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Biochemical Investigations on the In Vitro Effect Of Praziquantel on The Acetylcholine Esterase of the Cattle Parasite *Paramphistomum epiclitum*

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ABSTRACT

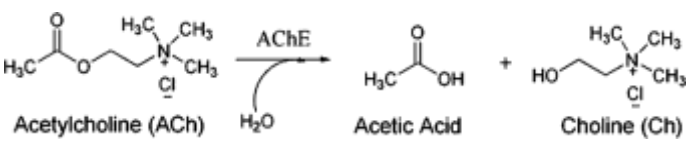
Paramphistomiasis or Amphistomiasis is a neglected tropical disease, caused by Paramphistomum species in livestock ruminants and wild mammals. Its symptoms include profuse diarrhea, anemia, and lethargy, and often result in death if untreated. It causes significant economic loss in livestock production, as it can reduce feed efficiency, increase weight, and affect milk production. The life cycle of a parasite is indirect, requiring a definitive host such as ruminants, and an intermediate host such as snails. To treat this disease, a wide range of Anthelmintic drugs with different types of mechanisms of action are available, among which Praziquantel (Biltricide, Droncit) is one of them. Understanding the mechanism of action of a drug on parasites gives better scope for the development of a wide range of effective drugs. Several drug target action sites are identified in parasites among which acetylcholine esterase (Ache) is one of them. It plays a crucial role in the regulation of nerve impulses by breaking down the neurotransmitter acetylcholine into acetic acid and choline in the synaptic cleft. In the current paper Praziquantel mode of action on Ache activity in *Paramphistomum epiclitum* is studied. the biochemical assay of Ache is estimated to be in control and treated with parasites at various drug concentrations over 1-hour long exposure. It has been observed that the drug significantly inhibits Ache activity by 4 ppm.

Key words: Praziquantel, Acetylcholine esterase, *Paramphistomum epicitum*, Anthelmintics, Ion channel.

INTRODUCTION

The helminth nervous system comprises neurons, nerves, nerve junctions, and the muscles they innervate. At any point in the neurological system, drugs can exert their effects. Presynaptic vesicles are present at neuromuscular connections (Dixon and Mercer, 1965). In general, trematodes contain four types of neurotransmitters: acetylcholine, 5-hydroxytryptamine, adrenaline, and Dopamine (Bueding and Bennett 1972).

Acetylcholinesterase is a neuronal enzyme responsible for the breakdown of acetylcholine to acetic acid and choline.



There are two types of esterase's. Specific esterase's are distinguished from nonspecific esterase's. The group of specialized esterase's or real cholinesterase's includes acetylcholinesterase. Pseudocholinesterase, also known as nonspecific esterase's, function on simple esters found in all nerve tissues. Red blood cells also contain acetylcholine, whereas butaryle esterase has been detected in the blood serum.

In Fasciola and Schistosoma, acetylcholine is produced in the presence of choline acetylase (Ch Ac) and destroyed by acetylcholinesterase (Budeing 1952).

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The physiological function of acetylcholine in the axon muscles of trematodes is as a key inhibitory neurotransmitter (Chance and Mansour 1953). It has an inhibitory effect on vertebrates and an accelerating effect on invertebrates.

Typically, neurons that release choline are known as cholinergic neurons. Acetylcholinesterase in Moniezia expansa was discovered to have a molecular weight greater than 300,000 (Gunn and Probert, 1981), in Neactor americanus between 380,000 and 400,000 (Yates and Ogilvine, 1976), and in Nippositronqylus brasiliensis between 65,000 and 75,000. Each subunit of the electrical eel enzyme (Ach E) has a molecular weight of 70,000.
Concanavalin mildly inhibits N. americanus and N. brasiliensis acetylcholinesterase. This indicates that these enzymes, like other cholinesterase's, are glycoproteins (Widmer et al., 1974). It is also inhibited between 10 and 20% by 5mm ATP.

It would appear that the enzymes are insensitive to the energy status of the host, as these effects are probably too small to be physiologically significant. Furthermore, phenyl trimethyl ammonium, N-Methyl pyridinium, N-Methyl quinolinium, etc. inhibited acetylcholinesterase.

Dale suggests that the enzymatic degradation of acetylcholine in nerve tissue is related to the activity of acetylcholinesterase (1914). Hutchinson and Probert (1962) reported the Synaptic transmission function of Acetylcholinesterase as well as its part in membrane transport and the release of sperms and eggs (Bogitsh 1967).

Lee (1970) and Ogilvine and Jone (1971) theorized that the enzyme works as a biochemical holdfast for the parasite by producing a local anesthetic effect on the gut wall of the host, so limiting parasite ejection. According to research on trematodes, esterase's exist in multiple forms (coles 1970, Dickinson and Johanson 1978). Edward et al. (1971) and Jones and Ogilvine (1972) found that this enzyme induces an immunological response in nippostrongylus brasiliensis-infected rats. This data demonstrates conclusively that Acetylcholinesterase is present in all trematode stages. The enzyme acetylcholinesterase contributes to the pathophysiology and immunology of infectious nematodes (Ogilvine et al., 1971).

According to AkPag et al. (1974), acetylcholine promotes glycogen production. Yeats and Oqilvine (1976) described the involvement of acetylcholine in the breakdown of glycogen into glucose, which aids the parasite in feeding. The enzyme acetylcholinesterase may deactivate peristaltic action (Nizami et al., 1977; Mamidala et al, 2020). The presence of Acetylcholinesterase in the tegument of Cotugnia digonopora has been linked to fatty acid hydrolysis (Rama Krishna et al., 1979). Gunn and Probert (1981) examined the enzyme activity in endoplasmic reticulum and ribosome fragments.

The localization of cholinesterase is useful for elucidating the details of the nervous system in parasitic helminths (Schardein and Waltz 1955, Jones et al. 1979). Rama Krishna (1979) in cestodes, Narsaiah (1979) in monogenetic trematodes, and Heather (1980) in digenetic trematodes localized the nervous system.

Kinetic and electrophoretic characteristics of acetylcholinesterase in Schistosome and Fasciola and their species are distinct (coles 1970). The high amount of Acetylcholinesterase in the secretory glands of Neactor americanus and Nippostrongylus brasiliensis has been found (Lee 1962 and Laren et al., 1974). It has been proposed that acetylcholinesterase is produced on ribosomes and delivered to nerve endings via the rough endoplasmic reticulum and Golgi apparatus (kasa 1968 and Pozdvakow 1968).

Compared to other species, Monezia expansa's Acetylcholinesterase appears to be less sensitive to the traditional inhibitors BW 284, C51. Similar insensitivity of helminth cholinesterase to traditional inhibitors has been observed before by Mc. Laren (1972) and Shields (1996). According to reports, M.expansa acetylcholinesterase is resistant to the majority of current anthelmintics (Gunn & Probert 1981). Although it is extremely susceptible to high doses of organophosphates, it is resistant to low concentrations (Govorova and polyakova 1971).

The literature review reveals that the biochemical assessment of Acetylcholinesterase in the parasite Paramphistomum epiclitum related the medicine praziquantel has not been performed.

MATERIALS AND METHODS

Before the assay, parasites were obtained from a local slaughterhouse, moved to a laboratory in a bottle containing Tyrode nutritional medium, and rinsed with saline with enough antibiotics to eliminate bacterial and intestinal debris. Various medication concentrations of PZQ solution were generated at 1, 2, and 4 ppm, and parasites were exposed in vitro for one hour under optimal laboratory conditions.

Enzyme Assay:

Acetylcholine esterase activity evaluated by the colorimetric method developed by Murali Krishna das (1967) and adopted by Metcalf.

RESULTS AND DISCUSSION

In this study, the author measured the biochemical activity of acetylcholinesterase in untreated and (PZQ)-treated *P. epiclitum*. The control parasite's acetylcholinesterase activity was 1.3538 PPM, while the

treated parasite's activity was 1.3522, 1.3467, and 1.2142 PPM, respectively. At high concentrations (4PPM), a reduction in acetylcholinesterase has been reported; specific values are listed in table 1.

Table-1. AcHE Activity in control and treated *P.epiclitum* (1 hr exposure)

S.No	Control	1PPM	2PPM	4PPM
1	1.164	1.354	1.321	1.241
2	1.768	1.321	1.344	1.244
3	1.465	1.36	1.388	1.222
4	1.263	1.328	1.362	1.206
5	1.109	1.407	1.367	1.178
MEAN	1.3538	1.3522	1.3467	1.2142
SD	0.268	0.036	0.0118	0.023
SE	0.12	0.016	0.008	0.21

** Values expressed as micro moles of Acetyl choline hydrolyzed 1mg protein/hr.*

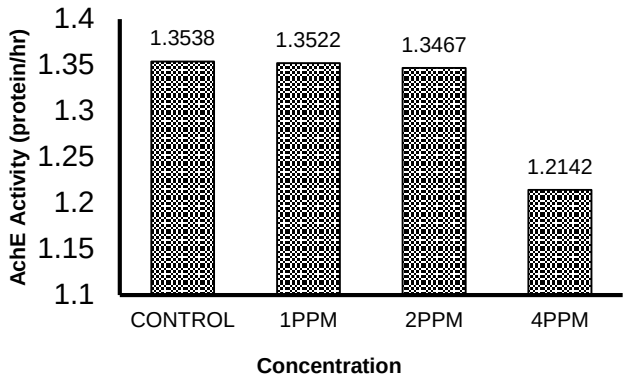

Figure-1. Graphical illustration of the AchE activity in control and treated *P. epicliteum* (1 hr exposure)

CONCLUSION

Due to the small size of trematode parasites, the entire parasite should be used for in vitro drug testing. The tegument of parasites is rich in neurotransmitters and has a high absorption capacity for diverse substances. When a chemotherapeutic drug absorbs on the surface of a parasite, it interferes with the parasite's metabolism by interacting with a specific substrate or by competing with an important metabolite for an enzyme or coenzyme, resulting in the parasite's death. The indirect effect of the medicine, on the other hand, alters the physical conditions of the parasite's habitat, such as pH, temperature, and ionic concentration, and causes the parasite's death. The locations of pharmacological action vary from parasite to parasite, species to species, and host to host. 30% of currently available anthelmintic medications are neuromuscular blocking medicines that impede the function of the enzyme. In this investigation, it was discovered that treated parasites at high medication concentrations experienced fewer aches (4ppm). The chemical mechanism of PZQ's mode of action is still partially understood. The majority of information suggests that the neuromuscular and tegument sites are its target areas. Andrews et al (1973). In vitro study of the medicine's activity reveals that the drug itself, not its metabolites, is active. (1977, Thomas and Andrewes). The initial effect of PZQ is the drug's interaction with lipid elements of the parasite tegument, which causes a rapid influx of calcium ions into the parasite body. Andrews (1985). The changed distribution of ions across the membrane raises the tension and depolarization of the membrane (Bricker et.al1983). In the present study experimental results coincides with the findings of Raymond et al. (1980), Gonnert and Andrews (1977), Gunn and Probert (1980), and Thomas and Andrewes (1980). (1977). Hence, a reduction in Acetyl choline esterase in treated *P.epiclitum* is expected.

Conflicts of Interest

Authors declare that there is no conflict of interests regarding the publication of this paper.

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