Research Article



The Therapeutic Effect of Coriander Aquous Extract and Garlic Juice Against Experimentally Infested Goats with *Haemonchus contortus*

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Abstract | In this study the therapeutic effect of garlic juice and coriander extract against experimental infection of Haemonchus contortus (H. contortus) in goat was evaluated. Goats were divided into 4 groups; the 1st one represented control negative (clinically normal). All animals of other groups were infected via stomach tube with 1750 of 3rd stage larvae of *H. contortus* (L3) and subsequently subdivided into positive infected untreated group (second group), positive infected and orally treated group with garlic juice, 5ml/animal, (third group) and positive infected and orally treated group with coriander extract, 0.9g/ Kg b.w. (fourth group). Fecal samples were collected weekly after 3 weeks post infection for Fecal Egg Count (FEC). One animal of each group was slaughtered at 7th week of experiment for sampling of abomasal specimens for histopathological examination and tissue estimation of Total peroxide, malondialdehyde (MDA), protein carbonyl (PC), Catalase, reduced glutathione (GSH) and superoxide dismutase (SOD). Garlic and coriander treated goats showed significant reduction of FEC as compared with control positive ones. Treated goats exhibit no changes in total peroxides while there were non significant changes of PC, MDA, Catalase and SOD, in contrast there was an obvious increase of GSH. Histopathologically, Abomasum tissue restored completely normal histological features in Garlic treated goats, however the abomasum was partially restored to the normal histological feature in coriander treated goats. In conclusion, the high flavonoid and phenol content of A. sativum ingredients have the ability to repair the degenerative hazards induced in a goat infested by *Haemonchus contortus* which aid in healing process. However, the time that Coriandrum sativum needs for repairing affected tissue is relatively more than that of Allium sativum.

Keywords | Goat, *Haemonchus contortus*, Garlic, Coriander and abomasum.

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INTRODUCTION

Ruminant livestock in Egypt are often attacked by various diseases originating from bacteria, parasites and poisoning. The parasitic disease that mostly attacks livestock from the past until now is haemonchosis caused by the worm *Haemonchus spp.* (Ahmad et al., 2012). All gas-

trointestinal nematode are pathogenic, some of them are blood-sucking and *Haemonchus contortus* is the most one (Mini 2012; Gidey 2017) which is found in the abomasum of goats and sheep (Pathak et al., 2013), while in large ruminants *Haemonchus placei* can be found (Saminathan et al., 2015; Sakti 2018) the morbidity rate of this nematode is 80% (Lastuti et al., 2006). While Suteky & Dwatmadji



(2010) stated that *H. contortus* mortality rate 66.7%. Parasitic diseases cause different pathological hazards in small ruminants if compared with other animal species (Iqbal et al., 1993). Haemonchosis harm the abomasal epithelium in the form of haemorrhages, in addition, mononuclear cells represented mostly by eosinophils were obviously observed in the glandular part of the stomach (Ahmed et al., 2007). Masamha et al. (2010) reported complete parasite eradication using garlic in goat while using chemical drugs are less by 71 % than garlic one. The pharmaceutical benefits obtained from coriander are anti-hyperlipidemic, antioxidant, and antimicrobial activities (Hilal, 2016).

The main scope of this research is to evaluate the Histopathological, antioxidants and regenerative effect of both Coriander and Garlic extracts on experimental infestation of goats with *Haemonchus contortus* larvae.

MATERIALS AND METHODS

STUDY AREA

Khashaba farm (Assiut province, Egypt) is located in mountainous area 27.18 latitude and 31.18 longitude and it is situated at elevation 56 meters above smjea level where the climate was continental with cold winter and hot summers. Goat reared under natural pasture resources that grown in the ground with supplementation of concentrates and some minerals.

ANIMALS

The experimental study was carried on 20 healthy Baladi goat 3-6 months old, weighing about 5-9 kg b.w. They kept inside the farm in a separate room with food and water ad libitum. Those goats were divided into 4 groups (5 in each one). The first and second groups were kept as control negative (C-ve) and control positive (C+ve) respectively. Each animal of the second, third and fourth groups were injected via a stomach tube with 1750 3rd stage larvae of Haemonchus contortus (L3); Those larvae were collected from infested goat eggs by egg culture (Eguale et al., 2007a). All of the third (H+G) and fourth (H+C) groups at 4th and 6th weeks post infection were treated with garlic juice (5 ml/ animal) (Worku et al., 2009) and coriander (0.9 g/kg b.w. of aqueous extract) (Eguale et al., 2007a). Faecal samples at 1st, 2nd, 3rd and 4th week post infestation, and at 1st, 2nd and 3rd week post treatment were collected for Fecal Egg Count (FEC) using McMaster technique.

One animal of each group was slaughtered at the 7th week of experiment for sampling of abomasal specimens for histopathological examination and tissue estimation of Total peroxide, MDA, PC, Catalase, GSH and SOD.

FECAL CULTURE FOR LARVAE RECOVERY AND IDENTIFICATION

Fecal cultures were done according to Coles (1980) to obtain larvae. Recovery and morphological identification of 3rd stage larvae (L3) of most parasitic nematodes were carried out using the keys (Gibbons et al., 2006; Van Wyket, Mayhew, 2013).

PLANT MATERIAL PREPARATION

The *Coriandrum sativum* (coriander) seeds were kept in the air for dryness, pulverized and preserved in dark glass bottle. A known weight quantity of preserved seeds powder was extracted in distilled water by shaking for three hours using electric shaker. The suspension was filtrated through muslin gauze and freeze at -20 C° for one day till lyophilization. Then collect in a completely sealed bottle after weighing then put in a desiccator for preservation from water evaporation till utilization (Eguale et al., 2007a).

The natural *Allium sativum* (garlic juice) obtained from Zoology Lab. Faculty of Science, Assiut University. By mincing 45 g of unpeeled garlic cloves in a juicer/blender device model no. MJ 176NR to give 15 ml of 100% garlic juice.

INFESTATION WITH PARASITES

Infective abomasums of goat were gathered from abattoir then examined macroscopically for obtaining females only of *Haemonchus contortus*. These worms were ground with a little amount of water for egg collection which is then put in a glass flask filled with autoclaved negative parasites goat faeces for 8 days at room temperature. At the 9th day, collect the 3rd stage larvae by pouring the side of the culture flask with a little amount of water. About 3000 larvae given orally to a parasite free goat that kept inside a single room along the experiment days. This goat considered as granter of *Haemonchus contortus* egg (Eguale et al., 2007b).

HISTOPATHOLOGY

The collected tissue samples of abomasum were preserved in Bouin's solution for 7 days. The histopathological slides were prepared and examined according modified procedure described by Lee G. Luna (1968).

PREPARATION OF HOMOGENATE

Ten percentage of homogenates (w/v) were made in phosphate buffer (pH 7.4) using homogenizer (model IKA-WERKE, D118 BASIC, Germany). The samples were centrifuged at 5,000 rpm for 15 min to separate the homogenate. All the rest samples were 250 ll-ali-quoted into Eppendorph tubes and stored at -40 C till used to avoid repeated freeze—thaw cycles in different assays (Blalock et al., 2001).



ESTIMATION OF ANTIOXIDANTS

Superoxide dismutase (SOD): SOD activity was estimated according to the method described by Misra and Fridovich (1972).

Estimation of catalase (CAT): CAT activity was measured by the method of Beers and Sizer (1952).

Estimation of reduced glutathione(GSH): GSH concentration was measured using the method of Beutler et al. (1963).

Estimation of Oxidants:

Estimation of total peroxide (T.peroxide):

following the method of Harmma et al. (2005).

Estimation of malondialdehyde (MDA):

Lipid peroxidation was determined as thiobarbituric acid reactive substances (TBARS) according to Placer et al. (1966).

Estimation of protein carbonyl (PC):

The DNPH (2,4 -dinitrophenylhydrazine) was used for the determination of carbonyl content of proteins as an index of protein oxidation (Levine et al., 1990).

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. "P" value of < 0.05 was assumed for statistical significance.

RESULTS

FECAL EGG COUNT

Result of fecal egg count (FEC) is shown in Tables (1). Determination of FEC revealed significant increase in H+garlic, H+coriander and control +ve groups at 3rd week till end of the experiment when compared with control -ve group. H+garlic and H+coriander groups showed significant decrease at 6th and 7th weeks of the experiment incomparable with control +ve group.

OXIDANTS / ANTIOXIDANTS RESULTS

Results of total peroxide (T.peroxide), malondialdehyde (MDA), protein carbonyl (PC) catalase, superoxide dismutase (SOD) and reduced glutathione (GSH) are shown in Table (2). Determination of total peroxide revealed no change in all infested groups compared to control –ve, however control +ve group showed significant increase incomparable with other groups. Estimation of MDA and PC exhibits non significant improvement in both H+Gar-

lic and H+Coriander groups if compared to C-ve while C+ve group revealed higher increasing effect incomparable to other groups. The obtained results for catalase and SOD values showed non significant decrease in treatment groups against C-ve group, while an obvious fall down of C+ve value in comparison with the rest groups. An advanced rise of GSH parameter was observed in the dual treatment groups in comparable to C+ve group.

HISTOPATHOLOGICAL RESULTS

In the histopathological observation,. Abomasum restored normal histological features and absence of the infective larvae in H+Garlic group (Figure 1), however in H+coriander group the abomasum partially restored the normal histological feature with the presence of degenerated larvae surrounded by inflammatory cells (Figure 2 a, b). In contrast, the C+ve group the abomasum was exhausted with different pathological lesions in the form of hemorrhages, edema and congestion in the blood vessels of the lamina propria, desquamation in the apical border of abomasal villi, inflammation and mucus secretions around the lesions with different degenerated larvae (Figure 3 a, b, c, d).

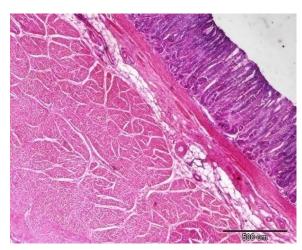
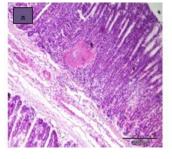


Figure 1: Histopathological changes in the abomasum of H+Garlic group: abomasum restored normal histological features and absence of the infective larvae.



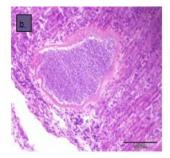


Figure 2: Histopathological changes in the abomasum of H+Coriander group: **a:** Abomasum partially restored the normal histological feature but degenerated larvae still present, **b:** high power revealed the degenerated larvae surrounded by moderate inflammatory cells.



Table 1: Fecal egg count (FEC, EPG) of different experimental groups (Means±SE)

Week of Infection Group	1 st	2 nd	3 rd	4 th	5 th	6 th	$7^{ ext{th}}$
C-ve	$0.00{\pm}0.00^{\mathrm{a}}$	$0.00{\pm}0.00^{\mathrm{a}}$	$0.00 {\pm} 0.00^{\mathrm{a}}$	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	$0.00 \pm 0.00^{\mathrm{a}}$	0.00 ± 0.00^{a}
C+ve	0.00 ± 0.00^{a}	$0.00\pm0.00^{\mathrm{a}}$	750±55.03 ^b	1383.3±169.2 ^b	1283.3±76.8°	1400±180.3°	1283.3±169.1°
H+Garlic	0.00 ± 0.00^{a}	$0.00{\pm}0.00^{\mathrm{a}}$	616.7±101.3 ^b	1183.33±120.1 ^b	583.33±44.09b	716.6±66.6 ^b	600±86.66 ^b
H+Coriander	0.00 ± 0.00^{a}	$0.00 \pm 0.00^{\mathrm{a}}$	650±57.73 ^b	1233.3±133.3 ^b	716.66±116.7 ^b	783.33±30.33 ^b	700±104.08 ^b

Group (C-ve) represents control negative (without infection and without treatment).

Group (C+ve) represents control positive (with infection and without treatment).

Group (H+Garlic) represents *Haemonchus contortus* infection with garlic juice treatment.

Group (H+Coriander) represents *Haemonchus contortus* infection with coriander (aquous extract) treatment.

Means with different superscripts (a,b,c) within a row are significantly different at P< 0.05.

Table 2: Total peroxide, malondialdehyde (MDA), protein carbonyl (PC) Catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH) of different experimental groups (Means±SE):

Group/ Parameter	C-ve	C+ve	H+Garlic	H+Coriander
T.peroxide nmole/mg protein	434.9±20.1 ^a	521.1±25.4 ^a	428.9±19.3 ^a	420.5±59.7 ^a
MDA nmole/mg protein	0.56±0.16ª	$1.72 \pm 0.10^{\rm b}$	1.29±0.34 ^{a,b}	1.12±0.31 ^{a,b}
PC μmole/mg protein	0.12 ± 0.04^{a}	$0.39 \pm 0.04^{\rm b}$	$0.16 \pm 0.1^{a,b}$	$0.22 \pm 0.07^{\mathrm{a,b}}$
Catalase U/mg protein	32.7±3.7 ^a	15.5±1.5 ^b	19.9±1.6 ^{a.b}	21.5±4.3 ^{a,b}
SOD U/mg protein	3.2±0.14 ^a	2.31 ± 0.14^{b}	$2.51 \pm 0.19^{a,b}$	$2.7 \pm 0.27^{a,b}$
GSH ng/mg protein	59.5±3.5 ^a	40.6±1.7 ^b	49.3±4.9 ^{a,b}	48.7±4.4 ^{a,b}

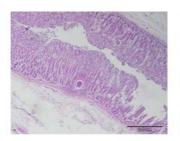
Group (C-ve) represents control negative (without infection and without treatment).

Group (C+ve) represents control positive (with infection and without treatment).

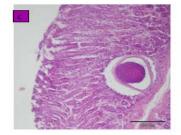
Group (H+Garlic) represents *Haemonchus contortus* infection with garlic juice treatment.

Group (H+Coriander) represents *Haemonchus contortus* infection with coriander (aquous extract) treatment.

Means with different superscripts (a,b,c) within a row are significantly different at P< 0.05.







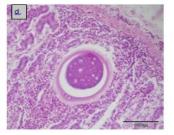


Figure 3: Histopathological changes in the abomasum of C+ve group:

a: Necrosis and sloughing of gastric glands with edema in lamina propria. b: Lamina propria showing a degenerated larvae with amorphous basophilic material c: Desquamation of of the gastic epithelium wth dense inflammatory cells infiltion in mucosa and submucosa d: High power of the degenerated larvae with amorphous basophilic material.

DISCUSSION

The main scope of most previous researches concerning gastrointestinal nematodes on goat was haematological alterations (Williamson et al., 2003) and histopathological changes (Mir et al., 2007) but there were no reports available about determination of oxidants / antioxidants parameters in tissue of goats.

A. sativum resembles any herbal plants, rises up the formation of non degradable complicated components with amino acids inside the stomach which provides high levels of supplemented protein, this followed up by improvement of the immune system of the affected species and the ability to overcome the parasitic infection. The active principle of anthelmintic effect in garlic is tannin component which highly represented, this substance can be reconciled the live functions as motor function, food metabolism and reproduction this concept is in harmony with Duval (2004) who mentioned garlic does not inhibit the production of egg but prevent the egg from developing into larvae. This lowering in larvae numbers will subsequently lower the worm burden in the hosts. The current study, supporting the previous concept, showed that A. sativum has the abili-

ty to lower the FEC. While mode of action of coriander is through propagation along cuticular membrane, this manner is widely known as a method of entry for any non food materials in nematodes (Geary et al., 1999). The present work showed that coriander is less effective on *Haemonchus contortus* infection in goat than garlic.

The first component of PUFA damage during lipid peroxidation is total peroxide while the end product is malondialdehyde (MDA), so that their high levels is considered as a mark of high production of free radicals and oxidative stress (Halliwell and Chirico, 1993). In addition, The increase in reactive hydroxyl radical (OH•) which is generated by high concentration of H2O2 is considered to be responsible for the formation of PC (Oliver, 1987), As well, The usage of PC is an indicator of early carbonylated protein formation and stability (Dalle-Donne et al., 2003). Dimitrijevi'c et al. (2012) reported that the level of MDA and PC were raised up in the same manner as Strongyloides papillosus infestation in goat increased, Supporting this, our work exhibited high degrees of T.pereoxide, MDA and PC values in C+ve group. Balasenthil et al. (1999) observed that treatment hamsters with DMBA (Dimethylbenz anthracene), a carcinogenic component administrated with of an aqueous extract of garlic exhibited diminished lipid peroxidation (T.peroxides, MDA) and PC as well as ameliorative effect in the levels of catalase, SOD and GSH in oral tumor tissue. Our data is in harmony with the previous author.

Seeds of coriander contain antioxidants (Wangensteen et al., 2004). The main antioxidant content in coriander is carotenoids. The carotenoids act as a scavenger of hydroxyl radicals, thereby protecting cells from oxidative damage (Peethambaran et al., 2012). These findings support the ameliorative action happened in Coriander treated group. As chung, 2006 mentioned that A. sativum contains high levels of powerful antioxidant agents represented in alliin, allyl cysteine, allyl disulfide, and allicin. In this fact, previous literatures explained illustrated that elevated antioxidant capacity in the damaged tissue area, spread the regeneration process (Rasik, Shukla, 2000; Farahpour et al., 2015). Moreover, the anti-inflammatory action of these components harry up regeneration process. The control of inflammation during regeneration will speed the proliferative action for more cell formation (Hemmati, Mohammadian, 2000; Rasik, Shukla, 2000). Polymorph nuclear cells play an important role in healing process as they act as scavenger for microorganisms and clear cellular debris (Guo, Dipietro, 2010). Regard to Shin et al. (2013), A. sativum has massive anti-inflammatory effects. Accordingly, our work showed high regenerative effect of garlic on abomasal tissue which in turn restoring the normal histological features.

The active substance of Coriandrum savitum is isoquer-(quercetin-3-O-β-d-glucopyranoside) is a one of the major glycosidic form of quercetin. It plays an important role to counteract oxidative damage, inflammation and apoptosis (Liang et al., 2020). It is the major bioactive component of the Coriander. Quercetin, can be an important dietry supplement to inhibit chronic degenerative diseases. Quercetin and other flavonoids can play an important role in modification of eicosanoid bioformation (antiprostanoid and anti-inflammatory responses) (Nambiar et al., 2010). In addition, some flavonoids act as precursor for enzymes needed for gene transcription and expression against oxidative damage insults (Rohrdanz et al., 2002). On this basis the degenerated larvae with a moderate inflammatory cells attributed to the antiinflammatory and antioxidant effect of coriander that counteract the action of parasitic propagation regardeless this effect is less than that of garlic.

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CONFLICT OF INTEREST

In relation to this manuscript, the authors state that they have no competing interests.

NOVELTY STATEMENT

As far as we know, there is no previous literatures have revealed the regenerative and antioxidant effects of coriander against *Haemonchus contortus*.

AUTHORS CONTRIBUTION

Al-Hassan M Mostafa: put Conceptualization, performed the experiment, laboratory works, supervision, writing, reviewing and editing. Gehan M Sayed performed the experiment, laboratory works and reviewing.

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