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Research Article



Inhibitory effect of aqueous extract of flowers *Dianthus chinensis* on the proteins and acetylcholinesterase for *Bothriocephalus acheilognathi*

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Abstract

Livestock plays a significant role in the national economy, making it crucial to safeguard the livestock population in order to achieve economic growth and increase exports. The current study aimed to determine whether or not the addition of an aqueous extract of Chinese carnation flowers to the tissues of the parasitic worm *Bothriocephalus acheilognathi* found in carp fish affected their total protein content and the enzyme's activity (*Cyprinus carpio*). Treatment with an aqueous extract of Chinese carnation flowers was found to raise both total routine concentration and acetylcholinesterase activity (AChE). The parasite's demise is ultimately caused by the host's behavior, reproduction, health, and other factors.

Keywords: *Bothriocephalus acheilognathi*, Clov, Proteins, Acetylcholinesterase, *Dianthus Chinensis*

Introduction

Bothriocephalus acheilognathi is a member of the Pseudophyllidea order. The head lacks sockets, which are substituted by two long sucking grooves termed Bothria on the ventral and dorsal sides, which become flat and serve as the pivot members. This tapeworm is a polyzoic worm since its length spans from 20 to 30 mm. The shape of the heads is elongated, rectangular, and occasionally spherical, or it resembles a club or heart. It has two sucking grooves on each side of the neck region. The body segments have a wrinkled appearance, and the genital orifices are positioned on the sides of the body segments, whilst the uterine orifices are located in the centre of the abdomen side. Both sides of the body segment include the vitelline glands (Yamaguti, 1959; Khalil et al., 1994).

Life cycle

This parasite's life cycle is indirect, as it involves two intermediary hosts. It begins with the introduction of the worm's eggs into the water via the feces of its final host.

Following the separation of the pregnant segments from the body via the anapolytic proglottids process. These eggs are distinguished by their thick shells and black hue. They contain Operculate egg coverings. The embryos develop in the eggs at a temperature between 10 and 32 degrees Celsius into a ciliated larva known as Coracidium. This larva can swim freely and typically emerges when the sun rises. It has a lifespan of (4-5) days. If this ciliated larva is ingested by a crustacean of the genus *Diaptoms cyclops* (the first intermediate host), it will grow in the host's intestines before shedding its coverings and breaching the intestinal wall. The parasite then enters the intestinal wall to reach the bodily cavity or the infective Proceroid stage, which contains six larval spines. When the second intermediate host (fish) consumes the infective larvae present in the crab, the proto-caudate penetrates the fish's intestines and matures into an infective larva, which is the complete or flat plerocercoid. When these fish are consumed by their final host, which may be other fish, cats, dogs, or pigs, these flatworms mature into adult tapeworms (Olsen, 1974).

Materials and Methods

Thirty-five *B. acheilognathi* were obtained from *Cyprinus carpio* accessible in local markets in Nineveh Governorate over the period from September 2021 to June 2022. Worms were collected and placed in petri dishes with distilled water. They were cleansed of plankton using a tiny brush and rinsed numerous times. The naked eye was utilized for the phenotypic evaluation, and then a dissecting microscope and a light microscope were employed to detect the worm's phenotype (Khalil et al., 1994).

Estimation of the total protein of *B. acheilognathi* has used the Total Protein Kit supplied by (Solarbio life Scienres). Acetylcholinesterase (AchE) activity was measured using an ELISA kit provided by Sunlong Biotech. LTD.

Results and Discussion

Estimation of total protein in current tapeworms

There was a variation in the number of total proteins in the present tapeworms. This could be related to the protein proportions in the parasite's body, which are based on the parasite's metabolic activity and associated critical actions, depending on the nature of the DNA. The parasite is also subjected to external or internal effects such as feeding, enzyme production, and amino acid absorption. It has an effect of this differs depending on the months of the study. The admission of nutrients into the parasite and the ensuing metabolic processes, as well as the host's immune system's response and the host's tissues' ability to respond to anti-parasitic compounds, which in turn encourage the synthesis of parasite immune substances. These drugs will alter the parasite's genetic traits and, as a result, the protein structure (Tomas et al., 2019).

As a result, the parasites have a qualitative and genetic relationship that is dependent on the genera and species, which differ according to the diverse adaptations in the hosts. They also differ according to the nature and physiology of the parasite tissues, as well as the type of host (Al-Kalak, 2001), which is the difference in fish hosts, their ages, and their distinct nutrition Table 1.

Table 1. Estimation of total protein of tapeworms by study months

Host	Tapeworm	April	May	June
<i>Cyprinus carpio</i>	<i>Bothriocephalus acheilognathi</i>	3.14 mg/mol	0.95 mg/mol	44.43 mg/mol

Small fish consume phytoplankton, but giant fish consume phytoplankton (2-4) years for aquatic creatures, including fish (Copp et al., 2009). According to Dorucu, proteins and fats are

the main sources of energy in fish, and there are several other elements influencing the amount of protein in fish, such as sex, age, variety, seasonal and genetic changes, as well as the nature of the aquatic habitat in which fish reside (Hassan et al., 2018; Hassan et al., 2020).

Because tapeworms do not have a digestive system, they dwell in the intestine and absorb nutrients contained in the lumen of the alimentary canal of fish by diffusion or efficient transfer through the body wall to meet their needs for growth, development, and survival in their host (Pawar, 2020).

The DNA and metabolic processes of modern tapeworms are different from those of their ancestors, which explains why their total protein concentration is lower than that found in earlier research (Al-Kalak, 2001; Al-Kalak et al., 2008; Al-Kalak & Muhammad, 2007; Pallewad et al., 2015; AL-Niaemi et al., 2019; Dawood et al., 2020; Fakri et al., 2021).

The variance in total proteins is not limited to helminthes, the nature of muscle tissue, contraction, and physiology in the current study, but there is also diversity in host adaptation, as well as in the transfer of materials between them and the host. This is what was implied by (Al-Mawla, 2010). Where he found a difference in protein concentrations in the larval stages of the threadworm *Contracecum sp.* Commenting on the importance of proteins in the body of an organism and, in particular, parasites, the research, and investigation of their protein content infers an understanding and how the parasite adapts to its host (Sulima et al., 2017). Nalamada and Kumar, 2018 investigated the protein content of *R. tetragona* body segments. The study's findings revealed that the protein concentration in the immature segments is higher than in the mature segments, which shows metabolic efficiency, high physiological activity, the maturity of the reproductive spores, and the parasite's development to ensure its survival within its host. (Al-Kalak and Muhammad, 2007; Al-Kalak et al., 2008; Saraf and Katyani, 2016), as well as knowledge of the tapeworm genetic map and tree (Paludo et al., 2020).

The specific activity of acetylcholinesterase enzyme

The results demonstrated a difference in the specific activity of the acetylcholinesterase enzyme for the current tapeworms and according to the months of the study as shown in Tables 2.

Table 2. The specific activity of acetylcholinesterase enzyme *B. acheilognathi* parasitoid in *Cyprinus carpio*, according to study months

Host	Tapeworm	Month	Qualitative effectiveness
<i>Cyprinus carpio</i>	<i>Bothriocephalus acheilognathi</i>	April	0.08 ng/ml
		May	0.72 ng/ml
		June	0.002 ng/ml

This decrease in the specific activity of the enzyme is compatible with the total protein estimation of the present tapeworms, as it varied among the tapeworms. These findings are comparable to those of (AL-Niaeemi et al., 2020b) and (Al-Nafajy, 2006). This variation in enzyme activity can be linked to the diverse fish hosts and habitats of the various tapeworm species. This may be related to the high-water temperature, which increases the enzyme's kinetic energy, especially in parasites, during the month of May. This corroborates what the study (AL-Sarraj and Jankeer, 2020) suggested regarding the enhancement of acetylcholinesterase (LAC) activity in *C. carpio* tissues.

According to a previous study, the specific activity of the enzyme acetylcholinesterase varied by body part and histological composition (Singh et al., 1985). This was ascribed to the eating and swimming patterns of fish, as well as the developmental stages of the brain map of *Thysanienzia giardia*. Commenting on the significance of finding the specific activity of this enzyme, it is still necessary to do additional studies in order to gain a deeper understanding of the various neurological mechanisms in helminths. Numerous scholars working on the study of parasites find the enzyme study to be an indispensable resource. The enzyme was considered a potential target for the production of antiparasitic medicines, resulting in the development of new treatment strategies for tapeworm infections (Hong et al., 2017; Joshi et al., 2018).

Effect of aqueous extract of Chinese carnation flowers on the vitality of tapeworms

The results of the current study showed that there is a clear effect of the aqueous extract of clove flowers to cause paralysis and death of tapeworms, and it depends on the amount of concentration used, as a concentration of 10g/100ml and 20g/100ml were used, as in Table 3.

Table 3. Duration of paralysis and death of tapeworms and treatment with aqueous extract of clove flowers at a concentration of 10g/100ml and a concentration of 20g/100ml

Host	Tapeworm	10g/100ml conc. Time	20g/100ml conc. Time
<i>Cyprinus carpio</i>	<i>Bothriocephalus acheilognathi</i>	1.30 hr.	45min.

The results showed that the duration of paralysis and death of worms (gradual killing) was less when the concentration was 20g/100ml than when the concentration was 10g/100ml.

Estimation of total protein of tapeworms after treatment with aqueous extract of clove flowers

The current investigation found a variation in the value of total protein in tapeworms treated with aqueous extract of clove

flowers at 10g/100ml and 20g/100ml concentrations, depending on the months of the study (Tables 4, 5).

Table 4. Estimation of total protein of tapeworms treated with a concentration of 10g/100ml of aqueous extract of clove flowers, according to the months of the study

Host	Tapeworm	April	May	June
<i>Cyprinus carpio</i>	<i>Bothriocephalus acheilognathi</i>	0.25 mg/mol	4.66 mg/mol	217.9 mg/mol

Table 5. Estimation of total protein of tapeworms treated with concentration and 20 g/100 ml of aqueous extract of clove flowers, according to study months

Host	Tapeworm	April	May	June
<i>Cyprinus carpio</i>	<i>Bothriocephalus acheilognathi</i>	0.19 mg/mol	1.95 mg/mol	91.21 mg/mol

It was revealed that the maximum value of total protein was in the month of June, whereas the values of total protein of tapeworms changed in the months of April and May. When these results were compared to the total protein values of tapeworms without treatment and killing them with clove flower extract, it was discovered that they were lower when treated with an aqueous extract of clove flowers at two concentrations of 10 g/100 ml and 20 g/100 ml. This is attributable to the present parasite kinds and the diversity of their fish hosts. The worms' defensive systems and histological structure allow them to withstand external environmental conditions such as the aqueous extract of clove blossoms. Clove blossoms also contain substances, such as tannins, which are polyphenolic compounds that bind to or precipitate proteins and amino acids (Daniel et al., 2014). The beneficial compounds of clove blossoms, their connection with the parasite's protein, and their sensitivity to breakdown resulted in shock. Shock and the emergence of defenses and immune processes, and so explains the considerable increase in the total protein levels of tapeworms.

It is also possible to attribute the significant increase in total protein concentration after treatment with an aqueous extract of clove flowers to the role of proteins in tapeworm physiology, the course of metabolic processes of cytoplasmic repair, and the growth of cells affected by the effect of the extract, with the difference in the histological structure of the current tapeworms and their protein content, as well as the layer of cytokine. Cytons in the parasite's epidermis (tegument) continuously generate protein and then travel through the cytoplasmic extensions to the distant cytoplasmic region in other tissues. Proteins are crucial to all interactions of biological activities (Al-Kalak, 2001). Differences in total protein concentration of contemporary tapeworms, are first reflected in differences in their fish hosts and food quality. These are crucial because they serve as the interface for the

host's immune system response. The continual change in the surface protection of the parasite's membranes, as it changes the shape of genes and their mode of action, has been pointed out as proof of the diversity in the form of specialized immunity.

It is known that the change in the total protein concentration of the present tapeworms depends mostly on the activity of the DNA in creating proteins and their types, and later the reflection of their relevance and active participation in the body of the parasite. It was noted (Maule and Marks, 2006; Tomas et al., 2019) that these important activities are the reaction of the host's tissues to the creation of anti-materials. In turn, this stimulates the parasite to create immunological components of protein origin in order to modify its genetic character. As a result, it explains the contemporary tapeworms' considerable increase in overall concentration. It is considered an acceptable condition when the total protein is high in the presence of the influence of a plant aqueous extract. Thus, this result was regarded the first topic of employing cloves against parasitic illnesses in order to resist the action of the aqueous

extract of clove flowers and its effects on tegument. Tapeworms and their modes of diffusion and penetration lead to a regulation of immunity, to resist and ensure the vitality of the parasite. (Nazeam et al., 2021) discussed the usage of clove plant as an antibiotic against *Pseudomonas lutzeri*, citing protein suppression of bacteria as a good medicinal alternative for treating skin bacteria.

Adding clove powder to sheep feed was seen as a potential and supplementary approach to controlling parasitic worms without resorting to veterinary chemical medications because of its efficacy in combating parasitic infestation through phytotherapy (Jezek et al., 2021).

Specific efficacy of acetylcholinesterase on tapeworms after treatment with aqueous extract of clove flowers

The current findings reveal a difference in the specific efficiency of the acetylcholinesterase enzyme for tapeworms and therapy with aqueous extract of clove flowers at concentrations of 10g/100ml and 20g/100ml, as shown in Table 6.

Table 6. Specific effectiveness of acetylcholinesterase for *B. acheilognathi* after treatment with aqueous extract of clove flowers and according to study months

Host	Tapeworm	Month	Qualitative effectiveness	
			10g/100ml conc.	20g/100ml conc.
<i>Cyprinus carpio</i>	<i>Bothriocephalus acheilognathi</i>	April	27.84ng/ml	1.01ng/ml
		May	0.13ng/ml	1.49ng/ml
		June	0.001ng/ml	0.001ng/ml

Based on these findings, it can be extrapolated that the effect of the aqueous extract of clove blossoms generated shock at the commencement of exposure to the conditional worms both directly and as a countermeasure to the effect of cloves. Cloves cause nerve burning, followed by inhibition and the resulting cholinergic stress and paralysis or inhibition of acetylcholinesterase during the slow suffocation of worms and their final demise. This tension is the primary cause of the gradual suffocation, paralysis, and death of worms. Enzyme function is known to have a neuronal mechanism and to be a required process for the orchestration of parasite neurotransmission (Tedla et al., 2019). The enzyme was described as an important tegument protein and a bio-stabilizer, and it is the main site of Metabolic interchange, as well as its contribution to the life of worms to escape the effect and the immune response of the host, such as external stimuli, including the effect of the aqueous extract of clove flowers, resulting in a significant increase in the specific activity of the enzyme after exposure of worms to the extract, corresponding to its secretion. For this enzyme to be resistant to the elements.

Due to a change in their neural structure, the specific activity of the enzyme in tapeworms differs from that in other animals (Al-Kalak, 1992; Al-Naftajy et al., 2009; Al-Kalak, 2008). A distinction in the organism's defensive systems and bodily structure enables it to resist the effects of the environment. Therefore, cloves can be viewed as an inhibitor of the parasite's life, the action of the enzyme Bioscavenger, the generation of ATP, and the subsequent destruction and death of parasites.

It is vital to conduct the following investigations linked to the separation, purification, and identification of the active chemical compounds of clove flowers and to demonstrate their effects on the biological components and the enzymatic system resulting from phenotypic and physiological alterations. The current study's findings indicate that the use of plant extracts against helminth infections, as well as the use of plant extracts as medication instead of anti-parasitic pharmaceuticals, is effective against parasites and helminths (Azadbakht et al., 2020). It is now time for parasites that are resistant to it to appear. Finding solutions to this is essential. This was the conclusion of (Batiha et al., 2019), who used a variety of therapeutic plants, including cloves, *Syzygium*

aromaticum, which proved lethal to the growth of Babesia and Theileria. I intended to employ them as therapeutic plant alternatives for parasitic and non-parasitic infections, according to studies and research. According to one study (Ayushi et al., 2020), cloves have antibacterial, antiviral, and anti-inflammatory properties, making them useful in the treatment of a variety of disorders. It is also excellent for liver function, anti-stress, and anti-inflammatory. In addition to being an anaesthetic in dental fillings, it is also an insect repellent and an exterminator of harmful larvae, whether for humans or livestock.

Clove oil helps keep crops protected from harmful pathogens like bacteria and mold. Clove oil was found to be effective, as it prolonged the freshness of cereal goods and improved the quality of food safety (Bhavaniramy et al., 2019). High results and benefits for clove oil against ticks, higher mortality, and low toxicity for bees that feed on plant flowers indicate that it is an excellent treatment for ticks and a means of protecting flowering plants from pathogenic pests that bees feed on. By finding Eugenol in beeswax, the researchers were able to extrapolate that clove oil plays a significant role in ridding plants of hazardous insects, as reported by (Li et al., 2017).

Similar findings were obtained when clove oil was used at various dosages to inhibit the activity of the enzyme acetylcholinesterase in both the spider-mite insect and the fly insect. The fruit is Drosophila sp. As a result, it was thought to be a treatment for insect infestations.

After concluding that the number of *Trypanosoma cruzi* parasites was significantly reduced, a number of researchers remained interested in studying the effect of clove oil against parasitic infections. Clove oil has proven its effectiveness and as a therapeutic alternative to the chemical substance (Berzidazole), which has side effects on laboratory animals (Sarto et al., 2021).

Therefore, it is necessary to undertake additional research on the effectiveness of clove oil against helminths and tapeworms, particularly those parasitic to fish. According to a study (Dawood et al., 2021), the use of plant extracts to combat bacterial and parasitic diseases of fish was considered an alternative approach to sustainable aquaculture due to the synergistic relationship between these extracts and their active components, which are hydrophobic and easily penetrate parasitic cells, causing deformities. Fish-infecting parasites experience cellular and organelle disruption, followed by death, resulting in growth modification and parasite immunity.

Numerous catalytic and non-catalytic signalling proteins drive the neural machinery of the nervous system, which is a strictly regulated process. It coordinates the movement into and out of the host, as well as feeding and reproduction, which are all necessary to the parasite's existence. All of these components are produced by the enzyme acetylcholinesterase and the major neurotransmitter acetylcholine (AL-Niaeemi et al.,

2020b). This enzyme was a valuable resource that piqued the curiosity of numerous parasitological experts, and it was also viewed as a potential target for advancing treatment approaches and tapeworm medication development (Hong et al., 2017).

Conclusions

At a concentration of 20g/100ml, an aqueous extract of Chinese clove blossoms was found to have a dramatic effect on the viability of tapeworms, resulting in rapid paralysis and death. Without causing any negative side effects, this extract can help get rid of these tapeworms in fish. The investigations show that the total protein value of the tapeworms under research varies by species and by the fish species serving as hosts. After being treated with Chinese carnation flower extract at two concentrations (10g/100ml and 20g/100ml), the values of total protein and the specific activity of the acetylcholinesterase enzyme change from month to month.

Conflict of Interest

The author hereby declares no conflict of interest.

Consent for publication

The author declares that the work has consent for publication.

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