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Physiological changes in the cestode of sheep *Avitellina centripunctata* treated with praziquantel drug

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Abstract
Host –The parasite relationship is a unique phenomenon, usually a dynamic equilibrium exists between the host and the parasite. *Avitellina centripunctata*, a cestode parasite in the intestine of sheep. The study observed Decreased glycogen. alkaline phosphatases and acetylcholinesterase levels in parasites treated with praziquantel drug. The levels were reduced significantly when compared with control parasites treated with 4 ppm praziquantel drug at different incubation periods. It acts on AchE, membrane-bound phosphatases and reduces the capacity of absorption from the host tissues. The drug acts on the Acetylcholinesterase paralyzing the parasites slowly in invitro treatment. This study proves that the drug praziquantel acts as an effective antihelminthic drug in sheep.

Keywords: Cestode parasite, Praziquantel anti-helminthic drug, Glycogen, Alkaline phosphatase, Acetylcholinesterase.

Introduction

Tapeworms of Avitellina species are among the gastro intestinal parasitic helminths which infect wild and domestic ruminants worldwide leading to various clinical manifestations in the ruminant hosts, thereby causing considerable economic losses in livestock production. While these worms are among the major constraints in ruminant livestock raising, very meagre molecula r information available making their identity error-prone. The genetic characterization of these economically important tapeworms were examined. About 480 guts of slaughtered goats (n = 413) and sheep (n = 67) of which 74 guts were found infected with anoplocephalid ces todes (sheep gut:18; goat gut:56). A total of 27 Avitellina lahorea worms (19 from goat and 8 fro m sheep) were isolated, fixed, relaxed and stained using Gower's carmine stain. (Susheel Kumar, *et al*., 2023) [13]. The parasites were collected and treated with the praziquantel drug Invitro and estimated different biochemical parameters mainly glycogen, Alkaline phosphates, and Acetylcholinesterase G.Y Bhargavi *et al*., (1987). The parasites were incubated in praziquantel 2ppm, 3ppm,4ppmand 5ppm concentration Roberson, *et al*., (1982) [10]. The lethal dosage observed is 6 ppm. 2ppm and 3ppm concentrations were found to have insignificant effects, hence 4ppm concentration is considered as standard for all the experiments. The glycogen content was decreased along with the incubation time increased when compared to the control group. Alkaline phosphatases were estimated and compared in control and treated parasites of *Avitellina centripunctata.* There was a decrease in Alkaline phosphatase in the treated group. The amount of acetylcholine estimated shows a significant reduction in the number of cholinesterase's when compared with control parasites treated with 4 ppm praziquantel drug at different incubation periods. The drug acted on the AchE and paralyzed the parasites slowly. This investigation concludes that the livestock in India can combat this cestode parasite.

Materials and methods

Collection of parasites

The parasites *Avitellina centripunctata* were collected from the slaughterhouse, Amberpet, immediately after removing the intestine of the decapitated host *Ovis Aries* (sheep) it was cut open and the parasites *Avitellina centripunctata were* collected, washed out by copious supplies of water to remove adhering food particles and mucous and transferred into the physiological saline. After cleaning the parasites were brought to the laboratory in physiological saline solution (Tyrode).

Estimation of Glycogen

Glycogen content is estimated by San Seifter method (1950). Parasites treated with 4ppm concentration of praziquantel for different incubation periods show a significant change in the glycogen content. The glycogen content decreased as the incubation time is increased when compared to a control group of *Avitellin centripunctata.* In stress conditions required energy was supplied through the glycogen reserves, thus the glycogen content of the treated group showed a reduced amount of glycogen, compared to the control. In the control condition, 50.51 ± 2.127 mg/gm & 48.593± 1.593 mg/gm in half an hour treated parasites. The glycogen content was estimated as 44.763± 1.285 mg/gm in one-hour treated parasites and 33.683 ± 2.448 mg/gm in two-hour drug-treated parasites respectively.
The glycogen content of *Avitellina centripunctata* in control and treated worms at various periods of exposure to praziquantel is summarized in Table 1.

Estimation of Alkaline phosphatase:

Alkaline phosphatase shows optimum activities at pH 9.0-9.6 and is distributed throughout the tissue and can be activated by magnesium, manganese, and cobalt ions M. Upendar *et al.,* (1985). Cyanide inhibits the enzyme activity, Alkaline phosphatase plays a key role in the transport of nutrients. The parasites were divided into two groups one group was kept as control, and the second group of parasites were treated with a 4ppm concentration of praziquantel and

incubated *et al.,* for different periods Sharma, *et al.,* (1987) [12]. An assay for alkaline phosphatase was then performed. Praziquantel inhibited the alkaline phosphatase activity by 45% in *Avitellina centripunctata.* The activity is too less in the 2-hour incubation period. This may be due to the stress of the drug. The energy was supplied through the reverse TCA cycle during stress. At an increased level of incubation, the praziquantel inhibited the alkaline phosphatase activity. Decreased alkaline phosphatase activity will reduce the absorption of nutrients. The activity of alkaline phosphatase in *Avitellina centripunctata* was 0.6186± 0.0455 µ moles in control, 0.522 ± 0.028 µ moles in half an hour and in 1-hour treated parasites 0.435± 0.044 µ moles, and 0.292± 0.031 µ moles of in 2 hours incubation period parasites.

The alkaline phosphatase activity of *Avitellina centripunctata* in control and treated at various incubation periods of exposure to praziquantel drug (4ppm) is summarized in Table 2.

Estimation of Acetylcholinesterase:

Calorimetric method was followed to estimate acetylcholinesterase. The Acetylcholinesterase is found in nervous tissue. The molecular weight of the enzyme has been investigated in Monteria expanse and found to exceed 30,000 Daltons (Gunn and Probert, 1981) in Nippostrongylus brasiliensis between 65,000 to 75,000 Daltons (Yeates and Ogiive, 1976). *Avitellina centripunctata* when treated with 4 ppm concentration of praziquantel at different incubation periods showed significant changes in AchE activity. Acetyl Cholinesterase was decreased in parasites treated with praziquantel drug compared to control worms.

The *amount of* acetylcholinesterase *was* 1.522 ± 0.025 µ moles in control. 1.442 ± 0.061 µ moles in half-an-hour treated parasites, 1.342 ±0.00332µ moles in 1 hour treated parasites, 0.568 ± 0.045 µ moles in 2 hrs treated parasites. The results tabulated in table no.3

Table 1: Glycogen content of *avitellina centripunctata* in control and treated worms at different incubation periods with praziquantel drug.

Sl.no	Control (mg/gm)	½ hour treated	1 hour treated	2 hrs treated
1	51.25	50.37	44.16	34.85
2	48.12	47.29	46.24	35.33
3	52.18	48.12	43.89	30.87
MEAN	50.5167	48.5933	44.7633	33.6833
SD	2.12702	1.59362	1.28594	2.44821

Values expressed as mg glucose/ gm fresh weight.

Table 2: Estimation of Alkaline Phosphotase In *Avitellina Centripunctata* In Control and Treated Worms at Different Incubation Periods with Praziquantel Drug.

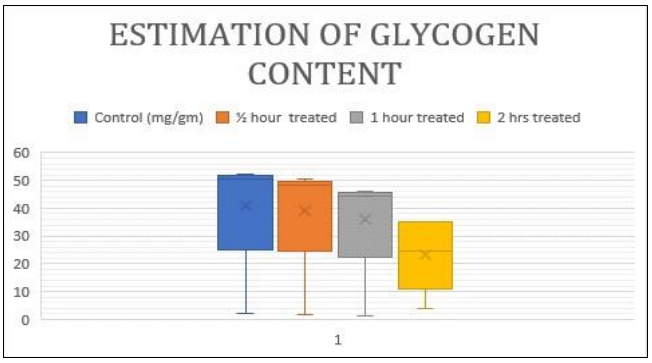
Sl.no	Control (mg/gm)	½ hour treated	1 hour treated	2 hrs treated
1	0.663	0.499	0.432	0.259
2	0.621	0.513	0.392	0.297
3	0.572	0.554	0.481	0.321
MEAN	0.618667	0.522	0.435	0.292333
SD	0.045545	0.028583	0.044576	0.031262

Values expressed as µ moles of pi/mg/ gm fresh weights

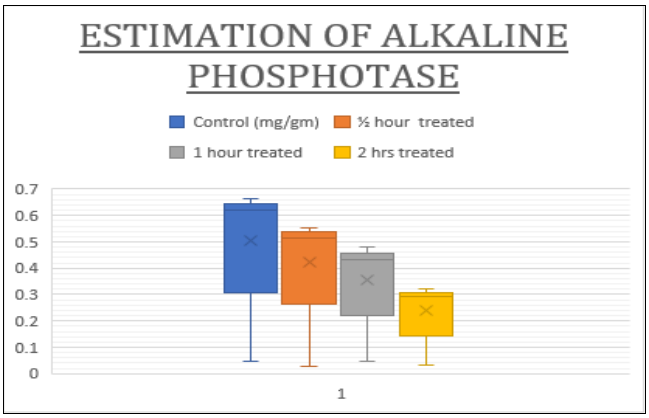
Table 3: Estimation of acetyl cholinesteras in *avitellina centripunctata* in control and treated worms at different incubation periods with praziquantel drug.

Sl.no	Control (mg/gm)	½ hour treated	1 hour treated	2 hrs treated
1	1.497	1.425	1.338	0.574
2	1.521	1.511	1.312	0.521
3	1.548	1.391	1.378	0.611
MEAN	1.522	1.44233	1.3427	0.56867
SD	0.02551	0.06185	0.0332	0.04524

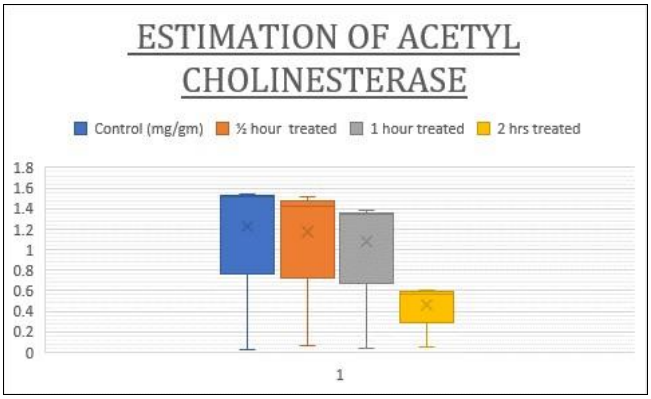
Values expressed as µ moles of acetylcholine /mg of protein



Graph 1: Glycogen content of *Avitellina Centripunctata* in control and treated worms at different incubation periods



Graph 2: Estimation of alkaline phosphotase in *Avitellina Centripunctata* in control and treated worms at different incubation periods with Praziquantel Drug.



Graph 3: Estimation of Acetyl Cholinesterase in *Avitellina Centripunctata* in control and treated worms at different incubation periods with Praziquantel Drug.

Discussion

The parasites were trying to utilize the reserve food during stressful conditions. Although the M.D.H and S.D.H activities increased for this purpose the parasites cannot survive for a longer period (Ramakrishna, *et al.,* 1980) [7]. The parasites became sluggish, this proves that the effect of the drug on Na^{+}, $K^{+,}$ and Ca^{+2} ATP ase activity, and microtubules were observed affected in such a way that the rupture of tegumental and microtubules of parasites could not absorb nutrients from the incubation medium (Upender *et al.,* 1985). Alkaline phosphatase which is present in the tegument and plays a very important role in the absorption of nutrients (Mc Manus, *et al.,* 1987). As a result, the esterase and Ca+ ATP ase which are much involved in neuromuscular action also reduced, the muscles in the holdfast organs like suckers observed to weak and lost their grip.

This study also proves that the drug praziquantel introduced in vivo condition against the infection of *Avitellina centripunctata simultaneously* acts on AchE, membrane-bound phosphatases and reduces the capacity of absorption from the host tissues (Rao, *et al.,* 1979) [5]. It also acts on tegument and microtubules and ruptures them which in a result affect the parasite, that it cannot take any nutrition from the host tissue. Finally, the drug effect the neuromuscular system and therefore esterase reduce the adherence power of suckers. So that the sluggish and paralyzed parasites will be released out from the host tissue and pass out of the host. Hence the drug praziquantel would be a better anthelminthic drug for cestodes.

Conclusion

This review article concludes that Praziquantel is a broad-spectrum, highly efficacious and safe, anthelmintic against trematode and cestode infections in humans and animals (Jong-Yil Chai *et al.,* 2013) [3]. The only limitations for its use in human infections include fascioliasis, hydatid disease, and sparganosis, in which triclabendazole, a combination of praziquantel and albendazole, and surgical removal of parasites, respectively, may be used as an alternative treatment for each infection. Emerging problems include the appearance of praziquantel-resistant strains or isolates in S. mansoni and S. japonicum in the laboratory or in the field. Occurrence in some patients of allergic, hypersensitivity, or anaphylactic reactions to treatment with praziquantel is another emerging problem.

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