







ORIGINAL ARTICLE

Evaluation of the *in vitro* and *in vivo* inhibitory effects of *Artemisia herba-alba* against the growth of piroplasm parasites

Rasha Eltaysh^{1,2} , Mohamed Abdo Rizk^{1,3} , Shima Abd El-Salam El-Sayed^{1,4} , Khaled Abouelnasr⁵ , Abdelnaser Ahmed Abdallah⁶ , Ikuo Igarashi¹ 

¹National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan

²Department of Pharmacology, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt

³Department of Internal Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt

⁴Department of Biochemistry and Chemistry of Nutrition, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt

⁵Department of Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt

⁶Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt

ABSTRACT

Objective: The effect of *Artemisia herba-alba* methanolic extract monotherapy and combination therapies on the *in vitro* growth of several *Babesia* and *Theileria* parasites *in vitro* and mice was investigated in this study.

Materials and Methods: Fluorescence assay using SYBR Green I stain was used to evaluate the antibabesial efficacy inhibitory of *A. herba-alba* either *in vitro* or *in vivo*. Hematological parameters in the treated mice were analyzed using a Celltac MEK-6450 computerized hematology analyzer.

Results: *Artemisia herba-alba* reduced the growth of *Babesia bovis*, *Babesia bigemina*, *Babesia divergens*, *Theileria equi*, and *Babesia caballi* *in vitro* in a dose-dependent manner. The *in vitro* inhibitory impact of *A. herba-alba* on *B. divergens* and *B. caballi* cultures was amplified when combined with either diminazene aceturate (DA). In *B. microti*-infected mice, a combination therapy consisting of *A. herba-alba* and a low DA dose inhibited *B. microti* growth significantly ($p < 0.05$) better than treatment with 25 mg kg⁻¹ DA.

Conclusions: These data show that *A. herba-alba*, when paired with a modest DA dose, could be a promising medicinal plant for babesiosis treatment.

ARTICLE HISTORY

Received February 09, 2022

Revised March 29, 2022

Accepted April 02, 2022

Published June 26, 2022

KEYWORDS

Babesia; *Theileria*; *Artemisia herba-alba*; *in vitro*; *in vivo*; combination therapy



© The authors. This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 License (<http://creativecommons.org/licenses/by/4.0>)

Introduction

Babesia and *Theileria* are tick-borne parasites that infect animals' erythrocytes, causing enormous economic losses in the agricultural industry and worldwide trade [1,2]. Clinical indicators of this infection include fever, malaise, jaundice, hemoglobinuria, and death [3,4]. The infection is mainly caused by either *Babesia bovis* (*B. bovis*) and *Babesia bigemina* (*B. bigemina*) in cattle [3] or *Theileria equi* (*T. equi*) and *B. caballi* in horses [5]. Because the inhibitory effects of recently developed antibabesial drugs should be evaluated in laboratory animals before they are used in the field, and because there are no acceptable laboratory

experimental animals for bovine and equine *Babesia* infections, a rodent *Babesia* model infected with *B. microti* or a gerbil infected with *Babesia divergens* is used for drug evaluation [6,7].

For many years, the standard therapies for babesiosis were diminazene aceturate (DA) and imidocarb dipropionate [8,9]. However, they have significant drawbacks, such as a long time to remove tissue, toxicity, and, in the case of DA, unavailability in certain regions [9]. Furthermore, new research has revealed that *Babesia* parasites may develop DA resistance [10,11]. As a result, finding more effective and safer antipiroplasm drugs has become a top objective.

Correspondence Mohamed Abdo Rizk ✉ dr_moh_abdo2008@mans.edu.eg 📧 National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan. **Correspondence** Ikuo Igarashi ✉ igarcpmi@obihiro.ac.jp 📧 National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan.

How to cite: Eltaysh R, Rizk MA, El-Sayed SAE, Abouelnasr K, Abdallah AA, Igarashi I. Evaluation of the *in vitro* and *in vivo* inhibitory effects of *Artemisia herba-alba* against the growth of piroplasm parasites. J Adv Vet Anim Res 2022; 9(2):267–274.

Natural phytochemicals could be a potential alternative in this scenario. In the same vein, *Artemisia herba-alba*, commonly known as desert or white wormwood, is used in folk medicine to treat various diseases [12,13]. Several studies have reported the wide pharmacological activities of *A. herba alba* as antidiabetic, antimicrobial, antimalarial, acaricidal [12,14,15], anticancer, and antioxidant [16,17]. However, *A. herba-alba* extract's antibabesial efficacy is yet to be determined. As a result, in the current investigation, we evaluated the antipiroplasm of *A. herba-alba* against the growth of *B. bovis*, *B. bigemina*, *B. divergens*, *B. caballi*, and *T. equi* *in vitro*, and *B. microti* in mice.

Materials and Methods

Ethical approval

The Animal Care and Use Committee at Obihiro University of Agriculture and Veterinary Medicine approved all of the study's experimental protocols (Approval No. 27-65). The trials followed the Fundamental Guidelines for the Proper Conduct of Animal Experiments and Related Activities at Academic Research Institutions published by the Ministry of Education (Culture, Sports, Science, and Technology, Japan).

In vitro growth inhibition assay

A. herba-alba was dissolved in 50 ml of 99.8% methanol (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and incubated at 30°C for 3 days [18]. The finished product was filtered using Whatman filter paper No. 1 and a rotary evaporator (BUICHI®RotavaporR-200/205, Flawil, Switzerland), and a freeze-drying vacuum system (Labconco, Kansas City, MO, USA) [19,20]. The crude extract was then dissolved in dimethyl sulfoxide (DMSO) at 100 mg/ml. *A. herba-alba* methanolic extract toxicity to bovine and equine erythrocytes was assessed using 25 mg/ml as previously published study [21].

The chemotherapeutic efficacy of *A. herba-alba* against *B. bovis* (Texas strain) [22], *B. bigemina* (Argentina strain) [23], *B. divergens* (German strain) [24], *B. caballi* [25], and *T. equi* (U.S. Department of Agriculture) [25] was investigated in the current study by a fluorescence assay using a nucleic acid stain SYBR Green I (Lonza, Rockland, ME) [8,22]. The concentrations of *A. herba-alba* utilized ranged from 0.025 to 30 mg/ml. The *in vitro* study used DA, a routinely used antibabesial medication, as a positive control agent with concentrations ranging from 0.1 to 10 µg/ml [22]. Cultures without the drug and cultures with only DMSO (0.3% for *A. herba-alba*) and DDW (0.02% for DA) served as negative experimental controls (Wako Pure Chemical Industries, Ltd., Osaka, Japan). RBCs infected with 1% parasitemia of bovine and equine Babesia/*Theileria* parasites were

cultured in 96-well plates for 4 days without daily medium replacement, using 2.5% hematocrit (HCT) for *B. bovis* and *B. bigemina* parasites and 5% HCT for other Babesia and *Theileria* parasites, as previously established [22,24]. All screened parasites' *in vitro* regrowth after ceasing *A. herba-alba* therapy was monitored using a viability assay, as described earlier in our study [24].

The combination therapy of *A. herba-alba* and DA was tested against *in vitro* cultures of bovine Babesia and horse piroplasm parasites having the highest IC₅₀ values, for *B. bovis* and *T. equi*, as previously detailed [6,26]. All *in vitro* tests were carried out three times.

In vivo chemotherapeutic effect of A. herba-alba

The *A. herba-alba* *in vivo* inhibition assay for *B. microti* (Munich strain) [27] in 25 female BALB/c mice (CLEA Japan, Tokyo, Japan) was performed twice using a fluorescence assay [28]. Five groups of mice (five animals per group) were employed. Simultaneously, with the drug inhibitory effect evaluation, 10 µl of blood was drawn from each mouse's tail every 4 days to examine hematological parameters using a Celltac MEK-6450 computerized hematology analyzer (Nihon Kohden Corporation, Tokyo, Japan).

Statistical analysis

A one-way analysis of variance test was used in GraphPad Prism to discover significant differences between the analyzed groups (GraphPad Software, Inc., San Diego, CA). Statistical significance was defined as *p*-value less than 0.05.

Results

Artemisia herba-alba effectively suppressed the in vitro growth of piroplasm

According to the computed IC₅₀s, *A. herba-alba* has the most significant impact on the growth of *T. equi* and *B. bigemina*, followed by *B. bovis* (Table 1). 0.025 mg/ml *A. herba-alba* effectively suppressed the development of *B. bigemina* and *B. bovis* *in vitro* (*p* < 0.05) (Fig. 1). 0.10 mg/ml *A. herba-alba* was found to be effective in inhibiting the growth of *T. equi* (Fig. 1). Furthermore, 0.50 mg/ml *A. herba-alba* treatments significantly reduced the development of *B. divergens* and *B. caballi* (*p* < 0.05) (Fig. 1).

Theileria equi and *B. bigemina* *in vitro* were suppressed at doses of 0.5 and 1 mg/ml, respectively, in the following viability test (Table 2). The parasite regrowth was suppressed *in vitro* when *B. bovis* was given 5 mg/ml *A. herba-alba* (Table 2). With 10 mg/ml *A. herba-alba*, *B. caballi* regrowth began to be reduced (Table 2). The lack of a significant difference (*p* > 0.05) between the DMSO-treated

Table 1. IC₅₀ values of *Artemisia herba-alba*, diminazene aceturate and other previously used herbal antibabesial drugs evaluated for bovine *Babesia* and equine *Babesia* and *Theileria* parasites

Organism	IC ₅₀ (µg/ml) ^a			
	<i>Artemisia herba-alba</i>	Diminazene aceturate	<i>Zingiber officinale</i> rhizome ^b	Turmeric (<i>Curcuma longa</i>) ^c
<i>B. bovis</i>	412.75±29.05	0.16 ± 0.02	588 ± 23.80	830 ± 78
<i>B. bigemina</i>	392.81±31.42	0.08 ± 0.003	14800 ± 1240	ND
<i>B. divergens</i>	566.56±37.33	0.046± 0.007	ND	375 ± 55
<i>T. equi</i>	303.50±26.50	0.28 ± 0.01	39350 ± 1340	1405 ± 575
<i>B. caballi</i>	633.33±34.11	0.012 ± 0.003	356.05 ± 34.71	720 ± 90

^a IC₅₀ values for *Artemisia herba-alba* and diminazene aceturate were calculated on the fourth day based on the growth inhibitions determined using fluorescence- based assay in three separate experiments. Each drug concentration was made in triplicate in each experiment, and the final obtained IC₅₀ represent the mean and standard deviation of three separate experiments. ND, not detected. ^b The IC₅₀ was reported in previous study (Rizk et al., 2021a). ^c The IC₅₀ was reported in previous study (Rizk et al., 2021b).

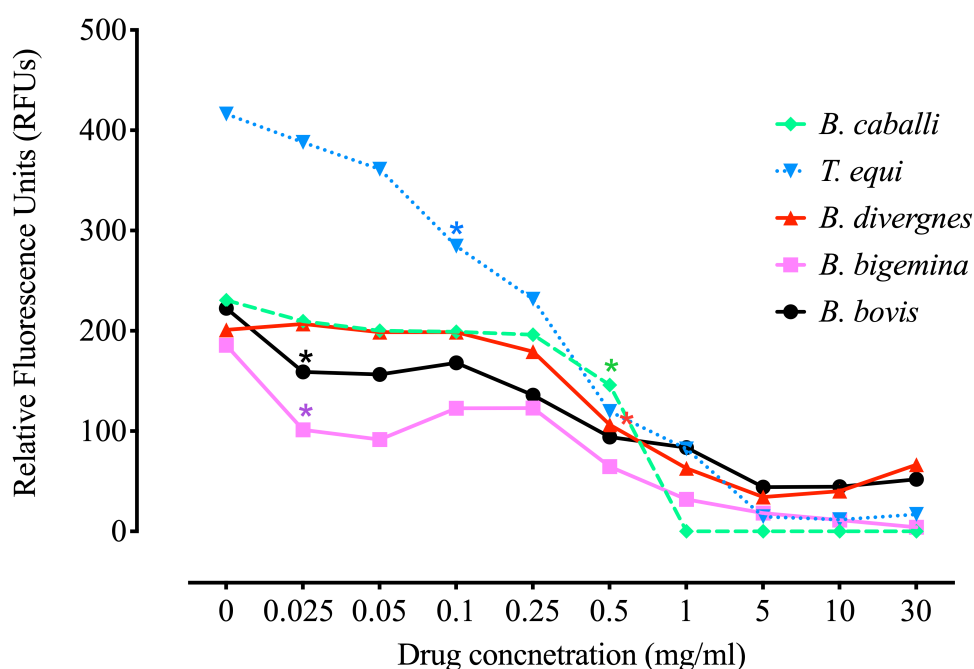


Figure 1. Antiprotozoal efficacy of *Artemisia herba-alba*. Each value represents the mean of three experiments. Asterisks indicate that the treated and control cultures differ significantly ($p < 0.05$).

positive control well and the untreated wells shows that the diluent did not affect the efficacy of the *A. herba-alba* methanolic extract. Furthermore, compared to nontreated erythrocytes, pretreatment of erythrocytes with a high dose of *A. herba-alba* methanolic extract at 25 mg/ml did not affect parasite growth pattern or erythrocyte morphology (data not shown).

DA enhanced the in vitro efficacy of *A. herba-alba*

On *B. divergens* and *B. caballi*, different combinations of *A. herba-alba* and DA were tested. On the growth of *B. divergens*, highest concentration of *A. herba-alba* (0.75 IC₅₀) demonstrated a synergistic interaction with high doses

of DA (0.75 and 0.50 IC₅₀) (Table 3). Low doses of *A. herba-alba* had an additive effect with DA in inhibiting the proliferation of bovine *Babesia* and *B. caballi* parasites (Table 3). Such findings validated *A. herba-alba*'s potential anti-*B. divergens* effect, especially when given in large doses combined with the regularly used antibabesial medication, DA.

Artemisia herba-alba suppressed the growth of *B. microti* in mice

A. herba-alba was tested in mice for its ability to suppress *B. microti* in vivo. Within the presence of 500 mg kg⁻¹ *A. herba-alba* monotherapy, the greatest fluorescence values

Table 2. Viability test results of *Artemisia herba-alba* evaluated for *Babesia* and *Theileria* parasite

Drug	Drug concentrations (mg/ml) ^a								
	0.025	0.05	0.1	0.25	0.5	1	5	10	30
<i>B. bovis</i>	+	+	+	+	+	+	-	-	-
<i>B. bigemina</i>	+	+	+	+	+	-	-	-	-
<i>B. divergens</i>	+	+	+	+	+	+	+	-	-
<i>T. equi</i>	+	+	+	+	-	-	-	-	-
<i>B. caballi</i>	+	+	+	+	+	+	+	-	-

^a Each value was calculated using fluorescence assay in three separate experiments. Each concentration of the drug was made in triplicate in each experiment. + = viable; - = dead

Table 3. Two drug interactions of *Artemisia herba-alba* in combination with diminazene aceturate on the *in vitro* growth of *Babesia divergens* and *Babesia caballi* parasites

Parasite	C ^a	FIC _{D1}	FIC _{D2}	ΣFIC	Degree of interaction ^b
<i>B. divergens</i>	0.75 + 0.75	0.21	0.11	0.32	Synergetic
	0.75 + 0.50	0.11	0.21	0.32	Synergetic
	0.75 + 0.25	0.31	0.42	0.73	Additive
	0.50 + 0.75	0.33	0.41	0.74	Additive
	0.50 + 0.50	0.31	0.39	0.7	Additive
	0.50 + 0.25	0.41	0.51	0.92	Additive
	0.25 + 0.75	0.34	0.62	0.96	Additive
	0.25 + 0.50	0.45	0.23	0.68	Additive
	0.25 + 0.25	0.46	0.47	0.93	Additive
	0.75 + 0.75	0.22	0.41	0.63	Additive
<i>B. caballi</i>	0.75 + 0.50	0.31	0.55	0.86	Additive
	0.75 + 0.25	0.41	0.33	0.74	Additive
	0.50 + 0.75	0.31	0.45	0.76	Additive
	0.50 + 0.50	0.41	0.41	0.82	Additive
	0.50 + 0.25	0.33	0.41	0.74	Additive
	0.25 + 0.75	0.36	0.38	0.74	Additive
	0.25 + 0.50	0.41	0.33	0.74	Additive
	0.25 + 0.25	0.39	0.44	0.83	Additive

^a C refer to the different concentrations of *Artemisia herba-alba* in combination with diminazene aceturate. ^b The degree of drug interaction was determined based on the following fractional inhibitory concentration (FIC) index: ≤ 0.5 (synergetic), and > 0.5–1 (additive). FIC_{D1} refers to the fractional inhibitory concentration of *Artemisia herba-alba*. FIC_{D2} refers to the fractional inhibitory concentration of diminazene aceturate. Three independent tests were performed after each combination was loaded in triplicate wells in 96-well plates. FIC_{D1+} inhibitory effect of D₁ in presence of D₂/inhibitory effect of D₁ alone, FIC_{D2+} inhibitory effect of D₂ in presence of D₁/inhibitory effect of D₂ alone, ΣFIC= FIC_{D1+} FIC_{D2}

within the *A. herba-alba*-treated groups reached a mean of 1702 at 12 days p.i. (Fig. 2). At 12 days p.i., 100 mg kg⁻¹ *A. herba-alba* with 15 mg kg⁻¹ DA demonstrated 991.15 mean fluorescence levels (Fig. 2). The peak fluorescence values in the positive control group, on the other hand, were 2033.65 at 12 days p.i (Fig. 2). Notably, when *A. herba-alba* was given at a low dose of DA, the suppression within the fluorescence values in mice was virtually identical to that shown in mice given 25 mg kg⁻¹ DA at peak parasitemia days (Fig. 2). At 10 and 12 days p.i., oral injections of 100 mg kg⁻¹ *A. herba-alba* in combination with a subcutaneous

dose of 15 mg kg⁻¹ DA inhibited parasite growth by 31.57% and 51.26%, respectively, compared to 54.64% and 73.24% inhibitions in the presence of 25 mg kg⁻¹ DA (Fig. 2).

The use of *A. herba-alba* in combination with a low DA dose normalized the hematological variables compared to those treated with 25 mg kg⁻¹ DA (Fig. 3). These findings indicated *A. herba alba*'s promising antibabesial activity when combined with a low DA dose. Such a regimen may aid in overcoming the toxic effects of high doses of the regularly used antibabesial medication, DA, and the parasite resistance resulting from this agent's prolonged use.

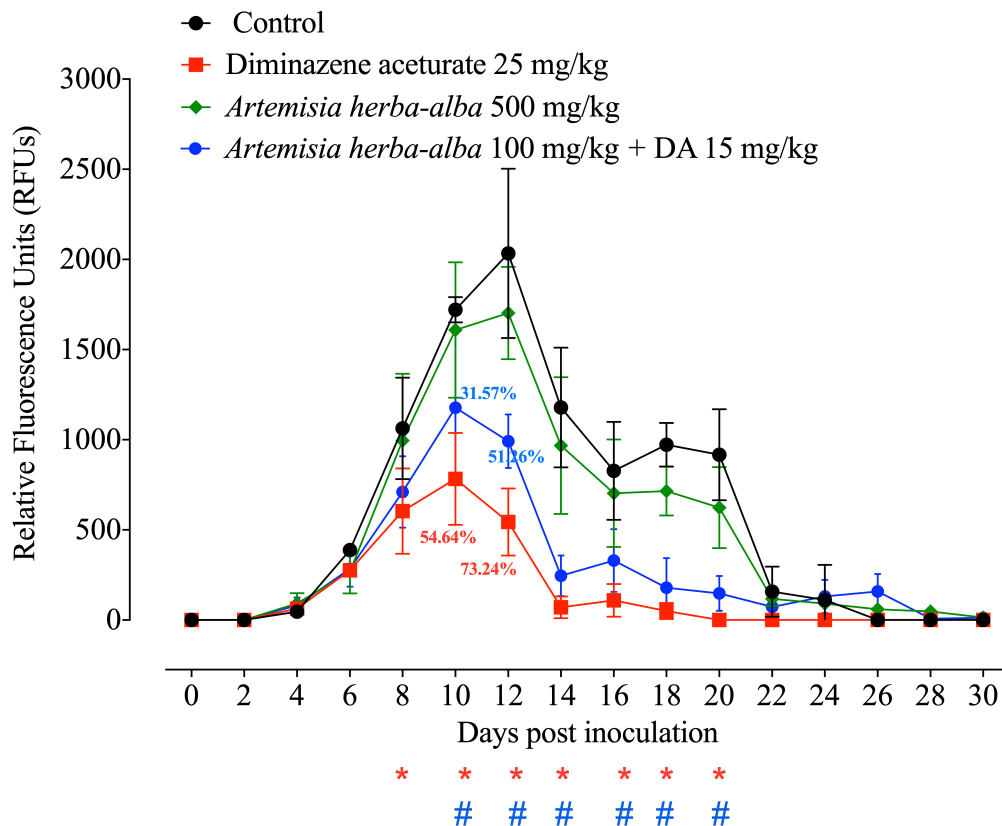


Figure 2. Anti-*B. microti* of *Artemisia herba-alba*. All the animals received 1×10^7 *B. microti* RBCs intraperitoneally. When parasitemia in the infected mice reached about 1%, the treatment began and lasted for 5 days. In the control group, mice were given I/P doses of DMSO in phosphate buffer saline (0.02%). DA and *Artemisia herba-alba* were used in subcutaneous and oral doses, respectively. In the combination therapy, the drugs were given at the same time as the inoculation. The mean and standard deviation of five mice per experimental group are represented by each value. # denotes significant differences ($p < 0.05$) between *Artemisia herba-alba*/DA or DA monotherapy-treated groups and the control group.

Discussion

This study looked at how *A. herba-alba* inhibited the growth of screened piroplasm parasites *in vitro* and *in vivo*. For *B. bovis*, *B. bigemina*, and *T. equi*, *A. herba-alba* had lower IC_{50} values than *Zingiber officinale* rhizome, a recently identified herbal antibabesial candidate [18]. Similarly, the IC_{50} for *B. bovis* in *A. herba-alba* was lower than those reported after *in vitro* treatment with turmeric (*Curcuma longa*) [29].

The efficacy of *A. herba-alba* as an agent with antimalarial activity [12,15] may explain the antibabesial efficacy of this herbal therapy because *Plasmodium* and *Babesia* parasites have striking biological similarities. Taken together, the antioxidant effect of *A. herba-alba* [17] may explain the antibabesial efficacy of this medicinal plant owing to the infection by *Babesia* is usually associated with increased levels of free radicals and oxidative stress markers [30], which is harmful to the infected host.

In the current investigation, very high concentrations of *A. herba-alba* exhibited no effect on bovine or horse RBCs. Additionally, *A. herba-alba* has been safely consumed for centuries without adverse effects. Previous studies reported the safe use of *A. herba-alba* in rats at >2 gm/kg [12,31]. Such findings were confirmed via histopathological analysis of animal organs [31]. Interestingly, the 50% lethal dose (LD_{50}) value of *A. herba-alba* in mice was greater than 5,000 mg/kg [32].

The *in vitro* inhibitory activity of *A. herba-alba* and its safety have prompted us to study the inhibitory effect of *A. herba-alba* when taken alone or in combination with DA in mice. In our investigation, the *in vitro* inhibitory effect of *A. herba-alba*, combined with DA, against the growth of *B. divergens* and *B. caballi* was strengthened. These results are similar to the *in vitro* inhibitory effects of myrrh oil/DA [21], allicin/DA [33], and thymoquinone/DA combinations [27]. In an *in vivo* study, the inhibition of *B. microti* growth caused by *A. herba-alba*/DA is nearly similar to 56.35%

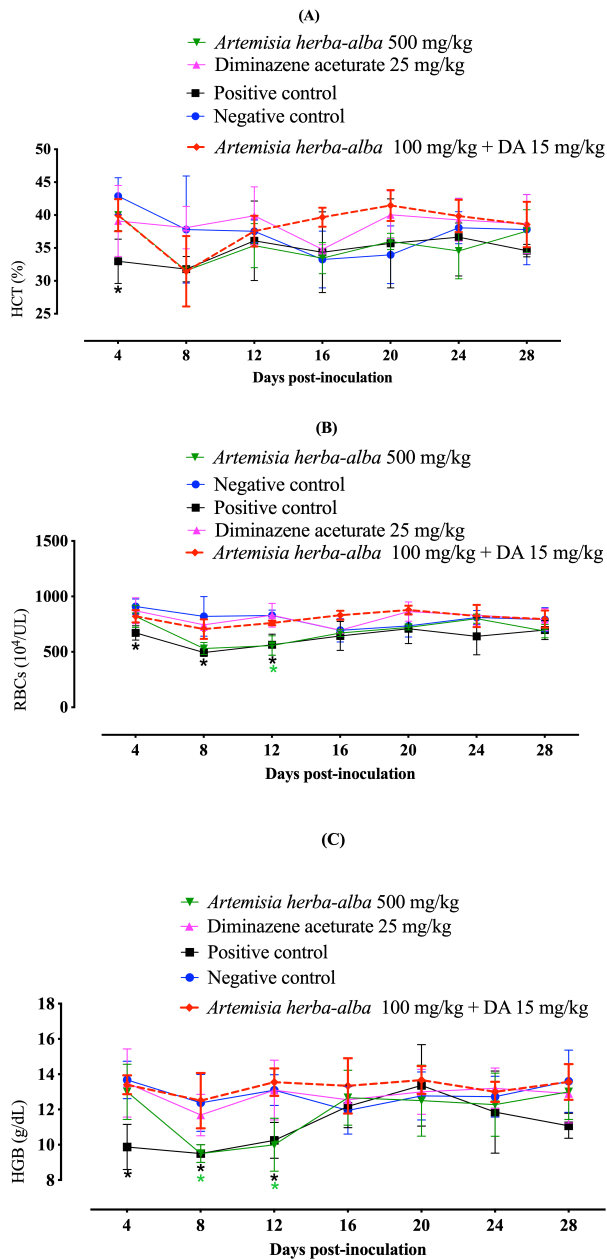


Figure 3. *Artemisia herba-alba* effect on the recovery from anemia associated with *B. microti* infection in mice. (a) HCT. (b) RBCs. (c) Hemoglobin (HGB). Each value represents the mean and standard deviation of five mice per experimental group. Asterisks indicate that the difference between treated or infected animals and uninfected mice is statistically significant ($p < 0.05$).

and 53.25% inhibition rates for 85 mg kg⁻¹ PYR combined with 10 mg kg⁻¹ DA, respectively [28]. Although the present study evaluated the inhibitory effect of *A. herba-alba*

when used as monotherapy or in combination therapy against the growth of *B. microti* in mice, further studies are required to determine the LD₅₀ of this herbal extract in cattle before its application under field conditions.

Conclusion

In conclusion, *B. bigemina* and *T. equi* were the most sensitive Babesia species to *Artemisia herba-alba*'s *in vitro* inhibitory action, followed by *B. bovis*. *A. herba-alba* was co-administrated with DA, a synergistic interaction against the *in vitro* growth of *B. divergens* was observed. The emitted fluorescence signal in the blood of mice treated with a combination therapy containing lower doses of *A. herba-alba* and DA was significantly reduced. Furthermore, a combination of *A. herba-alba*/DA therapy was used to correct hematological variables and treat hemolytic anemia caused by babesiosis. By overcoming the toxicity and resistance associated with long-term use of the antibabesial drug DA, *A. herba-alba* may be beneficial in treating animal piroplasmosis.

List of abbreviations

B. bovis: *Babesia bovis*; *B. bigemina*: *Babesia bigemina*; *T. equi*: *Theileria equi*; DA: Diminazene aceturate; DMSO: Dimethyl sulfoxide; HCT: Hematocrit; RBCs: Red blood cells; LD₅₀: 50% lethal dose.

Acknowledgments

Prof. Naoaki Yokoyama, National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido, Japan, provided invaluable scientific assistance and discussion to the writers.

Funding

This study was financed by the Japanese Ministry of Education, Culture, Sports, Science, and Technology. Egypt's Ministry of Higher Education backed Rasha Eltaysh. The Japan Society awarded Mohamed Abdo Rizk a research financing grant for the Promotion of Science (JSPS) (ID no. P18091).

Data and materials accessibility

The corresponding author will provide the data sets created and/or analyzed during the current work upon reasonable request.

Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contributions

Conceptualization: Mohamed Abdo Rizk and Ikuo Igarashi. Data curation: Rasha eltayesh and Mohamed Abdo Rizk. Formal analysis: Mohamed Abdo Rizk. Funding acquisition: Ikuo Igarashi. Investigation: Mohamed Abdo Rizk and Ikuo Igarashi. Methodology: Rasha eltayesh and Mohamed Abdo Rizk. Project administration: Ikuo Igarashi. Resources: Mohamed Abdo Rizk and Ikuo Igarashi. Software: Mohamed Abdo Rizk, Khaled Abouelnasr, and Abdelnaser Ahmed Abdallah. Supervision: Ikuo Igarashi. Validation: Ikuo Igarashi. Visualization: Mohamed Abdo Rizk and Ikuo Igarashi. Writing – original draft: Mohamed Abdo Rizk and Shimaa El-Sayed. Writing – review and editing: all authors.

References

- [1] Mosqueda J, Olvera-Ramirez A, Aguilar-Tipacamu G, Canto GJ. Current advances in detection and treatment of babesiosis. *Curr Med Chem* 2012; 19(10):1504–18; <https://doi.org/10.2174/092986712799828355>
- [2] Rizk MA, El-Sayed SAE, Eltaysh R, Igarashi I. MMV020275 and MMV020490, promising compounds from malaria box for the treatment of equine piroplasmiasis. *Ticks Tick Borne Dis* 2022; 13(2):101904; <https://doi.org/10.1016/j.ttbdis.2022.101904>
- [3] Rizk MA, El-Sayed SAE, El-Khodery S, Yokoyama N, Igarashi I. Discovering the *in vitro* potent inhibitors against *Babesia* and *Theileria* parasites by repurposing the malaria box: a review. *Vet Parasitol* 2019; 274:108895; <https://doi.org/10.1016/j.vetpar.2019.07.003>
- [4] Rizk MA, El-Sayed SAE, Eltaysh R, Igarashi I. *In vivo* antibabesial activity and bioinformatic analysis of compounds derived from the medicines for malaria venture box against *Babesia microti*. *Mol Biochem Parasitol* 2022; 247:111444; <https://doi.org/10.1016/j.molbiopara.2021.111444>
- [5] Bělková T, Bártová E, Řičařová D, Jahn P, Jandová V, Modrý D, et al. *Theileria equi* and *Babesia caballi* in horses in the Czech Republic. *Acta Trop* 2021; 221:105993; <https://doi.org/10.1016/j.actatropica.2021.105993>
- [6] Rizk MA, AbouLaila M, El-Sayed SAE, Guswanto A, Yokoyama N, Igarashi I. Inhibitory effects of fluorquinolone antibiotics on *Babesia divergens* and *Babesia microti*, blood parasites of veterinary and zoonotic importance. *Infect Drug Resist* 2018; 11:1605–15; <https://doi.org/10.2147/IDR.S159519>
- [7] El-Sayed SAE, Rizk MA, Eldoumani H, Sorour SS, Terkawi MA, AbouLaila M, et al. Identification and characterization of P0 protein as a vaccine candidate against *Babesia divergens*, Blood parasite of veterinary and zoonotic importance. *Front Vet Sci* 2021; 8:795906; <https://doi.org/10.3389/fvets.2021.795906>
- [8] Rizk MA, El-Sayed SAE, Nassif M, Mosqueda J, Xuan X, Igarashi I. Assay methods for *in vitro* and *in vivo* anti-Babesia drug efficacy testing: current progress, outlook, and challenges. *Vet Parasitol* 2020; 279:109013; <https://doi.org/10.1016/j.vetpar.2019.109013>
- [9] Tirosh-Levy S, Roth A, Leibovich B, Fleiderovitz L, Frid O, Yasur-Landau D, et al. Establishing *Babesia bovis*-free tick colony following treatment of the host with *Diminazene Aceturate* (Berenil). *Pathogens* 2021;10(5); <https://doi.org/10.3390/pathogens10050554>
- [10] Hwang SJ, Yamasaki M, Nakamura K, Sasaki N, Murakami M, Wickramasekara Rajapakshage BK, et al. development and characterization of a strain of *Babesia gibsoni* resistant to diminazene aceturate *in vitro*. *J Vet Med Sci* 2010; 72(6):765–71; <https://doi.org/10.1292/jvms.09-0535>
- [11] Yamasaki M, Watanabe N, Idaka N, Yamamori T, Otsuguro K, Uchida N, et al. Intracellular diminazene aceturate content and adenosine incorporation in diminazene aceturate-resistant *Babesia gibsoni* isolate *in vitro*. *Exp Parasitol* 2017; 183:92–8; <https://doi.org/10.1016/j.exppara.2017.10.016>
- [12] Moufid A, Eddouks M. *Artemisia herba alba*: a popular plant with potential medicinal properties. *Pak J Biol Sci* 2012; 15(24):1152–9; <https://doi.org/10.3923/pjbs.2012.1152.1159>
- [13] Réggami Y, Benkhaled A, Boudjelal A, Berredjem H, Amamra A, Benyettou H, et al. *Artemisia herba-alba* aqueous extract improves insulin sensitivity and hepatic steatosis in rodent model of fructose-induced metabolic syndrome. *Arch Physiol Biochem* 2021; 127(6):541–50; <https://doi.org/10.1080/13813455.2019.1659825>
- [14] Abdel-Ghany HSM, Abdel-Shafy S, Abuowarda M, El-Khateeb RM, Hoballah EM, Fahmy MM. Acaricidal activity of *Artemisia herba-alba* and *Melia azedarach* oil nanoemulsion against *Hyalomma dromedarii* and their toxicity on Swiss albino mice. *Exp Appl Acarol* 2021; 84(1):241–62; <https://doi.org/10.1007/s10493-021-00618-2>
- [15] El Naggar EMBA. *Artemisia herba alba* & *Artemisia monosperma*: The discovery of the first potential Egyptian plant sources for the pharmaceutical commercial production of artemisinin and some of its related analogues. *J Appl Pharm Sci* 2012;2:77-91.
- [16] Khelifi F, Sghaier RM, Amouri S, Laouini D, Hamdi M, Bouajila J. Composition and antioxidant, anti-cancer and anti-inflammatory activities of *Artemisia herba-alba*, *Ruta chalapensis* L. and *Peganum harmala* L. *Food Chem Toxicol* 2013; 55:202–8; <https://doi.org/10.1016/j.fct.2013.01.004>
- [17] Abid ZB, Feki M, Hédhili A, Hamdaoui MH. *Artemisia herba-alba* asso (*Asteraceae*) has equivalent effects to green and black tea decoctions on antioxidant processes and some metabolic parameters in rats. *Ann Nutr Metab* 2007; 51(3):216–22; <https://doi.org/10.1159/000104140>
- [18] Rizk MA, El-Sayed SAE, Igarashi I. Evaluation of the inhibitory effect of Zingiber officinale rhizome on *Babesia* and *Theileria* parasites. *Parasitol Int* 2021; 85:102431; <https://doi.org/10.1016/j.parint.2021.102431>
- [19] Al-Asmari AK AS, Athar MT, Khan AQ, Al-Shahrani H, Islam M. *Moringa oleifera* as an anti-cancer agent against breast and colorectal cancer cell lines. *PLoS One* 2015; 10(8):e0135814. <https://doi.org/10.1371/journal.pone.0135814>
- [20] Kamkar Asl M, Nazariborun A, Hosseini M. Analgesic effect of the aqueous and ethanolic extracts of clove. *Avicenna J Phytomed* 2013; 3(2):186–92.
- [21] AbouLaila M, El-Sayed SAE, Omar MA, Al-Aboody MS, Aziz ARA, Abdel-Daim MM, et al. Myrrh oil *in vitro* inhibitory growth on bovine and equine piroplasm parasites and *Babesia microti* of mice. *Pathogens* 2020; 9(3); <https://doi.org/10.3390/pathogens9030173>
- [22] Rizk MA, El-Sayed SA, Terkawi MA, Youssef MA, El Said el Sel S, Elsayed G, et al. Optimization of a fluorescence-based assay for large-scale drug screening against babesia and theileria parasites. *PLoS One* 2015; 10(4):e0125276; <https://doi.org/10.1371/journal.pone.0125276>
- [23] Rizk MA, El-Sayed SAE, AbouLaila M, Yokoyama N, Igarashi I. Evaluation of the inhibitory effect of N-acetyl-L-cysteine on *Babesia* and *Theileria* parasites. *Exp Parasitol* 2017; 179:43–8; <https://doi.org/10.1016/j.exppara.2017.06.003>
- [24] Rizk MA, El-Sayed SA, AbouLaila M, Tuvshintulga B, Yokoyama N, Igarashi I. Large-scale drug screening against *Babesia divergens* parasite using a fluorescence-based high-throughput screening assay. *Vet Parasitol* 2016; 227:93–7; <https://doi.org/10.1016/j.vetpar.2016.07.032>
- [25] El-Sayed SAE, Rizk MA, Terkawi MA, Mousa A, El Said ESES, Elsayed G, et al. Cocktail of *Theileria equi* antigens for detecting infection in equines. *Asian Pac J of Trop Biomed* 2015; 5 977–81. <https://doi.org/10.1016/j.apjtb.2015.09.001>

- [26] Rizk MA, El-Sayed SAE, Alkhoudary MS, Alsharif KF, Abdel-Daim MM, Igarashi I. Compounds from the medicines for malaria venture box inhibit *in vitro* growth of *Babesia divergens*, a blood-borne parasite of veterinary and zoonotic importance. *Molecules* 2021; 26(23); <https://doi.org/10.3390/molecules26237118>
- [27] El-Sayed SAE, Rizk MA, Yokoyama N, Igarashi I. Evaluation of the *in vitro* and *in vivo* inhibitory effect of thymoquinone on piroplasm parasites. *Parasit Vectors* 2019;12(1):37; <https://doi.org/10.1186/s13071-019-3296-z>
- [28] Rizk MA, El-Sayed SAE, AbouLaila M, Eltaysh R, Yokoyama N, Igarashi I. Performance and consistency of a fluorescence-based high-throughput screening assay for use in *Babesia* drug screening in mice. *Sci Rep* 2017; 7(1):12774; <https://doi.org/10.1038/s41598-017-13052-5>
- [29] Rizk MA, El-Sayed SAE, Igarashi I. Effects of methanolic extract from turmeric (*Curcuma longa*) against the *in vitro* multiplication of several *Babesia* species and *Theileria equi*. *Parasitologia* 2021; 1(4):188–96; <https://doi.org/10.3390/parasitologia1040020>
- [30] Kucukkurt I, Cigerci IH, Ince S, Kozan E, Aytekin I, Eryavuz A, et al. The effects of babesiosis on oxidative stress and dna damage in anatolian black goats naturally infected with *Babesia ovis*. *Iran J Parasitol* 2014; 9(1):90–8.
- [31] Lahna A, Benjelloun N, Seddik N, Farida M, Naya A, Oudghiri M. Toxicological study of the effect *in vivo* and *in vitro* of *Artemisia herba-alba* aqueous extract in rats. *Pharmacog Res* 2020; 12(3):207–11.
- [32] Asma BBM, Aicha TTM. A study of acute dermal toxicity of *Artemisia herba-alba* Asso essential oils. *Indian J Nat Prod Resour* 2021; 12(2):225–9.
- [33] Salama AA, AbouLaila M, Terkawi MA, Mousa A, El-Sify A, Allaam M, et al. Inhibitory effect of allicin on the growth of *Babesia* and *Theileria equi* parasites. *Parasitol Res* 2014; 113(1):275–83; <https://doi.org/10.1007/s00436-013-3654-2>