

Field Evaluation of Anthelmintic Efficacy of *Calligonum comosum* against Fasciolosis in Sheep at Taif, KSA

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Abstract: Fasciolosis is an endemic disease and causes severe economic losses and affecting both large and small animals even the human. The present study was designed for studying the *in vivo* effects of ethanolic extract of *Calligonum comosum* on fasciolosis in naturally infected sheep, through investigation of egg/g (EPG) in feces, hematological and serum and biochemical analytical changes. Comparison of its effects with the effects of Triclabendazole [TCBZ] which is the ideal anthelmintic against fasciolosis. Twelve sheep used in this study. Nine were infected with fasciolosis and divided into three groups. The first group treated with ethanolic extract of *Calligonum comosum* (3 g/kg BW), the doses of treatment given for 3 successive days and repeated at week 6 of the experiment, the second group treated with TCBZ (10 mg/kg BW) and repeated at week 6 of the experiment, the 3rd group kept infected non-treated. The 4th group was non-infected non-treated. Fecal egg counts were determined using sedimentation techniques before and after treatment weekly for 12 weeks. Hematological and biochemical analysis were done before and after treatment for 12 weeks. The results of this study indicated that using of ethanolic extract of *Calligonum comosum* treat infected animals with fasciolosis and improved their health condition. Through decreasing the number of egg Fasciola/g feces and improving the level of RBCs, Hb, total protein, albumin and A/G ratio, globulin, GGT, eosinophil, urea and creatinine levels than the infected sheep Compared to TCBZ.

Key words: Fasciolosis • Sheep • *Calligonum comosum* • Triclabendazole

INTRODUCTION

Fasciolosis is an important helminthes disease caused by two trematodes of the genus *Fasciola*; *Fasciola hepatica* (temperate liver fluke) and *Fasciolagigantica* (tropical liver fluke). The distributions of both species overlap in many areas of Africa and Asia [1].

The adult *Fasciola* inhabits the bile duct and gall bladder of infected animals causing severe damages which may lead to the death of the animals [2]. The sub-clinical and chronic disease usually results in decreased production of meat, milk and wool. Secondary bacterial infections, fertility problems and great expenses of anthelmintic should be considered [3]. It was shown that the estimated annual loss in livestock due to fasciolosis all over the world was more than 2000 million dollars [4]. It is stated that even low rates of fluke

infection in cattle can cause significant reduction in performance and infection with 54 flukes/animal resulted in 8-9% reduced weight gain. Even after the animals are cleared of fluke, the initial impaired performance remains until slaughter [5].

The effects of liver flukes include anaemia, weight loss and microbial infections to liver tissues. A serious consequence of the liver damage caused by fasciolosis is that latent *Clostridium novyi* spores can be activated by the low oxygen conditions in the damaged tracts the parasite forms in the liver; this can lead to "black disease", caused by "*Clostridium novyi*" type B or immune-mediated haemolytic anaemia (IMHA) leading to haemoglobinuria caused by *Clostridium novyi* type D [6].

Fasciolosis caused high morbidity and mortality in animals and hepato-biliary lesions [7]. Naturally infested sheep by fasciolosis led to lowering immunity of host, injuries of the liver tissues [8].

Using of ethanolic extraction of *Calligonum comosum* have highly efficacy in the treatment of Fasciolosis and Ascariasis. It had also a bacteriocidal effect [9]. *Calligonum comosum* had molluscicidal activity of *Schistosoma mansoni* and also, less expensive than other chemical drugs [10].

The aim of the study was to study the effects of ethanolic extract of *Calligonum comosum* in comparison with TCBZ on adult *F. gigantica*, as monitored by hematological and biochemical examinations.

MATERIALS AND METHODS

Experimental Animals: Twelve heads of sheep were used in this study. Nine were naturally infected with fasciolosis and divided into three groups. The first group was treated with ethanolic extract of *Calligonum comosum* (3 g/kg BW) [11], the doses of treatment were given for 3 successive days and repeated at 6th week of the experiment. The second group was treated with the recommended dose TCBZ (10 mg/kg BW) [Ciba-Geigy and repeated at 6th week of the experiment, the 3rd group was kept infected non-treated. The 4th group was non-infected non-treated.

Fecal Samples: Individual fecal samples were collected directly from the rectum of each animal. The collected samples were labeled and examined for determination of EPG feces. Fecal egg counts were determined using sedimentation techniques [12] before and after treatment weekly for 12 weeks.

Blood Samples: Blood samples (each of about 2 ml) were collected from experimental animals at zero day and weekly for 12 weeks. These samples were collected via jugular vein puncture in tubes containing EthylenDiamine Tetra acetic acid (EDTA) as anticoagulant, to be used for hematological investigation. The hemogram of all collected blood samples evaluated following the standard technique described by Keiser *et al.* [13]. The hemogram included the RBCs count, Hb concentration and WBCs count. The eosinophils count performed manually by microscopical examination of blood film stained by Fields stain according to the technique described by Tankey *et al.* [14].

Serum Samples: Blood samples (each of about 3 ml) were collected without anticoagulant for serum separation from the experimental animals. The serum was separated by

centrifuge, collected by Pasteur pipette and kept at -20°C till be used in the determination of liver and kidney functions (serum total protein [15], serum albumin [16], serum globulin, activity of GGT [17], blood urea nitrogen [18] and serum creatinine: [19].

Drugs: Ticlabendazole (TCBZ) “Fasinex®” was purchased from Ciba-Geigy Company.

Plants: *Calligonum comosum* fruits were purchased from Taif governorate, ethanolic extract of *Calligonum comosum* was prepared at Medicinal and Aromatic Research Department, National Research Center, Egypt: according to Tariq *et al.* [20].

Statistical Analysis: The obtained data were analyzed for the mean and standard deviation (SD). Statistical comparisons between the means of different groups in different weeks were made with two ways ANOVA, by groups during different weeks were made with different ways ANOVA by COSTAT program version I.P value of <0.05 was assumed for statistical significance.

RESULTS AND DISCUSSION

The fecal egg count considered as the most effective tools for judging the efficacy of the fasciolosis treatment [21].

Table (1) shows that the level of EPG feces revealed a higher decrease in the groups treated with ethanolic extract of *Calligonum comosum* (decrease EPG feces at 3rd day 2.8 and reached to zero EPG till the end of the experiment), followed by the groups treated with TCBZ (decrease EPG feces at 3rd day 4.3 reached to zero EPG feces till the end week of the experiment). High efficacy of *Calligonum comosum* in sheep naturally infected with fasciolosis agreed with some authors [22,23] who mentioned that *Balanites aegyptiaca* had schistosomicidal effect.

Moreover, Petti *et al.* [24] and Koko *et al.* [25] observed a fasciolocidal effect of *Calligonum comosum* which appeared more effective in treatment of fasciolosis than TCBZ.

Our results agreed with those of Keiser *et al.* [26] where they reported that, TCBZ is the only available drug for treatment of fasciolosis and is more efficient, especially in the area endemic in fasciolosis. In addition, it was reported that, TCBZ causes severe decrease of *Fasciola* eggs in EPG feces examined [4].

Table 1: Effect of different treatments on EPG feces at different weeks of experiment:

Groups	Weeks													
	Do	D3	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12
I	Ba	Cb	Cc	Bc	Cd	Ce	Ce	De	Cf	Cf	Cf	Cf	Cf	Cf
Treated with	5±	2.8±	1.2±	1±	0.83±	0.5±	0.5±	0.50±	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
Alco extract of	1	0.3	0.3	0.5	0.3	0.1	0.1	0.1						
<i>Calligonum comosum</i>														
II	Aa	Bb	Cc	Bc	Cd	Cd	Cd	Dd	Ce	Ce	Ce	Ce	Ce	Ce
Treated with	6.7±	4.3±	1.2±	1±	0.83±	0.17±	0.5±	0.5±	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
TCBZ	0.6	1.2	0.3	0.5	0.3	0.3	0	0.01						
III	Ab	Ab	Ab	Ab	Ac	Ac	Ac	Ab	Ab	Ab	Ab	Aa	Aa	Aa
C Control infected	6.7±	6.3±	6±	6.3±	5.17±	5.67±	5.5±	6.67±	6.67±	6.67±	7±	7.33±	7.50±	7.30±
non treated	1.5	0.6	1	0.6	1.3	1.2	1.3	0.6	1.50	0.60	1	0.8	1.0	0.5

Capital -letters Means within the same column of different letters are significantly different at (P < 0.01).

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D=day W=week G= groups TCBZ Triclabendazole EPG Egg per gram SD Standard deviation

Table 2: Effect of different treatments on Hbconc (g/dl) and PCV% at different weeks of experiment.

Weeks	Hbconc (g/dl)				PCV %			
	I Treated with Alc extract of <i>Calligonum comosum</i>	II Treated with TCBZ	III Control infected non-treated	IV Control non-infected non-treated	I Treated with Alc extract of <i>Calligonum comosum</i>	II Treated with TCBZ	III Control infected non-treated	IV Control non-infected non-treated
D0	De 7.5±0.4	Cd 7.9±0.2	Bd 7.2±1.5	Aa 12.5±0.6	Gc 18.4±0.5	Fd 17.9±0.9	Ce 16.1±0.9	Ba 29.8±2.1
W1	Dc 7.7±0.1	Cc 8.2±0.6	Ac 7.9±0.1	Aa 12.3±0.6	Fc 19.2±0.9	EFD 18.1±0.7	BCe 16.2±0.9	Ca 28.8±0.6
W2	Cc 8.2±0.3	Cc 8.2±0.5	Bd 7.2±0.3	ABa 11.7±0.7	Ec 20.7±0.5	Rd 18.3±0.4	Bf 16.6±0.4	Ca 28.8±0.8
W3	BCc 8.6±0.1	Cc 8.2±0.4	Ad 7.9±0.9	Ba 11.1±0.4	Dc 22.1±0.5	Ed 18.5±0.4	Be 16.8±0.6	Da 28.4±0.7
W4	Bc 8.8±0.1	Cc 8.3±0.4	ABd 7.5±0.7	Ba 11.5±0.8	Cc 23.3±9.7	Dd 19.2±0.4	Ae 17.3±0.7	Ca 28.9±0.3
W5	Bc 9.1±0.2	Bd 9.0±0.2	Ae 7.8±1.1	ABa 11.7±0.4	Ac 25.1±0.8	Dd 19.8±0.3	Bf 16.7±1.4	Ca 29.2±0.4
W6	Bc 9.2±0.1	Bc 9.1±0.2	Be 7.2±1.5	ABa 11.8±0.4	Ac 25.1±0.8	Cd 20.2±0.7	Bf 16.6±1.5	Ca 29.2±1.3
W7	Bc 8.8±1.3	Bc 8.9±0.4	ABd 7.6±1.0	ABa 11.8±0.5	Ac 25.1±1.5	Cd 20.0±1.1	Ae 17.1±1.7	Ba 29.7±0.7
W8	Bd 8.7±1.4	Bc 9.0±0.3	Be 7.2±1.7	ABa 11.9±0.4	Ac 25.1±1.4	Bd 23.5±0.5	Bf 16.6±1.6	Ba 29.6±0.7
W9	Bd 8.9±1.3	ABc 9.2±0.4	ABe 7.4±1.6	ABa 11.9±0.4	Ac 25.1±1.4	Bd 23.7±0.9	Ae 17.1±1.3	Ba 29.7±0.7
W10	Bc 9.1±1.4	Bc 9.1±0.3	Be 7.3±0.2	Aa 12±0.4	Ab 25.3±1.5	Bc 23.5±0.6	Ad 17.1±0.8	Ba 29.7±0.6
W11	Ab 9.8±0.7	Ab 9.4±0.1	Bd 7±2.4	Aa 12.1±0.4	Bc 23.8±2.4	Ab 24.3±0.3	Ad 17.3±2.4	Aa 30.00±0.3
W12	Ab 9.9±0.8	Ab 9.7±0.3	Bd 7.3±1.5	Aa 12.2±0.5	Bc 23.8±2.3	Ab 24.8±0.3	Ad 17.1±1.0	Aa 30.7±0.8

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Hb Hemoglobin

The results in Table 2 indicated that, the most efficient treatments on Hbconcentration were ethanolic extract of *Calligonum comosumas* it improve the Hbconc (from 7.5 at zero day till reached 9.9 at the end of the experiment), while TCBZ treated group (increase Hbconc from 7.9 at zero day till reached 9.7 at the end of the experiment). These results agreed with

those of Matanovic *et al.* [27], where they reported that, sheep infected with fasciolosis suffering from severe decrease in Hb level than the flock free from infection. In addition, Koko *et al.* [25] reported that goats infected with fasciolosis and treated with *Balanites aegyptiaca* showed a slight progress in Hbconc [27].

Table 3: Effect of different treatments on eosinophile levels ($10^3/\mu\text{l}$) at different weeks of experiment.

Weeks	Eosinophile ($10^3/\mu\text{l}$)			
	I	II	III	IV
	Treated with Alc extract of <i>Calligonum comosum</i>	Treated with TCBZ	Control infected non-treated	Control non-infected non-treated
D0	Ab 0.81±0.01	Ab 0.88±0.02	Aa 1.4±0.02	Ac 0.4±0.1
W1	Bb 0.6±0.03	Bb 0.54±0.03	Aa 1.4±0.1	Ab 0.4±0.1
W2	Bb 0.6±0.03	Cb 0.41±0.02	Aa 1.4±0.1	Ab 0.4±0.1
W3	Bb 0.6±0.1	Cb 0.4±0.01	Aa 1.5±0.1	Ab 0.4±0.1
W4	Bb 0.5±0.1	Cb 0.4±0.03	Aa 1.5±0.06	Ab 0.4±0.1
W5	Bb 0.5±0.05	Cb 0.4±0.02	Aa 1.4±0.1	Bb 0.33±0.1
W6	Dc 0.44±0.04	Cb 0.4±0.02	Aa 1.5±0.1	Bc 0.34±0.04
W7	Dd 0.32±0.02	Cc 0.4±0.01	Aa 1.4±0.03	Ad 0.32±0.02
W8	Dd 0.32±0.02	Cd 0.39±0.01	Aa 1.4±0.03	Ad 0.4±0.05
W9	Dd 0.35±0.02	Cc 0.42±0.04	Aa 1.5±0.06	Ac 0.4±0.04
W10	Dd 0.35±0.01	Cc 0.43±0.03	Aa 1.4±0.5	Ac 0.4±0.04
W11	Cdb 0.4±0.03	Cb 0.46±0.06	Aa 1.3±0.09	Ab 0.43±0.04
W12	Cdb 0.38±0.02	Cb 0.44±0.07	Aa 1.3±0.01	Ab 0.44±0.01

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Table 4: Effect of different treatments on albumin and globulin level at different weeks of experiment.

	Albumin				Globulin			
	I	II	III	IV	I	II	III	IV
	Treated with Alc extract of <i>Calligonum comosum</i>	Treated with TCBZ	Control infected non-treated	Control non-infected non-treated	Treated with Alc extract of <i>Calligonum comosum</i>	Treated with TCBZ	Control infected non-treated	Control non-infected non-treated
D0	Ec 1.8±0.1	Cb 2.1±0.2	Cb 2.2±0.2	Aa 3.5±0.3	Ab 3±0.1	Cb 2.7±0.2	Ca 3.5±0.2	Bb 3.1±1.3
W1	Cab 3.0±0.4	Ba 3.2±0.01	ABb 2.6±0.1	Aa 3.4±0.4	Bc 1.3±0.3	Dc 1.5±0.1	Aa 4.8±0.1	Bb 3.2±1.5
W2	Dc 2.5±0.3	ABa 3.5±0.1	Bc 2.4±0.4	Aa 3.7±0.2	Ac 2.9±0.3	Ab 3.6±0.2	Ba 4.2±0.2	Bc 3.1±1.0
W3	Ca 3.3±0.3	ABa 3.5±0.1	Bb 2.4±0.2	Aa 3.6±0.2	Ac 2.9±0.3	Ab 3.6±0.1	Ba 4.5±0.1	Bb 3.2±1.0
W4	Cbc 3.2±0.2	Aa 3.7±0.2	Ac 2.9±0.1	Aa 3.5±0.1	Ad 3.2±0.1	Bcd 3.4±0.1	Aa 4.8±0.1	Ab 3.7±0.2
W5	Ba 3.7±0.2	ABa 3.6±0.2	Bb 2.3±0.3	Aa 3.6±0.1	Ac 3.1±0.2	Ab 3.6±0.3	Ba 4.4±0.2	Ab 3.7±0.1
W6	Ba 3.8±0.2	Aa 3.7±0.2	Bb 2.4±0.3	Aa 3.6±0.1	Ac 3.0±0.2	Ab 3.6±0.3	Ba 4.3±0.2	Ab 3.8±0.2
W7	Aba 4.0±0.1	Aa 3.7±0.1	Bb 2.4±0.4	Aa 3.7±0.1	Ac 3.1±0.1	Aab 3.8±0.1	Ba 4.3±0.2	Aa 3.9±0.2
W8	Aa 4.1±0.1	Aa 3.7±0.1	Bb 2.5±0.4	Aa 3.7±0.1	Ac 3.1±0.1	Ab 3.8±0.1	Ba 4.3±0.2	Ab 3.8±0.2
W9	Aa 4.2±0.1	Aa 3.8±0.1	Bb 2.5±0.3	Aa 3.7±0.1	Ac 2.9±0.1	Ab 3.6±0.1	Ba 4.3±0.2	Ab 3.8±0.2
W10	Aa 4.1±0.1	Aa 3.7±0.3	Bb 2.5±0.3	Aa 3.7±0.1	Ac 3±0.1	Ab 3.6±0.1	Ba 4.3±0.1	ABb 3.5±0.5
W11	ABa 3.9±0.2	ABa 3.5±0.1	Bb 2.5±0.3	Aa 3.7±0.1	Ac 3.0±0.1	Ab 3.6±0.3	Ba 4.3±0.1	Ab 3.8±0.2
W12	ABa 4±0.3	ABa 3.5±0.1	Bb 2.5±0.3	Aa 3.7±0.1	Ac 3.0±0.1	Abc 3.3±0.1	Ba 4.3±0.1	ABb 3.5±0.5

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The results of TCBZ attributed to the TCBZ induced elimination of flukes and healing the hepatic lesions this results agreed with those of Matanovic *et al.* [27] TCBZ administration induced the elimination of flukes and healing of the majority of hepatic lesions but did not prevent severe hepatic damage produced by later infections. This will improve the body conditions of the sheep and regeneration of RBCs and improve the Hb%.

The results showed decreased the eosinophil level in ethanolic extract of *Calligonum comosum* (decrease from 0.81 till reached 0.38 at week 12 of the experiment) and the TCBZ treated group (decreased from 0.88 at zero day till reached 0.44 at the end of the experiment) compared to TCBZ treated group (Table 3).

The result also, indicated that the control infected non-treated group of higher eosinophil level than the sheep free from infection (Control non-infected non-treated). This results agreed with those of Matanovic *et al.* [27], where they reported that, sheep infected with fasciolosis showed a higher level of eosinophil level than the flock free from infection.

Concerning the effect of treatments on total protein level, Table (4) shows results indicated that, the groups treated with ethanolic extract of *Calligonum comosum* (increased from 4.8 at zero day reaching 7.0 at week 12), the extracts of *Calligonum comosum* causes increasing the level of total protein. The TCBZ improve the level of total protein (increased from 4.8 at zero day till reached 7.2 at week 11 of the experiment. This results agreed with those of Koko *et al.* [25] and Matanovic *et al.* [27], where they reported that, the sheep infected with fasciolosis suffering from severe decrease in total protein level than the flock free from infection.

With respect to the effect of different treatments of fasciolosis on albumin level at different weeks, the results indicated that, the most efficient treatments on albumin level were ethanolic extract of *Calligonum comosum* it improve the albumin level (increased from 1.8 at zero day till reached 4.0 at the end of the experiment). The results also indicated that, the group treated with TCBZ showed improved level of albumin (increased from 2.1 till reached 3.5 at week 12). In addition, the results indicated that, the control non-infected non-treated group of higher albumin level similar to the group treated with ethanolic extract of *Calligonum comosum*.

A lower globulin level was observed in the group treated with ethanolic extract of *Calligonum comosum* and TCBZ was agreed with those of Koko *et al.* [25] and Osama and Soliman [28], where they reported that, sheep

infected with fasciolosis showed a higher level of globulin than the flock free from infection.

The most efficient treatment on A/G ratio with ethanolic extract of *Calligonum comosum* it improves the A/G ratio (increased from 0.6 at zero days till reach 1.33 at week 12) as shown in Table (5).

These results agreed with those of Koko *et al.* [25] and EL-Shenawy *et al.* [29], where they reported that, sheep infected with fasciolosis suffering from severe decrease in A/G ratio.

Table (6) shows a lower level of urea observed in the group treated with TCBZ (decreased from 17.8 to 13.6 all over the experiment), while the minimum urea level observed in the group treated with ethanolic extract of *Calligonum comosum* (decreased from 18.1 at zero day till reach 11.5 at week 12 of the experiment). This results agreed with those of Koko *et al.* [25], where they reported that, sheep infected with fasciolosis suffering from severe increase in serum urea level than the flock free from infection. A lower level of creatinine observed in the group treated with TCBZ (decreased from 1.7 to 1.3 all over the experiment), while the minimum creatinine level observed in the group treated with ethanolic extract of *Calligonum comosum* (decreased from 1.7 to 1.1 all over the experiment).

This results agreed with those of Koko *et al.*, where they reported that, sheep infected with fasciolosis suffering from severe increase in blood creatinine level than the flock free from infection.

A lower level of GGT (Table 6) observed in the group treated with TCBZ and the level of GGT in it decreased gradually (decreased from 43 at zero day till reach 31 at the end of the experiment). While the minimum GGT level observed in the group treated with ethanolic extract of *Calligonum comosum* (decreased from 47.7 at zero days till reach 30 at the end of the experiment). These results agreed with those of Koko *et al.* [25], where they reported that, sheep infected with fasciolosis showed a higher level of GGT. Also, our results exactly similar to results of Osama and Soliman [28] where they observed that (Balanitaceae) fruit mesocarp water extract (traditionally used as an anthelmintic in the Sudan) causes the characteristic lesions of liver fasciolosis, egg/gm of faeces (EPG), packed cell volume (PCV), haemoglobin concentration, total red blood cells count (RBC), total white blood cells count (WBC) and eosinophil% were improved and significantly different from control and treated groups ($P < 0.05$).

Table 5: Effect of different treatments on Albumin/globulin ratio and BUN level at different weeks of experiment.

Weeks	Albumin/globulin ratio				BUN			
	I Treated with Alc extract of <i>Calligonum comosum</i>	II Treated with TCBZ	III Control infected non-treated	IV Control non-infected non-treated	I Treated with Alc extract of <i>Calligonum comosum</i>	II Treated with TCBZ	III Control infected non-treated	IV Control non-infected non-treated
D0	Jc 0.6±0.1.0	Db 0.78±0.30	Ac 0.63±0.03	Aa 1.13±0.30	Ad 18.1±3.4	ABc 17.90±2.55	Aa 17.5±4.17	Be 10.0±2.22
W1	Aa 2.31±0.20	Ab 2.13±0.30	Bc 0.54±0.04	Bb 1.06±0.20	Ad 16.3±2.6	ABc 16.5±2.44	Ga 18.5±4.15	Cd 11.2±2.11
W2	Id 0.86±0.10	Cc 0.97±0.12	Be 0.57±0.05	Ab 1.19±0.20	Ae 15.0±2.8	ABd 15.1±2.55	Fa 19.8±4.88	Ce 11.3±3.14
W3	Ga 1.14±0.10	Cb 0.97±0.12	Cc 0.53±0.03	Aa 1.13±0.10	Bf 13.2±1.12	Bd 14.7±2.18	Ea 20.9±4.99	Cc 11.2±2.13
W4	Ha 1.00±0.03	Ba 1.09±0.10	ABc 0.6±0.02	Cb 0.95±0.20	ABe 12.1±1.12	Bd 13.3±2.13	Da 22.4±4.44	Bf 10.3±3.15
W5	Ga 1.19±0.1	Bc 1.00±0.010	Cd 0.52±0.02	Cc 0.97±0.30	Ae 11.8±1.9	ABd 13.5±2.55	Ba 23.4±4.44	Bf 10.2±2.14
W6	Fa 1.27±0.10	Bc 1.03±0.03	Be 0.56±0.03	Cd 0.95±0.2	Ae 11.9±2.9	Ad 14±2.14	Aa 25.4±4.25	Ce 11.2±2.11
W7	Fa 1.29±0.20	Cb 0.97±0.20	Bc 0.56±0.02	Cb 0.95±0.20	Ae 11.2±3.2	ABd 13.5±2.55	Ca 22.3±3.22	Bf 10.3±2.17
W8	Fa 1.32±0.10	Ce 0.97±0.10	Bd 0.58±0.01	Ce 0.97±0.10	Bd 10.8±2.8	ABc 13.6±2.66	Ba 24.3±3.22	Ae 11.4±2.14
W9	Ba 1.45±0.3	Bb 1.06±0.10	Bc 0.58±0.10	Cb 0.97±0.2	Be 10.9±2.9	ABc 13.8±2.88	Ba 23.4±4.22	Ad 11.3±3.12
W10	Ca 1.37±0.20	Bc 1.03±0.10	Be 0.58±0.10	Dd 0.71±0.20	Bf 10.11±2.11	ABd 13.6±2.13	Ca 22.3±3.25	Ae 11.3±3.15
W11	Fa 1.3±0.20	Cd 0.97±0.10	Be 0.58±0.10	Cd 0.97±0.10	Ae 11±2.11	ABd 13.7±2.77	Ba 23.2±3.32	Ae 11.4±4.11
W12	DEa 1.33±0.30	Bc 1.06±0.20	Be 0.58±0.20	Bd 1.06±0.12s	Ae 11.5±2.55	ABd 13.6±2.66	Aa 25.5±5.55	Ae 11.5±5.11

Capital -letters Means within the same column of different letters are significantly different at (P < 0.01).

Small -letters: Means within the same raw of different letters are significantly different at (P < 0.01).

Table 6: Effect of different treatments on creatinine and GGT level at different weeks of experiment.

Weeks	Creatinine				GGT level			
	I Treated with Alc extract of <i>Calligonum comosum</i>	II Treated with TCBZ	III Control infected non-treated	IV Control non-infected non-treated	I Treated with Alc extract of <i>Calligonum comosum</i>	II Treated with TCBZ	III Control infected non-treated	IV Control non-infected non-treated
D0	Ad 1.7±0.20	Abc 1.7±0.2	Aa 1.8±0.2	Ad 0.9±0.3	Ab 47.7±2	Bd 43±1.5	Ia 50±2.5	Ae 33±1.5
W1	Abc 1.4±0.1	Ab 1.6±0.3	Aa 1.8±0.5	Ac 0.9±0.2	Bd 36±1	Ac 44±1	Jb 49±0.6	Ae 33±1
W2	Ac 1.5±0.1	Abc 1.4±0.2	Aa 1.7±0.3	Ac 1.0±0.1	Cd 33±2	Fd 32±1	Ha 51±1.2	Bd 32±1
W3	Ab 1.30.2	Ab 1.5±0.3	Aa 1.8±0.3	Ab 1.2±0.2	Ed 31±0.9	Gd 31±1.1	Ba 66±1.6	Cd 31±1.5
W4	Ab 1.1±0.1	Ab 1.3±0.1	Aa 1.8±0.2	Ab 1.2±0.2	Dde 32±1	Ed 33±1	Ga 56±4	Ce 31±2
W5	Ab 1.1±0.3	Ab 1.3±0.3	Aa 1.7±0.3	Ab 1.1±0.1	Ef 31±2	Dd 36±2	Fa 59±4.5	Ae 33±2
W6	Ab 1.1±0.1	Ab 1.4±0.4	Aa 1.8±0.5	Ab 1.3±0.3	De 32±2	Cd 37±2	Fa 59±2.5	Be 32±2
W7	Ad 1±0.2	Ac 1.3±0.2	Aa 1.8±0.2	Ac 1.2±0.2	Ed 31±1	Gd 31±4	Da 61±2	Dd 30±0.6
W8	Ab 1.2±0.2	Ab 1.2±0.2	Aa 1.9±0.3	Ab 1.1±0.1	Fc 30±1	Hc 30±3	Ea 60±2	Dc 30±1
W9	Abc 1.11±0.1	Ab 1.3±0.3	Aa 1.8±0.2	Ac 0.9±0.3	Ed 31±2	Ie 29±3	Da 61±3	Dd 30±2
W10	Ac 1.10±0.2	Ac 1.2±0.2	Aa 1.9±0.3	Ac 1.1±0.1	Fd 30±2	Hd 30±4	Ca 63±3	Ed 29±1.5
W11	Ab 1.1±0.1	Ab 1.2±0.2	Aa 1.8±0.4	Ab 1.2±0.2	Ed 31±1.5	Gd 31±1.5	Ba 66±1	Dd 30±1.2
W12	Ab 1.1±0.2	Ab 1.3±0.3	Aa 1.9±0.2	Ab 1.3±0.3	Fd 30±0.9	Gd 31±3.3	Aa 67±1	Dd 30±2

Capital letters: Means within the same column of different letters are significantly different at (P < 0.01). Small letters: Means within the same raw of different letters are significantly different at (P < 0.01).

Results of TCBZ agreed with those of Bashir *et al.* [22] on sheep, who mentioned that there is no any fasciolosis signs and no eggs of *Fasciola* with improvement of hematological and biochemical parameters of the treated sheep.

Concerning the anthelmintic effect of ethanolic extraction of *Calligonum comosum* in the treatment of fasciolosis, it had High efficacy in the treatment of sheep naturally infected with fasciolosis. Our results agreed with who reported that using of ethanolic extraction of some Plant Extracts of Saudi Arabia have highly efficacy in the treatment of Fasciolosis and Ascariasis. It had also a bacteriocidal effect and had molluscicidal activity of *Schistosoma mansoni* and also less expensive than other chemical drugs Bakray [10] and Mossa *et al.* [30].

CONCLUSION

Fasciolosis is considered as the most cause of liver condemnation and was responsible for total liver condemnation for sheep. Studying the effects of ethanolic extract of *Calligonum comosum* and TCBZ on adult *F. gigantica*, through hematological and biochemical examination revealed that, decreasing the number of egg of *Fasciola*/g feces and the blood parameter returned to its normal levels, leading to improved sheep health condition.

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