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Original Article

The potential effect of Allium Sativum and Coriandrum Sativum as natural sources of antioxidants on *Haemonchus contortus* infection in sheep

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Abstract

In this study the effect of garlic and coriander extract in comparison to albendazole against experimental infection of *Haemonchus contortus* (*H. Contortus*) in sheep was evaluated. Sheep were divided into 5 groups; the 1st one represented control negative. The remaining groups were infected orally with 1750 *H. contortus* infective larvae (L3) and were equally subdivided into control positive (infected untreated), infected and treated orally with garlic (5ml/animal), infected and treated orally with coriander extract (0.9g/ kg b.w.) and infected and treated orally with Albendazole (10 mg/ kg b.w). The treatments were done on the 4th and 6th week post infection. Fecal samples were collected weekly after 3 weeks post infection for Fecal Egg Count (FEC). Blood samples were collected at the 5th till the 8th week post infection. Coriander and garlic treated sheep expressed a significant reduction in FEC compared to the control positive. While in albendazole treated group, FECs was zero at the 7th and 8th weeks post infection. Erythrogram showed normocytic normochromic anemia at the 5thand 6th week and normocytic hypochromic anemia at the 7th and 8th week in infected groups. Significant increase in total peroxide, malondialdehyde (MDA), protein carbonyl (PC), Nitric oxide (NO), and total free amino acids (TFAA) levels with significant decrease in reduced glutathione (GSH) and superoxide dismutase (SOD) values of all treated groups when compared to control negative one. Garlic and coriander treatments showed ameliorative effect as they decrease the levels of total peroxide, MDA, PC, NO and TFAA. Also, garlic, coriander and albendazole showed an improving effect on the levels of SOD when compared to control positive group. In conclusion, sheep nematodiasisis accompanied by disturbances in protein synthesis combined with a general state of oxidative damage. Moreover, garlic and coriander showed animproving effect on the antioxidants alterations in experimentally infested sheep with haemonchosis.

Key words: *Haemonchus contortus*; sheep; coriander ; garlic; antioxidant

1. Introduction

Haemonchus contortus is considered a major blood feeding nematode of the abomasum affecting ovine and other small ruminants leading to lowering in the weight gain, haemorraghic anemia, high mortalities (**Schweizer et al., 2016**) and reduction of wool and milk production (**Vieira et al., 2017**). Haemonchus contortus has a high propensity to develop resistance towards anthelmintic drugs (**Kotze and Prichard 2016**); therefore, the anthelmintic efficacy for effective treatment of *Haemonchus Contortus* becomes lower, causing a significant economic impact. As new strategies for parasite control are urgently needed, recent studies have focused on the use of alternative approaches to parasite control. In this respect, the use of medicinal plants may be an alternative parasite control to reduce the frequency of anthelmintic treatmentsin addition to decrease anthelmintic residues in animal products (**Besier et al., 2016**).

Garlic (Allium sativum) has been reported to have antimicrobial, antioxidant, antihypertensive as well as, parasiticidal, larvicidal, and fungicidal properties (**Worku et al., 2009,Zhong et al., 2019**). Anti-parasitic and anthelmintic activities of garlic were studied in mice (**Erol et al., 2008)**, rabbits (**Toulah and Al-Raw 2007**), female Boer goats (**Worku et al., 2009**) and fish (**Hyun Kim et al., 2019 and Yildiz et al., 2019**)

Coriandrum sativum L., belongs to the Apiaceaefamily, is a herb that cultivated all over the world. Extract from seeds of Coriandrum sativum have many pharmacological effects. It has anti-diabetic, anti-hyperlipidemic, and antioxidant activities (**Sahib et al. 2013**).The alcoholic seed extract of C. sativum reported to have a protective effect against Hymenolepis nana infection in mice (**Hosseinzadeh et al., 2016**).

The aim of the current study was to investigate the anthelmintic potential of the *Allium sativum* and *Coriandrum sativum* aqueous extracts to control experimentally infected sheep with *Haemonchus contortus*.

2. Materials and methods

2.1. Commercial diagnostic kits supplied by Biodiagnostic pharmaceutical chemicals, Egypt were used for determination of total protein and hemoglobin concentration. Other kits and reagents were obtained from Sigma Aldrich Company, Germany.

Albendazole (2.5% oral suspension) was purchased from EVA Pharma, Cairo, Egypt

2.2. Larvae preparation

The infective larvae obtained by culturing *Haemonchus contortus* eggs collected from *Haemonchus contortus* infected donor sheep. Adult female worms of *Haemonchus contortus* were collected from abomasums of infected sheep obtained from abattoir. The worms were washed and crushed to liberate eggs. The eggs then were cultured in a glass jar filled with autoclaved negative parasites sheep feces for 8 days at room temperature. At the end of the 8th day, infective larvae were harvested by rinsing the side of the culture jar with drops of water. About 3000 larvae were inoculated orally to a worm free sheep that kept indoor in a separate house throughout the study period. This sheep served as *Haemonchus contortus* egg donors **(Eguale et al., 2007b)**.

2.3. Plant extract preparation

Seeds of *Coriandrum sativum* were purchased from local market; an aqueous extraction was performed according to **Eguale et al. (2007a)**. *Allium sativum* (garlic juice) was obtained from Zoology Lab., Faculty of Science, Assiut University. It was prepared by mincing 90 g of unpeeled garlic cloves in a juicer/blender device (model no. MJ 176NR).

2.4. Animals and experimental design

Twenty five male healthy Rahmani sheep about 4 months old (16-20 kg b.w) were purchased and kept indoor fed with hay and concentrate and provided with water ad libitum. The animals were acclimatized to animal house condition for 2 weeks before starting of the experiment. During the first two days of this period, all the animals were givenalbendazole at 10 mg/kg. All procedures were carried out according to the protocol approved by Institutional Animal Care and Use Committee, Beni-Suef University. The sheep were equally divided into 5 groups; the first was kept as a negative control, while sheep in the second; third; fourth and fifth groups were inoculated orally with 1750 *Haemonchus contortus* infective larvae (L3) according to **Eguale et al. (2007a)**. The sheep in the second group represented the positive control. On the 4th and 6th weeks after infection, all sheep of the 3rd , 4th and 5th groups were orally administrated garlic juice (5 ml/animal) **(Workuet al., 2009)**, coriander (0.9 g/kg b.w. of aqueous extract) **(Eguale et al., 2007a),** and albendazole (10 mg/kg b.w), respectively. Three weeks post infection till the end of the experiment;

individual fecal samples were collected weekly for Fecal Egg Count (FEC) by McMaster technique **(Coles et al., 1980)**. Moreover, blood samples were collected from the jugular vein once a week on the 5th, 6th, 7th, and 8th after infection. Blood was collected on EDTA-containing tube to obtain whole blood sample for erythogram analysis and plasma and serum separation (for total serum protein estimation). Plasma and serum were stored at -20 C ˚till analysis. For erythrocyte hemolysate preparation**,** there maining packed RBCs after plasma separation were washed 3 times with physiological saline thenthe washed packed RBCs were resuspended (v/v) in an ice-cold distilled water, and stored at -20 ˚C until analysis.

2.5. Hematological parameters

The RBCs count, hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC),total leucocytes and differential counts were determined by automatic blood cell counter (Telldyn 3700, Germany).

2.6. Estimation of hemoglobin homogenate

As parameters measured in hemolysate was expressed to hemoglobin parameter, the Hb was determined colorimetrically according to the kits obtained.

2.7. Oxidative parameters

Erythrocytic total peroxide (T. peroxide) was determined following the method of **Harma et al. (2005)**. Malondialdehyde (MDA) in RBCs hemolysate was determined according to **Placer et al. (1966)**. Nitric oxide (NO) was determined in plasma according to **Ding et al.(1988)**. Protein carbonyl as an index of protein oxidation was estimated according to **Levine et al. (1990)**. Plasma total free amino acids (TFAA) was determined according to **Rosen (1957)**.

2.8. Antioxidant parameters

Superoxide dismutase (SOD) and reduced glutathione (GSH) were estimatedin the RBCs hemolysateaccording to the method described by **Misraand Fridovich (1972)** and **Beutler et al. (1963)**,respectively.

Copper (Cu) and ceruloplasmin (CP)were determined according to **Skoog et al. (1998) and Houchin (1958)**, respectively.

2.9. Assessment of total protein

Total protein was determined in serum according to **Peters (1968)**

2.10. Statistical analysis

The present results were expressed as means ± standard error. Statistical analysis was performed using the Graph Pad Prism

5.0 Software, CA, USA. Statistical significance of differences of all examined parameters was determined by means of the one way ANOVA, followed by the Tukey's test. "P" value of < 0.05 was assumed for statistical significance

3. Results

3.1. Fecal egg count (FEC)

Result of FEC is shown in figure (1). Determination of FEC revealed significant increase in garlic,coriander and control positive groups at the 3rd week till end of the experiment while albendazole group showed significant increase at the 3rd, 4th, 5th and 6th weeks of the experiment when compared with control negative group. Garlic and coriander groups showed significant reduction in FECs at 7th and 8th weeks of the experiment compared to the control positive group.

3.2. Hematological parameters

In tables (1)**,** the results of RBCs; Hb concentration and PCV values in different experimental groups showed a significant decrease from the 5th week post infection till the end of experiment compared to the control negative group. At the 7th week post infection, the Hb and PCV values in groups treated with coriander and albendazole were significantly increased compared to the control positive group. While at the 8th week; the RBCs, Hb and PCV values were significantly increased in groups treated with coriander and albendazole compared to control positive but still lower than the control negative group.

According to the MCV and MCHC values, anemia was detected as normocytic normochromic at the 5th and 6thweek, and normocytic hypochromic at the 7th and 8th week in all groups except the group of albendazole, compared to control negative group.

The obtained data of leucogram, table (2), showed a significant increase in total leucocytic count at the 5th and 8th week of experiment in the control positive and coriander treated groups compared to control negative group. While at the 6th week, there was an increase of total leucocytic count in all groups except the albendazole group compared to the control negative one. A significant decrease of total leucocytic count was noticed in albendazole group from the 5th till the 8th week when compared to control positive, garlic and coriander treated groups. The lymphocyte count was significantly elevated in coriander treated group compared to the control negative one at the 5th and 6th week of experiment. Neutrophil count was significantly increased in control

positive, garlic and coriander treated group when compared to control negative at the 8th week. Eosinophil count showed a significant increase in the control positive group at the 5th till the 8th week compared to the control negative group. In treated groups with garlic, coriander and albendazole there was a significant decrease in eosinophil count during the experiment time when compared to the control positive group. Monocyte count revealed a significant increase in control positive group at the 5thand 6th week of experiment when compared to control negative group.

3.3.Serum oxidants parameters

Results of T. peroxide, MDA, NO and protein carbonyl are shown in table (3).The control positive group showed a significant increase in T. peroxide content at the 6th week till8th week compared to the control negative group. Garlic treatment at the 7thweek showed a significant decrease in T. peroxide value, while the decrease was detected in coriander treated group at the 7th and 8th week post infection compared to control positive group. Albendazole treated group showed a significant increase in T. peroxide value at the 5thtill the 7th week compared to the control negative group.

The present data showed a significant increase in MDA and PC values in control positive group when compared to control negative one throughout the experiment. There was a significant decrease in MDA value in garlic treated group at the 5th, 7th and 8th week, while in coriander and albendazole treated groups the decrease was noticed throughout the experiment when compared to the control positive group. Protein carbonyl results revealed no significant changes in garlic and coriander treated groups when compared to control negative. While in albendazole group, the PC value showed significant increase comparing to control positive and control negative groups from 5th week till the 7th week of experiment.

Throughout the experiment, nitric oxide results showed a significant increase in control positive group compared to control negative group. In groups treated with garlic, coriander and albendazole, the nitric oxide level showed a significant decreases at the 6th week till 8th week when compared to the control positive group.

The TFAA level was significantly elevated in the control positive group when compared to the control negative throughout the experiment. Its level in garlic treated group showed a significant

decrease at the 5th and 6th week of experiment when compared to control positive group. While, coriander and albendazole treated groups showed a significant decreases in TFAA value at the 5th week till8th week compared to control positive group.

3.4. Antioxidants status

Results of GSH and SOD are shown in table (4). Values of GSH revealed a significant decrease in control positive, garlic and coriander groups compared to control negative group throughout time of the experiment. However, albendazole treated group showed significant an increases in GSH values at 6th, 7th and 8th weeks when compared to the control positive, garlic and coriander treated groups.

Values of SOD showed a significant decrease in the control positive group all over the experimental period compared to the control negative group. The SOD data revealed a significant increase in garlic, coriander and albendazole groups from the 5th week till the end of the experiment when compared with the control positive group.

The values of Cu revealed a significant decrease in the control positive, garlic, coriander and albendazole treated groups compared to the control negative group at the 5th week till the end of the experiment. While garlic, coriander and albendazole treated groups exhibited an ameliorative effect on copper levels from the 6th week till the end of the experiment when compared to the control positive group.

The present data of ceruloplasmin revealed a significant decrease in the control positive group when compared to control negative one. Values of ceruloplasmin in garlic, coriander and albendazole groups showed a significant increases compared to control positive group at the 5th week post infection till the end of the experiment.

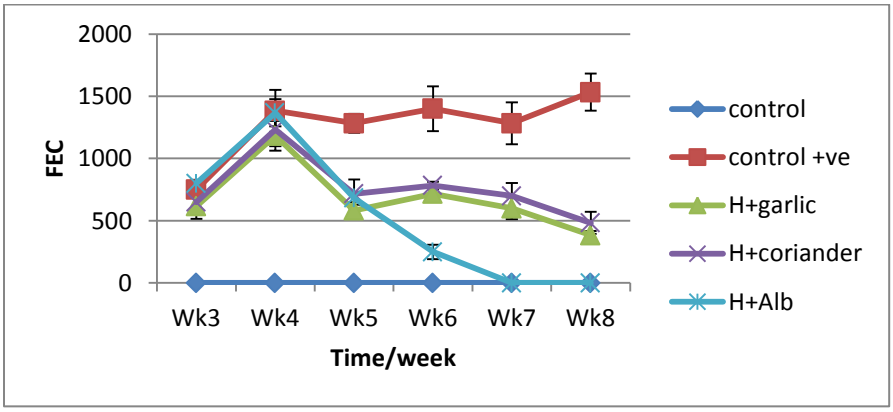

Figure (1): Fecal egg count of different experimental groups

Table (1): Erythrogram of the different experimental groups

Week of infection	Group	RBCs ($x10^6$/ul)	Hb (g/dl)	PCV (%)	MCV (fl)	MCHC (%)
5th	Control	8.3±0.05	13.3±0.06	35.9±0.87	43.3±1.30	37.0±0.81
	Control +ve	6.8±0.06[a]	11.3±0.40[a]	29.7±0.37[a]	43.8±0.34	37.9±0.90
	H+Garlic	6.9±0.34[a]	11.7±0.20[a]	35.7±1.30[b]	51.5±1.20	32.9±0.61
	H+Coriander	7.0±0.12[a]	12.5±0.15	35.5±1.90	50.8±3.64	35.5±2.15
	H+Albz	6.6±0.28[a]	12.3±0.37	33.5±1.24	50.9±2.13	36.8±2.10
6th	Control	8.1±0.03	14.8±0.26	37.4±0.66	46.6±0.67	39.5±1.39
	Control +ve	6.8±0.12[a]	10.9±0.49[a]	28.4±1.20[a]	43.0±1.50	37.1±0.93
	H+Garlic	6.2±0.35[a]	10.7±0.42[a]	29.1±3.12[a]	46.6±2.40	37.3±2.70
	H+Coriander	6.9±0.06[a]	11.7±0.09[a]	32.3±1.90	46.7±2.50	36.3±1.90
	H+Albz	7.2±0.03[a,c]	12.4±0.10[a,b,c]	35.1±0.63	48.47±0.94	35.5±0.90
7th	Control	8.6±0.08	13.7±0.12	37.0±0.44	43.3±0.64	37.1±0.44
	Control +ve	6.5±0.34[a]	8.8±0.17[a]	28.7±0.88[a]	44.6±1.50	30.9±1.45[a]
	H+Garlic	6.3±0.30[a]	9.3±0.17[a]	30.7±1.00[a]	48.6±2.90	30.3±1.23[a]
	H+Coriander	7.5±0.22[a,c]	10.7±0.32[a,b,c]	35.0±0.75[b,c]	46.9±1.41	30.7±1.3[a]
	H+Albz	7.1±0.10[a]	12.6±0.08[a,b,c,d]	34.1±0.60[b]	47.8±0.92	37.0±0.85[b,c,d]
8th	Control	8.7±0.19	13.9±0.07	36.4±0.46	41.9±1.10	38.1±0.51
	Control +ve	6.6±0.10[a]	9.6±0.35[a]	30.3±1.09[a]	46.4±2.20	31.5±0.67[a]
	H+Garlic	6.8±0.25[a]	10.3±0.06[a]	30.6±0.81[a]	44.9±1.90	33.7±0.80[a]
	H+Coriander	7.7±0.12[a,b,c]	11.9±0.15[a,b,c]	36.2±0.30[b,c]	47.1±0.50	33.1±0.65[a]
	H+Albz	7.4±0.02[a,b]	12.8±0.15[a,b,c]	34.93±0.92[b,c]	47.5±1.4	36.6±0.77[b,d]

Data are expressed as means ± SE with dissimilar superscript letters (significantly differing at $P < 0.05$): a) significantly different from control value; b) significantly different from the control +ve group; c) significantly different from the H+Garlic group and d) significantly different from the H+Coriander group. Groups: Control; Control +ve, represents sheep infected with *H.controtus*; H+Garlic group represents *Haemonchus contortus* infection with garlic treatment; H+Coriander group represents *Haemonchus contortus* infection with coriander treatment. H+Albz group represents *Haemonchus contortus* infection with Albendazole treatment

Table (2): Leucogram of different experimental groups.

Week of infection	Group	WBCs ($x10^3$/ul)	Neutrophil ($x10^3$/ul)	Lymphocyte ($x10^3$/ul)	Eosinophil ($x10^3$/ul)	Monocyte ($x10^3$/ul)
5th	Control	7.9±0.46	2.1±0.40	5.1±0.20	0.34±0.03	0.24±0.01
	Control +ve	10. 9±0.52[a]	2.7±0.30	6.1±0.41	0.76±0.09[a]	0.62±0.01[a]
	H+Garlic	9.4±0.26	3.1±0.22	6.0±0.50	0.47±0.03[b]	0.21±0.02
	H+Coriander	10.7±0.26[a]	2.9±0.34	6.9±0.27[a]	0.37±0.03[b]	0.25±0.03
	H+Albz	8.9±0.21[b,d]	2.8±0.20	5.3±0.33[d]	0.31±0.03[b]	0.40±0.01
6th	Control	7.9±0.50	2.3±0.40	5.19±0.43	0.10±0.03	0.24±0.02
	Control +ve	10.8±0.42[a]	2.8±0.44	6.8±0.39	0.60±0.11[a]	0.29±0.09
	H+Garlic	10.8±0.50[a]	2.2±0.50	7.6±0.55[a]	0.15±0.03[b]	0.78±0.04[a]
	H+Coriander	12.7±0.28[a]	3.4±0.17	8.7±0.18[a,b]	0.17±0.03[b]	0.23±0.02
	H+Albz	7.7±0.49[b,c,d]	1.9±0.43	4.9±0.43[c,d]	0.11±0.04[b]	0.32±0.05
7th	Control	8.6±0.63	3.0±0.20	5.1±0.28	0.12±0.10	0.69±0.02
	Control +ve	10.9±0.44	2.6±0.37	7.1±0.23[a]	0.75±0.09[a]	0.48±0.09
	H+Garlic	9.9±0.49	3.4±0.27	5.9±0.12	0.27±.080[b]	0.41±0.11
	H+Coriander	10.7±0.60	2.6±0.27	6.6±0.31	0.11±0.06[b]	0.63±0.10
	H+Albz	6.6±0.37[b,c,d]	2.1±0.24[c]	3.93±0.52[b,c,d]	0.12±0.02[b]	0.30±0.08
8th	Control	8.1±0.42	1.6±0.20	5.0±0.26	0.11±0.07	0.53±0.15
	Control +ve	11.0±0.49[a]	2.9±0.29[a]	6.9±0.38[a]	0.57±0.05[a]	0.45±0.08
	H+Garlic	9.40±0.37	2.8±0.27[a]	5.8±0.21	0.18±0.02[b]	0.49±0.01
	H+Coriander	10.7±0.15[a]	3.7±0.18[a]	5.9±0.10	0.33±0.04	0.68±0.04
	H+Albz	7.8±0.16[b,c,d]	1.8±0.09[b,d]	3.8±0.52[b,c,d]	0.23±0.05[b]	0.64±0.03

Data are expressed as means ± SE with dissimilar superscript letters (significantly differing at $P < 0.05$): a) significantly different from control value; b) significantly different from the control +ve group; c) significantly different from the H+Garlic group and d) significantly different from the H+Coriander group. Groups: Control; Control +ve, represents sheep infected with *H.controtus*; H+Garlic group represents *Haemonchus contortus* infection with garlic treatment; H+Coriander group represents *Haemonchus contortus* infection with coriander treatment. H+Albz group represents *Haemonchus contortus* infection with Albendazole treatment.

Table (3): Total peroxide, malondialdehyde (MDA), protein carbonyl (PC), nitric Oxide (NO), and Total free amino acids (TFAA) values of different experimental groups.

Week of infection	Group	T. peroxide (µmol/mg Hb)	MDA (nmole/mg Hb)	PC (µmole/mgHb)	NO (nmole/mg protein)	TFAA (µg/mg protein
5th	Control	0.8±0.04	30.1±1.71	0.24±0.05	0.14±0.04	3.7±0.39
	Control +ve	1.8±0.30	158.1±1.52[a]	0.89±0.11[a]	0.89±0.06[a]	11.8±1.39[a]
	H+Garlic	1.7±0.07	52.1±4.36[a,b]	0.45±0.13	0.32±0.08	4.7±0.64[b]
	H+Coriander	1.2±0.09	53.2±4.33[a,b]	0.24±0.02[b]	0.98±0.07[a,c]	5.4±0.54[b]
	H+Albz	2.9±0.52[a]	50.8±3.36[a,b]	1.4±0.16[a,b,c,d]	1.00±0.25[a,c]	4.5±0.37[b]
6th	Control	0.5±0.01	29.5±1.12	0.28±0.05	0.14±0.02	3.44±0.38
	Control +ve	1.8±.016[a]	85.4±2.70[a]	1.0±0.12[a]	0.77±0.06[a]	8.5±0.75[a]
	H+Garlic	1.2±0.19	74.0±4.54[a]	0.36±0.02[b]	0.23±0.05[b]	4.7±0.59[b]
	H+Coriander	1.0±0.14	66.3±0.48[a,b]	0.24±0.06[b]	0.36±0.07[b]	5.6±0.56[b]
	H+Albz	3.1±0.32[a,b,c,d]	46.5±2.7[a,b,c,d]	2.00±0.25[a,b,c,d]	0.25±0.06[b]	3.6±0.48[b]
7th	Control	0.5±0.03	40.4±1.4	0.25±0.06	0.12±0.02	3.3±0.63
	Control +ve	3.3±0.44[a]	116.7±8.82[a]	1.1±0.05[a]	0.75±0.05[a]	6.2±1.28[a]
	H+Garlic	1.4±0.10[b]	74.1±3.61[a,b]	0.39±0.05[b]	0.52±0.07[a,b]	5.6±0.45[a]
	H+Coriander	1.00±0.05[b]	50.4±1.8[b,c]	0.33±0.01[b]	0.48±0.01[a,b]	6.1±0.17[.a,b]
	H+Albz	2.3±0.30[a,d]	75.8±2.90[a,b,d]	2.01±0.27[a,b,c,d]	0.14±0.02[b,c,d]	2.8±0.49[b,c,d]
8th	Control	0.6±0.07	29.6±1.80	0.25±0.05	0.13±0.02	3.0±0.31
	Control +ve	2.5±0.31[a]	157.0±3.10[a]	2.3±0.31[a]	0.66±0.01[a]	7.1±0.35[a]
	H+Garlic	1.7±0.15[a]	65.7±3.50[a,b]	0.15±0.04[b]	0.28±0.01[a,b]	6.0±0.60[a]
	H+Coriander	1.1±0.14[b]	41.7±1.23[a,b,c]	0.33±0.04[b]	0.29±0.04[a,b]	4.8±0.36[b]
	H+Albz	1.8±0.23[a]	40.6±2.52[b,c]	1.35±0.19[a,b,c,d]	0.17±0.02[b]	3.1±0.30[b,c]

Data are expressed as means ± SE with dissimilar superscript letters (significantly differing at $P < 0.05$): a) significantly different from control value; b) significantly different from the control +ve group; c) significantly different from the H+Garlic group and d) significantly different from the H+Coriander group. Groups: Control; Control +ve, represents sheep infected with *H.controtus*; H+Garlic group represents *Haemonchus contortus* infection with garlic treatment; H+Coriander group represents *Haemonchus contortus* infection with coriander treatment. H+Albz group represents *Haemonchus contortus* infection with Albendazole treatment.

Table (4): Values of reduced glutathione (GSH), superoxide dismutase (SOD), copper (Cu) and ceruloplasmin (CP) from different experimental groups.

Week of infection	Group	GSH (mg/mg Hb)	SOD (U/mg Hb)	Cu (µg/dl)	Ceruloplasmin (mg/g protein)
5th	Control	1.24±0.18	2.0±0.14	45.3±2.7	7.6±0.43
	Control +ve	0.30±0.05[a]	1.2±0.19	20.3±1.6[a]	2.5±0.21[a]
	H+Garlic	0.33±0.02[a]	1.9±0.07	26.2±1.82[a]	6.7±0.55[b]
	H+Coriander	0.32±0.03[a]	2.00±0.26	26.4±1.61[a]	6.2±1.30[b]
	H+Albz	0.31±0.02[a]	2.00±0.36	19.4±3.33[a]	4.4±0.46[a,b,c]
6th	Control	0.97±0.09	1.7±0.03	44.6±1.23	8.2±0.57
	Control +ve	0.28±0.02[a]	0.60±0.16[a]	13.9±2.01[a]	3.2±0.33[a]
	H+Garlic	0.32±0.04[a]	1.6±0.15[b]	28.7±2.02[a,b]	7.8±0.76[b]
	H+Coriander	0.30±0.04[a]	1.8±0.21[b]	25.9±1.56[a,b]	7.8±0.39[b]
	H+Albz	0.73±0.07[b,c,d]	2.4±0.22[b]	28.1±1.57[a,b]	6.2±0.34[b]
7th	Control	1.1±0.05	1.8±0.15	44.20±2.34	8.6±0.28
	Control +ve	0.32±0.02[a]	0.94±0.05[a]	12.76±0.85[a]	3.7±0.17[a]
	H+Garlic	0.45±0.03[a]	2.0±0.11[b]	23.1±1.55[a,b]	7.6±0.57[b]
	H+Coriander	0.44±0.03[a]	1.8±0.11[b]	32.2±1.6[a,b,c]	6.8±0.19[b]
	H+Albz	0.96±0.07[b,c,d]	1.8±0.05[b]	29.7±1.8[a,b]	6.8±0.83[b]
8th	Control	1.1±0.09	1.8±0.04	44.6±1.65	8.8±0.22
	Control +ve	0.20±0.03[a]	0.85±0.06[a]	14.3±0.80[a]	4.2±0.01[a]
	H+Garlic	0.50±0.02[a]	2.2±0.21[b]	25.5±1.3[a,b]	8.7±0.42[b]
	H+Coriander	0.31±0.04[a]	1.7±0.23[b]	31.4±1.5[a,b]	7.2±0.51[b]
	H+Albz	1.09±0.12[b,c,d]	2.2±0.24[b]	39.9±1.6[b,c,d]	7.1±0.89[b]

Data are expressed as means ± SE with dissimilar superscript letters (significantly differing at $P < 0.05$): a) significantly different from control value; b) significantly different from the control +ve group; c) significantly different from the H+Garlic group and d) significantly different from the H+Coriander group. Groups: Control; Control +ve, represents sheep infected with *H.controtus*; H+Garlic group represents *Haemonchus contortus* infection with garlic treatment; H+Coriander group represents *Haemonchus contortus* infection with coriander treatment. H+Albz group represents *Haemonchus contortus* infection with Albendazole treatment.

4. Discussion

Haemonchus contortus is considered the main parasitic infestations in sheep **(Tak et al., 2017)**. In the current study, the coriander and garlic treated animals expressed a reduction in FECs levels

compared to the control positive, however, albendazole treatment was more effective as the FECs were zero for all sheep treated with albendazole at the 7th and 8th weeks post infection. *Haemonchus contortus* is the major hematophagous intestinal parasite of sheep **(Rouatbi et al., 2016).** In the present study, the reduction in PCV % were consistent with the high levels observed for FEC. Experimentally infected groups with *H. contortus* and those treated with garlic, coriander and albendazole showed a normocytic normochromic anemia at the 5th and 6th weeks, a finding which is in agreement with **Qasim (2015),** while at 7th and 8th weeks of the experiment they showed a normocytic hypochromic anemia which is on line with **Bordoloi et al. (2012)** who stated that, experimental haemonchosis in sheep caused a hypochromic anemia at 35-42 days post infection. The reduction in RBCs, Hb and PCV in infected groups may be due to the damage of the gastrointestinal mucosa and blood loss caused by *H. contortus* as it is a blood sucking parasite (**Shashank et al., 2019**). Garlic extract was not able to improve the PCV however, coriander extract and albendazole treatment were effective in improving and maintaining their PCV. The improvement in PCV %in albendazole treated group may be due to the complete removal of the parasite.

The leucocytosis that observed during the current experiment is due to the increase in the immune response against the parasite, as lymphocytosis, eosinophilia and monocytosis were detected in control positive group. Eosinophils have a major role for the immune response against nematodes infestations (**Balic et al., 2000**). Monocytosis may be due to increase the phagocytic activity to remove the parasitic debris (**Ahmed et al., 2015**).

Oxidative stress arises when there is an imbalance between radical-generating and radical-scavenging activity, resulting in an excessive production of reactive oxygen species (ROS) **(Urban-Chmielet al., 2009)**. The most sensitive part of the cell towards the action of ROS, primarily the HO, is polyunsaturated fatty acids (PUFA) of cell membranes.The present results showed an increase in total peroxide concentration in control positive as well as albendazole groups when compared to control. This finding is considered as an indicator of increased production of free radicals and oxidative stress as a result of infestation of sheep with gastrointestinal nematodes and chemical stress caused by albendazole

(**Dimitrijevi´c et al., 2012**). The increase of T. peroxide in garlic group at the 8th week of experiment may be due to the presence of S-allylcysteine (SAC); a water soluble constituent of garlic, which may induce increased value of total peroxides **(Balasenthil et al., 2000).**

Infected sheep treated with coriander extract showed no significant increase in total peroxide values during the experimental time; this may be due to their redox potential which important for adsorbing and neutralizing free radicals (**Balasundram et al., 2006)**.

The end product of lipid peroxidation is MDA, so that the increase of its concentration in tissues or biological fluids is considered as an indicator of increased oxidative stress **(Halliwell and Chirico, 1993).** A significant increased level of MDA in Akkaraman sheep infested with *Fasciola spp., Trichostrongylidae,* and *Eimeria spp*. was reported by **Dede et al. (2000)**. Also, **Simsek *et al.* (2006) and Dimitrijevi´c et al. (2012)** reported that MDA level increased with *Dicrocoelium dendriticum* and *Strongyloide spapillosus* infestation of sheep, respectively. The obtained data showed an increase in MDA values in all groups of experimentally infested sheep. This may be attributed to oxidative damage in the cell membrane which possibly resulting from overproduction of free radicals **(Dimitrijevi´cet al., 2012)**. The treatment with garlic, coriander extracts as well as the albendazole were able to lower the MDA level compared to control positive, but not able to return it to normal indicating improvement of the antioxiant status. Quercetin, a flavonoid thatpresent in *C. sativum* seeds, can reduce lipid peroxidation and enhance the activities of antioxidant enzymes (**Liu et al., 2010**).

It has been established in mammalian system that direct damage to proteins or chemical modification of amino acids in proteins during oxidative stress, can give rise to protein carbonyls **(Stadtman and Berlett, 1998)**. The usage of PC as biomarkers of oxidative stress has some advantages in comparison with the measurement of other oxidation products because of the relative early formation and the relative stability of carbonylated proteins **(Dalle-Donne et al., 2003)**. The highly reactive hydroxyl radical (OH•) which is generated by high concentration of H_2O_2 is considered to be responsible for the formation of PC **(Oliver, 1987)**.

Dimitrijevi´c et al. (2012) detected high level of PC in sheep infested with *Strongyloides papillosus*. In the present study, the increased erythrocytic PC in control positive and albendazole treated groups throughout the experiment suggests a rise in the oxidative damage of both cell membrane protein and hemoglobin confirming an enhancement of erythrocytic free radical overproduction.

The nitrite content represents a marker for increased reactive nitrogen species (RNS) production. In the present study, the highest nitrite concentration was shownin the infected groups, which indirectly demonstrates a stronger production of nitrogen (II)-oxide (NO).

Albendazole (ABZ) is the drug of choice for the therapy of haemonchosis and other parasitic infections **(Kassai, 1999)**. In domestic sheep after per oral administration, ABZ is metabolized through a two-step sulphoxidation **(Capece et al., 2009)**. During the process of drug biotransformation, ROS is generated and consequently its leakage from these systems could occur **(Guengerich, 2008)**. Although the majority of researchers state that practically the entire amount of the applied ABZ and its metabolites are eliminated from sheep blood plasma and gastrointestinal tract after about 60–70 h **(Moreno et al., 2004)**, it may be presumed that the amount of ROS generated during the biotransformation of ABZ is not to be neglected. In a 10-day trial period in rats treated with ABZ, **Locatelli et al. (2004)** have concluded that the treated animals seem to be unable to achieve adequate and persistent antioxidant compensation in relation to ROS/RNS generation and their deleterious effects on cell homeostasis. A number of authors state that ABZ is suspects of clastogenic, teratogenic or cytotoxic activity **(Dayan, 2003)**. Supporting the previous reports, the results of our research agreed with **(Dimitrijevi´cet al., 2012)** as the authors showed that the level of oxidative stress (T. peroxide and PC) is more intense after treatment of infected sheep with albendazole. In addition, the intense increase of carbonyl groups is probably due to the consequence of a more intense production of ROS and the cumulative effect caused by the biotransformation of ABZ **(Dimitrijevi´c et al., 2012)**. However, there were decreased values of MDA and NO which may be contributed

to that, the treated sheep with albendazole seem to be able to achieve adequate and persistent antioxidant compensation in relation to MDA and NO generation.

Proteins are the most abundant nutrients of the blood and therefore, the major digestive enzymes in blood-sucking parasites are thought to be proteases **(Dalton, 2003)**. Proteases facilitate the invasion of host tissues, aid in the digestion of host proteins and help parasites evade the host immune response**.** Proteases encompass a broad class of hydrolytic enzymes that play essential roles in digestive processes of proteins **(Williamson et al., 2003)** leading to protein degradation and increased formation of plasma total free amino acids (TFAA). Determination of TFAA can provide a useful information about the total pool of each free amino acid and also about protein metabolism **(Canepa et al., 2002)**. Infested sheep have lower food intake **(Vervelde et al., 2001)**. Malnutrition of the infested sheep causes increased protein breakdown and consequently increased plasma TFAA level to support gluconeogenesis **(Almeida et al., 2006)**. Similarly, in the present investigation there is a significant increase in plasma TFAA in control positive group. Such finding perhaps due to increased protein degradation through the action of proteases enzyme or due to decreased both uptake and absorption of nutritional amino acids caused by malnutrition and increased abomasal pH. Groups treated with coriander extract and albendazole showed a significant decrease of TFAA as a reason of increased protein metabolism which probably due to activation of gluconeogenesis.

Reduced glutathione is an important antioxidant protein enzyme, as well as a co-factor for various antioxidant enzymes **(Kidd, 1997)**. It acts as a substrate in the detoxification of peroxides such as hydrogen peroxide **(Rahman and MacNee, 1999)**. Super oxide dismutase activity has been proposed as the main reductant of oxygen in mitochondrial membranes **(Inoue et al., 2003)** and its activity may be increased in the case of a larger production of O_2^-or inhibited in the case of an increased production of H_2O_2, which arises by enzymatic oxidation of the radical superoxide anion **(Halliwell and Gutteridge, 1999)**. The authors added that nematodiasis of sheep may

cause excessive release of hydroxyl free radical rather than hydrogen peroxide. **Reznick and Packer (1994)** found that oxidative modification of proteins may lead to the structural alteration and functional inactivation of many enzyme proteins including GSH and SOD. Supporting these findings, the depletion of GSH is in harmony with the depletion of SOD which is obviously occurred in all infected groups is agreed with (**Deger et al., 2008)**. The current data exhibits that both GSH and SOD depletions may be at least in part to the excess protein carbonyl accumulation, which was probably concomitant with increased production of H_2O_2.

The radical scavenging activity of garlic, coriander and albendazole to overcome the increased production of H_2O_2 assists in increased activity of SOD enzymes, which were obviously clear in our data after treatment with the three previously mentioned treatments.

Ceruloplasmin acts as an extracellular scavenger of free radicals, thus it may protect the cells against ROS (**Saenko et al., 1994**). Activity of CP and the serum or plasma copper (Cu) concentration decreases with nutritional copper depletion of ruminants (**Blakley and Hamilton, 1985**). **Frandsen (1982)** reported that blood Cu levels are depressed in ruminants infested with nematodes. Most of the works reported on the relationship between endoparasitism and Cu deficiency have been based on the oral Cu supplementation **(Adogwa et al., 2005)**. The present data has shown that both plasma CP and Cu ions were significantly decreased in infected group. **Mulcahy et al. (2004)** reported that *H. contortus* increases pH value of abomasums. Consequently, it could be suggested that *H. contortus*affect Cu metabolism perhaps due to interference with Cu absorption from the gastrointestinal tract through increasing the pH of the abomasal environment. Supporting this finding, **Adogwa et al. (2005)** suggest that gastrointestinal parasites affect Cu metabolism probably by interference with Cu absorption from the gastrointestinal tract. Accordingly, *Haemonchus* infestation may cause Cu deficiency in blood which in turn, leads to reduction in plasma CP. Thus, it could be expected overproduction of hydroxyl free radicals and H_2O_2. Garlic extract treated group showed a significant decrease in the Cu level when compared

to control negative group, supporting this finding *A. sativum* causes increase in the pH of the gastric juice **(Ben Hadda et al., 2014)** and hence affected Cu absorption, also it may contributed to antioxidant powerful effect of the garlic on the liver to enhance the CP formation on account Cu ions and so increase CP and decrease Cu. While, albendzole treated group showed a significant increase in the Cu level at 8th week of the experiment, this may be attributed to increase the absorption of Cu from abomasum.

Coriander is a good source of polyphenols and phyto-chemicals due to its high antioxidant activity **(Msaada et al., 2017)**. Coriander seeds contain antioxidants **(Wangensteen et al., 2004)**, its antioxidant content is attributed to its high content of pigments particularly carotenoids. The carotenoids of its extract were found to show higher hydroxyl radicals scavenging potential thereby protecting cells from oxidative damage **(Peethambaran et al., 2012)**. These findings support the current results, as the coriander decreased the values of T.peroxide, MDA, PC and NO and has an ameliorative effect than the other treated groups especially at 7th and 8th week of the experiment.

The high tannin content in *A. sativum* may have a direct anthelmintic effect on the resident worm population disrupting the normal physiological functions like mobility, food absorption and reproduction, the later mode of action agrees with **Duval (2004)** who asserted that *A. sativum* did not prevent the production of egg but prevented the egg from developing into larvae. This reduction in larvae will subsequently reduce the worm burden in the hosts. The current study, supporting the previous concept, as the garlic extract was more effectively reducing the FEC than coriander extract.

While mode of action of both albendazole and coriander is through transcuticular diffusion which is, a common means of entry into helminth parasites for non-nutrient and non-electrolyte substances in nematodes **(Geary et al., 1999)**.

5. Conclusion

From the present study, it could be concluded that sheep nematodiasis is accompanied by protein oxidation and a state of oxidative stress. *Coriandrum sativum* may aid in improvement of the

antioxidants status of infested sheep with *Haemonchus contortus*. In addition, garlic juice and coriander enhance the reduction of the fecal egg count of the infested sheep with Haemonchosis, which results in reducing the intensity of infection. However, the detected efficacy of garlic and coriander extract are not to the therapeutically required level. This probably can be improved by repeating the dose or using it in a combination with anthelmintic drugs to reduce the number of anthelmintic treatment.

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الملخص العربي

التأثير المحتمل لمستخلص الثوم والكزبرة كمصادر طبيعية لمضادات الأكسدة على عدوى الهيمونكس كونتورتس في الأغنام

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أجريت هذه الدراسة لمعرفة تأثير الإصابة بالديدان الخيطية المعوية في الأغنام على المتغيرات الدموية، البيوكيميائية، مواد التأكسد/ مضادات الأكسدة وللمقارنة بين استخدام عصير الثوم والمستخلص المائى لنبات الكزبرة في مقاومة العدوى بطفيل الهيمونكس كونتورتس. تم تقسيم الأغنام إلى ٥ مجموعات ؛ الأول يمثل مجموعة الضابطة السلبية. تم إصابة المجموعات المتبقية عن طريق الفم بـ ١٧٥ .يرقات معدية (L3) وتم تقسيمها بالتساوي إلى الضابطة الايجابية (مصابة غير معالجة) ، أصيبت وعولجت عن طريق الفم بالثوم (٥ مل / حيوان) ، أصيبت وعولجت عن طريق الفم بمستخلص الكزبرة (٠.٩ جم / كجم من وزن الجسم) وأصيب ويعالج عن طريق الفم بألبندازول (١٠ ملغم / كجم من وزن الجسم). تم العلاج في الأسبوعين الرابع والسادس من العدوى. تم جمع عينات البراز أسبوعيا بعد ٣ أسابيع من الإصابة و ذلك لعد البيض FEC. تم جمع عينات الدم من الخامس حتى الأسبوع الثامن بعد الإصابة. أظهرت النتائج أن المعالجة بمستخلص الكزبرة والثوم أدي الي انخفاض كبير في FEC مقارنة مع الضابطة الإيجابية. أما في المجموعة المعالجة بالبيندازول كانت حالات FEC صفرًا في الأسبوعين السابع والثامن. أظهر الفحص الخلوي لكرات الدم الحمراء فقر الدم normocytic normochromic في الأسبوع الخامس و السادس . وفقر الدم normocytic hypochromic في الأسبوع السابع والثامن في المجموعات المصابة. وجد زيادة ملحوظة في إجمالي بيروكسيد ، مالونديالديهيد (MDA) ، بروتين الكربونيل (PC) ، أكسيد النيتريك (NO) وإجمالي مستويات الأحماض الأمينية الحرة (TFAA) مع انخفاض كبير في انخفاض قيم الجلوتاثيون (GSH) وفوق أكسيد الأكسيد الفائق (SOD) لجميع المجموعات المعالجة بالمقارنة مع السيطرة السلبية. أظهرت علاجات الثوم والكزبرة تأثيرًا تحسسيًا لأنها تقلل من مستويات البيروكسيد الكلي ، MDA، PC، NOو .TFAA. كما أظهر الثوم والكزبرة والألبندازول تأثيرًا محسنًا على مستويات SOD عند مقارنته بالمجموعة الضابطة الموجبة. من تلك الدراسة نستنتج أن الإصابة بالديدان الخيطية المعوية في الأغنام يؤدي إلى اضطرابات في تخليق البروتين مع حالة عامة من الأكسدة . بالإضافة إلى أنه كانت هناك تأثيرات واضحة تحسينية لنبات الثوم والكزبرة على تغيرات مضادات الأكسدة في عدوى الأغنام التجريبية.