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Abstract

Background: *Veronia anthelmintica* (Kaliziri) is a medicinally very important plant as being reported for remarkable therapeutic potential. The present research evaluates its antischistosomal activity.

Methods and Materials: 54 buffaloes naturally infected with Schistosomiasis were used for anthelmintic trails. All animals for the study were tagged so as to distinguish from others. The animals were checked up on routine basis by a veterinary doctor. Preparation of samples of herbal drug and experiment was design according to available literature.

Results: During present studies, buffaloes naturally infected with Schistosomiasis were treated with *Veronia anthelmintica* (Kaliziri), with dose 75 mg/kg, 150 mg/kg and 225 mg/kg body weight respectively, and their impact on various parameters like eggs reduction, milk production, weight gain and feed intake was noted. Furthermore, their efficiency (%) was compared with standard drug "Praziquantel" (allopathic drug) at "10mg/Kg" body weight dose level. The effectiveness (%) of "Praziquantel" was noted as hundred after first dose, while the effectiveness for the herbal drug get to this level after injecting of second dose of "225mg/kg body weight". Statistically significant (P<0.05 and P<0.001, respectively) increase in milk production was noted.

Conclusion: Keeping in view of our present findings, it is concluded that herbal treatment of bovine schistosomiasis with *Veronia anthelmintica* (seeds) is safe, easy, readily applicable and cheapest method and almost have no side effect.

Key words: Bovine schistosomiasis, *Veronia anthelmintica*, Medicinal Plant

Introduction

From Pakistan, around two thousands plant species are reported for medicinal uses, of these, however, only "400-600" plant species are reported for medicinal purposes (Hazrat et al., 2007). Medicinal plants are used from many centuries to fighting parasitism. In many part of the world, these medicinal plants are still in practice for the treatment of various diseases. Plants or plant extract that are appropriate are utilized to care for approximately every parasitic disease of the livestock (International Institute of Rural Reconstruction (IIRR), 1994). Medicinally, *Veronia anthelmintica* is reported to have anti-diabetic and antihyperlipidemic activity (Fatima et al., 2010). It is also effective against amoebiasis, stomachache, incontinence of urine, eczema, humed herpes, elephantiasis, ringworm, diarrhea and rheumatism as well as a valuable remedy for prolonging life, restoring youth and preventing the hair turning grey as well as also used in snake-bite and scorpion sting (Willis, 1996). Suteky et al. (2011) and Willis (1996) reported *Veronia anthelmintica* for its anti-helminthic activity - Bovine schistosomosis, which is caused by *Schistosoma bovis*. It is still a continuous serious problem for animals in different parts of the world (Aradaib and Osburn, 1995).

This study shows the effectiveness of *Veronia anthelmintica* against bovine schistosomiasis.

Method and Materials

The present study was conducted on 54 Babulus (buffaloes, both sexes) (5.0±1.15 years of age, infected with *Schistosoma bovis*. These selected animals were kept under similar feeding and environmental condition during the course of treatment. The present therapeutic trails were carried out in the month of September to October 2012, (temperature ranges "22.6 to 33.2° C"). The subject animals were tagged so as to differentiate them from each other. A veterinary doctor checked up the animals on regular basis.

Preparation of Herbal Drugs & Experimental Design

The herbs, *Veronia anthelmintica*, were got from the local marketplace and were cleaned from dust and other contamination, and then grind. The efficacy of the selected herb was then compared with "Praziquantel". The processed herb ("in capsules") was given orally to the subject animals, followed by method of (Jhangir et al. 2003). During therapeutic trails, the buffaloes were alienated into 3 groups "A, B and C". Animals in group A were further sub divided into 3 "sub groups", (A1, A2 and A3), each sub-group having 9 animals. Animals in groups A1, A2 and A3 were treated with *Veronia anthelmintica* at dosage rate of 75, 150, 225mg/kgb.w. Animals of group B were given 10mg/kgb.w of "Praziquantel" (PZQ) while group C animals were kept as untreated control. Group D (9 healthy animals) was kept as normal control group to

compare with infected groups. "Eggs per gram" in faeces (EPG) were examined by "Mc Master Egg counting technique" (Soulsby, 1982). All the Faecal samples were checked on "zero, 3rd, 7th and 18th" day of treatment. After 18th day, the positive animals were again treated with a second dose and their faecal samples were examined on 21st and 28th day. The percentage effectiveness of the drugs was calculated as described by (Moskey and Hardwood 1941).

Efficacy (%): $\frac{\text{Total no of eggs before treatment} - \text{Total no of eggs after treatment}}{\text{Total no of eggs before treatment}} \times 100$

Effects of the Herbal Treatment

Adverse effects of the drug if any were also noted i.e., "shivering, sweating, salivation and diarrhea" (Akhtar et al., 1987). In case of female lactating buffaloes, effect on milk production was also recorded before and after therapy (Maqbool et al., 2004). Effects of drugs on pregnant female buffaloes were also noted (Akhtar and Aslam, 1988).

Results

Comparative Efficacy (%) of *Veronia Anthelmintica* and Praziquantel

The drug's effectiveness (%) was calculated after treatment, on the basis of "egg reduction" given in (Table 2). The numbers of *Schistosoma*'s eggs (mean percentage) in the faecal samples of all treated groups were match up to the group kept in control (Table 1).

Efficacy of *Vernonia Anthelmintica* Seed Group A

V. anthelmintica seeds showed "3.70% and 42.40%" decline in EPG counts at 75 mg/kg dose on 18th and 28th day respectively. Furthermore, first high dose level (150 and 225 mg/kg of b.w) showed "45.0% and 78.03% reduction in egg count respectively while at second dose levels, the drug showed "92.5% and 100% effectiveness (Table 2). *V. anthelmintica* (p<0.05) significantly reduced EPG after treatment at all three dose levels and zero output was recorded in sub-group A₃ on 28th day (Table 1).

Efficacy of Praziquantel Group B

At one dose level the efficacy of Praziquantel at 10mg/kg body weight was 100% (Table- 2). Statistical analysis showed high significant (P<0.001) reduction in EPG after treatment and became "zero on 18th day" (Table 1). The EPG count was increased significantly (p<0.01) 35.0% in group C (untreated control). The lowest (75mg/kg) dose level of herb showed significant (p<0.01) efficacy difference as compare to PZQ, while 150 mg/kg and 225 mg/kg dose levels of the herb did not show any significant difference after therapy (Table 2).

Effects of the Herbal Treatment on Various Parameters

8.3% increase in milk production (non-significant) was noted at the end of treatment in sub group A1, whereas A2 & A3 subgroups showed 42.0% and 103.7% significant increase (p<0.05 and p<0.001 respectively) in milk production. 126% increase in milk production (highly significant P<0.001) was recorded in group B, while untreated buffaloes of Group C showed 53.1% decrease (significant, P<0.01) in milk production (Table 3). All groups of animals except group C showed increase in body weight non-significantly. The results showed that 30-96% increase (P<0.001, highly significant) in feed intake was recorded in all treated animals of group A & B, whereas healthy animals of group D showed slight increase 2.1% while infected animals of group C showed 32.4% decrease in feed intake. Three infected pregnant buffaloes were subjected to therapy, in which one was served with 225 mg/kg b.w of the herb and two were treated with 10mg/kg b.w of PZQ. No abortion and abnormality was noted during therapy (Table 3). All animals did not show any side effects.

Table 1: Comparative efficacy (%) of the seeds of *Vernonia anthelmintica* with Praziquantel against schistosomiasis in natural infected buffaloes

Groups	Sub-Group (n = 9)	Treatment	Dose (mg/kg bw)	Eggs per gram of feces (EPG)					
				1 st dose				2 nd dose	
				0 day	3 rd day	7 th day	18 th day	21 st day	28 th day
A	A1	<i>Vernoniaanthelmintica</i> (seeds)	75	867±88	833±88	833±88	833±88	800±100	500±58*
	A2		150	867±11	800±58	700±58	467±66*	367±66*	67±33***
	A3		225	900±58	700±0	500±58	200±58**	100±0	0±0***
B		Praziquantel (PZQ)	10	933±33	500±58	200±58	0±0***	0±0	0±0***
C (infected)		+ve control	-	767±33	800±58	833±33	900±58	967±33	1033±00**
D (normal)		-ve control	-	0	0	0	0	0	0

"Student's t-test: * = P<0.05, ** = P<0.01, *** = P<0.001, N.S. non-significant"

Table 2: Efficacy (%) of *V. anthelmintica* seed and PZQ comparison, in natural infected buffaloes with schistosomiasis

Groups	Sub-Group (n = 9)	Treatment	Dose (mg/kg bw)	Efficacy (%)				
				1 st dose			2 nd dose	
				3 rd day	7 th day	18 th day	21 st day	28 th day
A	A1	<i>Vernoniaanthelmintica</i> (seeds)	75	3.70±3.7	3.70±3.7	3.70±3.7	7.40±7.4	42.40±1.3 ^{bbb}
	A2		150	7.86±4.0	18.33±9.2	45.0±10.4	57.50±11.0	92.50±3.8
	A3		225	21.5±5.0	44.43±5.5	78.03±6.1	90.23±6.4	100±0.0
B		Praziquantel (PZQ)	10	46.63±4.6	78.83±5.4	100±0.0	100±0.0	100±0.0

“ANOVA: Comparison within group by Tukey’s test: a = P<0.05, aa = P<0.01, aaa = P<0.001, comparison between same doses of different groups N.S. = non –significant, comparison of all doses with PZQ = b = P<0.05, bb = P<0.01, bbb = P<0.001”

Discussion

The seeds of *V. anthelmintica* at 75 mg/kg dose (group A) caused a 3.7% and 42.4% decrease in egg counts on 18th and 28th day respectively. Higher dose of 150 mg and 225 mg/kg of body weight were “45%, 92.5% and 78%, 100%” effective at 1st and 2nd dose levels, respectively. *V. anthelmintica* also showed significantly decreased level in egg counts, and egg output becomes zero of sub-group A₃ at 28th day. (Javeed and Akhtar 1990) also used this herb against helminthes of goats with dose of 20 mg/kg bw and found 99.8% efficacy, which showed almost same efficacy as recorded in sub-group A₂ and A₃ at end of the treatment of this study. *Vernonia anthelmintica* has been tried for its anthelmintic activity and found positive in favor of treatment against parasitic infection i.e., “Nadkarni (1954), Chopra et al. (1958), Singh et al. (1985), Javeed and Akhtar (1990) and Hordegon et al. (2003). Certain plants are more useful against specific parasitic infections than others (Athanasiadou et al., 2005 and Tzamaloukas et al., 2005) and it has been recommended that plant have more satisfactory anthelmintic effectiveness to substantiate their use based on commercial threshold (Ketzi et al., 2006). Therefore, medicinal plants remain of great scientific interest because of their anthelmintic activities, despite the wide use of synthetic chemicals in modern medical practices globally (Akhtar et al., 2000). Therapeutic plants have been used to cure parasitic infection in human beings and animals (“Nadkarni, 1954; Chopra et al., 1956; Seid, 1969; Akhtar et al., 2000; Waller et al., 2001; Kashwaha et al., 2004, Min et al., 2004, Sheik et al., 2004, Chandrawathani et al., 2006, Lange et al., 2006, Athanasiadou et al., 2007). Bah et al. (2006) described 655 plants of 30 different families against schistosomiasis in Mali and found that some plants may induce useful result in treatment of schistosomiasis. Comparative efficacy (%) of praziquantel (PZQ) with highest dose level of all herbs (225 mg/kg) in buffaloes did not show any significant difference. No reference in this regard is available because these herbs have been used for the first time in Pakistan with useful outcome. Various researchers reported effectiveness of PZQ (100%) as a safe drug to treat schistosomiasis (Mutapi et al., 2003; Van-der-Werf et al., 2003; Chaiworaporn et al., 2005; Tianping et al., 2006; Ragaa 2007 and Dadara et al., 2008). However, the resistance risk of the PZQ cannot be overlooked, as reported by Alonso et al. (2006), Yu-Hua et al 2006 and Doenhoff et al 2008).

Conclusion

Keeping in view of our present findings, it is concluded that herbal treatment of bovine schistosomiasis with *Vernonia anthelmintica* (seeds) is safe, easy, readily applicable and cheapest method and almost have no side effect.

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Table 3: Comparison of milk production (liters), body weight (kg) and feed intake (kg)/day before and after treatment of buffaloes

Groups	Sub-Groups	Milk production (liters) (n = 2/3)			Feed (g)/day (n = 5)			Body weight (kg) (n = 5)		
		Before treatment	After treatment	Increase↑/decrease↓ (%)	Before treatment	After treatment	Increase↑/decrease↓ (%)	Before treatment	After treatment	Increase↑/decrease↓ (%)
A	A1	2.46±0.33	26.0±0.28	8.3 ↑	2.6±1.1	35.6±12	34.6 ^{**} ↑	165±3.1	175±2.9	6.1 ↑
	A2	3.13±0.21	4.43±0.27	42.0 ↑	23.0±0.3	37.67±0.8	61.0 ^{***} ↑	153±4.0	168±3.2	7.8 ↑
	A3	2.76±0.13	5.57±0.26	103.7 ^{***} ↑	28±0.5	47±1.0	68 ^{***} ↑	162±5.8	180±4.9	11.1 ↑
B		2.73±0.17	6.10±0.11	126 ^{***} ↑	24.6±0.6	47±1.0	96.0 ^{***} ↑	160±8.7	180±1	12.5 ↑
C		3.20±0.17	1.56±0.24	53.12 ^{**} ↓	37±1.1	25.7±0.3	32.4 ↓	169±0.3	157±6.7	7.6 ↓
D		6.73±0.31	6.73±0.31	0.0	46.6±1.1	47.6±1.1	2.1 ↓	232±12	232±12	0.0

“Student’s t-test: ^{**} = P<0.05, ^{*} = P<0.01, ^{***} = P<0.001, N.S. non-significant”

References

1. Akhtar, M.S., Z. Iqbal, M.N. Khan, and M. Lateef (2000). Anthelmintic activity of medicinal plants with particular reference to their use in animals in the Indo- Pakistan subcontinent. *Small Rumin. Res.*, 38: 99-107.
2. Akhtar, M.S., and S. Riffat (1987). Evaluation of anticestodal activity of *Lagenariasiceraria* (Kaddoo seeds) in sheep. *Pak. Vet. J.*, 7: 139-141.
3. Akhtar, M.S. and M. Aslam (1988). Anthelmintic efficacies of total alkaloids and glycosides isolated from *Punicagranatum* fruit rinds. *Pakistan J. Agric. Sci.*, 25: 161-8.
4. Alonso, D., J. Mounoz, J. Gascon, M.E. Valls, and M. Corachan (2006). Failure of standard treatment with praziquantal in travelers with *Schistosoma* infection. *Am. J. Trop. Med. Hyg.*, 74(2): 342-344.
5. Aradaib, I.E., and B.I. Osburn (1995). Vaccination of cattle against bovine schistosomiasis: current status and future prospects: A review. *Preventive Veterinary Medicine.*, 22(4): 285-291.
6. Athanasiadou, S., O. Tzamaloukas, I. Kyriazakis, F. Jackson, and L.R. Coop (2005). Testing or direct anthelmintic effect of bioactive forages against *Trichostrongylus* grazing sheep. *Vet. Para.* 127: 233-243.
7. Athanasiadou, S., J. Githiori, and I. Kyriazakis (2007). Medicinal plants for helminth parasite control. *Anim.*, 9: 1392-1400.
8. Bah, S., D. Diallo, S. Dembele, and B.S. Paulsen (2006). Ethnopharmacological survey of plants used for the treatment of schistosomiasis in Niono district Mali. *J. Ethnopharmacol.*, 105(3): 387-399.
9. Chaiworaporn, R., Y. Maneerat, W. Rojekkittikhun, P. Ramasoota, T. Janecharut, H. Matsuda, and V. Kitikoon (2005). Comparative studies on mortality in *S. mansoni* infected mice after treatment with artesunate and the current antischistosomal drugs. *South Asian J. Trop. Med. Pub. Hlth.*, 36: 1118-1124.
10. Chandrawathani, P., K.W. Chang, R. Nurulaini, P.J. Waller, M. Adnan, C.M. Zaini, O. Jamnah, S. Khadijah, and N. Vincent (2006). Daily feeding of fresh neem leaves for worm control in sheep. *Trop. Biomed.*, 23: 23-30.
11. Chopra, R.N., I.C. Chopra, K.I.L. Handa, and L.D. Kapur, L.D (1958). *Chopra's Indigenous drugs of India*. U.N. Dhur and Sons (P) Ltd., Calcutta. 303 p.
12. Da' dara, A.A., Y.S. Li, T. Xiong, J. Zhou, G.M. Williams, D.P. McManus, Z. Feng, X.L. Yu, D.J. Gary, and D.A. Harn (2008). DNA-based vaccines protect against zoonotic schistosomiasis in water buffalo. *Parasitol. Immunol.*, 30: 3617-3625.
13. Doenhoff, M.J., D. Cioli, and J. Utzinger (2008). Praziquantal: mechanism of action, resistance and new derivative for schistosomiasis. *Acta Trop.*, 108: 175-178.
14. Fatima, S.S., M.D. Rajasekhar, K.V. Kumar, M.T. Kumar, K.R. Babu, and C.A. Rao (2010). Antidiabetic and antihyperlipidemic activity of ethyl acetate: isopropanol (1:1) fraction of *Vernonia anthelmintica* seeds in streptozotocin induced diabetic rats. *Food. Chem. Toxicol.*, 48(2): 495-501.
15. Hazrat, A., J. Shah, M. Ali and I. Iqbal (2007). Medicinal value of *Ranunculaceae* of Dir valley. *Pak. J. Bot.*, 39(4): 1037-1044.
16. Hordegen, D., H. Hertzberg, J. Beilmann, W. Langhans, and V. Maurer (2003). The anthelmintic efficacy of five plant products against gastrointestinal helminths in artificially infected lambs. *Acta Vet.*, 55: 31-44.
17. International Institute of Rural Reconstruction (1994). *Ethnoveterinary medicine in Asia: an information kit on traditional animal health care practices*. International Institute of Rural Reconstruction (IIRR), second edition. Silang, Cavite, Philippines.
18. Javed and M.S. Akhtar (1990). Screening of *vernoniaanthelmintica* seeds against gastrointestinal nematodes. *Pak. J. Pharmacol. Sci.*, 3: 69-74.
19. Jhangir, M., A. Maqbool, A. Tanveer, and A. Mahfooz (2003). Therapy of Ancylostomiasis in dogs with *Nigella sativa* (Kalongi) and *Saussurea lappa* (Qust-e-Shireen). *Haryana Vet. J. India*, 40: 48-51.
20. Ketzis, J.K., J. Vercuryusse, B.E. Stromberg, M. Larsen, S. Athanasiadou, and J.G.M. Houdijk (2006). Evaluation of efficacy expectation for novel and non-chemical helminth control strategies in ruminants. *Vet. Parasitol.*, 139: 321-335.
21. Kushwaha, D.S., D. Kumar, and S.K. Tandan (2004). Effect of some indigenous medicinal plant extracts on *Fasciolagigantica* in vitro. *Ind. J. Anim. Sci.*, 74: 143-146.
22. Lange, K.C., D.D. Olcott, J.E. Miller, J.A. Mosjidis, T.H. Terril, J.M. Burke, and M.T. Kearney (2006). Effect of *Sericea lespedeza* fed on natural and experimental helminth infections in lambs. *Vet. Parasitol.*, 141(3-4): 273-278.
23. Maqbool, A., S.C. Hayat, and A. Tanveer (2004). Comparative efficacy of various indigenous and allopathic drugs against fasciolosis in buffaloes. *Vet. Arhiv*, 74: 107-104.
24. Min, B.R., W.E. Pomroy, S.P. Hart, and T. Sahl (2004). The effect of short term consumption of a forage containing condensed tannins on gastro-intestinal nematode parasite infection in grazing goats. *Small Ruminant Res.*, 51: 279-283.
25. Moskey, H.E. and P.D. Harwood (1941). Methods evaluating the efficacy of anthelmintics. *ARV. J. Vet. Res.*, 2: 55-59.
26. Mutapi, F., P. Hagan, M.E. Woolhouse, T. Mduluzi, and P.D. Ndhlovu (2003). Chemotherapy induced age-related changes in antischistosome antibody responses. *Parasite Immunol.* 25(2): 87-97.
27. Nadkarni, A.K. (1954). *Indian material medica*, 3rd edition. Popular Prakashan Pvt Ltd, Bombay, India. 23p.
28. Raso, G., P. Vounatsou, D.P. McManus, E.K. Goran, and J. Utzinger (2007). A Bayesian approach to estimate the age-specific prevalence of *Schistosoma mansoni* and implications for schistosomiasis control. *Int. J. Parasitol.*, 13: 1491-1500.
29. Said, M. 1969. *Hemdard pharmacopea of Eastern Medicine*. Hamdard National Foundation, Karachi, Pakistan.
30. Shaikh, S.A., T.H. Terrill, J.E. Miller, B. Kouakou, G. Kannan, R.K. Kallu, and J.A. Mosjidis (2004). Effects of feeding *Sericea lespedeza* hay to goats infected with *Haemonchus contortus*. *South Afr. J. Anim. Sci.*, 34: 248-250.
31. Singh, Sheela, N.A. Ansari, M.C. Srivastava, M.K. Sharma, and S.N. Sing (1985). Anthelmintic activity of *Vernonia anthelmintica*. *Indian Drugs*, 22: 508-511.
32. Soulsby, E.E. (1982). *Helminthes, arthropods and protozoa of domesticated animals*. 7th Ed. ELBS Bailliere Tindall and Cassel, London. 787-792p.

33. Suteky, T., and A.N.D. Dwatmadji (2011). Antihelmintic activity of *Melastomamalabatricum* extract on *Haemonchus contortus* activity in vitro, Asian. J. Pharm. Clin. Res., 4(1):68-70.
34. Tianping, W., S. Zhang, W. Wu, G. Zhang, D. Lu, N. Ornbjerg, and V.J. Maria (2006). Treatment and reinfection of water buffaloes and cattle infected with *S. japonicum* in Yangtze River valley, Anhui Province, China. J. Parasitol., 95: 1088-1099.
35. Tzamaloukas, O., S. Athanasiou, I. Kyriazakis, F. Jackson, and R.L. Crop (2005). The consequences of short-term grazing of bioactive forages on established adult and incoming larvae population in lambs. Int. J. Parasitol., 35: 329-335.
36. Van der Werf, M.J., A. Mbaye, S. Sow, B. Gryseels, and S.J. De Vlas (2003). Evaluation of staff performance and material resources for integrated schistosomiasis control in Northern Senegal. Trop. Med. Int. Health., 7: 70-79.
37. Waller, P.J., G. Bernes, S.M. Thamsborg, A. Sukura, S.H. Richter, K. Ingebrigtsen, and J. Hoglund (2001). Plants as de-worming agents of live stock in the Nordic countries. Acta. Vet. Scandinavica, 42: 31-44.
38. Willis J.C. (1966). Ed. A Dictionary of the Flowering Plants and Ferns, Cambridge Univ. Press, Cambridge.
39. Yu-Hua, C.G.W., J.F. Hua, and L. Yong (2006). Experimental observation on the relative side effects of praziquantal for schistosomiasis control in cattle and buffaloes. Chinese J. Vet. Med., 83: 130-135.