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Evaluation The Antioxidants Effect of Garlic Juice on Experimental Sheep Haemonchosis

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ABSTRACT

The present study aimed to examine the defensive mechanism of garlic juice (*A.sativum*) to counteract experimental *Haemonchus contortus* infection in sheep and its effect on hematological, some antioxidants and biochemical parameters. Nine male sheep were used in this experiment and divided into 3 groups 3 in each (first group was control negative, second group was infected and received garlic juice at dose 5ml/ animal and third group was infected and not treated). Fecal and blood samples were collected for analysis. Our data revealed that the both treated groups showed a significant decrease in RBCs count and hemoglobin concentration when compared to control -ve, in contrast, the *Garlic juice* group showed significant increase in PCV% when compared with control +ve. Our data revealed that there was a significant decrease of Protein Carbonyl (PC) and Catalase (CAT) in *Garlic juice* group when compared to control +ve group while Malondialdehyde (MDA) revealed significant increase in *Garlic juice* group when compared to control-ve group. Determination of Ceruloplasmin (CP), Superoxide dismutase (SOD) revealed significant increase in *Garlic juice* group in comparable with control+ve group. However, determination of reduced glutathione (GSH) revealed significant decrease in *Garlic juice* and control+ve groups when compared to control-ve. Determination of Total free amino acids (TFAA) revealed significant decrease in *Garlic juice* group when compared to control+ve. In addition, there was a significant decrease of both Iron (Fe) and Copper (Cu) in *Garlic juice* group when compared to control-ve. This study suggests the improving effect of garlic juice on the antioxidants alterations in experimental sheep haemonchosis.

Keywords: Sheep; Haemonchus contortus; Protein carbonyl; Allium sativum; Oxidative stress.

Introduction

Sheep are affected by many species of nematodes, cestodes and coccidia that ultimately lead to production losses. The most important parasites of sheep are gastrointestinal nematodes, out of these parasites are the genera of trichostrongylid nematodes that live in digestive tract of ruminants include

Haemonchus, *Trichostrongylus*, *Teladorsagia*, and *Cooperia***.** In terms of economics, these parasites cause reduction in growth and development of farm products (milk, meat, and wool). Prophylaxis and treatment expenses are massive burdens in animal husbandry **(Miller and Horohov, 2006)**.

Egypt is considered as one of the important endemic parts of the world for these parasites. The predominant species of nematodes affecting sheep belong to the Superfamily Trichostrongyloidea include *Haemonchus contortus (H. contortus)* and *Telodorsagia circumcincta*, located in the abomasum, and *Trichostrongylus colubriformis (T. colubriformis)*, *Cooperia* spp., *Nematodirus* spp. and *Oesophagostumum* spp., located in the small and large intestine respectively **(Miller and Horohov, 2006)**.

H. contortus is extremely pathogenic and the most economically devastating parasite of sheep and goats worldwide **(Mortensen *et al.*, 2003)**. *H. contortus* is commonly called the barber pole worm due to a distinct red and white striped appearance, caused by the helical winding of the white egg-filled uterus of the female adult worm wrapped around the digestive tract. The female adult worms are 18-30 mm long while the male adult worms are smaller and thinner than the females, at 10-16 mm long **(Zajac, 2006)**.

Oxidative stress arises when there is an imbalance between radical-generating and radical-scavenging activity; it may therefore cause an increase in the formation of oxidation products **(Gutteridge, 1995)**. The excessive production of reactive oxygen species (ROS) usually results from the excitation of O_2 to form free oxygen or from the transfer of one, two or three electrons to O_2 to form a superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) or a hydroxyl radical (HO), respectively **(Urban-Chmiel et al., 2009)**. The most sensitive part of the cell towards the action of ROS, primarily the hydroxyl radical, is polyunsaturated fatty acids (PUFA) of cell membranes. The end product of PUFA destruction during lipid peroxidation is malondialdehyde (MDA), so that the increase of its concentration in tissues or biological fluids is considered as an indicator of increased production of free radicals and oxidative stress **(Marnett, 1999).**

Protein oxidation by ROS is associated with the formation of many different kinds of inter- and intra-protein cross-linkages, including those formed by addition of lysine amino groups to the carbonyl group of an oxidized protein **(Valko et al., 2006)**. It has been established in mammalian system including humans that direct damage to proteins or chemical modification of amino acids in proteins during oxidative stress, can give rise to protein carbonyls (PC) **(Stadtman and Berlett, 1998).** The usage of PC as biomarkers of oxidative stress has some advantages in comparison with the measurement of other

oxidation biomarkers due to its relative early formation and the stability of carbonylated proteins **(Oliver, 1987; Dalle-Donne et al., 2003)**.

Ceruloplasmin (CP) is the main cupremic determinant in plasma and acts as an extracellular scavenger of free radicals, thus it may protect the cells against ROS **(Saenko et al., 1994).** Ceruloplasmin activity and the serum or plasma copper concentration decreases with nutritional copper depletion of ruminants **(Blakley and Hamilton, 1985)**. **Frandsen, (1982)** reported that blood copper levels are depressed in ruminants infested with nematodes. Most of the works reported on the relationship between endoparasitism and copper deficiency have been based on the oral copper supplementation **(Adogwa et al., 2005)**.

Control of gastrointestinal nematodes is almost entirely based on the use of anthelmintics **(Zajac and Gipson, 2000)**. Resistance of parasites to these traditionally used anthelmintics has become a serious problem in veterinary medicine, particularly in sheep husbandry **(Roos, 1997)**. It is evident that numerous medicinal plants have been evaluated *in vitro* and *in vivo* for their efficacy to control various parasitic diseases of livestock around the world particularly in Asian, African and South Latin American countries **(Waller *et al*., 2001)**.

In India, garlic (*A. sativum*) preparation is used for scabies in swine **(Josling, 2000). Orr (1998)** investigated the use of *A. sativum* as based herbal anthelmintic formula in dairy goat production.

The aim of this research is to examine the defensive mechanism of Garlic juice to counteract experimental *Haemonchus contortus* infection in sheep and its effect on some antioxidants and biochemical parameters.

MATERIALS AND METHODS

1. Animals:

Nine male healthy sheep weighing about 15-20 kg b.w. 4 months old were used. They kept indoor on concrete floor and fed with hay and concentrate provided with water *ad libitum* during the day time. The animals provided with an adaptation period of 3 weeks before initiation of the experiment, during the first 2 days of this period, all the animals were dosed orally with albendazole at 10 mg/kg (for eradication of any parasites may be present from any natural infection).

2. Parasites and Parasitological cultivation:

Adult female parasites of *Haemonchus contortus* were collected from abomasums of infected sheep obtained from Assiut abattoir. The worms washed and crushed to liberate eggs. The eggs then cultured in a glass jar filled with autoclaved sheep faeces for 8 days at room temperature. At the end of the 8th day, infective larvae harvested by rinsing the side of the culture jar with drops of water. About 3000 larvae inoculated to a worm free sheep that kept indoor in separate house

throughout the study period. This sheep served as *Haemonchus contortus* egg donor to infect the 2nd and 3rd groups (*Garlic juice* and control+ve groups respectively), each animal of the 2nd and 3rd groups were inoculated orally with 1750 infective larvae obtained from feces of *(Haemonchus contortus* egg donor sheep) **(Eguale *et al.*, 2007)**.

3. Plant material preparation:

The *garlic juice* obtained from Zoology lab. Faculty of science, Assiut University, garlic cloves were minced in a juicer/blender device model no. MJ 176NR and the Natural juice of garlic was obtained.

4. Experimental design:

Three groups of sheep in each group 3 sheep. 1st and 3rd groups were control negative and control +ve groups respectively while the second group is *Garlic juice* treated group. Each animal of the second, third groups were inoculated orally with 1750 *Haemonchus contortus* infective larvae (L3) **(Eguale *et al.*, 2007)**. All of the second group at 4th and 6th week post infection were treated with Garlic juice (5 ml) orally **(Worku *et al.*, 2009)**. Faecal and blood samples were collected for Fecal Egg Count (FEC) by McMaster technique and blood analysis for 8 weeks.

5. Sampling:

From each sheep, two blood samples were collected on tubes containing EDTA as anticoagulant from the jugular vein. The first was used for hematological investigations, while the other was centrifuged for separation of plasma and the packed red cells used for preparation of erythrocyte hemolysate. The sediment containing blood cells was washed three times by resuspending in isotonic phosphate-buffered saline, followed by re-centrifugation and removal of the supernatant fluid and the buffy coats. The crude red cells were lysed in nine volumes of ice-cold distilled water to prepare a 10% erythrocyte hemolysate **(Saleh, 2008)**.

Fecal samples were collected from all groups of sheep for isolation of nematode egg by sedimentation technique and Counting of egg by McMaster technique **(Coles, 1980)**.

6. Parasitological examination:

The standard flotation and McMaster techniques **(Coles, 1980)** were carried out on the fecal samples to ensure that the selected control sheep were free from any parasites and to count the egg of experimentally infected sheep with *Haemonchus contortus*.

7. Hematological investigations:

The hemogram were determined automatically by automatic blood cell counter (Telldyn 3700, Germany).

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8. Biochemical analysis:

Plasma values of Total Free Amino Acids (TFAA), Ceruloplasmin (CP), Copper and Iron (Fe) were determined in plasma according to **(Rosen, 1957), (Houchin, 1958), (Kolmer *et al.*, 1951) and (Bauer, 1984)** respectively.

Erythrocyte hemolysate was used for determination of Protein Carbonyl (PC), Lipid peroxidation, Reduced Glutathione (GSH), Superoxide dismutase (SOD) and Catalase (CAT) according to **Levine *et al.*, (1990) Placer *et al.*, (1966), Beutler et al. (1963), Misra and Fridovich (1972) and Beers and Sizer (1952)** respectively.

9. Statistical Analysis:

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 ANOVA, followed by the Newman-Keuls test. Data were expressed as means ± standard error. Differences between groups were determined by means of a Student t-test. Significance level was at $p< 0.05$ **(Zarj, 1984)**.

RESULTS AND DISCUSSION

Many literatures dealt with the effect of gastrointestinal nematodes on sheep focusing on its haematological alterations **(Williamson et al., 2003)** and histopathological changes **(Mir et al., 2007)**. In addition, trace literatures were conducted regarding gastrointestinal nematodes effect on the oxidative stress parameters and antioxidant defenses in the sheep **(Dede et al., 2002)** in spite of the exceeded exposure of sheep to *Haemonchus contortus* parasite now and later which considered amongst the main parasitic infestations in Egypt.

The mean values of the hematological parameters in all groups of sheep are presented in Table (1,2). There were no significant changes in RBCs count, PCV% and hemoglobin concentration during the whole experiment in Control -ve group, while in *Garlic juice* and control +ve groups there were a significant decrease of Hb and RBCs count at the 2^{nd} week till the end of the experiment. *Garlic juice* group showed significant decrease in PCV% at 2^{nd}, 3^{rd}, and 4^{th} weeks of the experiment when compared to control-ve and significant increase at 5^{th} week till end of the experiment incomparable with control +ve group. The obtained data of blood indices showed normocytic normochromic anemia at 3^{rd}, 4^{th}, 5^{th} and 6^{th} weeks in both *Garlic juice and* control +ve groups while macrocytic hypochromic anemia at 7^{th} and 8^{th} weeks of the experiment in both treated groups. The observed macrocytic hypochromic anemia in both treated groups is in agreement with **(Qamar and Maqbool, 2012)**. The obtained results of WBCs in *Garlic juice* and control +ve groups showed non significant increases throughout the experiment accompanied by

non significant neutrophilia and lymphocytosis while there was a significant decrease in eosinophils in *Garlic juice* group when compared to control +ve at 5th week till the end of the experiment, our results are in agreement with **Rahman and Collins (1990)** who stated that infection with *H. contortus* did not lead to significant changes in total white cell counts with marked eosinophilia. From these findings, our result assumed that eosinophilia in the response to haemonchosis probably is an immunological regulator against any excessive harmful parasitic effect on the host and/or may be as antibody reaction produced by the keratin of the parasites.

The mean values of the biochemical parameters in different experimental groups of sheep are presented in Table 3-5. **Dede et al., (2000)** determined that the MDA level increased significantly in Akkaraman sheep infested with *Fasciola spp.*, *Trichostrongylidae,* and *Eimeria spp*. Also, **Simsek et al., 2006 and Dimitrijevi´c et al., 2012** reported that MDA level increased with *Dicrocoelium dendriticum* and *Strongyloides papillosus* infestation of sheep respectively. Determination of MDA revealed significant increase in *Garlic juice* group when compared to control-ve group at 6th and 7th weeks. This may be attributed to oxidative damage in the cell membrane which possibly resulting from overproduction of free radicals exceeded the equalization power of garlic to counteract this damage.

There are no reports available about protein oxidation in gastrointestinal nematodiasis of sheep. However**, Dimitrijevi´c et al., 2012** reported that the level of Protein Carbonyl (PC) was increased with the intensity of *Strongyloides papillosus* infestation in sheep, reaching the maximum value in the group with the highest degree of infestation. Our results explained that PC revealed significant decrease in *Garlic juice* group in comparable to control+ve group at 5th week till end of the experiment. The increased erythrocytic PC in control+ve group suggests a rise in the oxidative damage of both cell membrane protein and hemoglobin confirming an enhancement of erythrocytic free radical overproduction in parasitized sheep, in contrary what happened in garlic group as garlic juice suppresses the formation of protein carbonyl.

The presence of parasites in the host spurs defense mechanisms. The first, unspecific line of defense represents activated macrophages **(Saleh, 2008).** Catalase (CAT) belongs to the cellular antioxidant enzyme system that counteracts the toxicity of ROS and facilitates the removal of H_2O_2, which is metabolized to molecular oxygen (O_2) and water **(Van der Oost et al., 2003)**. Determination of CAT revealed significant increase in control+ve group when compared to control-ve at 2nd week till end of the experiment while G*arlic juice* group showed significant increase at 2nd, 3rd and 4th weeks when compared to control-ve group and significant decrease at 5th week till end of the experiment when compared to control +ve group. **Baghshani et al., 2011 and Dimitrijevi´c et al., 2012** assigned that increased activity of CAT caused by parasitic infestation of sheep was due to overproduction of H_2O_2. The

current data, in agreement with the previous results, showed increased activity of CAT in control+ve group, and may point out to a moderate not severe intensity of parasitic infestation which counteracted by the host defense mechanism to overcome the overproduction of H_2O_2. Determination of SOD revealed significant increase in *Garlic juice* group compared to control+ve group at 5th week till the end of the experiment. SOD 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)**. This concept is in harmony with the depletion of erythrocytic superoxide dismutase activity (SOD) showing in the present study at 3rd and 4th week in *Garlic juice* group and at 3rd week till the end of the experiment in control +ve group. The radical scavenging activity of *Garlic juice* to overcome the increased production of H_2O_2 may assist in increased activity of SOD enzyme and decreased activity of catalase which were obviously clear in our data.

As nematodiasis of sheep may cause excessive release of hydroxyl free radical rather than hydrogen peroxide **(Rahman and MacNee, 1999)**. Supporting this finding, the present data showed a significant decrease of erythrocytic reduced glutathione (GSH) in *Garlic juice* and control +ve groups at all weeks of experiment incomparable to control-ve group which acts as a substrate in the detoxification of peroxides such as hydrogen peroxide.

In the current study, determination of ceruloplasmin (CP) revealed significant decrease in *Garlic juice* group at 2nd week till 5th week of the experiment compared to control –ve group and significant increase at 6th week till end of the experiment compared to control +ve group, in addition the values of Copper (Cu) revealed significant decrease in *Garlic juice* and control +ve groups when compared to control -ve at 3rd week till end of the experiment. **Mulcahy et al. (2004)** reported that *Haemonchus contortus* increases pH value of abomasums. Consequently, it could be suggested that *Haemonchus contortus* affect copper metabolism perhaps due to interference with copper 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 copper metabolism probably by interference with copper absorption from the gastrointestinal tract. Accordingly, *Haemonchus* infestation may cause Cu deficiency in blood which in turn, leads to reduction in plasma ceruloplasmin. While *Garlic juice* group showed significant increase in CP level but decrease in Cu level which may be due to increases pH of gastric juice as well as its powerful antioxidant effect to enhance CP formation.

A characteristic feature of sheep haemonchosis is the reduction of plasma protein concentrations **(Wallace et al., 1996)** due to blood loss **(Williamson et al., 2003)** and hemorrhagic gastritis, In addition, leakage of proteins to the gastric lumen

occurs as a result of the disruption of intercellular unions and increased gut permeability **(Baker et al., 2003).** 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, and help in the digestion of host proteins and parasites envade the host immune response **(Williamson et al., 2003).** Proteases encompass a broad class of hydrolytic enzymes that play essential roles in digestive processes of proteins **(Williamson et al., 2003)** that leading to protein degradation and increasing formation of plasma total free amino acids (TFAA). Determination of TFAA can provide 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)**. The present study showed a significant increase in plasma TFAA at 3rd, 4th and 8th weeks of the experiment in *Garlic juice* group, however control +ve group showed a significant increase at all weeks of the experiment if compared to control-ve group. Such finding may be due to increased protein degradation through the action of proteases enzyme and muscle breakdown which probably due to activation of gluconeogenesis as a compensatory mechanism and/or due to decreased both uptake and absorption of nutritional amino acids caused by malnutrition and increased abomasal pH respectively. In addition, the significant rise of plasma TFAA may be attributed to an increase in free hemoglobin released probably from the exploded RBCs after filling with H_2O_2 as result of severe depletion of GSH. While the decrease of TFAA after treatment with garlic may be as a reason of increased protein metabolism which probably due to activation of gluconeogenesis as a compensatory mechanism and/or due to increased both uptake and absorption of nutritional amino acids caused by decreased abomasal pH respectively.

Determination of Fe revealed significant decrease in *Garlic juice* group as compared to control-ve group at 1st, 2nd, 3rd, 4th, 5th, 6th and 7th weeks of the experiment, however control+ve group showed significant decrease at all weeks of the experiment compared to control-ve group. The reduction of serum iron level could be attributed to the expanded erythropiosis to compensate for blood loss leading to depletion of iron stores. These findings were in agreement with those conducted by **(Albers et al, 1990)**.

Result of fecal egg count (FEC) and FEC reduction test are shown in table (6, 7) respectively. There is a significant decrease FEC in *Garlic juice* group at 5th week till the end of the experiment when compared to control+ve group. *Allium sativum* like many other tanniferous plant, increases the supply and digestible protein by forming non degradable complexes with protein in the rumen, improves the host's

immunity and resistance to nematode infection. The high tannin content in *A. sativum* may have direct anthelmintic effect on resident worm population disrupting the normal physiological functions like mobility, food absorption and reproduction, the later mode of action reported by **Duval (2004)** who asserted that *A. sativum* does not prevent the production of egg but inhibit the egg development into larvae. This reduction in larvae will subsequently reduce the worm burden in the hosts. The current study, supporting the previous concept, showing that *A. sativum* has ability to reduce the FEC by 67.7%.

In conclusion, our results indicated that sheep haemonchosis is accompanied by protein oxidation and a state of oxidative stress which leads to disturbances in protein synthesis and may contribute to the pathogenesis of the disease and due to inappetance, gastrointestinal losses of protein and increased plasma TFAA. In addition, *Garlic juice* may improved the antioxidants status of infected sheep.

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Table(1): Effect of *Garlic juice* on sheep infected by *Haemonchus contortus* on Erythrogram of different experimental groups (Means±SE) (N=3)

Week of infection	Group	RBCs ($x10^6$/ul)	Hb (g/dl)	PCV (%)	MCV (fl)	MCHC (%)
WK1	C-ve	8.35±0.08[a]	13.60±0.06[a]	36.4±0.46[a]	43.59±0.1[a]	37.36±0.32[a]
	Garlic	6.59±0.73[b]	11.47±0.26[b]	31.7±2.9[a]	48.13±0.99[a]	36.6±2.5[a]
	C+ve	7.45±0.13[a]	12.20±0.21[a]	32.03±0.48[a]	43.0±0.71[a]	38.07±0.49[a]
WK2	C-ve	8.3±0.27[a]	14.13±0.38[a]	37.77±0.64[a]	45.57±1.1[a]	37.47±1.6[a]
	Garlic	6.68±0.69[b]	9.93±1.2[b]	28.6±3.6[a]	42.7±1.4[a]	34.82±2.2[a]
	C+ve	7.13±0.11[b]	11.97±0.24[b]	30.8±0.2[a]	43.23±0.43[a]	38.83±0.56[a]
WK3	C-ve	8.37±0.09[a]	14.07±0.07[a]	36.76±0.89[a]	43.93±0.64[a]	38.3±0.79[a]
	Garlic	6.99±0.41[b]	11.23±0.26[b]	29.03±0.92[b]	41.63±1.56[a]	38.73±0.61[a]
	C+ve	7.18±0.25[b]	12.0±0.29[b]	29.97±0.42[b]	41.8±1.7[a]	40.03±0.52[a]
WK4	C-ve	8.65±0.14[a]	13.63±0.35[a]	35.57±1.0[a]	41.2±1.9[a]	38.3±2.2[a]
	Garlic	7.12±0.38[b]	11.16±0.08[b]	28.1±0.55[b]	39.6±1.6[a]	39.73±0.48[a]
	C+ve	7.15±0.25[b]	11.3±0.35[b]	29.6±0.5[b]	41.5±1.3[a]	38.17±0.24[a]
WK5	C-ve	8.30±0.06[a]	13.8±0.06[a]	35.93±0.87[a]	44.3±1.3[a]	37.03±0.81[a]
	Garlic	6.90±0.35[b]	11.73±0.02[b]	35.7±1.2[a]	49.8±0.99[a]	32.9±0.61[a]
	C+ve	6.80±0.05[b]	11.3±0.36[b]	29.7±0.37[b]	43.77±0.34[a]	37.79±0.9[a]
WK6	C-ve	8.07±0.07[a]	14.6±0.26[a]	37.43±0.66[a]	46.47±0.83[a]	39.5±1.3[a]
	Garlic	6.20±0.35[b]	10.68±0.42[b]	29.1±3.12[b]	46.67±2.4[a]	37.3±2.7[a]
	C+ve	6.83±0.12[b]	10.9±0.49[b]	29.41±1.5[b]	43.0±1.5[a]	37.1±0.91[a]
WK7	C-ve	8.55±0.11[a]	13.86±0.06[a]	36.97±0.44[a]	44.27±0.65[a]	37.5±0.5[a]
	Garlic	6.35±0.31[b]	9.26±0.17[b]	32.33±1.91[a]	49.97±1.9[a]	28.87±2.1[b]
	C+ve	6.45±0.34[b]	9.67±0.32[b]	28.67±0.88[b]	44.6±1.5[a]	33.77±1.03[b]
WK8	C-ve	8.68±0.19[a]	13.7±0.12[a]	36.4±0.46[a]	43.97±1.1[a]	38.43±0.43[a]
	Garlic	6.50±0.21[b]	10.3±0.15[b]	31.97±1.6[a]	49.2±1.7[a]	31.37±1.4[b]
	C+ve	6.55±0.12[b]	9.53±0.35[b]	30.33±1.09[b]	46.37±2.2[a]	31.53±0.67[b]

Group (C-ve) represents control negative (without infection and without treatment).
Group (garlic) represents *Haemonchus contortus* infection with garlic juice treatment.
Group (C+ve) represents control positive (with infection and without treatment).
(Means±SE) with different superscripts (a,b,c) within a column are significantly different at $P< 0.05$.

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Table(2): Effect of *Garlic juice* on sheep infected by *Haemonchus contortus* on White Blood cells count (WBCs) and differential leukocytes (absolute count) of different experimental groups (Means±SE) (N=3)

Week of infection	Group	WBCs ($x10^3$/ul)	Neutrophil ($x10^3$/ul)	Lymphocyte ($x10^3$/ul)	Monocyte ($x10^3$/ul)	Eosinophil ($x10^3$/ul)
WK1	C-ve	7.8±0.51[a]	2.03±0.5[a]	5.34±0.21[a]	0.32±0.08[a]	0.07±0.01[a]
	garlic	10.34±2.5[a]	2.29±0.7[a]	7.02±1.5[a]	0.36±0.09[a]	0.56±0.25[a]
	C+ve	9.98±2.59[a]	2.59±0.2[a]	6.82±0.22[a]	0.17±0.02[a]	0.42±0.03[a]
WK2	C-ve	7.43±1.5[a]	1.69±0.3[a]	5.22±1.02[a]	0.34±0.01[a]	0.1±0.01[a]
	garlic	9.33±2.72[a]	3.36±0.2[a]	4.91±0.93[a]	0.37±0.02[a]	0.52±0.22[a]
	C+ve	9.32±2.72[a]	2.35±0.4[a]	6.05±0.25[a]	0.37±0.08[a]	0.51±0.01[a]
WK3	C-ve	6.94±0.66[a]	1.67±0.3[a]	4.85±0.41[a]	0.26±0.08[a]	0.07±0.01[a]
	garlic	11.56±1.8[a]	4.27±1.0[a]	5.88±0.69[a]	0.36±0.01[a]	0.95±0.29[b]
	C+ve	13.23±1.8[a]	3.71±0.6[a]	8.11±1.6[a]	0.41±0.01[a]	0.90±0.26[b]
WK4	C-ve	7.62±1.31[a]	2.05±0.4[a]	5.18±0.84[a]	0.20±0.09[a]	0.07±0.01[a]
	garlic	11.03±1.7[a]	3.82±0.1[a]	5.28±0.72[a]	0.83±0.09[a]	0.99±0.3[b]
	C+ve	11.31±1.3[a]	3.64±0.6[a]	6.15±1.37[a]	0.41±0.02[a]	1.02±0.2[b]
WK5	C-ve	8.43±1.2[a]	2.06±0.4[a]	5.79±0.82[a]	0.24±0.01[a]	0.24±0.01[a]
	garlic	10.77±1.6[a]	3.09±0.2[a]	7.02±1.35[a]	0.21±0.02[a]	0.40±0.07[a]
	C+ve	11.2±1.3[a]	2.70±0.3[a]	7.09±1.41[a]	0.58±0.01[a]	0.70±0.13[b]
WK6	C-ve	8.91±1.4[a]	2.31±0.4[a]	6.19±1.07[a]	0.24±0.02[a]	0.06±0.04[a]
	garlic	10.83±0.5[a]	2.23±0.5[a]	7.63±0.55[a]	0.78±0.01[a]	0.12±0.05[a]
	C+ve	10.77±0.4[a]	2.83±0.4[a]	6.76±0.39[a]	0.29±0.01[a]	0.75±0.19[b]
WK7	C-ve	8.65±0.63[a]	1.99±0.2[a]	5.76±0.89[a]	0.69±0.21[a]	0.10±0.01[a]
	garlic	9.87±0.49[a]	2.78±0.87[a]	6.30±0.45[a]	0.61±0.11[a]	0.27±.08[a]
	C+ve	10.9±0.4[a]	2.67±0.37[a]	7.09±0.23[a]	0.28±0.03[a]	0.75±0.09[b]
WK8	C-ve	8.12±0.42[a]	1.96±0.53[a]	5.35±0.59[a]	0.53±0.15[a]	0.11±0.07[a]
	garlic	9.4±0.37[a]	2.82±0.27[a]	5.82±0.21[a]	0.49±0.1[a]	0.18±0.02[a]
	C+ve	10.35±0.9[a]	2.58±0.49[a]	6.67±0.71[a]	0.45±0.08[a]	0.57±0.05[b]

Group (C-ve) represents control negative (without infection and without treatment).
Group (garlic) represents *Haemonchus contortus* infection with garlic juice treatment.
Group (C+ve) represents control positive (with infection and without treatment).
(Means±SE) with different superscripts (a,b,c) within a column are significantly different at $P< 0.05$.

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Table(3): Effect of *Garlic juice* on sheep infected by *Haemonchus contortus* on Lipid peroxide (MDA) and Protein carbonyl (PC) of different experimental groups (Means±SE) (N=3)

Week of infection	Group	Lipid peroxide (MDA, nmole/mgHb)	Protein Carbonyl (PC, µmole/mgHb)
WK1	C-ve	33.6±6.8[a]	0.18±0.04[a]
	Garlic	54.7±4.7[a]	0.23±.05[a]
	C+ve	45.0±13.2[a]	0.33±0.07[a]
WK2	C-ve	32.1±3.40[a]	0.18±0.02[a]
	Garlic	52.1±11.5[a]	0.35±0.04[a]
	C+ve	51.7±19.6[a]	0.28±0.06[a]
WK3	C-ve	31.6±3.3[a]	0.31±0.064[a]
	Garlic	44.2±5.9[a]	0.23±0.02[a]
	C+ve	46.5±11.9[a]	0.28±0.06[a]
WK4	C-ve	31.6±2.9[a]	0.23±0.04[a]
	Garlic	80.6±5.8[b]	0.55±0.03[a]
	C+ve	88.2±13.6[b]	0.64±0.03[a]
WK5	C-ve	33.4±4.30[a]	0.24±0.05[a]
	Garlic	56.1±7.0[a]	0.45±0.07[a]
	C+ve	150.1±23.3[b]	0.89±0.12[b]
WK6	C-ve	29.5±1.16[a]	0.28±0.05[a]
	Garlic	69.6±8.9[b]	0.30±0.08[a]
	C+ve	89.4±6.4[b]	0.92±0.19[b]
WK7	C-ve	35.6±5.11[a]	0.25±0.07[a]
	Garlic	86.7±10.3[b]	0.33±0.11[a]
	C+ve	177.5±25.4[b]	1.02±0.03[b]
WK8	C-ve	29.6±1.8[a]	0.25±0.01[a]
	Garlic	62.3±11.3[a]	0.15±0.04[a]
	C+ve	163.7±9.4[b]	2.09±0.28[b]

Group (C-ve) represents control negative (without infection and without treatment).
Group (garlic) represents *Haemonchus contortus* infection with garlic juice treatment.
Group (C+ve) represents control positive (with infection and without treatment).
(Means±SE) with different superscripts (a,b,c) within a column are significantly different at $P< 0.05$.

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Table(4): Effect of *Garlic juice* on sheep infected by *Haemonchus contortus* on Catalase, Ceruloplasmin (CP), Superoxide dismutase (SOD) and reduced glutathione (GSH) of different experimental groups (Means±SE) (N=3)

Week of infection	Group	Catalase (U/mg Hb)	SOD (U/mg Hb)	GSH (mg/mg Hb)
WK1	C-ve	2.73±0.49[a]	1.86±0.12[a]	0.85±0.11[a]
	Garlic	5.63±0.31[a]	1.13±0.1[a]	0.25±0.08[b]
	C+ve	7.82±0.52[a]	1.75±0.13[a]	0.29±0.03[b]
WK2	C-ve	2.94±0.55[a]	1.61±0.05[a]	0.76±0.02[a]
	Garlic	10.59±2.19[b]	1.22±0.22[a]	0.22±0.05[b]
	C+ve	10.22±3.22[b]	1.18±0.21[a]	0.23±0.07[b]
WK3	C-ve	2.74±0.57[a]	1.85±0.21[a]	0.99±0.15[a]
	Garlic	9.51±0.35[b]	1.07±0.23[b]	0.18±0.03[b]
	C+ve	10.77±0.3[b]	1.15±0.2[b]	0.17±0.01[b]
WK4	C-ve	4.48±0.39[a]	1.95±0.11[a]	1.04±0.14[a]
	Garlic	12.24±1.1[b]	0.98±0.13[b]	0.27±0.08[b]
	C+ve	7.45±0.88[b]	0.91±0.15[b]	0.19±0.06[b]
WK5	C-ve	3.37±0.36[a]	1.99±0.14[a]	1.24±0.18[a]
	Garlic	4.85±0.63[a]	1.85±0.07[a]	0.33±0.02[b]
	C+ve	16.06±2.82[b]	1.09±0.29[b]	0.30±0.06[b]
WK6	C-ve	2.59±0.63[a]	1.67±0.05[a]	0.97±0.09[a]
	Garlic	4.21±0.53[a]	1.63±0.14[a]	0.32±0.04[b]
	C+ve	11.28±1.75[b]	0.60±0.16[b]	0.28±0.02[b]
WK7	C-ve	2.98±0.56[a]	1.81±0.15[a]	1.11±0.05[a]
	Garlic	4.02±0.28[a]	2.04±0.11[a]	0.46±0.03[b]
	C+ve	23.24±2.12[b]	0.94±0.07[b]	0.38±0.06[b]
WK8	C-ve	2.60±0.52[a]	1.81±0.04[a]	1.09±0.09[a]
	Garlic	4.39±0.72[a]	2.16±0.21[a]	0.43±0.05[b]
	C+ve	14.24±1.44[b]	0.85±0.1[b]	0.24±0.06[b]

Group (C-ve) represents control negative (without infection and without treatment).
Group (garlic) represents *Haemonchus contortus* infection with garlic juice treatment.
Group (C+ve) represents control positive (with infection and without treatment).
(Means±SE) with different superscripts (a,b,c) within a column are significantly different at $P< 0.05$.

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Table (5): Effect of Garlic juice on sheep infected by Haemonchus contortus on Ceruloplasmin (CP), Total Free Amino Acids (TFAA), Total Iron (Fe) and Copper (Cu) of different experimental groups (Means±SE) (N=3)

Week of infection	Group	Ceruloplasmin (CP, mg/g protein	TFAA (µg/mg protein	Fe (µg/dl)	Cu (µg/dl)
WK1	C-ve	7.64±1.09[a]	3.52±0.35[a]	178.68±29.5[a]	50.2±1.6[a]
	Garlic	5.36±0.89[a]	3.92±0.34[a]	69.69±5.3[b]	47.72±0.77[a]
	C+ve	4.42±0.44[a]	4.87±0.6[a]	93.42±11.7[b]	38.1±8.85[a]
WK2	C-ve	8.91±0.56[a]	3.19±0.31[a]	171.5±48.5[a]	47.7±6.7[a]
	Garlic	3.6±0.51[b]	5.91±0.53[b]	82.63±3.9[b]	30.4±1.92[a]
	C+ve	2.78±0.46[b]	6.37±0.33[b]	69.84±15.1[b]	35.4±9.62[a]
WK3	C-ve	9.44±0.63[a]	3.72±0.23[a]	211.84±45.4[a]	44.8±3.8[a]
	Garlic	3.44±0.17[b]	5.36±0.79[b]	78.9±2.8[b]	17.31±3.46[b]
	C+ve	2.26±0.19[b]	6.59±0.96[b]	72.1±5.7[b]	11.16±2.69[b]
WK4	C-ve	7.97±0.54[a]	3.64±0.46[a]	191.83±32.4[a]	48.3±3.1[a]
	Garlic	4.62±0.35[b]	7.31±0.74[b]	64.33±11.6[b]	23.09±3.07[b]
	C+ve	3.15±0.4[b]	6.63±0.51[b]	51.7±8.4[b]	20.01±6.15[b]
WK5	C-ve	8.6±0.79[a]	3.73±0.39[a]	197.89±41.1[a]	44.8±4.8[a]
	Garlic	4.73±1.42[b]	4.74±0.64[a]	79.48±23.2[b]	24.63±1.53[b]
	C+ve	2.71±0.07[b]	11.16±1.39[b]	47.39±2.52[b]	20.39±2.69[b]
WK6	C-ve	8.53±0.86[a]	3.44±0.38[a]	208.1±49.1[a]	43.7±1.5[a]
	Garlic	8.15±1.52[a]	4.72±0.59[a]	99.06±10.1[b]	30.4±1.92[a]
	C+ve	3.18±0.33[b]	8.48±0.75[b]	36.2±5.1[b]	14.23±3.46[b]
WK7	C-ve	8.83±0.44[a]	3.28±0.6[a]	209.0±49.5[a]	46.3±1.9[a]
	Garlic	7.31±0.88[a]	5.63±0.45[a]	99.41±13.0[b]	21.93±1.92[b]
	C+ve	3.74±0.15[b]	6.89±1.28[b]	41.79±3.9[b]	13.08±2.3[b]
WK8	C-ve	8.76±0.23[a]	3.36±0.51[a]	177.29±32.2[a]	46.3±1.9[a]
	Garlic	8.75±0.42[a]	6.02±0.6[b]	103.03±14.6[a]	25.4±2.3[b]
	C+ve	4.21±0.01[b]	6.79±0.67[b]	43.22±2.1[b]	13.85±0.01[b]

Group (C-ve) represents control negative (without infection and without treatment).
Group (garlic) represents *Haemonchus contortus* infection with garlic juice treatment.
Group (C+ve) represents control positive (with infection and without treatment).
(Means±SE) with different superscripts (a,b,c) within a column are significantly different at $P< 0.05$.

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Table (6): Fecal egg count (FEC, EPG) of different experimental groups (Means±SE) (N=3)

Week of infection / Group	WK1	WK2	WK3	WK4	WK5	WK6	WK7	WK8
C-ve	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Garlic	0.00±0.00[a]	0.00±0.00[a]	616.67±101.3[b]	1183.33±120.1[b]	583.33±44.09[b]	716.66±66.67[b]	600±86.6[b]	383.3±33.3[b]
C+ve	0.00±0.00[a]	0.00±0.00[a]	750±55.03[b]	1366.7±208.8[b]	1283.3±76.8[c]	1400±180.27[c]	1283.3±169.14[c]	1533.3±148.13[c]

Group (C-ve) represents control negative (without infection and without treatment).
Group (garlic) represents *Haemonchus contortus* infection with garlic juice treatment.
Group (C+ve) represents control positive (with infection and without treatment).
(Means±SE) with different superscripts (a,b,c) within a column are significantly different at P< 0.05.

Table (7): Percentage efficacy based on fecal egg count reduction test of different experimental groups

Group	% of reduction			
	WK5	**WK6**	**WK7**	**WK8**
Garlic	50.7	39.43	49.29	67.6

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