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Efficacy of some Egyptian native plant extracts against *Haemonchus contortus* in vitro and in experimentally infected sheep along with the associated haematological and biochemical alterations

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Abstract

Background: Haemonchosis is a serious disease affecting ruminants' productivity worldwide. Medicinal plants are deemed one of the most natural bio-products safely used as alternatives to the synthetic anthelmintics. In the present study, comparative efficacy of crude ethanolic extracts (CEEs) of *Artemisia herba-alba* (*A. herba-alba*), *Balanites aegyptiaca* (*B. aegyptiaca*) and *Allium sativum* (*A. sativum*) as alternative treatments was tested on *Haemonchus contortus* (*H. contortus*). An in vitro test to evaluate the anthelmintic efficacy of various concentrations of extracts at 25, 30 and 50 mg/ml was accomplished on motility and viability of adult worms in comparison with albendazole, reference drug at 10 µg/ml at various time intervals. An in vivo test was carried out in lambs experimentally infected with *H. contortus* to detect anthelmintic activity of CEEs of *A. herba-alba* and *B. aegyptiaca* compared to albendazole. Fifteen parasite-free Baladi Egyptian lambs aged 4–8 months old were categorized into five groups, each of three lambs as follows: G1 was kept as uninfected untreated one, G2 was utilized as infected untreated group, G3 was given CEE of *A. herba-alba*, G4 was received CEE of *B. aegyptiaca*, and G 5 was treated with albendazole.

Results: The in vitro test revealed that CEE of *B. aegyptiaca* had the most significant anthelmintic activity on adult *H. contortus* followed by *A. herba-alba,* while *A. sativum* was of the lowest effect. The in vivo test showed that the CEE of *B. aegyptiaca* achieved an excellent faecal egg reduction (100%) at the 7th day post-treatment. The most efficient treatments that improved the haematological parameters and regained the level of serum total protein, albumin and A/G ratio, serum globulin, SGoT, SGPT, urea and creatinine to the almost normal levels were CEE of *B. aegyptiaca*, albendazole and CEE of *A. herba-alba*, respectively.

Conclusions: This study highlighted the marked anthelmintic potency of the CEEs of *B. aegyptiaca* and *A. herba-alba* on *H. contortus* and the superiority of CEE of *B. aegyptiaca* as a talented anti-parasitic medicinal plant for sheep.

Keywords: *Haemonchus contortus*, Sheep, *Artemisia herba-alba*, *Balanites aegyptiaca*, *Allium sativum*, Haematological and biochemical changes

Background

Baladi sheep are accounted as beneficial and valuable food animals reared all over rural Egypt (Hanelein and Abdellatif 2003; El-Dakhly et al. 2012). Helminthiasis is deemed a great constraint against small ruminants

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husbandry (El-Ashram et al. 2017). Haemonchosis is one of the most serious widespread, infectious life threaten that negatively affected the livestock performance and productivity (Brik et al. 2019; Hassan et al. 2020). The blood-sucking barber pole worm, *H. contortus*, is the causative agent of the disease and considers the most critical and pathogenic abomasal nematode affecting ruminants worldwide including Egypt (Hassan et al. 2019; Arsenopoulos et al. 2021). The average daily blood loss in infected sheep is about 0.03 ml/parasite (Awad et al. 2016). The infection causes pronounced effects in animal health involving mainly anaemia, inappetence to off-food and death in acute infection in particular young animals (Mannan et al. 2017). For decades, chemotherapeutics were utilized substantially to control parasitic diseases in ruminants. The excessive and inappropriate use of the anthelmintic drugs for prolonged period has resulted in the development of multi-drug-resistant gastrointestinal nematodes (GINs) (Shalaby 2013). Moreover, the risks of drug residues in food animal and the cost-effectiveness of the anthelmintics have motivated to seek about potent alternatives for sustainable parasitic control (Albadawi 2010). It was documented that the first helminth that developed the multi-drug resistance was *H. contortus* against various anthelmintic classes, particularly benzimidazole, levamisole and the macrocyclic lactone (Kebede 2019). Direct life cycle besides the high egg-laying rate of *H. contortus* is considered the main biological factor that could facilitate anthelmintic resistance to occur (Coles 2005). Medicinal plants containing promising bioactive constituents afford significant economic and environmentally acceptable solutions against parasitic infection in animals (Torres-Acosta et al. 2012). *A. herba-alba* plant, known also as desert worm wood (in Arabic as shih), is one of the family Asteraceae and commonly utilized in folk medicine and has various anti-parasitic impacts (Idris et al. 1982; Ahmed et al. 2020). *B. aegyptiaca* Del. (L.) (Family: *Balanitaceae*) is recognized as "desert date"; the fruits are utilized traditionally in the treatment of parasitic infection, gastrointestinal disturbance, syphilis and fever (Doughari et al. 2007; Vijigiri and Sharma 2010). Furthermore, *A. sativum* or garlic has been documented as potent natural agents against parasitism and fungal infection and reported as immuno-stimulant compound (Duke 2002; Orengo et al. 2016). Thus, the current study is designed to firstly investigate the comparative in vitro potency of crude ethanolic extracts of *A. herba-alba, B. aegyptiaca* and *A. sativum*. Moreover, it aims to use the most efficient extracts in vivo, as alternative treatments for haemonchosis among sheep.

Methods

Plants

Whole plant (stem and leaves) of *A. herba-alba*, *B. aegyptiaca* fruits and cloves of *A. sativum* were brought from local markets in Cairo Governorate, Egypt. The plant materials were identified at the laboratory of Medicinal and Aromatic Plants Research Department, National Research Center, then cleaned, shade-dried and mechanically ground utilizing a laboratory mortar and pestle. The ethanolic extracts were prepared according to Harborne (1984) as follows: the plant materials were pounded and then extracted with 70% ethanol. Plant materials were macerated at room temperature in dark place, and the percolate was collected by filtering through cotton wool. The process of maceration/percolation was repeated three times for 3 weeks. The combined filtrate was completely evaporated in a vacuum rotary evaporator (Heidolph-Germany) under pressure at 50 °C to obtain a semi-solid crude ethanolic extract. The extract was scraped off, transferred to container, and kept airtight; it was stored at 4 °C until further use.

Parasite

Adult *H. contortus* worms were freshly assembled from abomasa collected from abattoir. The worms were thoroughly washed utilizing phosphate-buffered saline to remove debris and mucus (Soulsby 1986).

In vitro bio-assay

For the assessment of in vitro anthelmintic impacts of the crude ethanolic extract prepared from the three plants on adult *H. contortus*, the worm motility inhibition assay was achieved.

Twelve adult *H. contortus* worms were exposed separately to CEEs of *A. herba-alba, B. aegyptiaca* and *A. sativum* at three various concentrations 25, 30 and 50 mg/ml dissolved in DMSO (0.1%). Albendazole was employed at 10ug/ml as the standard drug (The Egyptian Company for Chemicals and Pharmaceuticals (ADWIA) 10th of Ramadan City). DMSO (0.1%) was utilized as negative control. Three replicates were applied for each treatment. The worms' vitality was watched through detection complete inactivity and mortality at 0, 2, 4, 6 and 8 h of intervals. After 8 h, the revival of the worm motility was tested as the extracts and albendazole were removed away and parasites were dropped again in PBS for 30 min. Per cent worm motility inhibition (% WMI) was assigned according to Rabel et al. (1994) by the following formula:

$$\%\mathrm{WMI} = \frac{\left(\text{Number of mobile worms in negative control Petri dish} - \text{Number of mobile worms in treatment Petridish}\right) \times 100}{\left(\text{Number of mobile worms in negative controls Petri dish}\right)}.$$

The mortality index was estimated as mentioned by Tariq et al. (2009) by the following formula:

$$\text{Mortality index (MI)} = \frac{\text{Total number of immobile worms (dead)}}{\text{Total number of worms per Petri dish}}.$$

In vivo bioassay

Animals

Seventeen Baladi lambs of 15–18 kg BW were bought from a private farm at Monieb, Giza Governorate.

Larval donors

Two worm-free lambs were utilized as *H. contortus* larval donors. Larval culture was carried out according to Solusby (1986). The female adult worms of *H. contortus* were used freshly and disintegrated for liberation of eggs. The culture was obtained by addition of sand charcoal, sterilized faeces and few drops of water. Daily mixing is required for aeration with maintaining of humidity for 12 days. Then, infective larvae were harvested and counted. Each lamb was infected using 10.000 third-stage larvae L_3. Three weeks post-infection, faecal samples were confirmed positive for *H. contortus* using concentration floatation technique. Donors' faecal samples were used as source of monospecific *H. contortus* infective larvae for further infection.

Experimental animals

A total of fifteen Baladi lambs of about 4–8 months old were employed. Lambs were clinically and parasitologically examined to confirm the absence of infection. The animals were categorized into five groups, each of three lambs. Group 1 was kept as uninfected untreated one. Group 2 was utilized as infected untreated group. Group 3 was given a single dose of CEE of *A. herba-alba* 2 g /kg BW followed by booster dose after 12 days (Tariq et al. 2009). Group 4 was received 3 g/kg BW orally of CEE of *B. aegyptiaca* for 3 successive days (Koko et al. 2000). Group 5 was treated with a single dose of albendazole (0.5 g/20 kg BW) as described by the manufacture company. Each lamb in groups 2 to 5 was infected by giving 10,000 L_3 orally, at zero day of experiment. The treatment regimes were started 3 weeks post-infection. Blood samples were taken every 3 weeks till the end of the experiment, with EDTA for determination of haematological parameters and without anticoagulant for serum separation for assigning some significant biochemical parameters. Individual anal faecal samples were assembled for faecal egg counting by McMaster technique.

Faecal egg count reduction per cent (FECR %) was estimated utilizing the formula reported by Tariq et al. (2009) as follows:

$$\mathrm{FECR}\% = \frac{\text{Pre-treatment egg count per gram} - \text{Post-treatment egg count per gram}}{\text{Pre-treatment egg count per gram}}.$$

Statistical analysis

Data are presented as mean ± standard error. Differences between means in the different groups were tested for significance by one-way analysis of variance (ANOVA) and Duncan's multiple range tests to detect the significance among means in between different experimental groups and weeks (Snedecore and Cochran 1984). SPSS (version 16) computer program was used.

Results

In vitro bioassay

The three used plant extracts exhibited anthelmintic potency on the adult worms in comparison with the negative control (DMSO 0.1%). There was a concentration-dependent effect on adult *H. contortus* worms. In other words, CEE of *A. herba-alba*, *B. aegyptiaca* and *A. sativum* at 50 mg/ml was the most effective concentration. The CEE of *B. aegyptiaca* revealed the fastest and greatest anthelmintic affectivity in comparison with the other extracts at the same concentration in terms of mortality/paralysis of the worms. It was noticed that the motility of the worms diminished gradually from the start of experiment especially, in treatment with CEEs of *A. herba-alba, B. aegyptiaca* and *A. sativum* at 50 mg/ml concentration, whereas the worms which revealed motility 8 h post-exposure were 3 ± 0.57, 3 ± 1.0 and 5.33 ± 0.88, respectively. After the re-suspension in PBS for 30 min, the worms which re-attained their motility were 2 ± 1.1, 1 ± 0.57 and 3 ± 1.15, respectively (Table 1). The mean %WMI was of 81.8, 90.9 and 72.7, and the MI was of 0.83, 0.91 and 0.75, respectively (Table 2).

Table 1 Effect of CEEs of *A. herba-alba, B. aegyptiaca* and *A. sativum* on mean number of *H. contortus* worms (± SE) showing motility post-exposure to various treatments

H	CEE of *A. herba-alba*			CEE of *B. aegyptiaca*			CEE of *A. sativum*			DMSO 0.10%	AZL 10 µg/ml
	25 mg	30 mg	50 mg	25 mg	30 mg	50 mg	25 mg	30 mg	50 mg		
0	12 ± 0.00^{A}	12 ± 0.00^{A}	12 ± 0.00^{A}	12 ± 0.00^{A}	12 ± 0.00^{A}	12 ± 0.00^{A}	12 ± 0.00^{A}	12 ± 0.00^{A}	12 ± 0.00^{A}	12 ± 0.00^{A}	12 ± 0.00^{A}
2	10.67 ± 0.33^{Ab}	10.33 ± 0.33^{Ab}	9 ± 0.57^{Bc}	10 ± 0.57^{Bb}	10 ± 0.57^{Bb}	9.3 ± 0.33^{Bbc}	11.33 ± 0.33^{ABab}	11.33 ± 0.33^{Aab}	10.67 ± 0.33^{ABb}	12 ± 0.00^{Aa}	8 ± 0.57^{Bc}
4	7 ± 1.0^{Bb}	6.67 ± 0.33^{Bb}	6 ± 0.00^{Cb}	9 ± 0.57^{Bb}	7.67 ± 0.33^{CDbc}	7.6 ± 0.33^{BCb}	9.67 ± 0.33^{BCb}	9.33 ± 0.67^{Bb}	8.67 ± 0.3^{Bb}	11.67 ± 0.3^{ABa}	1 ± 0.57^{Cc}
6	6 ± 1.0^{BCb}	6.7 ± 0.33^{Bb}	5 ± 0.57^{Cb}	8.33 ± 0.88^{BCb}	7.67 ± 0.33^{CDbc}	6.33 ± 0.88^{Cc}	8.33 ± 0.33^{CDb}	7.33 ± 0.67^{Cbc}	6.33 ± 0.8^{Cc}	11.67 ± 0.3^{ABa}	0 ± 0.00^{Cc}
8	5.67 ± 1.3^{BCb}	5 ± 0.57^{Bbc}	3 ± 0.57^{Dc}	6.67 ± 0.67^{Cb}	6.3 ± 0.33^{Db}	3 ± 1.0^{Dc}	7 ± 0.57^{Db}	6 ± 0.57^{Cb}	5.33 ± 0.88^{Cb}	11.33 ± 0.3^{ABa}	0 ± 0.00^{Cd}
PB S	4 ± 0.57^{Cb}	3 ± 1.1^{Cb}	2 ± 1.1^{Dbc}	$3 \pm o.57^{Db}$	3 ± 0.57^{Eb}	1 ± 0.57^{Ec}	5 ± 1.15^{Eb}	4 ± 0.57^{Db}	3 ± 1.15^{Db}	11 ± 0.00^{Ba}	0 ± 0.00^{Cc}

S.E., standard error of mean; CEE, crude ethanolic extract; DMSO, dimethyl sufoxide indicates negative controls; AZL, albendazole

*Indicates that worms were exposed to PBS for 30 min after exposure to different treatments to confirm their mortality

Different capital letters indicate significance between hours for each dose in *CEEs* (column). Different small letters indicate significance between doses for each hour in *CEEs* (row)

Table 2 Mean percent of worm motility inhibition and mortality index of different CEE-treated worms at various treatments

	CEE concentration								
	A. herba-alba			*B. aegyptiaca*			*A. sativum*		
	25 mg/ml	30 mg/ml	50 mg/ml	25 mg/ml	30 mg/ml	50 mg/ml	25 mg/ml	30 mg/ml	50 mg/ml
WMI%	63.6	72.7	81.8	72.7	72.7	90.9	54.5	63.6	72.7
MI	0.67	0.75	0.83	0.75	0.75	0.91	0.58	0.67	0.75

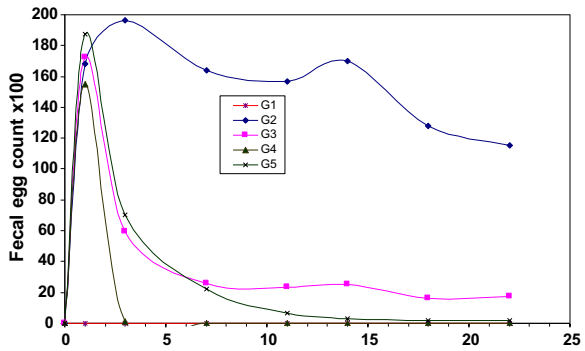

Fig. 1 Mean faecal egg counts for different sheep groups under experiment; infected untreated (G1), *A. herba-alba* (G2), *B. aegyptiaca* (G3) compared to albendazole one (G4)

Table 3 The FECR% recorded in G1 (uninfected untreated), G2 (infected untreated), G3 (*A. herba-alba*), G4 (*B. aegyptiaca*) versus G5 (albendazole) at different days post-treatment

Days post-treatment	FECR%				
	G1 (%)	G2 (%)	G3 (%)	G4 (%)	G5 (%)
3	0	− 17.0	65.5	99.0	62.6
7	0	2.57	85.1	100	88.1
14	0	− 1.2	85.4	100	98.6
22	0	3.97	89.9	100	99.1

In vivo experiment

The infection was detected for all lambs groups except uninfected untreated group at 21 days post-infection by detecting *H. contortus* eggs in faeces through faecal examinations. The results of preclinical *in vitro* experiment revealed a low potency of anthelmintic effect of *A. sativum* CEE on *H. contortus* adult worms. So, the *in vivo* study concerned on the evaluation of anthelmintic activity of CEE of *A. herba-alba, B. aegyptiaca* compared to albendazole in the experimentally *H. contortus*-infected lambs. A significant reduction has been detected in the mean FECs for G4 (*B. aegyptiaca* CEE) and G5 (albendazole-treated) followed by G3 (*A. herba-alba* CEE) compared to G2 (infected untreated) 3 days post-treatment (PT) till the end of the experiment. The FECs of G3, G4 and G5 at 3 days PT were 59.3 ± 3.5, 1.5 ± 0.28 and 70.17 ± 5.4, respectively, while FEC of the infected untreated one was 196.7 ± 41 as demonstrated in Fig. 1. The CEE of *B. aegyptiaca* (G4) has recorded 99% FECR at 3 days PT. At the 7th day PT, the FECR was 100%. The effect of CEE of *A. herba-alba* and albendazole on the FECR was comparable as illustrated in Table 3.

Haematological parameters

Significant variations in haematological values among groups were detected. The experimental infection with haemonchosis caused a decrease in RBCs count, Hb g/dL and PCV% in all the groups. Moreover, it gave arise to increment of WBCs and eosinophil levels in all the groups compared to uninfected untreated one. The control infected untreated group recorded the lowest RBCs count, Hb g/dL and PCV% and highest WBCs and eosinophil levels compared to uninfected untreated one which was within the normal range in the whole experimental period. The result revealed that the most efficient treatments of haemonchosis in sheep, which improve the RBCs count, Hb concentration and PCV% and cause a gradual decrease in WBCs and eosinophil levels to return it to almost normal level, were recorded in the groups treated with *B. aegyptiaca* CEE, albendazole and *A. herba-alba* CEE successively (Table 4).

Biochemical parameters

The results declared that the experimental infection of lambs with haemonchosis induced a decrease in total protein, albumin levels, serum globulin and albumin/globulin (A/G) ratio in all the groups. Moreover, it causes a higher level of SGOT, SGPT, BUN and creatinine level compared to the uninfected untreated one. The control infected untreated group showed the lowest level of the total protein, albumin levels, albumin/globulin and (A/G) ratio besides high level of SGOT, SGPT, BUN and creatinine level compared to the uninfected untreated one. The results revealed that the most efficient treatments that improved total protein, albumin levels, A/G ratio, level of SGOT, SGPT and BUN to almost the normal level were

Table 4 Effect of various treatments on haematological parameters of different experimental lamb groups

Parameter	Week	Experimental animals				
		G1	G2	G3	G4	G5
RBCs count (10^6/µl)	0	$9.05 \pm 0.10^{Da\,b}$	9.4 ± 0.41^{Aab}	$8.7 \pm 0.26^{A\,b}$	$9.5 \pm 0.24^{Aa\,b}$	9.9 ± 0.52^{Aa}
	3	$9.2 \pm 0.14^{CD\,a}$	5.3 ± 0.21^{Bb}	$5.0 \pm 0.36^{E\,b}$	5.2 ± 0.10^{Cb}	5.4 ± 0.29^{Cb}
	6	10.2 ± 0.26^{ABa}	4.0 ± 0.45^{Cd}	5.8 ± 0.44^{CDEc}	9.2 ± 0.21^{ABa}	7.1 ± 0.44^{Bb}
	9	7.4 ± 0.10^{Bc}	9.93 ± 0.19^{Aa}	9.0 ± 0.12^{Ab}	$4.3 \pm 0.44B^{Cd}$	10.2 ± 0.26^{ABa}
Hb (g/d)	0	$10.6 \pm 0.68^{Ab\,c}$	$11.3 \pm 0.23^{Aa\,b}$	$10.6 \pm 0.32^{A\,bc}$	$9.6 \pm 0.31^{CD\,c}$	12.0 ± 0.14^{Aa}
	3	11.5 ± 0.60^{Aa}	7.3 ± 0.43^{Bb}	6.9 ± 0.39^{Cb}	7.1 ± 0.17^{Eb}	7.7 ± 0.44^{Eb}
	6	11.2 ± 0.36^{Aa}	5.1 ± 0.16^{Cd}	10.17 ± 0.08^{Ab}	10.4 ± 0.30^{BCb}	8.2 ± 0.20^{CDEc}
	9	11.27 ± 0.43^{Aa}	5.26 ± 0.37^{Cc}	9.8 ± 0.16^{ABb}	11.5 ± 0.16^{Aa}	9.1 ± 0.15^{Bb}
PCV%	0	$28.28 \pm 0.51^{C\,b}$	28.57 ± 0.32^{Ab}	$30.0 \pm 0.08^{A\,a}$	$29.0 \pm 0.18^{ABa\,b}$	28.8 ± 0.61^{Aab}
	3	$^{Ca}29.05 \pm 0.22^{AB}$	18.45 ± 0.32^{Bbc}	$^{b}18.59 \pm 0.58^{D}$	17.2 ± 0.24^{Fc}	18.4 ± 0.41^{Dbc}
	6	29.8 ± 0.43^{ABa}	14.7 ± 0.63^{Dc}	25.0 ± 0.32^{Bb}	26.16 ± 0.53^{CDb}	25.6 ± 0.60^{Bb}
	9	28.8 ± 0.36^{BCb}	15.6 ± 0.91^{CDcd}	25.59 ± 0.31^{Bc}	30.78 ± 0.22^{Aa}	26.5 ± 0.22^{Bc}
WBCs count (10^3/µl)	0	12.0 ± 0.08^{Ba}	12.6 ± 0.49^{Ba}	12.3 ± 0.51^{DEa}	12 ± 0.49^{Ea}	12.0 ± 0.14^{Aa}
	3	18.1 ± 1.4^{Aab}	16.2 ± 0.55^{Ab}	19.3 ± 1.1^{Aa}	18.0 ± 0.69^{BCab}	12.0 ± 0.26^{Ac}
	6	12.6 ± 0.68^{Bbc}	10.7 ± 0.40^{Cc}	$13.3 \pm 0.55C^{Db}$	17.8 ± 0.86^{BCa}	12.3 ± 0.45^{Abc}
	9	11.0 ± 1.1^{Bbc}	9.4 ± 0.34^{Cc}	10.4 ± 0.20^{Ebc}	15.4 ± 0.72^{Da}	11.9 ± 0.20^{Ab}
Eosinophil (10^3/µl)	0	0.6 ± 0.66^{Ba}	0.3 ± 0.33^{BCa}	$0.3 \pm 0.33^{C\,a}$	0 ± 0.00^{Da}	0.67 ± 0.33^{AB}
	3	7.3 ± 2.8^{Aa}	4.7 ± 0.88^{Aa}	$5.3 \pm 0.33^{A\,a}$	5.6 ± 0.88^{ABa}	0 ± 0.00^{Bb}
	6	0.3 ± 0.3^{Bb}	0.33 ± 0.33^{BCb}	0.67 ± 0.33^{BCb}	3.67 ± 1.2^{BCa}	0.67 ± 0.33^{ABb}
	9	1.3 ± 0.3^{Bab}	0 ± 0.00^{Cb}	0.67 ± 0.67^{BCab}	2.3 ± 0.88^{CDa}	0.33 ± 0.33^{ABb}

G, lambs group

Capital letters: means within the same column of different letters are significantly different at ($P < 0.01$). Small letters: means within the same row of different letters are significantly different at ($P < 0.01$)

B. aegyptiaca CEE, albendazole and *A. herba-alba* CEE, respectively (Table 5).

Discussion

Haemonchus contortus infection is an urgent disease among ruminants. Herbal medicine has global offered safe, economic and effective products of anthelmintic properties. This study was firstly implemented to assess the comparative in vitro anthelmintic potency of crude ethanolic extracts of native Egyptian medicinal plants, namely *A. herba-alba*, *B. aegyptiaca* and *A. sativum*, versus the standard drug albendazole. After that, it aims to in vivo test the most talented plant extracts among sheep experimentally infected with *H. contortus* as alternative era of treatment. The three tested plant extracts provided an in vitro anthelmintic and a concentration-dependent effect on the adult *H. contortus* worms compared with negative controls and drug standard group. They had an inhibitory effect on the motility and viability of the adult worms. The CEE of *B. aegyptiaca* was of the greatest per cent of WMI and MI followed by *A. herba-alba* and *A. sativum*. Similarly, previous finding confirmed that a potent in vitro effect on the motility and vitality of *H. contortus* adult worms through using various plant species had been reported *Artemisia absinthium* (Tariq et al. 2009), *Cissus quadrangularis* L and *Schinus molle* L. (Zenebe et al. 2017) and *Punica granatum* (Hassan et al. 2020).

The study evoked the significant in vivo anthelmintic impact of CEEs of *A. herba-alba, B. aegyptiaca* in comparison with albendazole on lambs experimentally infected with *H. contortus*. A marked reduction in FECs after 3 days PT and continued throughout the experimental period was recorded in the group treated with CEE of *B. aegyptiaca* followed by *A. herba-alba* CEE-treated one. A great FECR (100% and 85.1%) was recorded for CEE of *B. aegyptiaca and A. herba-alba*, respectively, at the 7th day PT till the end of the experiment, while albendazole caused 88.1% FECR. These results agree with the results reported by Idris et al. (1982) who proved a successful impact of *A. herba-alba* on goat infected with haemonchosis; El-Ghazaly et al. (1997) who concluded that *B. aegyptiaca* had antischistosomicidal effect; and Koko et al. (2000) who observed the curative effect of *B. aegyptiaca* on liver fasciolosis. Furthermore, Jaheed et al. (2019) documented a potent anthelmintic activity of *B. aegyptiaca* ethanolic extract where FECR reached 88.10% on 4th week PT.

Table 5 Effect of various treatments on biochemical parameters of different experimental lamb groups

Parameter	Week	Experimental animals				
		G1	G2	G3	G4	G5
Serum total protein	0	$7.03 \pm .23^{Aab}$	6.53 ± 0.26^{Bb}	6.97 ± 0.23^{Aab}	7.5 ± 0.14^{Aa}	6.53 ± 0.26^{ABb}
	3	$5.87 \pm .067^{Ba}$	6.43 ± 0.23^{Ba}	4.9 ± 0.14^{Cb}	4.47 ± 0.37^{Cb}	5 ± 0.36^{Db}
	6	$7.03 \pm .23^{Aab}$	6.53 ± 0.26^{Bb}	6.97 ± 0.23^{Aab}	7.5 ± 0.14^{Aa}	6.53 ± 0.26^{ABb}
	9	$4.9 \pm .057^{Dc}$	$7.53 \pm .14^{Aa}$	6.9 ± 0.20^{Ab}	7 ± 0.10^{Aab}	6.87 ± 0.29^{ABb}
Serum albumin	0	3.8 ± 0.23^{Aa}	3.5 ± 0.26^{Ba}	3.7 ± 0.26^{ABa}	3.9 ± 0.088^{ABa}	4.1 ± 0.32^{Aa}
	3	2.57 ± 0.17^{Bb}	4.47 ± 0.35^{ABa}	1.9 ± 0.08^{Cbc}	2.0 ± 0.13^{Cbc}	1.9 ± 0.10^{Cc}
	6	1.73 ± 0.12^{Db}	4.1 ± 0.17^{ABa}	3.97 ± 0.088^{Aa}	4.07 ± 0.14^{ABa}	4.17 ± 0.088^{Aa}
	9	1.8 ± 0.033^{Db}	4.77 ± 0.26^{Aa}	4.37 ± 0.21^{Aa}	4.47 ± 0.37^{Aa}	4.2 ± 0.20^{Aa}
A/G ratio	0	1.2 ± 0.20^{Aa}	1.2 ± 0.31^{Aa}	1.2 ± 0.18^{Aa}	1.09 ± 0.00^{ABa}	1.8 ± 0.44^{Aa}
	3	0.79 ± 0.11^{Bb}	2.6 ± 0.68^{Aa}	0.64 ± 0.05^{Ab}	0.90 ± 0.20^{Bb}	0.63 ± 0.09^{Bb}
	6	0.53 ± 0.04^{Bb}	1.5 ± 0.23^{Aa}	1.4 ± 0.12^{Aa}	1.5 ± 0.22^{ABa}	1.8 ± 0.19^{Aa}
	9	0.59 ± 0.02^{Bb}	1.8 ± 0.36^{Aa}	1.8 ± 0.32^{Aa}	1.8 ± 0.43^{Aa}	1.6 ± 0.31^{Aa}
SGOT	0	11.07 ± 0.38^{Da}	12.73 ± 2.14^{Aa}	12.57 ± 1.18^{Ba}	12.53 ± 1.92^{Ba}	11.50 ± 0.52^{Ba}
	3	13.00 ± 0.23^{Cab}	11.97 ± 1.27^{Ab}	15.93 ± 1.56^{Aa}	15.73 ± 1.4^{Aab}	13.73 ± 0.66^{Aab}
	6	16.60 ± 0.35^{Aa}	11.73 ± 0.26^{Abc}	11.43 ± 0.23^{Bbc}	10.87 ± 0.35^{Bc}	12.50 ± 0.50^{ABb}
	9	13.60 ± 0.32^{BCa}	11.63 ± 0.69^{Ab}	11.57 ± 0.40^{Bb}	10.70 ± 0.20^{Bb}	11.83 ± 0.12^{Bb}
SGPT	0	24.6 ± 4.4^{Ba}	31.4 ± 11.9^{Aa}	25 ± 1.8^{Ca}	28.97 ± 4.7^{Ba}	33.4 ± 2.5^{Ba}
	3	42.8 ± 7.7^{Aab}	30.1 ± 9.8^{Ab}	47.7 ± 6.17^{Aab}	54.23 ± 4.0^{Aa}	56.2 ± 4.1^{Aa}
	6	43.3 ± 3.6^{Aa}	32.4 ± 9.4^{Aa}	32 ± 3.30^{BCa}	28.7 ± 5.8^{Ba}	34.4 ± 1.2^{Ba}
	9	40.7 ± 0.93^{ABa}	29.6 ± 9.2^{Aa}	27.4 ± 1.43^{Ca}	28.27 ± 4.9^{Ba}	33.6 ± 5.0^{Ba}
BUN	0	15.47 ± 2.0^{Ca}	13.1 ± 1.10^{Aa}	14.1 ± 1.9^{Aa}	13.57 ± 1.2^{Ba}	15.3 ± 1.4^{BCa}
	3	18.1 ± 2.26^{BCa}	11.2 ± 0.23^{Bb}	17.6 ± 2.0^{Aa}	17.3 ± 0.75^{Aa}	19.9 ± 1.2^{Aa}
	6	21.5 ± 0.36^{ABa}	10.8 ± 0.47^{Bc}	15.0 ± 0.86^{Ab}	10.7 ± 0.12^{Dc}	15.6 ± 0.26^{BCb}
	9	23.47 ± 0.87^{Aa}	11.0 ± 0.31^{Bc}	14.37 ± 1.09^{Ab}	10.9 ± 0.26^{CDc}	13.7 ± 0.79^{Cb}
Serum creatinine	0	0.95 ± 0.08^{Ca}	1.0 ± 0.16^{ABa}	1.07 ± 0.08^{Ba}	1.1 ± 0.10^{Ca}	1.2 ± 0.03^{BCa}
	3	1.8 ± 0.05^{Ba}	0.93 ± 0.06^{Bc}	1.6 ± 0.05^{Aab}	1.5 ± 0.06^{Ab}	1.5 ± 0.07^{Ab}
	6	2.1 ± 0.35^{ABa}	1.27 ± 0.06^{Ab}	1.1 ± 0.05^{Bb}	1.1 ± 0.12^{BCb}	1.0 ± 0.06^{Db}
	9	1.8 ± 0.05^{Ba}	1.06 ± 0.12^{ABb}	1.0 ± 0.04^{Bb}	1.0 ± 0.09^{Cb}	1.0 ± 0.06^{Db}

G, lambs group

Capital letters: means within the same column of different letters are significantly different at ($P < 0.01$). Small letters: means within the same row of different letters are significantly different at ($P < 0.01$)

The potent anthelmintic activity of the used ethanolic extracts might be related to the fact that alcohol-soluble active anthelmintic phyto-components had easy, efficient and rapid transcuticular absorption in the body of the worms due to the lipid-soluble nature of the ethanolic extracts (Eguale et al. 2007; Tariq et al. 2008). Moreover, Chothani and Vaghasiya (2011) reported that *B. aegyptiaca* contained very important bio-components including saponins, flavonoids, terpenoids, and phenolic. Besides, Meda et al. (2010) recorded significant antioxidant properties of *B. aegyptiaca*. The anti-parasitic affectivity of these phytochemical constituents had been also reported by Al-Shaibani et al. (2008) and Wang et al. (2010). It was mentioned that worm wood is also a rich source of various bioactive constituents, e.g. polyphenols, flavonoids and condensed tannins that had anti-parasitic effect (Mohamed et al. 2010; Akkari et al. 2014). In the present study, the CEE of *A. sativum* showed the lowest *in vitro* anthelmintic effect compared to the other used extracts. This acceptable anthelmintic activity might be attributed to the characteristic sulphur compounds in garlic (Itakura et al. 2001). Moreover, Chung (2006) documented antioxidant properties of garlic compounds due to the presence of alliin, an alkyl derivative of cysteine alkyl sulfoxide, allyl disulfide and allicin**.** A gradual improvement in RBCs count, Hb g/dL and PCV% in CEE of *B. aegyptiaca*, albendazole, and CEE of *A. herba-alba*-treated groups, respectively, was disclosed in comparison with the infected untreated group. These results coincided with Koko et al. (2000), Khalid et al. (2005),

Albadawi (2010) and Parmar et al. (2019). Meanwhile, these might be disagreed with that obtained by Bordoloi et al. (2012) who concluded that PCV per cent remained constant between *H. contortus*-infected and uninfected control group of lambs. The findings of the present study revealed that there was a significant decrease in Hb value, PCV% and RBCs counts, in sheep infected with *H. contortus* due to the blood-sucking activities of the developing larval and young adults of *H. contortus*. The parasite secretes cathepsin L-like cysteine proteases that could potentially assist the helminths access to the host blood (Rhoads and Fetterer 1995).

Although the infection induced elevation of the WBCs count and eosinophils level, the used treatments CEE of *B. aegyptiaca,* albendazole and CEE of *A. herba-alba* had successes to return the values to their normal extent (Koko et al. 2000; Albadawi 2010; Hassan et al. 2013; Parmar et al. 2019). Oeosinophilia is well recognized in *H. contortus*-infected sheep, as a mean of protection (Huang and Appleton 2016).

Moreover, the biochemical analysis of the experimentally infected sheep showed a significant decrease in total serum protein with a consequent decrease in serum albumin and the A/G ratio (Soulsby 1986; Bahrami et al. 2011; Khan et al. 2012; Parmar et al. 2019).

In the current study, the infected untreated group showed a higher level of serum GOT/AST, GPT/ALT, serum urea and creatinine level (Bordoloi et al. 2012. It documented that in muscle atrophy and diseases without liver damage, ALT is elevated. Plasma AST is also increased with both muscle and liver damage (Allen and Randell 1993). Furthermore, Al-Zubaidy et al. (1987) deduced that traumatic damage to the lining of abomasal mucosa due to 4th-stage larvae and adult *H. contortus* caused high level of serum ALT and AST. The most significant and efficient treatments which improved serum GOT/AST, GPT/ALT, serum urea and creatinine level to be returned to almost normal level were the CEE of *B. aegyptiaca* and the CEE of *A. herba-alba,* respectively. So the used extracts were quite efficient to improve the animal health conditions; these more or less resemble that obtained by Idris et al. (1982) and Bordoloi et al. (2012).

Conclusions

It could be concluded that the *in vitro* anthelmintic activity assay declared the successful anthelmintic effect of CEEs of *B. aegyptiaca*, *A. herba-alba* and *A. sativum* compared to albendazole on *H. contortus. The in vivo* test exposed that the CEE of *B. aegyptiaca* was the most effective followed by CEE of *A. herba-alba* in competing the infection and improving the animal health conditions. It is suggested that the CEEs of *B. aegyptiaca* and *A. herba-alba* might have liver and kidney protective effects and could be considered as unique alternative treatments to avoid or delay the anthelmintic drug resistance in ruminants.

Abbreviations

A. herba-alba: *Artemisia herba-alba*; *B. aegyptiaca*: *Balanites aegyptiaca*; *A. sativum*: *Allium sativum*; CEE: Crude ethanolic extract; BUN: Blood urea nitrogen.

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Authors' contributions

AZ, NMTA and MN designed, supervised and directed the experiment. NMFH prepared the plant extracts. NMFH, NMTA, MN and AS carried out the in vivo and in vitro experiment. NMFH, AZ, NMTA, MN and AS analysed and discussed the resultant data. NMFH edited the manuscript. NMFH revised and reviewed the manuscript for publication. All authors read and approved the final manuscript.

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Availability of data and materials

All data and material are available inside the manuscript.

Declarations

Ethics approval and consent to participate

The experiments were conducted in compliance with the requirements and recommendations of the Ethical Committee of the National Research Centre and the current Egyptian Law and Regulations that are assigned for the protection of the experimental animals to minimize the negative states (harms) and improve feeding and housing conditions under code number (16229). All authors read and approved the final manuscript. We had obtained written informed consent to use the animals in our study from the owner(s) of the animals. After the experiment had ended, owners of the Baladi lambs had regained the used animals.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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