World Journal of Zoology 10 (2): 118-123, 2015
ISSN 1817-3098
© IDOSI Publications, 2015
DOI: 10.5829/idosi.wjz.2015.10.2.93241

Therapeutic Efficacies of *Fumaria parviflora, Caesalpinia crista* and Oxyclozanide against Fasciolosis in Naturally Infected Sheep in Rawalakot-Azad Jammu and Kashmir, Pakistan

[1]Anisa Mushtaq, [2]Nisar Ahmad, [2]Haroon Akbar, [3]Aneela Zameer Durrani, [1]Muhammad Waqas, [1]Mohsin Nawaz, [1]Zulfiqar Ahmad, [1]Sajid Mahmood Sajid, [1]Muhammad Akhtar Qureshi and [1]Abid Hussain

[1]Faculty of Veterinary and Animal Sciences, The University of Poonch, Rawalakot-Azad Jammu and Kashmir, Pakistan
[2]Department of Parasitology, Faculty of Veterinary Science, University of Veterinary and Animal Sciences, Lahore-Punjab, Pakistan
[3]Department of Clinical Medicine and Surgery, Faculty of Veterinary Science, University of Veterinary and Animal Sciences, Lahore-Punjab, Pakistan

Abstract: Therapeutic trial was conducted to check the efficacies of *Fumaria parviflora*, *Caesalpinia crista* in comparison with oxyclozanide (Standard drug) against fasciolosis in sheep. Eighty sheep naturally infected with fasciolosis were randomly divided in to four groups viz A, B, C and D. Groups A and B were further divided in to three sub-groups viz A_1, A_2, A_3 and B_1, B_2, B_3 comprised of 10 animals each. Groups C and D were also incorporated with 10 animals in each group. Group A (A_1, A_2, A_3) was treated with dried leaves of *Fumaria parviflora* (Shahterah) while Group B (B_1, B_2, B_3) was treated with finely grinded seeds of *Caesalpinia crista* (Karanjwa). Group C was treated with oxyclozanide (Zanil-ICI Pakistan Pvt. Ltd), while group D was kept as infected untreated control. The efficacies of the applied materials were recorded on 3rd, 7th, 18th, 21st and 28th days post treatment. The efficacy of oxyclozanide in terms of reduction in faecal egg count was found 100% on 18th day post treatment. *Fumaria parviflora* and *Caesalpinia crista* showed 59.4%, 91.8% and 58.7%, 87.3% efficacy at the dose of 80 mg/kg on 18th and 28th day post treatment, respectively. It was concluded that *Fumaria parviflora* and *Caesalpinia crista* were comparable with that of standard drug oxyclozanide possessing significant *($p<0.01$)* anthelmintic activity against ovine fasciolosis.

Key words: Sheep · Fasciolosis · *Fumaria parviflora* · *Caesalpinia crista* · Oxyclozanide

INTRODUCTION

Parasitic diseases are one of the major health constraints in production and development of sheep [1]. Fasciolosis is one of the major parasitic diseases of sheep. It can affect a wide range of hosts mainly sheep, goat, cattle and humans as well. The disease is caused by two species of liver flukes, *Fasciola hepatica* and *Fasciola gigantica*, with a major variance that *Fasciola hepatica* is preponderant in temperate zones and *Fasciola gigantica* being in tropical zones of Asia and Africa [2]. Major clinical signs in sheep affected with fasciolosis include anorexia, right upper quadrant pain, vomiting, hepatomegaly, jaundice, biliary obstructions, cholangitis, bottle jaw, fever, anemia, lethargy, weight loss and emaciation [3]. Wasted carcasses, reduction in milk revenue, growth retardation, abortions, hepatic dysfunction and huge mortalities are the direct results of fasciolosis [4] leading to huge economic losses [5]. Fasciolosis affect 250 million of sheep worldwide [2, 7] and is important disease from zoonotic point of view affecting a large human population as well [7, 8].

Corresponding Author: Muhammad Waqas, Faculty of Veterinary and Animal Sciences, The University of Poonch, Rawalakot-Azad Jammu and Kashmir, Pakistan.
Tel: +92-345-9847205.

Medicinal plants are a significant therapeutic aid for relieving the ailments of animals as well as human beings. Today, there is an increased interest in the use of medicinal plants primarily due to the fact that "green medicine" is safe and more reliable than the synthetic drugs which are costly and having untoward side effects [9]. *Fumaria parviflora* is widely used as an antipyretic, liver tonic, diuretic, expectorant, laxative, appetizer, anti-scabies [10] and can also be used as hypoglycemic in Diabetes mellitus [11]. In traditional medication system, *Caesalpinia crista* leaves, stem, root and seeds are used as anthelmintic, febrifugal, tonic, vesicant and for the treatment of palsy, colic, convulsions and leprosy. The oil of *Caesalpinia crista* seed has the properties of being skin softener and pimple remover [12]. *Caesalpinia crista* is useful in maladies like dementia and various neurodegenerative ailments [13].

Livestock is a leading wing of an Agriculture sector of Pakistan. According to the economic survey of Pakistan 2013-14; livestock contribution to agriculture value added stood at 55.9% while it contributes 11.8% to the national Gross domestic product during 2013. The total sheep population of Pakistan is 29.1 million with 0.23 million sheep in Azad Jammu and Kashmir, Pakistan [14]. Keeping in view the importance of sheep and the impact of fasciolosis on the production and performance of sheep, the proposed study was designed to evaluate the therapeutic efficacies of *Fumaria parviflora* (Shahterah) and *Caesalpinia crista* (Karanjwa) in comparison with standard drug oxyclozanide against naturally infected sheep with fasciolosis.

MATERIALS AND METHODS

Herbs and Standard Drug: *Fumaria parviflora* and *Caesalpinia crista* were used in comparison with standard drug oxyclozanide against naturally infected sheep with fasciolosis.

Source of Samples: A total of 300 faecal samples from sheep of various age groups and both sexes were collected and examined for fasciolosis. Samples were collected from various private and public sheep farms located in and around Rawalakot, Azad Jammu and Kashmir (Pakistan). Moreover sheep from the Livestock Research Station, Khaigala (Faculty of Veterinary and Animal Sciences, The University of Poonch, Rawalakot) were also incorporated in this study.

Collection of Faecal Samples: Fresh faecal samples were collected directly from the rectum of each animal. Faecal samples were collected in separate clean sterilized plastic bags and were labeled properly. Collected faecal samples were then transported to Parasitology laboratory (Faculty of Veterinary and Animal Sciences, The University of Poonch, Rawalakot) for further studies.

Faecal Examination: Faecal samples were processed for the presence of trematode eggs using direct smear technique. Samples found positive for *Fasciola* spp. were further subjected to McMaster egg counting technique for counting the number of eggs per gram (EPG) of the faeces [15].

Therapeutical Trials: Eighty positive animals were randomly divided into four groups viz, A, B, C and D. Groups A and B were further divided into three sub-groups viz A_1, A_2, A_3 and B_1, B_2, B_3. A total of 30 animals were kept in group A with each sub-group comprised of 10 sheep while the same pattern was also followed in group B. Groups C and D also comprised 10 affected sheep in each group.

Group A: Sheep in Group A were treated with oral dosing of dried grinded leaves of *Fumaria parviflora* mixed with wheat bran at the rates of 60, 70 and 80 mg/kg B.W to A_1, A_2 and A_3, respectively. The mean EPG were noted on 3rd, 7th and 18th day post treatment. Animals still found positive on 18th day were given the second dose and EPG was noted again on 21st and 28th day.

Group B: Sheep in Group B were treated with oral dosing of finely grinded seeds of *Caesalpinia crista* mixed with wheat bran at the rates of 60, 70 and 80 mg/kg B.W to B_1, B_2 and B_3, respectively. The mean EPG were noted on 3rd, 7th and 18th day post treatment. Animals still found positive on 18th day were given the second dose and EPG was noted again on 21st and 28th day.

Group C: Sheep in this group were treated with oral drench of Oxyclozanide (Zanil, ICI-Pakistan Limited, Animal Health division) at the dose rate of 10 mg/kg B.W.

Group D: This group was kept as positive control (Infected-Untreated).

Drug Efficacy: The efficacies of herbs and standard drug were calculated in treated groups A, B and C on the basis of reduction in egg per gram (EPG) of faeces [16].

$$Efficacy\,\% = \frac{EPG(before\,treatment - EPG\,after\,treatment)}{EPG(before\,treatment)} \times 100$$

Obtained mean EPG of treated groups were compared with control Group D (Infected-untreated).

Statistical Analysis: One way ANOVA was used to analyze the data obtained from the therapeutic trials using Statistical Package for Social Sciences (SPSS) considering $p<0.01$ being significant.

RESULTS

Group A

Group A_1: Group A_1 was treated with *Fumaria parviflora* at the dose rate of 60 mg/kg body weight. The mean EPG recorded on day 0 was 310 and then on 3rd, 7th and 18th day, it was 275, 225 and 150, respectively. The mean EPG on 21st and 28th day post treatment was 65 and 50, respectively (Table 1). The efficacy of *Fumaria parviflora* at 60 mg/kg on 3rd, 7th, 18th, 21st and 28th day was given in Table 2.

Group A_2: Group A_2 was treated with *Fumaria parviflora* at the dose rate of 70 mg/kg. The mean EPG recorded on day 0 was 320 and then 3rd, 7th and 18th day, it was 275, 220 and 145, respectively. The mean EPG on 21st and 28th day was 55 and 40 (Table 1). The efficacy of *Fumaria parviflora* at 70 mg/kg BW on 3rd, 7th, 18th, 21st and 28th day was given in Table 2.

Group A_3: Group A_3 was treated with *Fumaria parviflora* at the dose rate of 80 mg/kg. The mean EPG recorded on day 0 pre-medication was 345 and then on 3rd, 7th and 18th day post-medication was 280, 225 and 140, respectively. The mean EPG on 21st and 28th day post treatment was 45 and 30 (Table 1). The efficacy of *Fumaria parviflora* at 80 mg/kg BW on 3rd, 7th, 18th, 21st and 28th day was given in Table 2.

Group B

Group B_1: Group B_1 was treated with *Caesalpinia crista* at the dose rate of 60 mg/kg body weight. The mean EPG recorded on day 0 was 380 and then on 3rd, 7th and 18th day, it was 355, 315 and 260, respectively. The mean EPG on 21st and 28th day was 200 and 185 (Table 1). The efficacy of *Caesalpinia crista* at the dose rate of 60 mg/kg BW on 3rd, 7th, 18th, 21st and 28th day was given in Table 2.

Group B_2: Group B_2 was treated with *Caesalpinia crista* at the dose rate of 70 mg/kg. The mean EPG recorded on day 0 was 350 and then on 3rd, 7th and 18th day, it was 315, 270 and 205, respectively. The mean EPG on 21st and 28th day was 140 and 125 (Table 1). The efficacy of *Caesalpinia crista* at the dose rate of 70 mg/kg body weight on 3rd, 7th, 18th, 21st and 28th day was given in Table 2.

Group B_3: Group B_3 was treated with *Caesalpinia crista* at the dose rate of 80 mg/kg. The mean EPG on day 0 was 315 and then on 3rd, 7th and 18th day it was 260, 210 and

Table 1: Mean EPG for Groups A, B, C and D on different days

		Mean EPG on different days							
			EPG after 1st dose			After 2nd dose			
Drug used	Dose mg/kg	Day 0	Day 3	Day 7	Day 18	Day 21	Day 28	SE of mean	SE of difference between two means
Fumaria									
Parviflora	60	310 A	275 A	225 B	150 C	65 D	50 D	14.02	19.8
	70	320 A	275 B	220 C	145 D	55 E	40 E	16.03	22.6
	80	345 A	280 B	225 C	140 D	45 E	30 E	9.17	12.9
Caeselpinia									
Crista	60	380 A	355 B	315C	260D	200 E	200 E	7.21	10.16
	70	350 A	315 B	270 C	205 D	140 E	125 E	13.3	18.9
	80	315 A	260 B	210 C	130 D	55 E	40 E	14.6	20.4
Oxyclozanide	10	280 A	205 B	65 C	0 D	-	-	20.3	28.7
Control group	-	235 C	240 C	250 C	265 BC	275 AB	290 A	16.2	22.9

Means followed by same letters do not differ significantly (p value 0.0001)

EPG=Egg per gram, SE=Standard Error

Table 2: Efficacy (%) of *Fumaria parviflora*, *Caeselpinia crista* and Oxyclozanide against fascioliasis on different days

		Efficacy (%) on different days				
		After 1st dose			After 2nd dose	
Drug used	Dose mg/kg	3rd day	7th day	18th day	21st day	28th day
Fumaria parviflora	60	11.2	27.4	51.6	79.6	83.8
	70	14	31.2	54.6	82.8	87.5
	80	18.8	34.7	59.4	86.9	91.8
Caeselpinia crista	60	6.57	17.1	31	47.3	51.3
	70	10	22.8	41.42	58.8	63.2
	80	17.4	33.3	58.7	82.5	87.3
Oxyclozanide	10	32.1	76.7	100	-	-

130, respectively. The mean EPG on 21st and 28th day was 55 and 40 (Table 1). The efficacy of *Caesalpinia crista* 80 mg/kg body weight on 3rd, 7th, 18th, 21st and 28th day is given was Table-2.

Group C: Sheep in Group C were treated with oxyclozanide at the dose rate of 10 mg/kg BW. The mean EPG on day 0 was 280 and then on 3rd, 7th and 18th day post-medication, it was 205, 65 and 0 (Table 1). The efficacy of oxyclozanide on 3rd, 7th and 18th day was given in Table 2. A single dose of oxyclozanide was much more effective against fascioliasis with a 100% reduction in EPG on 18th day post-medication.

Group D: The mean EPG of control group recorded on 0, 3rd, 7th, 18th, 21st and 28th days was 235, 240, 250, 265, 275 and 290, respectively (Table 1). There was a gradual increase in EPG on different days as this group was kept as infected untreated control group.

No side effects were observed in each of the treated groups during treatment trials.

DISCUSSION

Fumaria parviflora and *Caesalpinia crista* were used in comparison with Oxyclozanide. The active constituents of *Caesalpinia crista* include carbohydrates, alkaloids, Glycosides, tannins, flavonoids and Coumarins [9]. Ten new furanocassane-type diterpenes [caesalpinins H-P (1-9) and norcaesalpinin F (10)], along with 13 known diterpenes have been isolated from the CH_2Cl_2 extract of the seed kernels of *Caesalpinia crista* [17]. *Fumaria parviflora* possesses alkaloids, saponins, tannins and anthraquinones [18].

Sheep in Group A were treated with *Fumaria parviflora*. Results of groups A_1, A_2, A_3 indicated that the efficacies of a single dose of *Fumaria parviflora* at the dose rate of 60, 70 and 80 mg/kg BW on 18th day post-medication were 51.6%, 54.6% and 59.4%, respectively. After the administration of 2nd dose of *Fumaria parviflora* on 18th day, the efficacies were 79.6%, 82.8% and 86.9%, respectively, on 21st day post treatment and 83.8%, 87.5% and 91.8%, respectively, on 28th day post treatment. These results were comparable to the previous studies which stated that *Fumaria parviflora* was more effective drug with high antifasciolic properties against fascioliasis as compared to *Nigella sativa, Caesalpinia crista and Saussurea lappa* [19, 20]. Different herbal extracts for treatment of fascioliasis had been used; among them the efficacy of *Fumaria parviflora* was slightly more at different dose rates [21]. Furthermore, in one of the previous study, *Fumaria parviflora* at the same dose levels was used with nearly the same results obtained with the efficacy to be 41.66%, 45.71%, 63.88% and 83.33%, 91.42% and 97.22% after first and second doses [22].

Sheep in Group B were treated with *Caesalpinia crista* at the dose rate of 60, 70 and 80 mg/kg BW. Results of groups B_1, B_2, B_3 indicated that the efficacies of a single dose of *Caesalpinia crista* on 18th day post medication were 31%, 41.42% and 58.7%, respectively. After the administration of 2nd dose on 18th day, the efficacies recorded on 21st and 28th days were 47.3%, 58.8%, 82.5% and 51.3%, 63.2%, 87.3%, respectively. The findings of our research was in line with the previous studies which showed that *Caesalpinia crista* seeds at the dose rate of 50 mg/kg BW was the most effective anthelmintic ($p<0.001$) [23]. *Caesalpinia crista* was 87±1.7% effective at the rate of 40 mg/kg BW [19]. *Caesalpinia crista* at the dose rate of 60, 70 and 80 mg/kg BW was found to be 38.23%, 45.71% and 51.42% effective [22].

Group C was treated with oxyclozanide at the dose rate of 10 mg/kg BW. It was found that oxyclozanide was more effective drug against *Fasciola* infection at single dose of 10 mg/kg body weight as animals showed complete recovery after 18th day post treatment with 100 percent reduction in faecal egg count. Previous studies stated that oxyclozanide was effective against the adult fluke [24]. Oxyclozanide treated sheep showed lower E.P.G up to 0 percent at 28th day post treatment [25]. Oxyclozanide against fasciolosis in hill sheep flock was highly effective drug at a single dose [26, 27]. Similarly a single dose of Oxyclozanide was effective against all bovine helminthes [28]. One previous study reported the same results that oxyclozanide with single dose was most effective anthelmintic against fascioliasis as treated animals recovered completely [29].

CONCLUSION

It is concluded that *Fumaria parviflora* and *Caesalpinia crista* possess significant therapeutic efficacies against ovine fasciolosis comparable with that of standard oxyclozanide. Furthermore, it can be concluded that *Fumaria parviflora* and *Caesalpinia crista* can be used as alternate therapeutic agents against ovine fasciolosis. However, studies pertaining clinical trials on larger scale are recommended to further explore their efficacies on scientific basis.

REFERENCES

1. Gadahi, J.A., M.J. Arshed, Q. Ali, S.B. Javaid and S.I. Shah, 2009. Prevalence of gastrointestinal parasites of sheep and goats in and around Rawalpindi and Islamabad. Pakistan. Vet World, 2: 51-53.
2. Mas-Coma, M.S., S. Bargues and M.A. Valero, 2005. Fascioliasis and other plant borne trematode zoonoses. Int. J. Parasitol., 35: 1255-1278.
3. Aksoy, D.Y., U. Kerimoglu and A. Oto, 2006. *Fasciola hepatica* infection: Clinical and computerized tomographic findings of ten patients. Turk J Gastroenterol., 17: 40-45.
4. Phiri, I.K., A.M. Phiri and L.J. Harrison, 2006. Serum antibody isotype responses of Fasciola infected sheep and cattle to excretory and secretory products of *Fasciola species*. Vet Parasitol., 141(3-4): 234-42.
5. Khan, M.K., M.S. Sajid, M.N. Khan, Z. Iqbal and M.U. Iqbal, 2009. Bovine Fascioliasis: prevalence, effects of treatment on productivity and cost benefit analysis in five districts of Punjab, Pakistan. Res. Vet Sci., 87: 70-75.
6. Charlier, J., L. Duchateau, E. Claerebout, D. Williams and J. Vercruysse, 2007. Association between anti-Fasciola hepatica antibody levels in bulk-tank milk samples and production parameters in dairy herds. Prev Vet Med., 78: 57-66.
7. Itagaki, T., M. Kikawa, K. Sakaguchi, J. Shimo, K. Terasaki, T. Shibahara and K. Fukuda, 2005. Genetic characterization of parthenogenetic *Fasciola spp*. in Japan on the basis of the sequences of ribosomal and mitochondrial DNA. Parasitol., 131: 679-685.
8. Le, T.H., N.V. De, Agatsuma, Nguyen, Q.D. Nguyen, D.P. McManus and D. Blair, 2008. Human fascioliasis and the presence of hybrid/introgressed forms of *Fasciola* in Vietnam. Int. J. Parasitol., 38: 725-730.
9. Suryawanshi, H.P. and M.R. Patel, 2011. Traditional uses, medicinal and Phytopharmacological properties of *Caesalpinia Crista* linn-an overview. Int J. Res. Pharm Chem., 1(4): 1179-83.
10. Gilani, A.H., S. Bashir, K.H. Janbaz and A. Khan, 2005. Pharmacological basis for the use of *Fumaria indica* in constipation and diarrhea. J. Ethnopharmacol., 96(3): 585-9.
11. Fathiazad, F., S. Hamedeyazdan, M.K. Khosropanah and A. Khaki, 2013. Hypoglycemic Activity of *Fumaria parviflora* in Streptozotocin-Induced Diabetic Rats. Adv Pharm Bull., 3(1): 207-210.
12. D'Souza, M., 1998. Tribal Medicine; Social center, Ahmednagar, India, 1st Ed, pp: 300.
13. Kshirsagar, S.N., 2011. Nootropic Activity of dried Seed Kernels of *Caesalpinia crista* Linn against Scopolamine induced Amnesia in Mice. Int. J. Pharm Tech Res., 3(1): 104-109.
14. Anonymous, 2013-14. Economic survey of Pakistan, Finance division, economic advisors wing, ministry of finance," Government of Pakistan. Islamabad, pp: 37.
15. Urquhart, G.M., J. Duncan, J. Armour, A.M. Dun and F.W. Jennings, 1996. Veterinary Parasitology, 2nd Ed. Blackwell Science Ltd, pp: 42-47 and 276-277.
16. Moskey, H.E. and P.D. Harwood, 1941. Methods of evaluating the efficacy of Anthelmintics. Amer J. Vet Res., 2: 55-59.

17. Awale, S., T.Z. Linn, Y. Tezuka, S.K. Kalauni, A.H. Banskota, F. Attamimi, J.Y. Ueda and S. Kadota, 2006. Constituents of *Caesalpinia crista* from Indonesia. Chem Pharm Bull (Tokyo), 54(2): 213-8.
18. Rehman, N.U., M.H. Mehmood, A.J. Al-Rehaily, R.A.A. Mothana and A.H. Gilani, 2012. Species and tissue-specificity of prokinetic, laxative and spasmodic effects of *Fumaria parviflora.* BMC Comp Alt Med., 12: 16.
19. Kailani, S.R., M.S. Akhtar and M. Ashraf, 1995. Antifasciolic efficacy of indigenous plant drugs: kalonji, shahterah and karanjwa in buffaloes. Pak J. Pharm Sci., 8(1): 17-27.
20. Maqbool, A. and C.S. Hayat, 2000. Epidemiology and Chemotherapy of Fascioliasis in Cattle and buffaloe. Pak J. Agri Sci., 37(3-4): 201.
21. Maqbool, A., C.S. Hayat and A. Tanveer, 2004. Comparative efficacy of various indigenous and allopathic drugs against Fasciolosis in buffaloes. Veterinarski arhiv, 74(2): 107-114.
22. Kakar, M.U., A. Maqbool, J.U. Khan, A. Kakar, M.S. Khan and M. Lateef, 2013. Comparative efficacy of various indigenous and allopathic drugs against Ovine Fascioliasis. Pak J. Zool., 45(4): 1170-1172.
23. Javed, I., M.S. Akhtar, Z.U. Rahman, T. Khaliq and M. Ahmad, 1994. Comparative anthelminthic efficacy and safety of *Caesalpinia crista* seed and piperazine adipate in chickens with artificially induced *Ascaridia galli* infection. Acta Vet Hung, 42(1): 103-9.
24. Coles, G.C. and K.A. Stafford, 2001. Activity of oxyclozanide, nitroxynil, clorsulon and albendazole against adult triclabendazole-resistant Fasciola hepatica. Vet Rec., 148: 723-724.
25. Yildirim, A., V. Güneş, A. Iça, S. Sariözkan, O. Düzlü, A. Inci and H. Albasan, 2008. The investigation of short term efficiency of oxfendazole + Oxyclozanide paste and tablet formulations against gastrointestinal nematodes in sheep. Turk Parazitol Derg, 32: 134-38.
26. Mooney, L., B. Good, J.P. Hanrahan, G. Mulcahy and T. de Waal, 2009. The comparative efficacy of four anthelmintics against a natural acquired *Fasciola hepatica* infection in hill sheep flock in the west of Ireland. Vet Parasitol, 164(2-4): 201-5.
27. Paraud, C., C. Gaudin, I. Pors and C. Chartier, 2009. Efficacy of oxyclozanide against the rumen fluke *Calicophoron daubneyi* in experimentally infected goats. Vet J., 180(2): 265-7.
28. Athar, L.A., M.N. Khan, M.S. Sajid, T. Rehman and I.A. Khan, 2011. Cost benefits analysis of Anthelmintics treatment of Cattle and Buffaloes. Pak Vet J., 31(2): 149-152.
29. Shokier, K.A., S.M. Aboelhadid and W.M. Arafa, 2013. Efficacy of five anthelminticss against a natural *Fasciola* Species Infection in Cattle. Beni-Suef Univ J. App Sciences, 2(1): 59-65.