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Studies on the Acetyl Cholinesterase Activity in Hamsters (*Mesocricetus Auratus*) Infected with *Ancylostoma Ceylanicum*

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Abstract: ***Helminthic infections are among the most prevalent parasitic diseases, primarily found in tropical and subtropical areas. These infections are characterized by an endoparasitic hookworm affecting the intestinal region of the host. Reports of hookworm infections have been reported in many laboratory animals. The hookworm has a distinctive hook-like structure at its anterior end, which draws out nutrients from the host. Hookworm infections lead to metabolic disorders and significant disturbances in tissue structure and function. Acetyl cholinesterase, an enzyme related to the nervous system, plays a vital role in the hydrolysis of acetylcholine into acetic acid and choline. In the current study, the hookworm Ancylostoma ceylanicum was experimentally introduced into the host, Mesocricetus auratus. The infected host was subsequently examined by measuring the acetylcholinesterase levels in various tissues. The acetylcholinesterase content in M. auratus was analyzed biochemically in both infected and control samples. The biochemical estimation of acetylcholinesterase levels in the hamsters revealed that its activity was significantly higher in the brain, muscle, spleen, and lung tissues, adversely affecting muscular and neuronal functions. The concentration of acetylcholinesterase in the host tissues of M. auratus can provide insights into the pathogenicity of the A. ceylanicum infection. This paper explicates the levels of acetylcholinesterase in infected hamster and reveals its significance in the field of enzyme biochemistry.***

Keywords: Acetylcholinesterase, *Ancylostoma ceylanicum, Mesocricetus auratus,* pathogenicity, enzyme biochemistry

1. Introduction

Hookworm infections rank among the most common helminthic infections, especially in tropical and subtropical areas, where they cause substantial morbidity (Pearson et al., 2012). The lifecycle of hookworms necessitates intestinal colonization, during which they adhere to the mucosa through hook-like structures situated at their anterior end. This attachment helps to withdraw nutrients from the host, resulting in tissue impairment and metabolic disruptions. Among the various species of hookworms, *Ancylostoma ceylanicum* is noted for its zoonotic potential, affecting both humans and animals. Abuzeid *et al*. (2020) examined the hookworm excretory/secretory products, focusing on their significance in parasite biology and host-parasite interactions. They found hookworm excretory/secretory products as targets for vaccines and pharmaceuticals, while also examining the gaps in research for future studies. Mendez *et al.* (2005) investigated the host antibody responses during the infection of *A. ceylanicum* in Syrian hamsters.

Acetyl cholinesterase is an enzyme linked to the nervous system, playing a crucial role in the hydrolysis of acetylcholine into acetic acid and choline. Acetylcholine serves as an interneuronal end plate neurotransmitter, which is swiftly eliminated through hydrolysis, resulting in the formation of acetic acid and choline. Two distinct types of cholinesterases have been identified; one type is located in erythrocytes, while the other is present in serum. The enzyme found in erythrocytes hydrolyses acetylcholine significantly faster than butyrylcholine, thus it is referred to as acetylcholinesterase, or specific or true cholinesterase. In contrast, the enzyme present in serum hydrolyses butyrylcholine much more efficiently than acetylcholine, and is termed pseudocholinesterase or non-specific cholinesterase. Acetylcholinesterase is present in the nervous tissues of nearly all animal species. The significance of acetylcholinesterase has been an inquisitive component, to undertake the study on the biochemical activity of acetylcholinesterase in *M. auratus* following experimental infection with *A. ceylanicum*. It aimed to investigate the metabolic impact of the infections on host tissues.

2. Literature Review

The literature review shows a range of research work done on the acetylcholinesterase activity in various organisms. Some of the important are highlighted here.

Gunn and Probert (1981) investigated the enzyme's distribution within subcellular fractions by staining acetylcholinesterase at the ultrastructural level, revealing that the enzyme is exclusively localized on the fragments of rough endoplasmic reticulum and ribosomes. It has been proposed that the enzyme is synthesized at ribosomes and subsequently transported in the cisternae of the rough endoplasmic reticulum through the Golgi apparatus to the nerve endings (Kasa, 1968; Pozdyakov, 1968). The importance of acetylcholinesterase as a neurotransmitter at interneuronal and end plate sites in vertebrates is widely recognized. Given that this enzyme is present in the nervous system, it can be inferred that it serves a crucial function as a neurotransmitter. In mammals, acetyl cholinesterase is present in the nervous system, while cholinesterase is found in the plasma. Both acetyl cholinesterase and cholinesterase have been identified in various helminths. A significant number of intestinal nematodes possess a high concentration of acetylcholine esterase within their bodies.

These nematodes seem to release substantial amounts of acetylcholinesterase *in vitro*, as noted by Yeates and Ogilvie (1976) in *T. colubriformis, T. axei, Trichostrongylus retortaeformis, O. radiatum, Nippostrongylus americanus,* and by Pritchard et al. (1991) in *Nippostrongylus americanus* and *Ancylostoma ceylanicum*. Similar to the specific protease from *Schistosoma mansoni*, the synthesis of acetylcholine esterase by these nematodes is noteworthy for two reasons. Firstly, since invertebrate and helminth acetylcholine esterases are typically more susceptible to inhibition by organophosphates compared to the mammalian enzyme, this enzyme represents a potential target for chemotherapy (Reiner *et al*., 1978).

Secondly, the release of significant quantities of acetylcholinesterase by these nematodes results in the generation of specific antibodies by the host. The role of acetylcholinesterase secretion by intestinal nematodes remains unclear. Acetylcholinesterase may inhibit the peristaltic movement of the host's intestine or potentially diminish the host's immune response. In the cases of *Nippostrongylus trasiliensis* and *Nippostrongylus battus*, the production of acetylcholinesterase fluctuates according to the immune status of the host. Conversely, acetylcholinesterase may promote glycogen breakdown and elevate free glucose levels by lowering acetylcholine esterase levels in the intestine. Acetylcholine is typically found in intestinal secretions and may play a role in regulating absorption mechanisms. Graff and Read (1967) conducted a specific study on the activity of acetyl cholinesterase in *Hymenolepis diminuta*. Krishna *et al.* (1980) examined the histochemical localization of acetylcholinesterase activity in helminths. Probert and Durrant (1977) performed research on the characteristics of total cholinesterase and the effects of specific inhibitors in helminths. Sanderson (1972, 1974) investigated the release of cholinesterase in nematodes. Murugan and Kaleysa Raj (1995) analyzed acetyl cholinesterase activity in Seteria digitata. Martin (1981), Sharma and Singh(1995), and Tamizhselvi (1985) researched acetyl cholinesterase activity in rats. Chafer Dolz et al. (2025) conducted a study on the use of Artificial Intelligence (AI) for screening the Drug Bank (DB) database to discover new acetylcholinesterase (AChE) inhibitors, which is a target that has not been previously characterized in the American cockroach (Periplaneta americana). The application of Molecular Mechanics to Generalized Born Surface Area (MMGBSA) is utilized to identify the most promising compounds for inhibiting the AChE enzyme.

Given the limited studies on the levels of acetyl cholinesterase activity in the various tissues of the infected host hamster, an effort has been made to investigate the enzyme activity in *Mesocricetus auratus* infected with *Ancylostoma ceylanicum.*

3. Materials and Methods

Adult male Syrian golden hamsters,*Mesocricetus auratus* of 4-5 weeks weighing around 40-50 gms were used in all the experiments. They were infected with the larva of *Ancylostoma ceylanicum.* After infection, hamsters were maintained under similar conditions of diet and environment. On day 15 microscopic examination of faecal pellets was done for the presence of *A.ceylanicum* ova. Mostly, all the hamsters were found positive for *A. ceylanicum* infection. The strain was maintained by regular passage in hamsters once in a month. The hamsters were anaesthetized with ether by placing them in a container. The small intestines were removed for observing the *Ancylostoma ceylanicum* infection. The tissues like liver, intestine, spleen, kidney, lungs, muscle, brain and serum, were collected and homogenated in 0.25M sucrose solution. The homogenate was used for enzyme assay by the colorimetric method (Metcalf,1951).

To 1 ml of buffer substrate solution, 1 ml of homogenate (uncentrifuged) was incorporated. The mixture was incubated for 30 minutes at 37 ± 1°C, after which the reaction was halted by the addition of 2 ml of alkaline hydroxylamine hydrochloride. The tubes were shaken thoroughly, and 1 ml of HCl (1:1 HCl: H_2O) was introduced with additional shaking. The contents were then filtered. To 2.5 ml of the filtrate, 0.5 ml of ferric chloride solution was added, and the tubes were shaken thoroughly once more. Zero- time controls were established by adding 2 ml of alkaline hydroxylamine hydrochloride before the homogenate was introduced. The color of the hydroxymic acid produced was measured at 540 nm against a blank. The blank consisted of 1 ml of buffer instead of the buffer substrate mixture. The enzyme activity was assessed by measuring the amount of unreacted acetyl choline chloride remaining after 30 minutes of incubation. The intensity of the color at the conclusion of the incubation indicates the quantity of acetyl choline that remained unhydrolyzed. By subtracting this amount from the known initial substrate concentration, the actual enzyme activity level was calculated. The enzyme activity was reported in µ moles of acetyl cholinesterase hydrolyzed per mg of protein per hour.

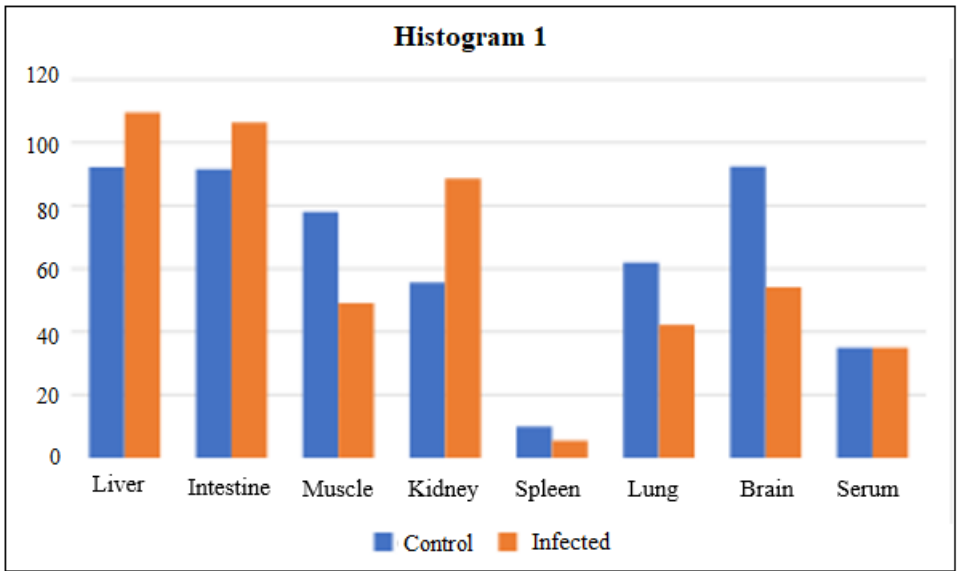
4. Results

Table 1: Acetocholinesterase content in the different tissues and serum of *Mesocricetus auratus* induced with *Ancylostoma ceylanicum* infection

S. No.	Tissues	Group	Mean ± S.D.	% Change
1.	Liver	Control	93.200 ± 2.993	17.425%
		Infected	109.44 ± 5.589	
2	Intestine	Control	91.60 ± 98.305	16.157%
		Infected	106.40 ± 12.027	
3	Muscle	Control	78.00 ± 5.657	37.026%
		Infected	49.12 ± 7.081	
4	Kidney	Control	55.600 ± 10.911	61.295%
		Infected	89.680 ± 2.296	
5	Spleen	Control	9.920 ± 1.866	44.556%
		Infected	5.500 ± 1.153	
6	Lung	Control	62.000 ± 9.121	28.710 %
		Infected	44.200 ± 4.583	
7	Brain	Control	92.320 ± 5.463	4.334%
		Infected	54.160 ± 1.488	
8.	Serum	Control	34.960 ± 2.858	0 %
		Infected	34.960 ± 2.858	

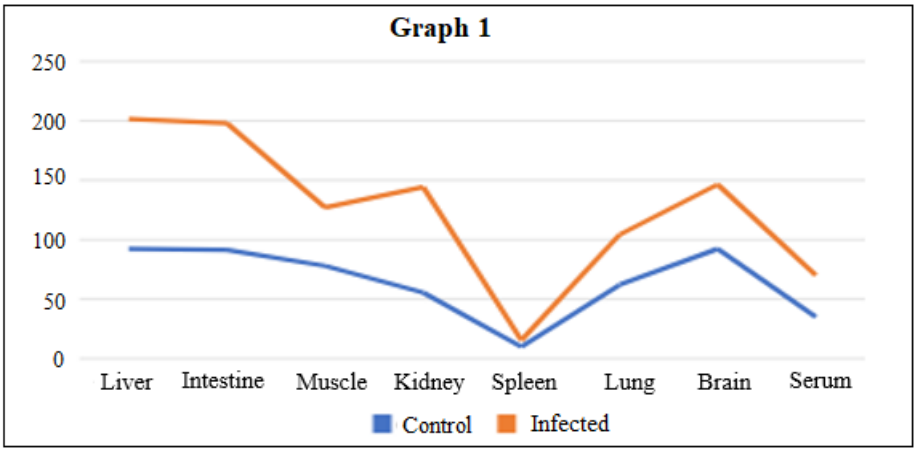
For tissues, values are expressed as µ moles of acetylcholinesterase hydrolysed/ mg protein/ hr.
For serum, values are expressed as µ moles of

acetylcholinesterase hydrolysed/ ml protein/ hr.
± Indicates standard deviation for control and experimental values Figures in parenthesis is percent change over control.



Histogram 1

Acetocholinesterase content in the different tissues and serum of *Mesocricetus auratus* induced with *Ancylostoma ceylanicum* infection



Graph 1: Acetocholinesterase content in the different tissues and serum of *Mesocricetus auratus* induced with *Ancylostoma ceylanicum* infection

The acetyl cholinesterase activity was estimated in various tissues and serum of hamster infected with a hookworm and in uninfected control. The results are given in the Table1 and are also expressed through the Histogram 1 and Graph 1.

The results obtained in the various tissues of the control animals are indicated as liver 93.200 ± 2.993, intestine 91.600 ± 98.305, muscle 78.000± 5.657, kidney 55.600 ± 10.911, spleen 9.920 ± 1.866, lung 62.000 ± 9.121, brain 92.320 ± 5.463 µ moles of acetyl choline hydrolysed / mg protein /hr. and in serum 34.960 ± 2.858 µ moles of acetyl choline hydrolysed / ml/hr.

The values in the different tissues of the infected host as indicated in liver 109.440 ± 5.569. intestine 106.400 ±12.027, muscle 49.120 ±7.081, kidney 89.680 ± 2.296, spleen 5.500 ± 1.153, lung 44.200 = 4.583, brain 54.160 ± 1.488, µ moles of acetyl choline hydrolysed / mg protein / hr. and in serum 34.960 ± 2.858 µ moles of acetyl choline hydrolysed / ml/hr.

The acetyl cholinesterase activity has been found to be increased in liver, intestine and kidney by 17.425%, 16.157%, and 61.295% respectively. However, the levels of enzyme activity have been found to be decreased in muscle, spleen, lung. and brain by 37.026%, 44.556%. 28.710%, 4.334% respectively. No alteration has been observed in the serum of the infected host in comparision with control.

5. Discussion

According to Akpan *et al*. (1974), acetylcholine promotes the production of glycogen. Acetylcholinesterase breaks down glycogen, which aids in the parasite's feeding, according to Yeates and Ogilive (1975). Reduced acetylcholine levels in the gut, liver, and kidney of infected hamsters show an increased level of acetyl cholinesterase,

which is indicative of a high intestinal worm burden. Increased levels of acetyl cholinesterase indicate that the host becomes weak and senile, which leads to the breakdown of glycogen. Since these metabolically significant tissues store glycogen, acetylcholinesterase is used for glucose synthesis to meet the energy requirement due to parasitism. Intestinal secretions typically contain acetylcholine, which may have a role in controlling the absorption mechanism.

According to Yeates and Ogilivie (1976), Reiner *et al*. (1978), and Pritchard et al. (1991), nematodes emit a significant amount of acetyl cholinesterase, which causes the host to produce certain antibodies and may lessen intestinal peristalsis or the host immune response. Acetylcholine buildup and impaired muscle and neural activity are indicated by decreased enzyme activity in the brain, muscle, spleen, and lung. Glow and Richardson (1967) and Carlton (1969) consider that a reduction in brain acetylcholinesterase in vertebrates can lead to physiological and behavioral changes in animals. The reduction in acetyl cholinesterase activity was more significant in the brain, muscle, spleen, and lung tissues, resulting in impaired impulse transmission. This was due to the buildup of acetyl cholinesterase, which in turn compromised the stability of the nervous system and serum. In serum, the levels of acetyl cholinesterase activity have shown no change

6. Conclusion

The current research on *Ancylostoma ceylanicum* infection in the host *Mesocricetus auratus* offered experimental insights into the biochemical impacts of *A. ceylanicum* on acetylcholine metabolism in *M. auratus*. The notable changes in acetylcholine levels in different tissues emphasize the widespread metabolic disturbances triggered by hookworm infections. These results have consequences for comprehending the pathophysiology of helminthic infections and creating specialized therapeutic approaches.

7. Recommendation:

In light of the findings and conclusions, the following recommendations are given:

1) Employers of the respondents may undertake an in-depth study of the results of this research and disseminate the findings to the manufacturers related to anti-helminthic drugs.
2) It may provide additional information to the researchers to work on the studies on acetylcholinesterase activity on the parasite-host evolution.

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Author Profile

Rajani, P. S. received PHD (Zoology), M.A. (Eng), B.Ed. The research work focuses on Parasitology. Author has researched on the Acetylcholinesterate activity in the host *Mesocricetus auratus* infected by *Ancylotoma ceylanicum*. Author has been a prominent researcher in Helminth biology and a faculty with more than a decade of experience in teaching graduate students in Government Degree College, Begumpet. The research on acetylcholinesterase content fascinated me to study its content on the host, *Mesocricetus auratus* infected with *Ancylostoma Ceylanicum*. The results were astonishing. The crux of present research has been curiosity in exploring the enzyme dynamics of *Ancylostoma Ceylanicum* infection in *Mesocricetus auratus*. The interesting veterinary and medical importance of this parasite has initiated the present research study.

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