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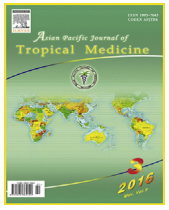

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Achillea fragrantissima, rich in flavonoids and tannins, potentiates the activity of diminazine aceturate against *Trypanosoma evansi* in rats

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ABSTRACT

Objective: To evaluate activity of methanol extract of *Achillea fragrantissima* (meth) (*A. fragrantissima*) alone or in combination with diminazine aceturate (DA) against *Trypanosoma evansi* (*T. evansi*) in experimentally infected rats.

Methods: Sixty adult male Wister albino rats were divided equally into 6 groups (A–F). Rats in groups A–E were experimentally infected with *T. evansi* and those in group F were uninfected. The groups were treated respectively as follows: group A-with 3.5 mg/kg DA; group B- with 1 000 mg/kg meth *A. fragrantissima*; group C-3.5 mg/kg DA plus 500 mg/kg meth *A. fragrantissima*; group D-3.5 mg/kg DA plus 1 000 mg/kg meth *A. fragrantissima*. Group E was left untreated. Parasitemia, survivability, packed cell volume, hemoglobin concentration, total leucocytes count, lymphocyte count, and serum malondialdehyde and reduced glutathione (GSH) levels were estimated. Phytochemical screening of meth *A. fragrantissima* was also performed.

Results: The phytochemical analysis of the meth *A. fragrantissima* indicated a higher content from polyphenolic tannins and non tannins and flavonoids. The efficacy percentage against trypanosomiasis in groups A – E was respectively as follows 80, 40, 90, 100, 0. The administration of meth-*A. fragrantissima* (1 000 mg/kg b.wt.) produced a moderate efficacy against trypanosomiasis. Untreated rats in group E died between 25 and 30 d post infection. The rats given DA and meth *A. fragrantissima* combinations (C and D) showed faster and higher recovery rates than the uninfected control and groups A and B. The initial reduction in packed cell volume, hemoglobin, total leucocytes count, increases in serum malondialdehyde and decreases in GSH levels were reversed by the treatments.

Conclusion: The administration of the methanol extracts of *A. fragrantissima* and DA combination therapy was more effective than each product alone in the treatment of rats infected with *T. evansi* and further studies are required to isolate more active ingredients.

1. Introduction

Trypanosomiasis is an important protozoan disease of domestic animals and man [1,2]. The disease is characterized by a rapid decrease in red blood cell, hemoglobin (Hb) concentration and packed cell volume (PCV) confirming that anemia is a critical feature in the pathogenesis of trypanosomiasis [2,3]. Tissue damage has been indicated in the pathophysiology of trypanosomiasis [4]. Oxidative stress plays an important role in the pathogenesis of sleeping sickness [4–8]. It has been established that infections caused by the *Trypanosoma* spp. alter the antioxidant defense of the host [4,6,7] and thus, increase the susceptibility of the erythrocytes to oxidative hemolysis [9,10].

Achillea fragrantissima (*A. fragrantissima*) is one of the Asteracaea Family and is widely used in traditional medicine for gastrointestinal disorders [11]. Authors reported its activity as

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carminative, anthelmintic and antiseptic to various infections for the urinary tract [12,13]. Moreover, insecticidal, rodenticidal, antiviral and antioxidant activities of *A. fragrantissima* were demonstrated [14–16]. Neither acute nor subchronic toxicity were noticed in mice and rats with the different extracts of *A. fragrantissima* [17,18]. In most countries control of trypanosomiasis relies mainly on chemotherapy and chemoprophylaxis using salts of three compounds – diminazine, homidium and isomethamidium. Many trials has been done to investigate combination therapy [4,7]. It is therefore conceivable that co-administration of diminazine with plants known to have immunostimulatory and antioxidant properties may potentiate its therapeutic activity.

2. Materials and methods

2.1. Animals

Wister albino mature male rats [(180 ± 20) g] were obtained from the Animal House of the College of Agriculture and Veterinary Medicine, Qassim University, Kingdom of Saudi Arabia and housed at a temperature of 22 °C – 28 °C and relative humidity of 50%–60%, with artificial light from 5:00 a.m. to 4:00 p.m. Animals had free access to tap water and standard rat chow, used for the study. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by US National Institutes of Health (NIH publication no. 85-23, revised 1996). The local ethics committee approved the study.

2.2. Preparation of plant material

The plant *A. fragrantissima* was collected at the flowering stage, in and around Al-Gouf and Qassim Districts, Kingdom of Saudi Arabia. The collected plant species were identified and confirmed at the Department of Botany, College of Agricultural and Veterinary Medicine, Qassim University, Al Qassim, Kingdom of Saudi Arabia and a voucher specimen was deposited in the Department of Botany, College of Agricultural and Veterinary Medicine, Qassim University, Al Qassim, Kingdom of Saudi Arabia for further reference.

2.3. Phytochemical analysis of the methanolic extract of *A. fragrantissima*

Shade dried and powdered plant materials were successively extracted. About 300 g of the powdered plant were soaked in 3000 mL methanol. It was left for 72 h, with intermittent shaking till obtain methanolic extract. The extract was filtered through Whatman No. 1 filter paper and concentrated until obtaining paste under vacuum using the rotary evaporator (Rotavapor R-215, Bũtchi, Switzerland), yield 16.5% (w/w). The quantitative phytochemical constituents were analyzed using the standard methods [19–21].

2.4. Trypanosomes and inoculation of donor rats

A trypanosome strain *Trypanosoma evansi* (*T. evansi*) obtained from the infected animals (camels blood). The diluted infected blood (0.1 mL) contains 1 or 2 parasite per field (microscopic field), inoculated into healthy rats intraperitoneally to serve as the donor. Infection monitored every morning by microscopic examination of blood samples taken from the tail of the infected rats.

2.5. Infection of experimental rats

Blood collected by cardiac puncture with an EDTA coated syringe from the heavily infected rats and immediately diluted with physiological saline to serve as the inoculums. Healthy rats were injected intraperitoneally with 0.2 mL of the inoculums containing about 1×10^6 trypanosome cells, as described previously [22,23] and parasitemia monitored every day by microscopic examination.

2.6. Experimental design and grouping

Six groups (A – F) of 10 rats each distributed into a well ventilated cages. All rats in group A–E were infected with 1×10^6 trypanosome cells intraperitoneally, while the group F was left as uninfected control. Rats in groups A–E experimentally infected with *T. evansi* and those in group F uninfected. The groups were treated respectively as follows: group A-given 3.5 mg/kg diminazine aceturate *im.* (Diminazene®, Montlant Pharmaceuticals, Saudi Arabia), (DA); group B- given 1000 mg/kg meth *A. fragrantissima*; group C- given 3.5 mg/kg DA plus 500 mg/kg meth *A. fragrantissima*; group D-given 3.5 mg/kg DA plus 1000 mg/kg meth *A. fragrantissima*. Group E-was left untreated. Rats in group E were left untreated as infected control. Treatments were given every morning for 5 consecutive days (The paste of the plant extract was weighed and diluted, in a gum acacia solution 1% as a vehicle and the volume given equals 10 mL/kg b. wt. by gavage).

2.7. Parasitemia

Parasitemia monitored every day under the microscope at ×40 magnification as described by Herbert and Lumsden [24].

2.8. Measurement of hematological parameters

Basal blood samples were taken, then every five days of survivor rats under light ether anesthesia inside an anesthetic chamber to perform collection of blood samples (from the inner canthus of the eye). Approximately 10 min after the procedure, all animals showed signs of recovery. A volume of 0.5 mL was used to estimate hematological parameters including PCV, Hb, total leucocytes count (TLC) and lymphocyte count (LC) according to Dacie *et al.* [25] and 3 mL was used to obtain serum for the measurement of lipid peroxidation (MDA) and reduced glutathione (GSH) levels.

2.9. Determination of serum MDA concentration

Serum malondialdehyde concentration was determined by the method of Draper and Hadley [26] and modified by Altuntas *et al.* [27]. Briefly, 2.5 mL of 100 g/L trichloroacetic acid solution was added to 0.5 mL of serum in a centrifuge tube and placed in a boiling water bath for 15 min. After cooling in tap water, the mixture was centrifuged at 1000 *g* for 10 min, and 2 mL of the supernatant was added to 1 mL of 6.7 g/L thiobarbituric acid in a test tube and placed in a boiling water bath for 15 min. Then the solution was cooled in tap water and its absorbance measured using a UV

spectrophotometer (Jenway, 6405 Model, Japan) at 532 nm. A total of 1 mL of 10% trichloroacetic acid and 1 mL of 0.67% thiobarbituric acid were used as the blank.

2.10. Determination of GSH in serum

GSH was assayed by spectophotometric technique according to the method described by Sedlak and Lindsay [28]. Briefly, the method based on the reduction of 5,5 dithiobis (2-nitrobenzoic acid) with GSH to produce a yellow compound. The reduced chromogen directly proportional to GSH concentration and its absorption can be measured at 405 nm.

2.11. Statistical analysis

Data were analyzed by the General Linear Model procedure [29]. The Least Square Mean ± standard errors were calculated and tested for significance using the *t* test.

3. Results

3.1. Parasitemia

An average pre patent period of four days was recorded in all infected rats. The clinical signs observed were anorexia, starry hair coat, weakness and depression. These signs disappeared gradually following treatment in groups A, C, D while the signs remained in the untreated group E in addition to the inability to move, enlarged abdomen and death. All rats in group E died between 20 and 30 post infection. The efficacy percentage against trypanosomiasis in groups A – E was respectively as follows 80, 40, 90, 100, 0. The administration of meth-*A. fragrantissima* (1 000 mg/kg b.wt.) produced a moderate efficacy against trypanosomiasis. Untreated rats in group E died between 35 and 40 d post infection. The rats given DA and meth *A. fragrantissima* combinations (C and D) showed faster and higher recovery rates than the uninfected control and groups A and B. The administration of meth-*A. fragrantissima* (group B) produced a moderate efficacy against trypanosomiasis. No relapse infection in the treated groups (Table 1).

3.2. PCV

The percent of PCV of rats in the infected groups was significantly lower than those of the uninfected untreated group F at day 5 post infection. The values of PCV of rats in group E 5 d post treatment was significantly lower than those of the uninfected control and other treated groups. This continued till the death of all rats in group E. The values of PCV of rats in groups C, D (treated with meth *A. fragrantissima* + DA) were significantly increased comparing with those of rats in group A (treated with DA alone) and group B (treated with meth *A. fragrantissima* alone) at days 5, 10, 15, 20 post treatment while not significantly different compared with the uninfected control group F (Table 2).

3.3. Hb concentration

The Hb concentration of rats in the infected groups was significantly lower than those of the uninfected untreated group F at day 5 post infection. The values of Hb concentration of rats in group E at 10 d post treatment was significantly lower than those of the uninfected control and other treated groups. This continued till the death of all rats in group E. The values of Hb concentration of rats in groups C, D (treated with meth *A. fragrantissima* + DA) were significantly increased comparing with those of rats in group A (treated with DA alone) at days 15, 20 post treatment while not significantly different compared with the uninfected control group F (Table 3).

Table 1
Parasitemia in rats experimentally infected with *T. evansi* and treated with diminazine aceturate alone, or combination with methanolic extract of *A. fragrantissima*.

Days	Group A	Group B	Group C	Group D	Group E	Group F
0	0/10	0/10	0/10	0/10	0/10	0/10
5*	10/10	10/10	10/10	10/10	10/10	0/10
10	2/10	6/10	1/10	0/10	10/10	0/10
15	2/10	6/10	1/10	0/10	10/10	0/10
20	2/10	3/7	1/10	0/10	4/4	0/10
25	0/8	0/4	1/10	0/10	2/2	0/10
30	0/8	0/4	0/9	0/10	1/1	0/10
35	0/8	0/4	0/8	0/10	0/0	0/10
40	0/8	0/4	0/9	0/10	0/0	0/10

Numerator: Number of rats positive, Denominator: Number infected and surviving. *Day of treatment. Group A: infected and treated with 3.5 mg/kg DA; Group B: infected and treated with 1 000 mg/kg of meth *A. fragrantissima*; Group C: infected and treated with 3.5 mg/kg DA + 500 mg/kg of meth *A. fragrantissima*; Group D: infected and treated with 3.5 mg/kg DA + 1 000 mg/kg of meth *A. fragrantissima*; Group E: infected and untreated; Group F: uninfected and untreated.

3.4. TLC

The TLC of rats in the infected groups was significantly lower than those of the uninfected untreated group F at day 5 post infection. The values of TLC of rats in group E 10 d post treatment was significantly lower than those of the uninfected control and other treated groups. This continued till the death of all rats in group E. The values of TLC concentration of rats in groups C, D (treated with meth *A. fragrantissima* + DA) were significantly increased comparing with those of rats in group A (treated with DA alone) and group B (treated with meth *A. fragrantissima* alone) at days 5, 10, 15, 20 post treatment while not significantly different compared with the uninfected control group F (Table 4).

3.5. LC

The LC of rats in the infected groups was significantly lower than those of the uninfected untreated group F at day 5 post infection. The values of LC of rats in group E 10 d post treatment was significantly lower than those of the uninfected control and other treated groups. This continued till the death of all rats in group E. The values of TLC concentration of rats in group D (treated with 1 000 mg/kg meth *A. fragrantissima* + DA) were significantly increased comparing with all treated groups at days 5, 10, 15 post treatment while not significantly different compared with the uninfected control group F (Table 5).

Table 2
Mean packed cell volume (%) of rats experimentally infected with *T. evansi* and treated with diminazine aceturate alone, or combination with methanolic extract of *A. fragrantissima*.

Days	Group A	Group B	Group C	Group D	Group E	Group F
0	46.4 ± 2.0^{a}	48.0 ± 1.0^{a}	46.6 ± 1.3^{a}	46.4 ± 1.0^{a}	47.7 ± 2.0^{a}	46.2 ± 2.0^{a}
5*	34.2 ± 1.5^{b}	36.5 ± 2.0^{b}	34.0 ± 1.2^{b}	32.0 ± 1.0^{b}	34.2 ± 1.1^{b}	46.3 ± 3.0^{a}
10	38.0 ± 3.0^{b}	35.3 ± 1.1^{b}	43.0 ± 1.1^{a}	44.2 ± 1.2^{a}	32.2 ± 2.0^{c}	44.0 ± 1.1^{a}
15	35.4 ± 1.3^{b}	36.1 ± 1.0^{b}	42.3 ± 1.7^{a}	43.5 ± 2.0^{a}	31.1 ± 1.1^{c}	44.1 ± 2.0^{a}
20	37.0 ± 1.6^{b}	38.4 ± 1.3^{b}	45.0 ± 1.0^{a}	43.0 ± 1.6^{a}	31.5 ± 3.1^{c}	46.5 ± 1.0^{a}

*Treatment day. Different superscript in a row indicates significant difference between the group mean at ($P < 0.05$).

Table 3
Mean hemoglobin concentration (g/dL) of rats experimentally infected with *T. evansi* and treated with diminazine aceturate alone, or combination with methanolic extract of *A. fragrantissima*.

Days	Group A	Group B	Group C	Group D	Group E	Group F
0	14.2 ± 0.4^{a}	14.4 ± 0.4^{a}	16.0 ± 0.5^{a}	15.2 ± 1.3^{a}	14.7 ± 1.0^{a}	15.4 ± 1.0^{a}
5*	10.1 ± 0.9^{b}	9.2 ± 0.4^{b}	11.0 ± 1.0^{b}	10.4 ± 1.0^{b}	10.2 ± 1.1^{b}	14.8 ± 1.2^{a}
10	09.2 ± 0.4^{c}	10.8 ± 0.3^{bc}	13.0 ± 1.2^{b}	12.1 ± 1.0^{b}	09.2 ± 0.6^{c}	14.2 ± 1.1^{a}
15	10.5 ± 0.7^{bc}	11.8 ± 0.7^{bc}	12.3 ± 1.1^{b}	13.2 ± 1.3^{b}	09.1 ± 0.6^{c}	14.2 ± 0.4^{a}
20	10.2 ± 0.3^{c}	13.6 ± 0.2^{a}	14.0 ± 1.2^{a}	14.3 ± 1.3^{a}	08.5 ± 0.1^{c}	16.2 ± 1.0^{a}

*Treatment day. Different superscript in a row indicates significant difference between the group mean at ($P < 0.05$).

3.6. Levels of MDA in serum

The MDA levels of rats in the infected groups were significantly higher than those of the uninfected untreated group F at day 5 post infection. The values of MDA levels of rats in group E 5 days post treatment was significantly higher than those of the uninfected control and other treated groups. This continued till the death of all rats in group E. The values of MDA levels concentration of rats in groups C, D (treated with meth *A. fragrantissima* + DA) were significantly decreased comparing with those of rats in groups A and B (treated respectively with DA alone or meth *A. fragrantissima* 1 000 mg/kg alone) at days 5, 15 post treatment while not significantly different compared with the uninfected control group F (Table 6).

3.7. Levels of GSH in serum

The GSH content of rats in the infected groups was significantly lower than those of the uninfected untreated group F at day 5 post infection. The values of GSH content of rats in group E 5 d post treatment was significantly lower than those of the uninfected control and other treated groups. This continued till the death of all rats in group E. The values of GSH content of rats in groups C and D (treated with meth *A. fragrantissima* + DA) were significantly increased comparing with those of rats in groups A and B (treated respectively with DA alone and meth *A. fragrantissima* 1 000 mg/kg alone) at days 5 and 15 post treatment while not significantly different compared with the uninfected control group F (Table 7).

Table 4
Mean total leukocyte count ($\times 10^3/mm^3$) of rats experimentally infected with *T. evansi* and treated with diminazine aceturate alone, or combination with methanolic extract of *A. fragrantissima*.

Days	Group A	Group B	Group C	Group D	Group E	Group F
0	13.3 ± 0.3^{a}	2.6 ± 1.0^{a}	13.2 ± 1.1^{a}	12.4 ± 1.0^{a}	13.8 ± 0.8^{a}	12.2 ± 0.5^{a}
5*	10.1 ± 0.5^{b}	09.4 ± 0.4^{b}	09.0 ± 1.2^{b}	09.2 ± 1.0^{b}	10.1 ± 0.6^{b}	13.5 ± 0.3^{a}
10	09.2 ± 0.4^{b}	09.2 ± 1.1^{b}	11.7 ± 1.1^{a}	10.6 ± 1.2^{a}	08.4 ± 1.0^{b}	12.0 ± 0.5^{a}
15	10.4 ± 0.3^{b}	10.1 ± 1.0^{b}	12.3 ± 1.0^{a}	13.5 ± 0.6^{a}	06.1 ± 0.3^{c}	11.9 ± 0.2^{a}
20	11.0 ± 0.6^{b}	10.4 ± 0.3^{b}	13.0 ± 1.0^{a}	14.0 ± 0.6^{a}	06.5 ± 0.2^{c}	12.3 ± 0.5^{a}

*Treatment day. Different superscript in a row indicates significant difference between the group mean at ($P < 0.05$).

Table 5
Mean lymphocyte count (%) of rats experimentally infected with *T. evansi* and treated with diminazine aceturate alone, or combination with methanolic extract of *A. fragrantissima*.

Days	Group A	Group B	Group C	Group D	Group E	Group F
0	36.5 ± 2.0^{a}	38.2 ± 1.0^{a}	37.1 ± 1.3^{a}	36.9 ± 1.0^{a}	41.0 ± 2.0^{a}	37.6 ± 2.0^{a}
5*	21.3 ± 1.5^{b}	23.1 ± 2.0^{b}	21.0 ± 1.2^{b}	22.0 ± 1.0^{b}	23.4 ± 1.1^{b}	39.4 ± 3.0^{a}
10	19.0 ± 3.0^{b}	21.6 ± 1.1^{b}	23.2 ± 1.1^{b}	31.8 ± 1.2^{a}	12.9 ± 2.0^{c}	34.0 ± 1.1^{a}
15	25.4 ± 1.3^{b}	26.1 ± 1.0^{b}	26.4 ± 1.7^{b}	33.5 ± 2.0^{a}	14.1 ± 1.1^{c}	34.1 ± 2.0^{a}
20	24.1 ± 1.6^{b}	22.6 ± 1.3^{b}	24.0 ± 1.0^{b}	33.2 ± 1.6^{a}	14.5 ± 3.1^{c}	38.7 ± 1.0^{a}

*Treatment day. Different superscript in a row indicates significant difference between the group mean at ($P < 0.05$).

Table 6
Mean serum malondialdehyde level (nmol/dL) of rats experimentally infected with *T. evansi* and treated with diminazine aceturate alone, or combination with methanolic extract of *A. fragrantissima*.

Days	Group A	Group B	Group C	Group D	Group E	Group F
0	15.6 ± 2.0[a]	16.1 ± 1.2[a]	14.9 ± 0.8[a]	13.8 ± 0.4[a]	14.2 ± 0.6[a]	16.4 ± 0.5[a]
5*	32.6 ± 3.1[a]	36.2 ± 2.3[a]	32.7 ± 1.6[a]	36.4 ± 1.2[a]	34.9 ± 3.3[a]	14.8 ± 0.4[c]
10	25.3 ± 2.2[b]	24.3 ± 2.0[b]	18.3 ± 2.2[c]	16.3 ± 2.0[c]	31.3 ± 2.9[a]	16.3 ± 0.2[c]
20	21.3 ± 2.4[b]	22.3 ± 2.3[b]	17.3 ± 1.9[c]	15.3 ± 0.8[c]	33.3 ± 2.0[a]	16.7 ± 0.2[c]

*Treatment day. Different superscript in a row indicates significant difference between the group mean at ($P < 0.05$).

Table 7
Mean serum reduced glutathione (mg/dL) of rats experimentally infected with *T. evansi* and treated with diminazine aceturate alone, or combination with methanolic extract of *A. fragrantissima*.

Days	Group A	Group B	Group C	Group D	Group E	Group F
0	6.2 ± 0.4[a]	7.2 ± 0.5[a]	6.9 ± 0.2[a]	6.4 ± 0.2[a]	5.3 ± 0.2[a]	6.6 ± 0.2[a]
5*	24 ± 0.2[b]	2.7 ± 0.3[b]	2.3 ± 0.2[b]	5.6 ± 0.3[b]	2.4 ± 0.1[b]	6.7 ± 0.3[a]
10	4.3 ± 0.7[b]	4.2 ± 0.2[b]	6.2 ± 0.3[a]	6.3 ± 0.4[a]	3.1 ± 0.1[b]	6.5 ± 0.1[a]
20	3.8 ± 0.1[b]	3.9 ± 0.2[b]	6.4 ± 0.4[a]	6.1 ± 0.2[a]	2.3 ± 0.2[c]	5.9 ± 0.1[a]

*Treatment day. Different superscript in a row indicates significant difference between the group mean at ($P < 0.05$).

3.8. Phytochemical analysis of the methanolic extract of A. fragrantissima

The phytochemical constituents (mean ± SE, $n = 5$) present in methanolic extract of *A. fragrantissima* indicated respectively the presence of total phenolics, tannins, non tannin phenolics and total flavonoids (25.57 ± 3.16) mg/g, (16.62 ± 1.45) mg/g, (8.98 ± 0.48) mg/g, (10.4 ± 0.84) mg/g of methanolic residue.

4. Discussion

Parasitemia in rats in this experiment was associated with clinical signs of anorexia, pyrexia, depression, swollen abdomen and similar to those in mice, dogs and rabbits infected with *Trypanosoma brucei* (*T. brucei) brucei* [30,31] and in cattle infected with *Trypanosoma congolense* [32]. Following treatment, the clinical signs were gradually disappeared. All rats in the infected untreated group were died between 25 and 30 d post infection, due to the progress of the parasitemia. The efficacy percentages against trypanosomiasis in groups A to E were respectively as follows 80, 40, 90, 100, 0. The administration of meth *A. fragrantissima* (1 000 mg/kg b.wt.) produced a moderate efficacy against trypanosomiasis. Recently, many authors recorded the efficacy of certain plant extracts against trypanosomiasis [33–40]. The rats given DA and meth *A. fragrantissima* combinations (C and D) showed faster and higher recovery rates than the uninfected control and groups A and B. At the same direction, Chekwube *et al.* [41] studied the effect of DA alone or in combination with either levamisole and/or vitamin C in albino rats experimentally infected with *T. brucei brucei* and found that levamisole and/or vitamin C combination with DA were more effective in the treatment of infected rats. da Silva *et al.* [42] reported that selenium supplementation decreases the parasitemia of various Trypanosome infections and reduces important parameters associated with diseases such as anemia and parasite-induced organ damage.

The quantitative phytochemical analysis of the methanolic extract of *A. fragrantissima* indicated the presence of a high content from flavonoids, polyphenolics and tannins and confirmed also from other studies [15,16]. Previous studies strongly correlate between the trypanocidal action of plant extracts and their contents of certain bioactive constituents especially flavonoids, tannins and others. da Rocha *et al.* [43] isolated 3 flavonoids from the roots of *Arrabidaea brachypoda*, compound 1 exhibited no activity toward *Trypanosoma cruzi,* while flavonoids 2 and 3 exhibited selective activity against these trypomastigotes. Gallic acid is a plant phenolic and well known hydrolyzable tannins, Koide *et al.* [44] reported its trypanocidal activity against *T. brucei* in both the long slender bloodstream forms and the procyclic forms, *in vitro*. Moreover, there are several studies investigated the relation of the herbal trypanocidal activity and their contents from flavonoids, polyphenolics and tannins [45–47]. So, the presence of flavonoids and tannins with higher concentrations may at least in part explain the observed activity against trypanosomiasis.

Our study showed the incidence of anemia (decreased PCV and Hb values), leucopenia and lymphopenia in all infected rats 5 d post infection. By 10th day post treatment, rats treated with DA and meth *A. fragrantissima* (in a dose-dependent manner) combination therapy recovered early than rats treated with DA alone. It could be seen that the previous parameters of groups that received DA plus meth *A. fragrantissima* were not significantly different with the uninfected control group and were bitter than group A (DA alone). Group D shows rapid return to normal values than other treated groups. At the same direction, there was an increase in serum MDA and decrease in GSH levels in all infected rats 5 d PI. By 10th day post treatment, rats treated with DA and meth *A. fragrantissima* (in a dose-dependent manner) combination therapy restored the level of GSH and diminished the level of MDA than rats treated with DA alone. It could be also seen that the previous parameters of groups that received DA plus meth *A. fragrantissima* were not significantly different with the uninfected control group and were bitter than group A (DA alone). Group D shows rapid return to normal values than other treated groups.

Moreover, it was shown that treatment with methanolic extract of *A. fragrantissima* reduced the lipid peroxidation, increased the level of reduced glutathione and keep the hematological parameters normal and indicated that there is a

correlation between MDA levels and induction of anemia in trypanosomiasis. The proposed mechanisms are erythrophagocytosis, destruction of the erythrocytes by the immune system, erythrocyte osmotic fragility, hemodilution and erythropoietic depression [48,49]. It is [9,50] found that erythrocytes of *T. brucei brucei*-infected mice and *Trypanosoma vivax* in cattle produced significantly greater amounts of by-products of lipid peroxidation than the erythrocytes of the control animals. This suggests that the infected animals may have reduced ability in the prevention of free radicals-mediated lipid peroxidation in the erythrocyte membrane. This may contribute to the pathogenesis in anemia in trypanosomiasis. It is believed that antioxidant treatment might prevent the erythrocyte destruction, since *A. fragrantissima* is a potent antioxidant as indicated from the present study. The chemical analysis of the methanolic extract of *A. fragrantissima* indicated the presence of several compounds which are potent as antioxidant activity (it is rich in flavonoids, and polyphenolics) and confirmed also from other studies [51,52].

The administration of flavonoids, possessing antioxidant activity, may reduce the cellular injury caused by trypanosome-induced generation of free radicals [53]. At the same direction, treatment with a flavonoid mixture and diminazene aceturate significantly reduced trypanosome-induced increases in erythrocyte osmotic fragility and lipoperoxidative changes, suggesting possible antioxidant properties of this mixture and its therapeutic value in trypanosomosis [54]. Do Carmo *et al.* [55] mentioned that some plant forms improved the hematological and biochemical parameters in rats experimentally infected with *T. evansi.*

Based on our results, we conclude that coadministration of diminazene aceturate and methanolic extract of *A. fragrantissima* was more active against experimentally induction of parasitemia and more potent in prevention of PCV, Hb and GSH reduction, leucocytopenia, lymphopenia and MDA elevation than administration of diminazene aceturate alone. These potentiating activities of methanolic extract of *A. fragrantissima* against trypanosomiasis might be due to its phytochemical constituents of total flavonoids and total polyphenolics a point needs further investigations.

Conflict of interest statement

The authors have declared that there is no conflict of interest related to this paper.

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