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In vitro and in vivo anthelmintic efficacy of condensed tannins extracted from the seeds of alfalfa (*Medicago sativa* L.) against *Haemonchus contortus* infection

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Abstract

This study was designed to examine *in vitro* and *in vivo* anthelmintic efficacy of condensed tannins (CT) extracted from seeds of *Medicago sativa* on *Haemonchus contortus* in sheep. CT's *in vitro* anthelmintic effect was assessed at a 300 μg/ml concentration compared with albendazole (reference drug) at 10 μg/ml. The results showed that CT had a nematocidal effect on *H. contortus*, and the cuticle of the adult worm appeared to be its initial target. For the *in vivo* experiment, nine 3-month-old helminths-free lambs were distributed into three groups. Group 1 (n=3) was challenged only as infected untreated controls; Group 2 (n=3) was treated with condensed tannin, and Group 3 (n=3) was treated with albendazole. Fecal and blood samples were collected every 3 days until the end of the experiment; for fecal egg count (FEC) and anti- *H. contortus* IgG titers determination, respectively. The lambs treated with the CT in G2 exhibited a pronounced decrease of mean FEC with great FECR% detected from the first-week post-treatment (PT) until the end of the experiment compared with G1 animals. The antibody levels gradually increased in G2 following the 2nd dose of CT treatment compared to other groups. A brilliant consistent relation between the elevation of IgG response and reduction of FEC was observed following the second booster dosing of the CT in G2. In conclusion, the CT evoked strongly *in vitro* and *in vivo* anthelmintic activity against *H. contortus* and could be used as a natural alternative treatment of high potency against haemonchosis in sheep.

Introduction

Among small ruminants, sheep are essential livestock industry source worldwide. Haemonchosis substantially affect ruminants' health and production because of the significant economic losses, severe clinical signs, and mortalities (1). *Haemonchus contortus* is the causative nematode of the disease, which can penetrate the abomasal mucosa of the host for nutrition through sucking blood. This hematophagous activity causes hemorrhages, gastritis, anemia, edema, and associated complications, resulting in death in severely affected animals, especially young animals (2,3). Anthelmintics have been widely used to combat the infection; however, the resistance of *H. contortus* to various anthelmintics has become a growing global phenomenon. The causative impetus might be due to the direct life cycle and the high egg-laying rate of the worm, in addition to the excessive and frequent intensive use of anthelmintics (4,5). Anthelmintic resistance is a heritable change among the worm populations resulting in the continuous reduction of

the anthelmintics' efficacy and progeny, so exploring and developing novel alternative treatments that might control the infection becomes an urgent need (6).

Medicinal plants are considered one of the most significant natural eco-friendly and biodegradable anthelmintics (7). Efficient anthelmintic properties of various herbal bio-products against different helminths have been reported worldwide (8-10). Alfalfa (*Medicago sativa* L.) is one such significant and widespread legume crop (11) that has been investigated to have potent bioactive components such as tannins, pectin substances, saponins, and flavones and phenolics (12). Tannins are considered a significant category of polyphenols that are widely spread in the bark of trees, leaves, stems, fruits, and seeds. There are two kinds of tannins type compounds: hydrolyzable, derived from simple phenolic acids, and condensed, which come from flavan-3-ols (catechin). Condensed tannins (CT) are polymers of two or more called flavan-3-ol catechetical molecules or leucoanthocyan flavan-3,4-diols (13). Many reports have documented the remarkable *in vitro* anthelmintic activity of CT through egg hatching ability, exsheathment, and larval motility of gastrointestinal nematodes (14).

Moreover, Hoste (15) and Hamad (16) detected anti-parasitic activity of the CT on *H. contortus* in small ruminants. Besides, Tibe (17) displayed the potentiality of CT to promote the host's innate immune response. However, the data on the anti-parasitic activity of the CT derived from the Egyptian native plants on adult *H. contortus* are limited. Thus, the goal of the present study was to examine *in vitro* and *in vivo* anthelmintic efficacy of CT extracted from the seeds of Alfalfa (*Medicago sativa* L.) on adult *H. contortus* worms as a safe, dependable alternative control method of haemonchosis.

Materials and methods

Ethical approval

The experiments were conducted in compliance with the requirements and recommendations of the International Animal Ethics Committee and the Ethical Committee of the National Research Centre, and the current Egyptian Law and Regulations for the protection of experimental animals to minimize negative states (harm) and improve feeding and housing conditions, under certificate number 19150.

Plant extraction and fractionation

The seeds of *Medicago sativa* L were purchased from the Agriculture Research Center, Cairo, Egypt. The collected sample (2.0 Kg) was grounded to a fine powder and defatted with 5 liters of chloroform and chloroform/ methanol (3:1 v/v). The residue of seeds was further extracted by percolation with aqueous acetone (75 % v/v) containing 0.1 % w/v of ascorbic acid (18). The acetone was evaporated under vacuum, and the aqueous layer was fractionated with chloroform (3×500 ml) and ethyl acetate (3×500 ml). The ethyl acetate fraction was selected for further working up due to promising activity against *H. contortus*. This fraction was tested for the presence of tannins using the ferric chloride test (19). A dark green color was formed and indicated the presence of tannins. The ethyl acetate fraction was applied to silica gel PSQ 100 column chromatography and eluted with a gradient solvent system of CH_2Cl_2: Me OH with increasing polarity. Fourteen fractions (1A-14A) were obtained and checked for the presence of tannins by the $FeCl_3$ test. Fractions 9A to 14A were collected together due to a positive test with $FeCl_3$ and analyzed by HPLC-ESI-MS/MS to identify compounds.

HPLC-ESI-MS/MS analysis

The combined fractions 9A-14A of the ethyl acetate extract were analyzed by HPLC-ESI-MS/MS (20). The fraction (100 μg/mL) was dissolved in methanol, filtered using a membrane disc filter (0.2 μm), and subjected to analysis. Sample (10 μL) was injected into the ACQUITY-UPLC™ system (Waters, Milford, USA) equipped with a reverse phase C-18 column (BEH-C18, 1.7 μm particle size and 50×2.1 mm Column). Elution was carried out with a gradient of mobile phase consisting of H_2O containing 0.1% formic acid (A) and methanol (B), also acidified with 0.1% formic acid at a flow rate of 0.2 ml/min. Mass spectra were detected in the ESI between *m/z* 100-1500. The analysis parameters were carried out using the ESI-negative ion mode: source temperature 150°C, cone voltage 30 eV, capillary voltage 3 kV, dissolution temperature 440°C, cone gas flow 50 L/h, and dissolution gas flow 900 L/h. The peaks and spectra were processed using the MAs lynx 4.1 software and identified by comparing their mass data with the literature. Adult worms of *H. contortus* were assembled from the abomasa of naturally infected sheep after slaughtering at El-Moneib abattoir in Giza Governorate.

In vitro assay
The percent inhibition of *H. contortus* motility

Under sterile conditions in a laminar flow cabinet, thirty fresh active motile worms were put in Ringer solution and divided into three groups; ten worms for each as follows: normal untreated, treated with CT fraction at a concentration of 300μg/ml (21) and treated with albendazole 10μg/ml; (Evazole®- EVA Pharma, Cairo, Egypt); as a standard anthelmintic drug dissolved in DMSO at 0.1%. Then, the worms were kept for 24 h at 37°C in an atmosphere of 5% CO_2. The activity of worms was examined at the start of the test till 24 h incubation via visual investigation and, if necessary, by gently agitating the worms in the culture media. The percent inhibition of motility of treated *H. contortus* was calculated by utilizing the following formula; Inhibitory activity= (C-E)/C*100, were *C* mean number of control motile worms; and *E* mean number of exposed motile worms.

Light microscopy

Fixation of untreated control worms was carried out immediately following the first washing. After the incubation period, the mid-body region of treated and untreated *H. contortus* adult worms was dissected into 5-mm samples, fixed at 10% buffered formol saline, and processed as described by Bancroft (22). The worm samples were checked and photographed by utilizing an Olympus CX41 microscope.

In vivo study

Locally lambs were kept in a hygienic isolated pen and offered a balanced ration and tap water. The animals had undergone a parasitological investigation. Fecal samples were collected and examined using a concentration floatation test to ensure that the infection of lambs was free from helminths. Female *H. contortus* worms were selected and homogenized for egg liberations and then cultured according to Soulsby to obtain a sufficient number of infective L3 for experimental infection (23). One locally lamb was infected at 2-3 months of age using 20.000 *H. contortus* L3. Then, after 17 days of infection, a fecal examination of the lamb was performed to detect the characteristic egg of *H. contortus*. Collection of the required L_3 for infection of the experimental lambs was accomplished through culturing from fecal samples of the larval donor.

Experiment design

Nine 3-month-old helminths-free lambs were housed in separate pens. The animals were categorized as follows: Group 1 (n=3), challenged only, control positive; Group 2 (n=3), treated with the fraction of condensed tannin (two doses); Group 3 (n=3), treated with albendazole. At 0 week of the experiment, each lamb was infected with 20000 L_3 from the donor lamb. GI was served as control infected. The 0 day of treatment was at the 18th day post-infection (PI). Group 2 was treated utilizing an oral dose of 150mg/kg BW of condensed tannin (two doses) with an interval of 2 weeks. Group 3 received albendazole at 10μg/ml (24).

Coprological examination

Fecal samples of the lambs under the experiment were extracted directly from the rectum of each animal. Fecal examination for detection and counting of *H. contortus* eggs per gram (EPG) was performed on day 17 post-infection (pre-treatment) and every 3 days (post-treatment) until the end of the experiment. Fecal egg count (FEC) was assigned through the Modified Mac-Master technique (23). The number of eggs per gram of feces (EPG) was calculated through the following equation were EPG= (Total number of eggs in both chambers/2)*100. The fecal egg count reduction percent (FECRT %) as follow FECR%= Pretreatment egg count per gram-post treatment egg count per gram/ Pretreatment egg count per gram*100 (25).

IgG response of the infected and the treated lambs

Serum samples of the infected and the treated lambs were collected every three-day post-infection (DPI) to 68 DPI, following the standard methods, and they were preserved at -20°C until used. Crude *H. contortus* somatic antigen (CSA) was prepared as the method mentioned by Johnson (26).

Standardization of the enzyme-linked immunosorbent assay (ELISA)

Indirect ELISA was optimized by serial checkerboard titration to the following setup to determine *H. contortus* specific IgG levels against an adult somatic antigen in the serum samples of the experimentally infected lambs treated with condensed tannin fraction and albendazole, according to the method of Kandil (27). The optimal concentration of the coating antigen (4 μg/well), dilution of the sera (1:50) as well as donkey anti-sheep IgG (whole molecule) horseradish peroxidase conjugate (Sigma-Aldrich, USA) diluted at 1:1000, and the optimal test conditions respectively were determined by checkerboard dilution assay using flat-bottom 96-well ELISA microplates (Grainger, Germany). Optical densities were read at a wavelength of 450 nm with an ELISA reader (BIOTEK, INC., ELx, 800UV).

Statistical analysis

Data are shown as mean ± SE. Differences between means in the various groups were checked for significance by one-way analysis of variance (ANOVA) and Duncan's multiple range tests to reveal the significance between means among various experimental groups and days. SPSS (version 16) computer program was utilized.

Results

HPLC-ESI-MS/MS analysis

Detection of condensed tannins and other phenolic compounds in the ethyl acetate extract of seeds of *Medicago sativa* L was investigated using HPLC-ESI-MS/MS. Total ion chromatograms using the negative ion mode exhibited the following natural compounds (Figures 1 and 2): procyanidin C (1), isoquercetin (2), ellagic acid (3), rutin (4), and luteolin (5). Compounds were identified by searching in the Dictionary of Natural Products (DNP), confirmed by their fragmentation patterns, and compared with literature data (Table 1).

In vitro effects

Through the whole period of incubation (24 h), the untreated *H. contortus* adult worms remained alive with no loss of activity, while albendazole sulfoxide, active form (ABZ-SO), caused a full stoppage of motility or death of the worms. CT fraction revealed less activity on the worms at a time, where at the beginning of exposure, the worms showed vigorous movement that declined gradually with time till

70% of the treated worms became paralyzed in the test medium after 24 h of incubation.
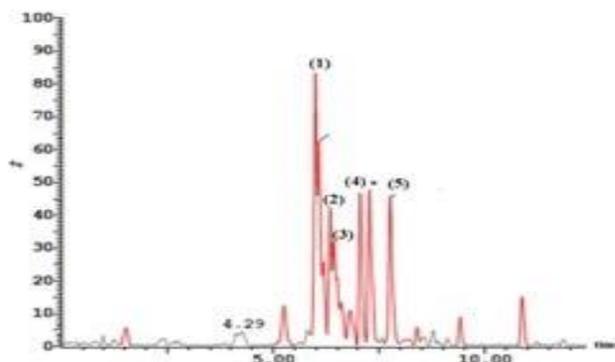

Figure 1: Total ion chromatogram recorded by the negative-ion HPLC-ESI-MS/MS for fractions 9A-14A of the alfalfa seeds extract. * means compound was not identified.
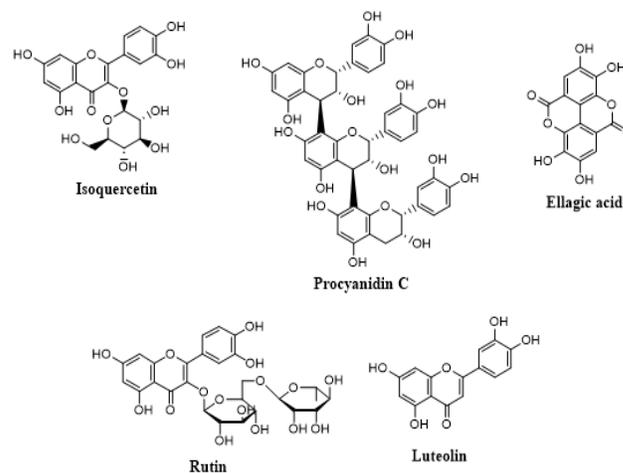

Figure 2: Chemical structure of some significant compounds identified using HPLC-ESI-MS/MS.

Table 1: Some identified compounds in the alfalfa seed extract

No.	Compound name	R_t (min)	$[M-H]^{-1}$ m/z	Product ions
1	Isoquercetin	5.99	463.18	300, 271, 179, 151
2	Procyanidin C	6.44	865.33	733, 695, 425, 407, 289
3	Ellagic acid	6.78	301.19	283,185, 157
4	Rutin	7.26	609.24	300, 271, 255, 243, 151
5	Luteolin	7.76	285.11	255, 201, 151, 107

Light microscopic examination

The whole-body cuticle of the CT-treated worms revealed a significant swelling in the mid-body region. Some specimens displayed cuticular damage with protrusion of viscera visible to the naked eye and expulsion of genital tubules at the vulvar region. A short description of cuticular features of adult worms utilizing light microscopy was necessary to assign the changes following exposure *in vitro* with CT (Figures 3 and 4).

Histological observations

The body wall of untreated *H. contortus* adult worm consists of three layers; cuticle, hypodermis, and an inner layer of muscle cells. The hypodermis, present beneath the cuticle, was relatively thin and comprised of a syncytium of cells. Interior to the hypodermis were several layers of longitudinally arranged striated muscles. These were closely tied with the hypodermis and the cuticle by fibers passing from the contractile part of each muscle cell. After 24 h incubation with ABZ-SO (reference drug), the worms' cuticles seemed thinner and pale than the normal control. Small areas could be detected for connection loss between the cuticle and muscle layer. Most specimens revealed deformity of the subcuticular region and disarrangement of the cuticular musculature. After 24 h incubation with a fraction of the CT, cuticular distortion resembled that caused by ABZ-SO and showed small isolated parts of cuticular cutting. Additionally, degenerative alterations in the underlying muscle cells could be noticed in some cuticle areas (Figure 5).

Effect of CT fraction on fecal egg count

On the 17th day post-infection, fecal examination revealed that all lambs were infected with *H. contortus*. The average count of EPG of G1 (infected untreated animals), G2 (infected animals and treated with CT), and G3 (infected animals and treated with albendazole) are demonstrated in table 2 (. Marked reduction of mean FEC had started to be noticed from the 8th day PT until the end of the experiment; the 50th day PT, for both groups G3 and G2, respectively ($P \leq 0.001$), compared with mean FEC of G1. The mean FEC of G2 and G3 lambs on day 8 PT was 68.67 ± 15.2 and 19.33 ± 1.0. They reached 3.5 ± 2.0 and 0 ± 0 at the end of the experiment, respectively. A remarkable reduction of EPG was observed from day 8 PT until the end of the experiment in G2 compared with G1 animals. Following the 2nd dose of CT, on the 32nd day of the experiment (2 weeks after the first dose), a significant descending decline of the mean FEC until the end of the experiment was detected compared to G1 (Table 2). The maximum FECR was 96.9% and 100% at 50th day PT in lambs of G2 and G3, respectively (Table 3).
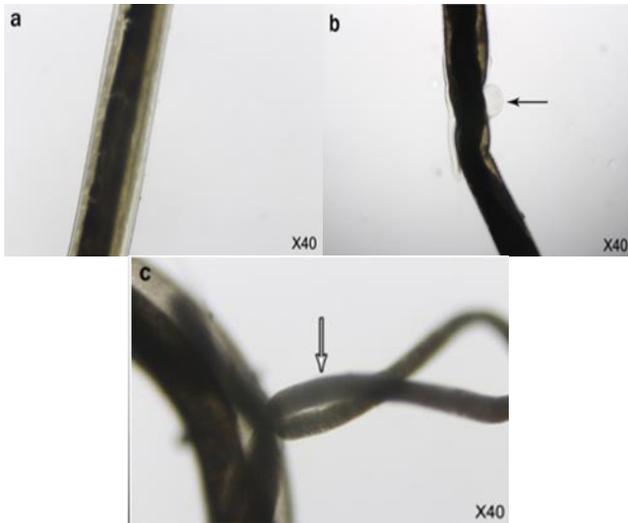
Figure 3: Light micrographs of the mid-body region of *H. contortus* adult worm a) regular fresh worm reveals no change in the wholeness of the body wall. (b, c) Following 24 h incubation with CT. Notice the focal area of swelling of the cuticle (black arrow) and cuticular damage associated with the bulge of uterine tubules (white arrow).
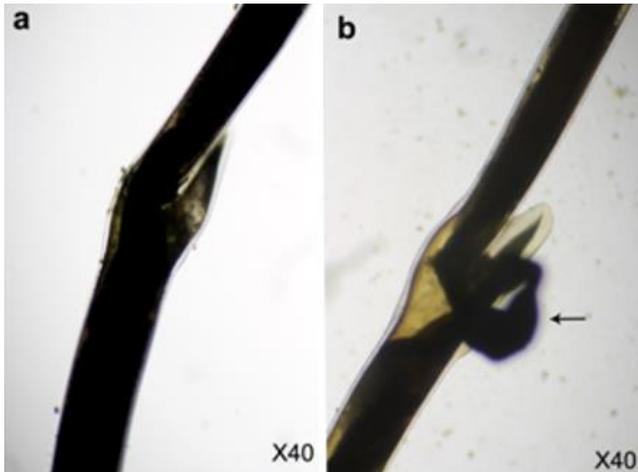

Figure 4: Light micrographs of the female vulvar region. (a) Normal fresh worm. (b) CT treated worm showing expulsion of genitalia.

H. contortus specific IgG response

The anti- *H. contortus* IgG titers in all groups from day zero and infection of all lambs with L_3 till day 68 (the day 50 PT) were displayed in (Figure 6). The antibody titers increased gradually after receiving the 1st dose of treatment in all groups. Furthermore, the greatest titer was noted on day 41 after giving the 2nd dose of CT in G2 compared to other groups. The antibody levels were also gradually increased in

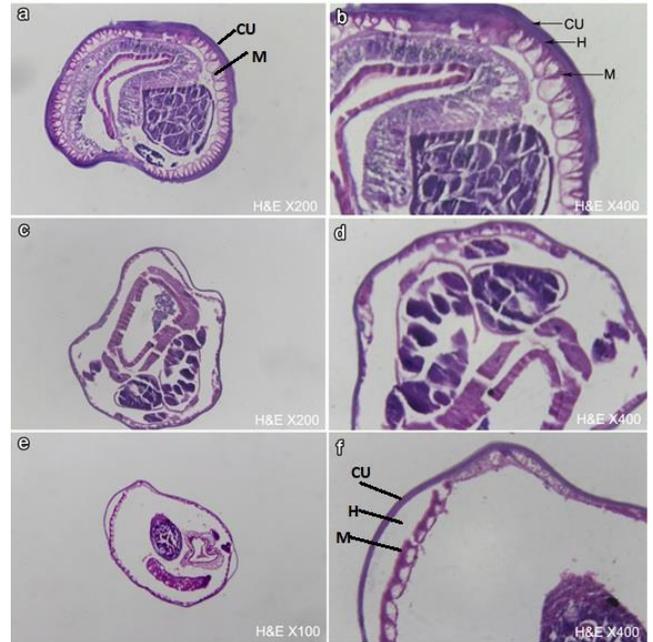G2 following the 2nd dose of treatment with CT fraction compared to other groups.

Figure 5: Light micrographs of the body wall cross-section of *H. contortus* adult worm. (a, b) Normal fresh worm. (c, d) After 24 h incubation *in vitro* with ABZ-SO (reference drug), the micrographs display cuticular dividing and disarrangement of the cuticular musculature. (c, d) After 24 h incubation *in vitro* with CT. Notice the absence of connection between the cuticle and the muscle layer and degenerative alterations in the underlying muscle cells such as *CU* cuticle, *H* hypodermis, *and M* muscle layer .
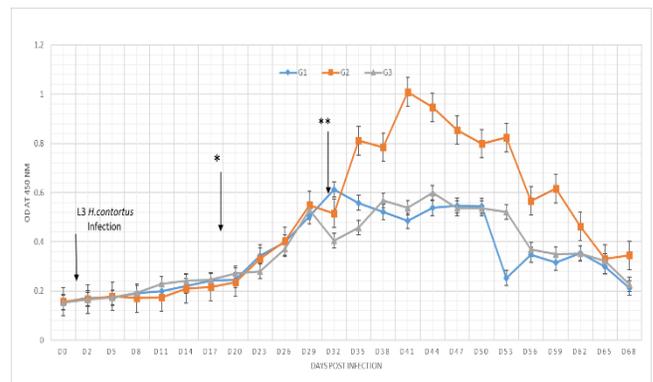

Figure 6: Levels of *H. contortus* IgG against CSA in serum regarding different treatment regimes. *Treatment with the 1st dose of CT for G2 and Albendazole for G3 on the day 18; ** Treatment with the 2nd dose of CT for G2 at the day 32.

Table 2: Mean fecal egg count ±S.E. in lambs infected with *H. contortus* and treated with CT and Albendazole

Days	Mean fecal egg count ±S.E. of eggs per gram of feces ×100			F value	P-value
	G1	G2	G3		
pre-treatments	110 ±2.887 [b]	112.50±10.492 [a]	122.33±13.173 [a]		
2 days post-treatment	115.83 ±3.283 [bA]	110.83±11.077[aA]	99.33±3.320 [bA]	1.486	.299
5 days post-treatment	122 ±1.893[aA]	73.167±15.698 [bB]	86±6.807 [cB]	6.489	.032
8 days post-treatment	113±4.769 [bA]	68.667±15.210 [bB]	19.33±1.0138 [dC]	25.815	.001
11 days post-treatment	102.3±3.346 [cA]	63.67±9.6104 [bB]	9.16±0.601d[eC]	63.250	.001
14 days post-treatment	102.16±2.186 [cA]	57.67±4.4752 [bB]	8.66±0.6001[deC]	260.732	.001
17 days post-treatment	100.67±1.424[cA]	52.83±2.1667 [bcB]	8±0.7638[eC]	881.875	.001
20 days post-treatment	99.5±1.528 [cA]	33.50±2.179[cdB]	7.16±0.882[eC]	863.420	.001
23 days post-treatment	98.5±1.00 [cA]	19.5±2.254[deB]	5.83±0.333[eC]	1211.964	.001
26 days post-treatment	98.3±1.093 [cA]	17.16±5.238[deB]	4.16±0.333 [e]	271.729	.001
29 days post-treatment	98.17±1.0138[c]	11±2.8867[e]	3.16±0.333[eC]	880.701	.001
32 days post-treatment	98.3±1.0138 [cA]	10.50±0.288 [eB]	2±0.5 [eC]	6269.531	.001
35 days post-treatment	98±0.867[cA]	8±1.1547 [eB]	1.83±0.6667[eC]	3439.000	.001
38 days post-treatment	97.33±0.726 [cA]	7.83±1.3017 [eB]	1.83±0.6667[eC]	3218.719	.001
41 days post-treatment	97.33±1.0138 [cA]	6.33±1.878 [eB]	1.16±0.601 [eC]	1785.328	.001
44 days post-treatment	97±0.866 [cA]	6.25±3.608[eB]	0.66±0.441 [eB]	628.231	.001
47 days post-treatment	97±0.866 [cA]	4±2.309 [eB]	0±0[eB]	1485.534	.001
50 days post-treatment	97±0.866[cA]	3.5±2.0207 [eB]	0±0[eB]	1878.983	.001
F value	13.779	26.674	111.703		
P-value	0.001	0.001	0.001		

The small letters in the same column indicate a significant difference between days. Different capital letters in the same row indicated a significant difference between treatment groups ≤0.001.

Table 3: FECR % in lambs after treatment with CT or albendazole in comparison with infected untreated lambs

Days	G1	G2	G3	F value	P-value
2 days post-treatment	-5.4933 ±4.69466[cA]	1.5767±1.10252 [GA]	16.6267±10.16473[dA]	3.025	.123
5 days post-treatment	-11.0333±2.83221[bcB]	36.3733±7.56755 [fA]	29.2200±3.26523[cA]	25.799	.001
8 days post-treatment	-2.6400±1.64147[bC]	40.1233±7.91515 [efB]	83.7267±2.22191[bA]	79.603	.000
11 days post-treatment	6.7800±4.47131[aC]	43.6467±5.26406 [defB]	92.2600±1.17462 [aA]	112.354	.000
14 days post-treatment	6.9833±3.26410[aC]	48.3300±3.91081[deB]	92.6767±1.13452[aA]	202.298	.000
17 days post-treatment	8.3533±2.78506[aC]	52.4500±3.63154[dB]	93.2033±1.20274[aA]	241.275	.000
20 days post treatment	9.4367±2.45840[aC]	69.3733±4.60397[cB]	93.8967±1.18668[aA]	197.694	.000
23 days post-treatment	10.3333±2.48141[aC]	82.7200±0.56536[bB]	95.1767±0.27339[aA]	961.070	.000
26 days post-treatment	10.4933±2.38161[aC]	85.3100±3.06857[abB]	96.5267±0.44857[aA]	429.223	.000
29 days post-treatment	10.6567±2.08138[aC]	90.2333±2.59419[abB]	97.3033±0.51641[aA]	613.052	.000
32 post-treatments	10.5133±1.93950[aC]	90.4933±0.99494[abB]	98.3967±0.34338[aA]	1456.283	.000
35 days post-treatment	10.8100±2.02865[aC]	92.8367±1.04923[abB]	98.5567±0.49482[aA]	1323.948	.000
38 days post-treatment	11.4133±1.97919[aC]	93.0100±1.14001[abB]	98.5567±0.49482[aA]	1307.539	.000
41 days post-treatment	11.4233±1.91804[a]	94.4000±1.68405[ab]	99.1167±0.44213[aA]	1087.679	.000
44 days post-treatment	11.7167±2.00776[a]	94.5700±3.35878[ab]	99.4567±0.38602[aA]	471.714	.000
47 days post treatment	11.7167±2.00776[aB]	96.5233±2.15123[aA]	100 ±0.00[aA]	866.055	.000
50 days post-treatment	11.7167±2.00776[aB]	96.9600±1.88128a[A]	100±0.00[aA]	995.310	.000
F value	6.592	57.669	82.228		
P-value	0.001	0.001	0.001		

The small letters in the same column indicate a significant difference between days. Different capital letters in the same row indicated a significant difference between treatment groups.

Discussion

Haemonchosis is one of the utmost critical threats, which affects sheep growth and production. *H. contortus* is the most prevalent, multiple drug-resistant gastric nematodes (28). The tannin-rich plants constitute a non-conventional, natural alternative treatment for gastrointestinal nematodes in ruminants. Condensed tannins (CT) are a diverse group of secondary plant metabolites that have proved essential effects as an anti-*Haemonchus* anthelmintic; through tests of egg hatchability and larval development (29-31), while its efficacy on *H. contortus* adult worms is poorly studied. The present study detected procyanidin C by HPLC-ESI-MS/MS in the CT fraction. Ellagic acid, which belongs to the tannin family, and other phenolic compounds were identified. As per the literature, several tannins and polyphenolic compounds were identified in *Medicago sativa* L (32,33). The role of CT has been explored against the adult *H. contortus* by assessing its *in vitro* effects on the adult worm cuticle, which has a protective function. The CT showed nematocidal effects *in vitro* on adult *H. contortus*. Besides, the treatment efficiently caused paralysis in 70% of the treated worms and severe cuticle damage and alterations. Acevedo-Ramirez (34) reported similar results when checking the *in vitro* anthelmintic effect of hydrolysable tannins on the adult worm of *H. contortus*. Williams (35) mentioned that tannins caused a rupture in the cuticle of *Ascaris* spp. Worms. The cuticular deformities and alterations might be due to CT's capability to produce complexes with proteins of the cuticle, altering its physical properties (36). Moreover, Athanasiadou (37) explained that the ingested tannins could link protein of the internal mucosa resulting in internal disruption and expulsion of viscera. The cuticle in nematodes has a substantial role in the selective absorption of nutrients, secretion of glycoproteins for immunoprotection, osmoregulation, and sensory perception (38). Besides, Mottier (39) reported that transcuticular passive diffusion had been deemed the primary mechanism of anthelmintic entry into the nematode. Thus, it had been considered that destruction of the worm cuticle indicated potency for any anthelmintic (40). The *in vivo* test revealed that the CT had noticeable anthelmintic activity through diminished mean FEC post-treatment, particularly following the second dose, till the end of the experiment, with excellent FECR (96.9%), in comparison with infected untreated controls. Similarly, Cenci (41) reported the anti-parasitic effect of CT derived from *Acácia Negra* at receiving 18g of Acácia Negra containing 18% of CT/animal/week as an alternative treatment for different helminths control in sheep. Moreover, Pathak (30) proved that supplementation of CT at 1.5 % of diet dietary through a mixture of *Ficus infectoria* and *Psidium guajava* leaves had induced a significant decrease of mean FEC in sheep infected with *H. contortus*. The CT anthelmintic effect might be owing to its direct effect on physiological and metabolic functioning of GI parasite; through suppression of oxidative phosphorylation (42). The CT might also counteract helminths by improving protein nutrition and activating specific immune responses against parasitic infection (43). It is evident that following *H. contortus* infection, the immune system creates a strong Th2 type response, which causes a cascade of immunoglobulins production. The immune response against GIN infection is distinguished by a decrease in FEC and worm burden and can be affected by factors like age, sex, dietary intake, and physiological status (44). In the current study, the IgG response gradually elevated in G2 after the 2nd dose of tannin treatment compared to other groups. Besides, it was noticed that the specific IgG response is consistently related to the decrease of the helminth fecundity and the decline of egg shedding. These go parallel with the findings of Dutta (45), who mentioned perceptible encouraging effect on growth, antioxidant condition, and humoral immune response in goats who received a diet containing 1-2 % CT. The impact of tannins on the host immune response might be attributed to their antioxidant activity via the removal of lipid peroxidation (46).

Conclusion

The study indicated special anthelmintic effects of CT derived from the seeds of Alfalfa (*Medicago sativa* L.) on adult *H. contortus*. It exhibited an *in vitro* nematocidal effect on *H. contortus* in the form of significant alterations and deformities of the cuticle of the adult worm. CT's *in vivo* anthelmintic effect has also been proved by the marked reduction of FEC and enhancement of the IgG response. Thus, the CT might be suggested as a talented alternative for controlling *H. contortus*.

Acknowledgments

Conflict of interest

The authors declare that there is no conflict of interest.

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فعالية التانينات المكثفة في المعمل وداخل الجسم الحي والمستخلصة من بذور البرسيم (ميديكاجو ساتيفا) كمضاد للديدان ضد الاصابة بالهيمونكس كونتورتس

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١قسم الطفيليات وأمراض الحيوان، معهد البحوث البيطرية، المركز القومى للبحوث، الدقى، ٢قسم الكيمياء، كلية العلوم، جامعة حلوان، القاهرة، مصر

الخلاصة

الدراسة الحالية تتناول الفحص المختبري والحيوي للفاعلية المضادة للديدان للتانينات المكثفة المستخرجة من بذور ميديكاجو ساتيفا على ديدان الهيمونكس كونتورتس، وهي ديدان خيطية المعوية الممرضة والمتغذية على الدم في الأغنام. تم تقييم تأثير التانينات المكثفة المضاد للديدان مختبريا بتركيز ٣٠٠ ميكروجرام / مل مقارنة مع البيندازول (دواء مرجعي) عند ١٠ ميكروجرام / مل. أظهرت النتائج أن التانينات المكثفة كان لها تأثير فعال كمضاد لديدان الهيمونكس كونتورتس، تبين أن الغشاء الخارجي للدودة البالغة كان هدفها الأول. ولأجراء تجارب العدوى التجريبية لدراسة تأثير التانينات المكثفة المضاد للديدان حيويا، تم توزيع تسعة حملان خالية من الديدان عمرها ٣ أشهر إلى ثلاث مجموعات. المجموعة الأولى (عدد = ٣) مجموعة ضابطة مصابة غير معالجة؛ المجموعة الثانية (عدد = ٣) عولجت بالتانين المكثف، والمجموعة الثالثة (عدد = ٣)، عولجت بالألبيندازول. تم جمع عينات الدم والروث كل ٣ أيام (بعد العلاج) حتى نهاية التجربة، لمتابعة عدد البيض في الروث وتحديد مستوى الأجسام المضادة المناعية لديدان الهيمونكس كونتورتس، على التوالي. أظهرت الحملان التي عولجت بالتانين المكثف في المجموعة الثانية انخفاضا واضحا في متوسط عدد البيض في الروث مع نسبة انخفاض عدد البيض في الروث كبيرة تم اكتشافها من الأسبوع الأول بعد العلاج حتى نهاية التجربة مقارنة مع حيوانات المجموعة الأولى. زادت مستويات الأجسام المضادة تدريجيا في المجموعة الثانية من الحيوانات بعد الجرعة الثانية من التانين المكثف مقارنة بالمجموعات الأخرى. لوحظ وجود علاقة تناسقية بين ارتفاع مستوى الأجسام المضادة وقلة عدد البيض بعد الجرعة الثانية من التانين المكثف. نستنتج إن التانينات لها تأثير مضاد لديدان الهيمونكس كونتورتس مختبريا وحيويا ويمكن استخدامه كعلاج بديل طبيعي ذو فعالية عالية ضد مرض الهيمونكوزس في الأغنام.