Comparative studies on efficacy of ethanolic extracts of *Calligonum comosum* plant and triclabendazole (TCBZ) "Fasinex on treatment of goats fascioliasis in Taif Governorate.


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**ABSTRACT**

This study was planned for studying the effect of some plant extracts in vitro on fascioliasis in goats, and comparison between the plant extracts and drug (TCBZ) that commonly found in the market used for fascioliasis treatment. In vitro, determination the susceptibility of adult flukes *Fasciola spp.* to ethanolic extracts of *Calligonum comosum* at different concentrations (10, 20, 30, 40 and 50 μg/ml) and *triclabendazole* (TCBZ 20 μg/ml) for 24 h incubation period by using light microscope and scanning electron microscope. Result revealed that, all four concentrations caused tegumental alterations such as, swelling of oral and ventral suckers, swelling of the tegument, disappearance of spines, appearance of spine socket, folding and furrowing of the tegument depending on the concentration level and the high efficacy observed first in *Calligonum comosum* followed by TCBZ.

**KEYWORDS:** *Calligonum comosum*, fascioliasis, TCBZ, Helminthes, triclabendazole and goat.

**INTRODUCTION**

*Calligonum comosum* a plant with rich ethnomedical applications, has been also introduced as potential anti-microbial, anti-helminthic agent, possesses strong antioxidant, anti-inflammatory properties Mossa, J.S.; *et al.* [1].Niaz *et al.* [2] showed that it may be a good herbal medicine to treat animals infected with schistosomiasis with no side effect and the cheapest one. Koko *et al.* [3] and Liu *et al.* [4] observed a fasciolicidal effect of *Calligonum comosum* which appeared more effective in treatment of fascioliasis than triclabendazole (TCBZ). They studied anti-inflammatory and anti-ulcer activity of *calligonum comosum* in rats, results indicated the ethanolic extract of the aerial parts of *Calligonum comosum* significantly reduced the increase in hind paw oedema induced by carrageenan in rats. Furthermore, a pre-treatment with the extract produced a significant dose-dependent inhibition to the acute gastric ulcers induced by phenylbutazone indomethacin. Badria *et al.* [5] were tested also for their anticancer activity using Ehrlich ascites. Indeed, anti-inflammatory, anti-ulcer and anti-cancer activities of *C. comosum* have been reported in rat and shrimp animal models. Moreover, tar resulting from stem combustion is used to cure dromedary scabies. Stem bark and leaf-bath serves as leather tanning and milk wineskin disinfectant. Bakray [6] recorded that, *C. comosum* had molluscicidal activity of *Schistosomum mansoni* and also, less expensive than other chemical drugs. Tariq *et al.* [7] used of ethanolic extraction of *Calligonum comosum* and recorded that, it have highly efficacy in the treatment of Ascariasis. Nabila S. Degheidy *et al.* [8] studied the effects of ethanolic extract of *C. comosum* and TCBZ on adult *F. gigantica,*
through hematological and biochemical examination and 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. Also it had a bactericidal effect and less expensive than other chemical drugs. Concerning the anthelmintic effect of ethanolic extraction of *Calligonum comosum* in the treatment of fascioliasis, it had highly anthelmintic activity against hepatic worm among goats.

Nabila S. Degheidy et al. [8] also recorded that the effects of ethanolic extract of *Calligonum comosum* more efficient than TCBZ in the treatment of *Fasciola gigantica* among goats and less expensive.

In this study, experiments were carried out for the first trials to show anti-parasitic activity of *Calligonum comosum* in the treatment of *Fasciola* spp., and comparison between the plant extracts and drug (TCBZ) that found in the market and used commonly for fascioliasis treatment.

**MATERIALS AND METHODS**

*Identification of Fasciola Worms:*

One hundred adult worms were randomly collected from bile duct and livers of goats, which had active infection, for identification of *Fasciola gigantica* were done according to Periago et al. [9] and Soulsby [10].

*Drugs:*

Ticlabendazole (TCBZ) “Fasinex®” was purchased from Ciba-Geigy Company, and used at dose 20 mg per ml.

*Plant Material:*

*Calligonum comosum* seedlings (35 – 40 cm height) were provided from Dirab near Riyadh city, Saudi Arabia. *Calligonum comosum* plants were obtained from the local market of Taif City, KSA.

The leaves, stem and roots of *Calligonum comosum* were used for testing their anthelmentic efficacy against *Fasciola* spp.

The plant extract (Calligonum comosum plant) was prepared at the laboratory of Medicinal and Aromatic Plants Research Department, National Research Center, Egypt, according to the method of Tariq et al. [7]. It used at different doses (10, 20, 30, 40 and 50 mg per ml).

In *vitro* determination of the anthelmentic efficacy of *Calligonum comosum* plant and TCBZ on adult *F.gigantica*: (death rate) was done by Ibarra and Jenkins [11] and Sanyal [12].

Samples for scanning electron microscope examinations were made according to method proposed by Valero et al. [13], using a computer image analysis system (ELICA QWin 500, Cambridge, England). The measurements included spine characters as well as area of tegumental swelling around the ventral sucker.

*Statistical analysis:*

The obtained data were analyzed were made with different ways ANOVA by COSTAT program version I.P value of <0.05 was assumed for statistical significance. Data were analysed using Epi Info version 6 statistical software and for further compared using Chi-square test at critical probability of p<0.05.

*Experiment I:*

In *vitro* determination of the anthelmentic efficacy of *Calligonum comosum* plant and TCBZ on adult *F. gigantica*: (death rate).

The present study was designed for comparative study on efficacy of ethanolic extract of *Calligonum comosum* plant and triclabendazole (TCBZ) on adult flukes, among goats in Taif, through investigate death rate of adult worms. Results are shown in table (1 and 2).

*Experiment II:*

Scanning electron microscopy (SEM) of normal fresh adult *F. gigantica*.

Results are shown in Fig.1 (a, b, c, d, e and f).

*Experiment III:*


Results are shown in Fig.2 (a, b, c, d, e and f), Fig.3 (a, b, c, d, e and f), Fig. 4 (a, b, c, d, e and f) And Fig.5 (a, b, c, d, e and f)
RESULTS AND DISCUSSION

Table (1) and (2) C. comosum and TCBZ showed significant effect on adult worms of Fasciola gigantica after 24 h in vitro cultivation revealed that 100% of worms were died with Ethanolic extract of C. comosum and TCBZ. The results showed that the different concentrations of C. comosum plant and TCBZ have an impact outside the host body and worms started to stop motion animation worms and paralysis leading to death.

Scanning electron microscopy (SEM) of normal fresh adult Fasciola gigantic flukes:

following 24 hrs incubation in solvent; 70% ethanol, revealed that, the oral and ventral sucker showing smoothness with thick rims covered with transverse folds. The entire surface of the tegument, with the exception of the rims of both suckers, was seen to be covered in spines. Fig (a and b). Lateral margin and posterior to the ventral sucker showed that the size and shape of these spines were dependent on the area of the fluke on which they were present. Spines were larger on the anterior and anterior mid body regions than other parts of the tegument Fig.1 (c, d and e). Posterior to the ventral sucker, the tegumental surface of the fluke between the spines seen to be made up of tiny meshwork of ridges and pits Fig 1 (d). This meshwork was present on all areas of the fluke, except the rims of both suckers.

Concerning Scanning electron microscopy (SEM) of treated adult Fasciola gigantic fluke with TCBZ:

show different changes in Fasciola gigantic adult fluke after 24 hrs incubation with 20 μg/ml TCBZ, the oral sucker showing sever swollen tegument with sloughing in which the tegument had been stripped off to expose the basal lamina beneath Fig2 (a). The ventral sucker showing sever swelling with smoothness of the tegument Fig. 2 (b). Ventral mid-body region, the tegument showing sloughing and furrows Fig. 2 (c). The tegument showing submerged spines which either lied very flat against the surface or had become submerged in the tegument by the swollen tegument around them leaving deep furrows Fig. 2 (d and f). The tegument of the tail region showed deep furrows and folding. Fig. 2 (f). The results of SEM revealed a potency of studied ethanolic extracts and TCBZ on adult F. gigantica. The high efficacies were observed in ethanolic extract of Calligonum comosum plant. In addition, the high concentration of extract resulted in more tegumental alterations in adult flukes.

Table 1: In vitro effect of C. comosum ethanolic extract on adult worms, after 24 hours incubation (Death rate of adult worms).

<table>
<thead>
<tr>
<th>Concentration of C. comosum</th>
<th>Worm suspended in C. comosum ethanolic extract</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mg/ml</td>
<td>All worms dead</td>
<td>100</td>
</tr>
<tr>
<td>20 mg/ml</td>
<td>All worms dead</td>
<td>100</td>
</tr>
<tr>
<td>30 mg/ml</td>
<td>All worms dead</td>
<td>100</td>
</tr>
<tr>
<td>40 mg/ml</td>
<td>All worms dead</td>
<td>100</td>
</tr>
<tr>
<td>50 mg/ml</td>
<td>All worms dead</td>
<td>100</td>
</tr>
<tr>
<td>control (DMSO) added to media</td>
<td>All live for 48 hours</td>
<td>-----------</td>
</tr>
</tbody>
</table>

Table 2: In vitro effect of TCBZ on adult worms, after 24 hours incubation (Death rate of adult worms).

<table>
<thead>
<tr>
<th>Concentration of TCBZ</th>
<th>Worm suspended in TCBZ</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mg/ml</td>
<td>All worms dead</td>
<td>100</td>
</tr>
<tr>
<td>20 mg/ml</td>
<td>All worms dead</td>
<td>100</td>
</tr>
<tr>
<td>30 mg/ml</td>
<td>All worms dead</td>
<td>100</td>
</tr>
<tr>
<td>40 mg/ml</td>
<td>All worms dead</td>
<td>100</td>
</tr>
<tr>
<td>50 mg/ml</td>
<td>All worms dead</td>
<td>100</td>
</tr>
<tr>
<td>control (DMSO) added to media</td>
<td>All live for 48 hours</td>
<td>-----------</td>
</tr>
</tbody>
</table>

Scanning electron microscope (SEM) of treated adult Fasciola gigantic fluke with ethanolic extracts of Calligonum comosum, revealed that:

the changes in F. gigantic adult fluke after 24h incubation with different doses of ethanolic extracts of Calligonum comosum plant concerned, had provided evidence for the greater anthelmintic activity of C. comosum plant on the in vitro treatment of naturally infected with fasciolasias.

SEM micrographafter 24 hrs incubation with 10 μg/ml ethanolic extracts of Calligonum comosum plant, the oral and ventral suckers appeared to be slightly more swollen than normal Fig3 (a and b). Lateral margin and posterior to the ventral sucker, the tegument showing submerged spines by the swollen tegument around them Fig. 3(c and d). The tegument of the tail region showed furrows, embedding of spines and sloughing in which the tegument had been stripped off to expose the basal lamina beneath Fig. 3 (f).
Fig. 1: Scanning electron micrographs (SEMs) of adult *F. gigantic* following 24 hrs incubation in solvent. (a) SEM of the oral sucker (Os) showing smooth oral sucker with thick rim. (b) SEM of the ventral sucker (Vs) showing smoothness with a with thick rim. (c, d and e) SEMs of lateral margin and posterior to the ventral sucker region, the tegument at higher magnification showing ridgedtegument with numerous spines (S).The spines showing finger-like protrusions at their tips. (f)SEM of the tail region.
Fig. 2: Scanning electron micrographs SEMs of adult *F. gigantic* following 24 hrs incubation in 20 μg/ml trilabendazole. (a) Oral sucker (Os) showing severe swollen tegument with sloughing (Sl) in which the tegument had been stripped off to expose the basal lamina beneath. (b) The ventral sucker (Vs) showing swelling around the ventral sucker with smoothness of the tegument. (c) Ventral mid-body region, the tegument showing sloughing (Sl) and furrows (F). (d and f) The tegument showing submerged spines (S) which either lied very flat against the surface or had become submerged in the tegument by the swollen tegument around them leaving deep furrows (F). (e) Tail region. Tegument showed deep furrows (F) and folding.
Fig. 3: Scanning electron micrographs SEMs of adult *F. gigantic* following 24 hrs incubation in 10μg/ml ethanolic extracts of *Calligonum comosum* (a) Oral sucker (Os) showing slightly more swollen tegument than normal. (b) The ventral sucker (Vs) showing swelling around the ventral sucker and cirrus (c). (c and d) Lateral margin and posterior to the ventral sucker, the tegument showing submerged spines (S) by the swollen tegument around them. (e) Tail region. (f) Tegument of tail region showed furrows (F), embedding of spines (S) and sloughing (Sl) in which the tegument had been stripped off to expose the basal lamina beneath.

After 24hrs incubation with 20 μg/ml ethanolic extracts of *Calligonum comosum* plant, the oral and ventral suckers appeared to be more swollen tegument than normal Fig. 4 (a and b). Posterior to ventral sucker, the tegument showing the spines either lied very flat against the surface with swelling Fig. 4 (d), lateral margin, the tegument showing submerged spines by the swollen tegument around them with appearance of spine socket Fig. 4 (c and e). Tail region showed furrows with deep folding in the tegument and completely disappearance of spines. Fig. 4 (f).
After 24 hrs incubation with 40 μg/ml ethanolic extracts of Calligonum comosum plant, the oral sucker showing sloughing apart from swelling of the tegument leaving a basal lamina beneath Fig. 5 (a). The ventral sucker appeared to be severely more swollen than normal Fig. 5 (b). Lateral margin, the tegument showing submerged spines by the swollen tegument around them leaving spine socket Fig. 5 (c). Ventral mid-body region, severe swelling of the tegument showed furrows, submerged spines which appeared sunken with their tips protruding from swollen and blebbed bases Fig. 5 (e). Tail region, swelling of the tegument led to completely submerged spines leaving spine socket, furrows and deep folding Fig. 5 (d,f). Tegumental changes depend on the concentrations of extracts. The severity of tegumental alterations observed in 50, 40 and 20 μg/ml, respectively.

**Fig. 4:** Scanning electron micrographs SEMs of adult F. gigantic following 24 hrs incubation in 20μg/ml ethanolic extracts of Calligonum comosum plant (a) Oral sucker (Os) showing more swollen tegument than normal. (b) The ventral sucker (Vs) showing swelling around the ventral sucker and cirrus (c). (c) Lateral margin, the tegument showing submerged spines (S) by the swollen tegument around them with appearance of spine socket (Ss). (d) Posterior to ventral sucker, the tegument showing the spines either lied very flat against the surface or had become submerged in the tegument (e) lateral margin. Sever swelling of the tegument led to submerging of spines (S) leaving spinesocket (Ss). (f) Tail region showed furrows (F) with deep folding (Fo) in the tegument and completely disappearance of spines.
Fig. 5: Scanning electron micrographs SEMs of adult *F. gigantic* following 24 hrs incubation in 40μg/ml ethanolic extracts of *Calligonum comosum* plant. (a) Oral sucker (Os) showing sloughing (Sl) apart from swelling of the tegument leaving a basal lamina beneath. (b) The ventral sucker (Vs) showing swelling around the ventral sucker and sloughing (Sl) of the tegument. (c) Lateral margin, the tegument showing submerged spines (S) by the swollen tegument around them leaving spine socket (Ss). (d) Tail region. (e) Ventral mid-body, severe swelling of the Tegument showed furrows (F), submerged spines (S) which appeared sunken with their tips protruding from swollen and blabbed bases. (f) Tail region, swelling of the tegument led to completely submerged spines leaving spine socket (Ss), furrows (F) and deep folding.

The tegument of trematodes has a number of important roles, including osmoregulation, protection, secretion or synthesis, and hence represents a primary drug target. SEM has proved to be a useful tool for evaluating surface changes on the tegument and suckers of trematode resulting from anthelmintics action [14].
Differences in response to ethanolic extracts of *Calligonum comosum* plant and TCBZ action observed, depending on the used concentration. The swelling and blebbing of the tegument induced by the studied extracts had been described for different schistosome species as reported previously by Jennifer and Norson [15], Singh et al. [16], Al-Shaal et al. [17]. The effect in vitro susceptibility of different concentrations (10, 20 and 40 μg/ml) of ethanolic extracts of *Calligonum comosum* plant and 20 μg/ml of TCBZ using SEM were studied by Halferty et al. [14], the obtained results revealed a high efficacy of studied extract and TCBZ on the tegument, which revealed swelling, disappearance of spines, swelling of the oral and ventral suckers, furrowing and folding of the tegument depending on the concentrations of these extracts. The highest efficacies on adult worm was the ethanolic extracts of *Calligonum comosum*. Similar results were obtained by many authors Al-varez et al. [18], Meaney et al. [19], Halferty et al. [14] and Jennifer and Norson [15] who found that the effect of TCBZ on adult flukes revealed sloughing, blebbing and eruptions in the tegument. In addition, these results similar to that recorded by Al-Shaal et al. [17] who found that the fixed oil of *B.aegyptiaca* fruits had anthelmintic activity against hepatic worm (*S. mansoni* and *F. gigatca*). Also, Singh et al. [16] and Ebeid et al. [20] revealed that, alchoholic extract of some medical plants produced significant reduction in the frequency and amplitude of contractile activity of adult fluke.

The effect of ethanol extracts of *Calligonum comosum* planton adult flukes may be attributed to the constituent of alkaloids as mentioned by Al-Shaal et al. [17]. In addition mentioned that it contains unsaturated fatty acids and sterols which had antimitugenic activity against *F.gigantica*. Moreover, it had a potent free radical scavenging and antioxidant properties, anti-inflammatory and anti-ulcer seems to be a highly promising agent in protecting hepatic tissue against oxidative damage [4, 21]. Finally, TCBZ is a benzimidazole that binds to tubulin impairing intracellular transport mechanisms and interfering with protein synthesis [22].

Concerning the anthelmintic effect of ethanolic extraction of *Calligonum comosum* invitro, the treatment of fascioliasis, had highly anthelmintic activity against hepatic worm *F. gigantica* among goats. Our results agreed withwho reported that using of ethanolic extraction of some plant extractsof Saudi Arabia have highly efficacy in the treatment of fascioliasis and Ascarasis and also less expensive than other chemical drugs [1,6].

Results obtained in this study strongly coincided with recorded byNabila S. Degheidy et al. [8]. They recorded that the effects of ethanolic extract of *Calligonum comosum* plant and TCBZ on adult *F. gigantica*, through hematological and biochemical examination, revealed that, decreasing the number of eggof Fasciolal/g feces and the blood parameter returned to its normal levels, leading to improved sheep health condition. Nabila S. Degheidy et al. [8] also recorded that the effects of ethanolic extract of *Calligonum comosum* more efficient than TCBZ in the treatment of *Fasciola gigantica* among goats and less expensive.

Conclusion:

In conclusion, concerning the anthelmintic effect of ethanolic extraction of *Calligonum comosum* the treatment of fascioliasis, it had highly anthelmintic activity against hepatic worm among goats. Also, the effects of ethanolic extract of *Calligonum comosum* more efficient than TCBZ in the treatment of *Fasciola gigantica* among goats and less expensive. Further studies are required to validate our findings and improve our knowledge on the potential of the *Calligonum comosum* extract as anthelmintic in relation to its chemical composition.

REFERENCES


