100, 50, 10, and 0.5 µM for N-acetyl-L-cysteine were used
94 for the cultivation of pRBCs in double 96-well plates (Nunc, Denmark) at 2.5 % hematocrit
95 (HCT) for *B. bovis* and *B. bigemina* parasites or 5 % HCT for other *Babesia* and *Theileria* parasites
96 for four days without daily replacement of the medium in triplicate wells for each concentration of
97 the drug. Non-parasitized bovine or equine RBCs were loaded into each well in triplicate and
98 used as blank controls. First culture plate was used for evaluation the antibabesial effect of NAC.
99 On the fourth day of culture, 100 μl lysis buffer containing 2× SG I was added to each well on the

100 first plate that contain either 97.5 µl or 95 µl of the specific medium in the assay with 2.5% or
101 5% HCTs, respectively. Next, plates were incubated for 6 hours in a dark place at room
102 temperature, and fluorescence values were determined using a fluorescence plate reader
103 (Fluoroskan Ascent, Thermo Electron Informatics, Philadelphia, PA, USA) at 485 nm and 518
104 nm excitation and emission wavelengths. Gain values were set to 100. Each experiment was
105 repeated three times.

106

107 *2.4. Viability test*

108 The viability of bovine *Babesia* and equine *Babesia* and *Theileria* parasites was assessed
109 on the second plate after four days of treatments, as previously described by Rizk et al. (2016).
110 On the fourth day of culture treatment, 0.75 µl of the control or drug-treated infected RBCs from
111 the culture with 2.5% HCT was mixed with 1.75 µl of parasite-free RBCs. While, for *Babesia*
112 and *Theileria* culture with 5% HCT, 1.5 µl of each of the control and drug- treated (at the various
113 indicated concentrations) infected RBCs was mixed with 3.5 µl of parasite-free RBCs. Next,
114 pRBCs were suspended in fresh growth specific medium without drug supplementation. Then,
115 the plates were incubated at 37°C for the next four days without daily replacement of the
116 medium. Afterward, 100µl lysis buffer containing 2× SGI was added to each well on the plate and
117 fluorescence values were determined as previously mentioned. The amount of parasite DNA was
118 measured using a fluorescence spectrophotometer and used as an indicator of parasite
119 recrudescence. Each experiment was repeated three times.

120

121 *2.5. In vitro drug combination test*

122 To examine the possible synergistic effect between N-acetyl-L-cysteine and diminazene
123 aceturate, different concentrations of both drugs were tested in the in vitro cultures of *B. bovis*
124 and *B. caballi* as bovine and equine *Babesia* models, respectively. Combinations of both drugs
125 (M1, M2, M3, and M4) were based on the calculated IC_{50} values obtained from the in vitro *B*FA
126 (Table 1) and prepared as previously described (Salama et al., 2014) with some modifications.
127 Non-treated cultures and cultures containing only diminazene aceturate IC_{50} of *B. bovis* and *B.*
128 *caballi* parasites were used as controls. Each drug combination was applied in triplicate in three
129 separate trials over a period of four days using *B*FA. On the fourth day of culture, the
130 fluorescence values were calculated after adding lysis buffer containing 2× SG I to each well on
131 the 96-well plate.

132

133 *2.6. Statistical analysis*

134 A one-way ANOVA test (GraphPad Prism version 5.0 for Windows; GraphPad Software,
135 Inc., San Diego, CA, USA) was used for calculating the significant differences between
136 examined groups. A P value < 0.05 was considered to be statistically significant. For a viability
137 test, the regrowth of the parasite was determined based on the statistically significant differences
138 between the drug-treated and the positive-control group (Rizk et al., 2016).

139

140

141

3. Results

3.1. The in vitro inhibitory effect and viability test

The in vitro growth of *B. divergens* and *B. caballi* was significantly inhibited ($P < 0.05$) by 10-µM and 0.5-µM of N-acetyl-L-cysteine, respectively. Meanwhile, 100-µM treatments of N-acetyl-L-cysteine were sufficient for the significant inhibition ($P < 0.05$) of the in vitro growth of *B. bigemina*. On the contrary, the in vitro growth of *B. bovis* and *T. equi* was significantly inhibited ($P < 0.05$) by 400-µM treatments of N-acetyl-L-cysteine (Fig. 1). *B. divergens* and *B. caballi* were the *Babesia* parasites most susceptible to the in vitro inhibition effect of N-acetyl-L-cysteine (Table 2). Subsequently, to determine the ability of N-acetyl-L-cysteine to suppress the regrowth of *Babesia* and *Theileria* parasites after four days of in vitro treatment, viability test was performed. There was a statistically significant inhibition ($P < 0.05$) in the regrowth of *B. bovis*, *B. bigemina*, and *T. equi* parasites treated with 400 µM N-Acetyl-L-cysteine in comparison with non-treated parasites. Meanwhile, such inhibition in *B. caballi* or *B. divergens* parasites was observed at 10 µM N-Acetyl-L-cysteine (Table 3). On contrary, treatment with the lowest concentration of diminazene aceturate (0.25 µM) was sufficient for statistically significant inhibition ($P < 0.05$) the regrowth of bovine *Babesia* and equine *Babesia* and *Theileria* parasites in comparison with non-treated parasites (Table 4).

3.2. Drug combination testing

Combination therapy from N-acetyl-L-cysteine and diminazene aceturate enhanced the inhibitory effect in *B. bovis* and *B. caballi* in vitro cultures even with combination M4, which

163 consisted of 1/2 the IC_{50s} of both drugs (Table 5). Furthermore, treatment of *B. caballi* parasites
164 with combined therapy significantly enhanced the inhibition of parasite growth as compared with
165 those observed with the diminazene aceturate IC_{50} even at M3, which represents 1/2 and 3/4 the
166 IC_{50s} of N-acetyl-L-cysteine and diminazene aceturate, respectively (Table 5).

167

168 **4. Discussion**

169 N-acetyl cysteine is a potent antioxidant agent and a precursor of the hepatic synthesis of
170 reduced glutathione (GSH) (Quadros Gomes et al., 2015). Recently, the role of reactive oxygen
171 and nitrogen species (ROS and RNS) in the pathogenesis of parasitic infections has been
172 demonstrated (Oliveira et al., 2002; Bildik et al., 2004). Indeed, parasitic infestations are
173 accompanied by various oxidant-generating enzymes, which subsequently activate the
174 production of a variety of inflammatory cells to kill intra-cellular and extra-cellular parasites
175 (Kocyigit et al., 2005). These ROS and RNS are generated mainly to attack invading
176 microorganisms by nitration, oxidation, and chlorination reactions (Ince et al., 2010). However,
177 excess amounts of such reactions can injure host cells and damage tissue (Kocyigit et al., 2005;
178 Ince et al., 2010). For *Babesia*, infection increases the production of free radicals and oxidative
179 stress markers, including malondialdehyde (MDA), protein carbonyl content (PCO), and the
180 plasma concentration of nitric oxide metabolites (NOx), with decreased total antioxidant activity
181 (AOA) and reduced glutathione (GSH) (Küçükkurt et al., 2014). Therefore, this study evaluated
182 the inhibitory effect of NAC against the in vitro growth of *Babesia* and *Theileria* parasites. *B.*
183 *divergens* and *B. caballi* were the most sensitive to N-acetyl-L-cysteine, followed by *B. bovis*, *B.*

184 *bigemina*, and *T. equi*. The IC_{50} values of N-acetyl-L-cysteine for *Babesia* and *Theileria*
185 parasites were higher than those of previously developed antibabesial drugs listed in Table 6. On
186 the other hand, the IC_{50} values of N-acetyl-L-cysteine for *Babesia* and *Theileria* parasites were
187 lower than those of allicin against bovine *Babesia* and equine *Babesia* /*Theileria* parasites
188 (Salama et al., 2014), metronidazole against *B. gibsoni* (Matsuu et al., 2008) and of clodinafop-
189 propargyl against *B. bovis* and *B. bigemina* (Bork et al., 2003) as shown in Table 6. The IC_{50}
190 values of N-acetyl-L-cysteine for *B. divergens* and *B. caballi* parasites were nearly similar to
191 those of clindamycin phosphate on *B. gibsoni* (Matsuu et al., 2008). Therefore, a viability test
192 was applied, and the results showed N-acetyl-L-cysteine's ability to suppress the regrowth of
193 *Babesia* and *Theileria* parasites at lower concentrations as compared with those observed with
194 allicin (Salama et al., 2014). Next, combination therapy involving the candidate with diminazene
195 aceturate was performed on the in vitro growth of *B. bovis* and *B. caballi*. The results confirmed
196 the potential antibabesial effect of N-acetyl-L-cysteine, especially when administrated
197 simultaneously with diminazene aceturate. In the same way, the administration of high and
198 multiple doses of commonly used antibabesial drugs is required to produce their maximal
199 inhibitory effects. Subsequently, drug toxicity and parasite resistance emerge, which suggests the
200 use of N-acetyl-L-cysteine in combination with low dose of diminazene aceturate for babesiosis
201 treatment. To date, although the in vitro growth of *Babesia* and *Theileria* parasites was
202 moderately inhibited in the presence of a high concentration of N-acetyl-L-cysteine, its
203 inhibitory effects were enhanced when used in combination with diminazene aceturate. Taken
204 together, the antibabesial effect of this hit with its potential role as mucolytic (Poole and Black,

205 2001), nephroprotective (Tepel et al., 2000), antioxidant (Quadros Gomes et al., 2015), and
206 antibacterial agents (Buijtels and Petit, 2005) encourage us to recommend using this candidate to
207 treat babesiosis under field condition.

208 In conclusion, N-acetyl-L-cysteine showed potential antibabesial activity, particularly
209 against *B. divergens* and *B. caballi* parasites, and its inhibitory effect was enhanced when
210 combined with diminazene aceturate. These results indicate that N-acetyl-L-cysteine might be
211 useful as a drug for treating babesiosis, particularly when used in combination with diminazene
212 aceturate. However, other studies are needed to evaluate the inhibitory effect of N-acetyl-L-
213 cysteine/diminazene aceturate combination therapy in mice infected by *B. microti*.

214

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310

311 **Figure Legend**

312 **Fig. 1. Correlation between relative fluorescence units (RFUs) and the log concentrations of**
313 **N-acetyl-L-cysteine (nM) on bovine *Babesia* parasites on the fourth day of treatment.** Each
314 value represents the mean of triplicate wells ± standard deviation (SD) after the subtraction of
315 background fluorescence for non-parasitized RBCs. Statistically significant differences are
316 indicated by asterisks (*$P < 0.05$) between the drug-treated cultures and the control cultures.

317

318 **Fig. 2. Correlation between relative fluorescence units (RFUs) and the log concentrations of**
319 **N-acetyl-L-cysteine (nM) on equine *Babesia* and *Theileria* parasites on the fourth day of**
320 **treatment.** Each value represents the mean of triplicate wells ± standard deviation (SD) after the

321 subtraction of background fluorescence for non-parasitized RBCs. Statistically significant
322 differences are indicated by asterisks ($*P < 0.05$) between the drug-treated cultures and the
323 control cultures.

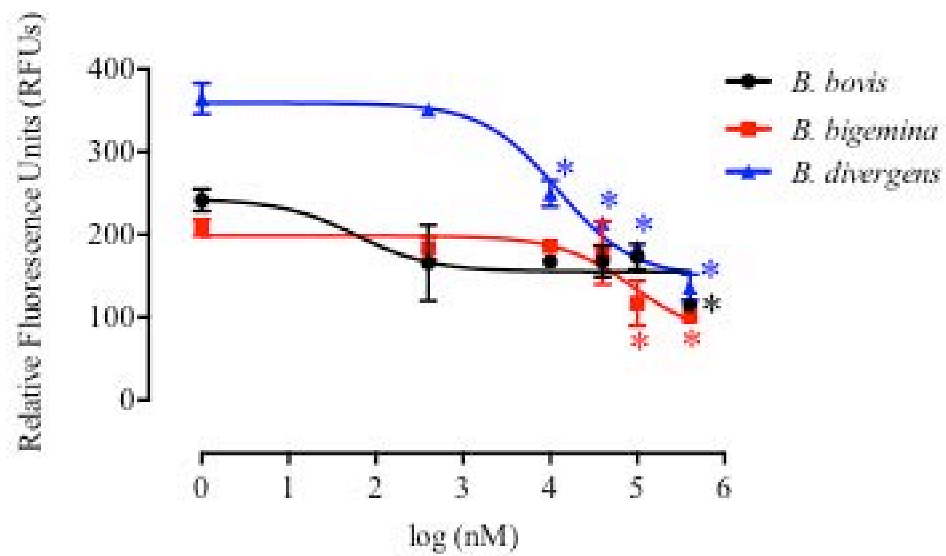

Fig. 1. Rizk et al., 2017

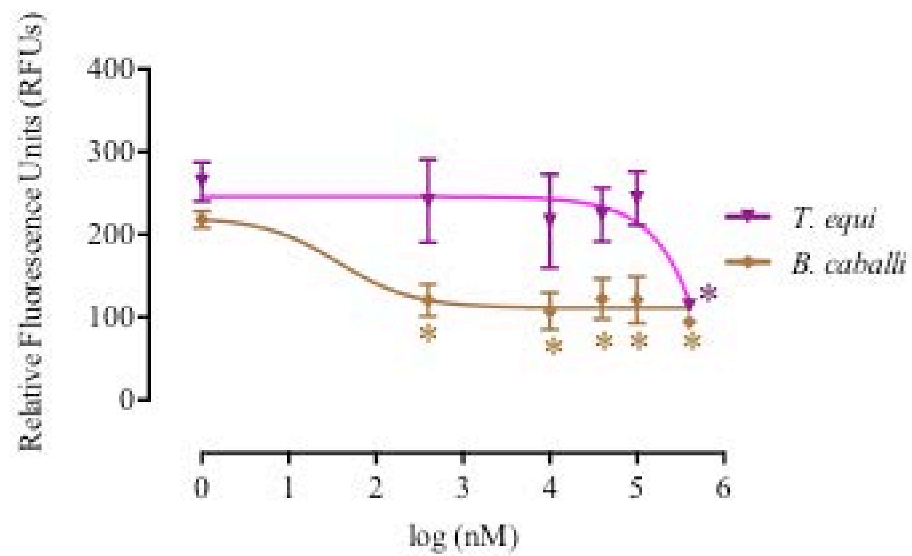

Fig. 2. Rizk et al., 2017

Table 1. Concentrations of N-Acetyl-L-cysteine combined with diminazene aceturate applied to the cultures of *B. bovis* and *B. caballi* parasites.

	IC_{50}	
	N-Acetyl-L-cysteine	**Diminazene aceturate**
M1	¾	¾
M2	¾	½
M3	½	¾
M4	½	½

[a] Combinations were based on the calculated IC_{50} values obtained from the *in vitro* fluorescence- based assay

Table 2. IC_{50} values of N-Acetyl-L-cysteine and diminazene aceturate drugs evaluated for *Babesia* and *Theileria* parasites

Drug	IC_{50} values (μM) [a]	
	N-Acetyl-L-cysteine	**Diminazene aceturate**
B. bovis	332.11 ± 33.11	0.48 ± 0.09
B. bigemina	229.16 ± 37.51	0.21 ± 0.06
B. divergens	117.23 ± 8.04	0.14 ± 0.03
T. equi	349.05 ± 16.21	0.72 ± 0.05
B. caballi	114.53 ± 28.58	0.005 ± 0.0002

[a] IC_{50} values for each drug were calculated on the fourth day of the in vitro culture using *Babesia* fluorescence assay (*B*FA) in three separate experiments. Each drug concentration was made in triplicate in each experiment, and the final obtained IC_{50}s were the mean ± SD of values obtained from three separate experiments.

Table 3. Viability test results of N-Acetyl-L-cysteine drug evaluated for *Babesia* and *Theileria* parasites

Drug	Drug concentrations (µM) [a]					
	PC	**400**	**100**	**50**	**10**	**0.5**
B. bovis	232.72 ± 96.60	22.07 ± 8.42*	160.92 ± 43.71	165.32 ± 40.81	176.77 ± 15.85	186.57 ± 31.94
B. bigemina	218.19 ± 68.59	58.92 ± 15.37*	114.99 ± 26.25	175.74 ± 39.07	184.17 ± 15.22	182.09 ± 14.54
B. divergens	367.17 ± 51.73	34.51 ± 12.55*	70.37 ± 15.02*	91.50 ± 14.63*	85.12 ± 17.28 *	291.47 ± 36.18
T. equi	301.70 ± 83.29	72.58 ± 26.98*	202.55 ± 48.71	200.72 ± 20.68	210.15 ± 62.72	241.12 ± 57.87
B. caballi	200.91 ± 28.74	95.88 ± 4.70*	105.21 ± 26.65*	114.66 ± 23.04*	119.01 ± 24.95*	158.73 ± 28.02

[a] Each value was calculated using fluorescence- based assay in three separate experiments. Each concentration of the drug was made in triplicate in each experiment, and the final obtained fluorescence value represents the mean and standard deviation (SD) of three separate experiments after subtraction of the background fluorescence for non-parasitized RBCs and multiplied by 100. * $P < 0.05$ statistically significant differences between the N-Acetyl-L-cysteine -treated group and control groups.

Table 4. Viability test results of diminazene aceturate drug evaluated for *Babesia* and *Theileria* parasites

Drug	Drug concentrations (µM) [a]					
	PC	**10**	**5**	**1**	**0.5**	**0.25**
B. bovis	133.82 ± 87.43	0.36 ± 0.02*	2.57 ± 0.06*	1.44 ± 0.98*	9.29 ± 4.30*	11.44 ± 3.07*
B. bigemina	137.10 ± 56.87	1.25 ± 0.57*	10.78 ± 6.45*	17.87 ± 8.09*	20.62 ± 4.85*	24.91 ± 6.88*
B. divergens	394.61 ± 30.47	14.30 ± 0.76*	25.99 ± 2.95*	30.60 ± 5.14*	41.34 ± 12.70*	43.35 ± 3.75*
T. equi	337.11 ± 90.17	6.59 ± 1.82*	10.60 ± 3.21*	15.71 ± 5.03*	53.23 ± 13.82*	52.96 ± 9.82*
B. caballi	151.03 ± 48.19	0.59 ± 0.08*	9.19 ± 3.74*	12.18 ± 2.15*	17.31 ± 3.60*	23.93 ± 8.87*

[a] Each value was calculated using fluorescence- based assay in three separate experiments. Each concentration of the drug was made in triplicate in each experiment, and the final obtained fluorescence value represents the mean and standard deviation (SD) of three separate experiments after subtraction of the background fluorescence for non-parasitized RBCs and multiplied by 100. * $P < 0.05$ statistically significant differences between the diminazene aceturate -treated group and control groups.

Table 5. Growth inhibition effect of diminazene aceturate and N-Acetyl-L-cysteine combinations on *B. bovis* and *B. caballi* parasites on the fourth day of treatment

Group	Fluorescence value (mean ± SD) [a]	
	B. bovis	***B. caballi***
Control	234.55 ± 19.77	259.43 ± 21.89
Diminazene aceturate IC_{50}	111.62 ± 7.45	143.88 ± 12.45
M1	111.87 ± 30.10*	159.15 ± 16.02*
M2	152.52 ± 36.55*	155.28 ± 14.95*
M3	91.27 ± 28.77*	113.53 ± 11.10**
M4	122.74 ± 33.94*	125.68 ± 11.55*

[a] Each value was calculated using fluorescence- based assay in three separate experiments. Each concentration of the drug combination was made in triplicate in each experiment, and the final obtained fluorescence value represents the mean and standard deviation (SD) of three separate experiments after subtraction of the background fluorescence for non-parasitized RBCs and multiplied by 100. * $P < 0.05$ statistically significant differences between the combined-drug-treated group and control groups. ** $P < 0.05$ statistically significant differences between the combined-drug-treated group and both the diminazene aceturate and control groups.

Table 6. IC_{50} values of previously developed antibabesial drugs

Antibabesial drug	IC_{50} (µM)						References
	Parasite						
	B. bovis	*B. bigemina*	*T. equi*	*B. caballi*	*B. divergens*	*B. gibsoni*	
Pyronaridine tetraphosphate	4.31	0.70	2.14	0.44	4.25	ND	(Rizk et al., 2015, 2016)
Luteolin	5.20	0.30	2.40	0.96	2.50	ND	(Rizk et al., 2015, 2016)
Nimbolide	6.17	2.96	6.71	0.99	ND	ND	(Rizk et al., 2015)
Gedunin	17.86	19.95	12.40	11.18	ND	ND	(Rizk et al., 2015)
Enoxacin	38.04	18.00	24.70	12.63	ND	ND	(Rizk et al., 2015)
MMV396693	0.40	0.059	0.057	0.048	ND	ND	(Van Voorhis et al., 2016)
MMV666093	0.05	0.083	0.21	0.13	ND	ND	(Van Voorhis et al., 2016)
MMV006706	0.75	0.10	0.06	0.38	ND	ND	(Van Voorhis et al., 2016)
MMV073843	0.06	0.06	0.63	0.04	ND	ND	(Van Voorhis et al., 2016)
MMV665875	0.07	0.08	0.10	0.48	ND	ND	(Van Voorhis et al., 2016)
Clofazimine	4.50	3.00	0.29	4.30	ND	ND	(Tuvshintulga et al., 2016)
Fusidic acid	144.80	17.30	56.25	33.30	ND	ND	(Salama et al., 2013)
Epoxomicin	0.021	0.004	0.021	0.009	ND	ND	(AbouLaila et al., 2010)
Ciprofloxacin	8.30	15.80	2.50	2.70	ND	ND	(AbouLaila et al., 2012)
Thiostrepton	11.50	8.20	6.40	2.70	ND	ND	(AbouLaila et al., 2012)
Rifampicin	12.00	8.30	4.10	4.70	ND	ND	(AbouLaila et al., 2012)
Allicin	818	675	742	470	ND	ND	(Salama et al., 2014)
Metronidazole	ND	ND	ND	ND	ND	>1000	(Matsuu et al., 2008)
Clodinafop-propargyl	265	390	ND	ND	ND	ND	(Bork et al., 2003)
Clindamycin phosphate	126.6	206	27.2	4.7	ND	108.83	(Matsuu et al., 2008; AbouLaila et al., 2012)

ND, not detected