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Effect of *Artemisia* extract on *Argulus coregoni* and *Lernaea cyprinacea* infestation in carp fish

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ABSTRACT

Different concentrations of *Artemisia* extract were experimentally tested to treat Lernaea and *Argulus* infestations in common carp (*Cyprinus carpio*) fish with different methods. A total number of 210 carps (50 ±10 g) were collected from private farms at Kafr El-Sheikh Governorate, Egypt, and divided into 7-groups in triplicate: G1 negative-control (apparently healthy carps), G2 positive-control (infested carps with Lernaea and *Argulus*), G3: infested carps treated with dipping in a NaCl solution (5g /L for 5 min. for 3 consecutive days), G4-G5: infested carps treated with dipping in a solution of *Artemisia* extract with concentration 50% and 60% respectively, for 5 minutes, then fishes were accommodated at a recovery tank and inspected for 10 min., G6-G7: infested carps were placed in aquarium supplied with *Artemisia* extract (3 mg/ L and 6mg/L respectively, for 15 consecutive days). The results revealed that *Artemisia* extract had a noticeable positive effect on survival, recovery of infested carps and the parasitic elimination% which increased proportionally with increasing the concentration of the extract, whereas the best results were obtained at G5. Also, there were an improvement in serum biochemical parameters (increased total protein, albumin and globulin with reduced AST, ALT, creatinine and urea), which were proportionally improved with increasing both concentration and the duration of exposure to the extract, (especially at G7). Gross and histopathological examination and alterations of skin, gills, liver and intestine also revealed a noticeable improvement to the normal in the groups treated with *Artemisia* extract in comparison with G2 especially G6 and G7. Consequently, application of *Artemisia* extract is a safe and optimal method for treatment of *Argulus* and Lernaea parasitism in cultured carp.

Introduction

Fish is an important source of animal protein for human consuming. Among various fish species, carps (family; *Cyprinidae* which is the largest family of freshwater fishes), are widely spread around the world (Nelson *et al*., 2016). several microbes (bacteria, virus, and parasites) infect fish (Mhaisen, 2018). The parasitic infestation in Egypt represent more than 80% of diseases affecting freshwater fish (Nofal *et al*., 2016). It is mostly affecting fish growth performance, health, survival and deteriorate their food value. Whereas parasitic infestation hazards on fish health can be mechanical, or affecting reproduction and physiology of fish, or may even lead to mortality (Hamzah *et al*., 2017).

Ectoparasites are considered the most common parasites affecting cultured fishes. *Argulus* species (fish lice) and *Lernaea cyprinacea* (anchor worm) are common members of these ectoparasites that can infest and cause disease in fish. *Argulus* species (fish lice) which are belonging to family Argulidae, are members of the large group of branchiura parasites that infest fish and cause disease. *Argulus* infestations are most common in wild and cultured freshwater fish, particularly koi, goldfish, and other cyprinids (carps and minnows) (Khan *et al*., 2017). The anchor worm (*Lernaea cyprinacea* Linnaeus, 1758) is belongs to family Lernaeidae, order Cyclopoida, class Hexanauplia, subphylum Crustacea of the phylum *Arthropoda* (WoRMS, 2021). This worm is a common ectoparasite in freshwater fish, especially family Cyprinids and other fishes (Attia *et al*., 2022), it adheres to the fish body with their anchor (Rohlenová *et al*., 2021). Causing intense inflammation and injuries, which leading to secondary bacterial and fungal infections (Al-Dulaimi *et al*., 2021). *Argulus* and Lernaea are obligate parasites attach themselves to the external body parts and gills of fish, and suck blood, mucous and body fluids of the host. Both of these parasitic infestation in fish causing several sings such as repeated rubbing of fins, lethargy, restlessness, off food, reduction in the body weight, reduced pigmentation, damage to skin and underlying musculature causing severe injuries and ulcerations, haemorrhages, fibrous nodules were found on the body surface of parasitized fishes and even leading to mortality (Raissy *et al*., 2013; Abbas *et al*., 2014; Ahamad *et al*., 2016). Larger numbers of the parasites on gills could be interfere with respiration, causing asphyxia and death (Hossain *et al*., 2018). The secondary infections caused by these infestations sometimes worsen and kill the infested fish.

The conventional treatment of ectoparasite with chemicals has been used for many years, however the threats of bioaccumulation and residual formation in the host tissue caused by frequent use of these chemicals have led to the need of other alterative control methods (Klinger and Floyd, 2002).

Artemisia is a short shrub medicinal plant usually located at Northern Africa and the Middle East, which used as an antimicrobial, antihypertensive, antidiabetic and antispasmodic agents (Moufid and Eddouks, 2012). *Artemisia herba-alba* is also used for controlling and treatment of parasitic infestation of several parasites such as roundworms, hookworms, pinworms, tapeworms and flukes, because of its content of certain chemicals which have antiparasitic effect (Soliman *et al*., 2017). It is also useful in health supplements because of its high content of phenolic acids and flavonoids (Vagi *et al*., 2005).

The present study is aimed to use the alcoholic extract of *Artemisia* to treat common carps (*Cyprinus carpio*) infested with argulosis and lernaeosis, via different methods; as this plant extract is available, cheap and its use is generally considered harmless to the environment.

Materials and methods

Extraction of artimizia

Artemisia air dried leaves were subjected to exhaustive extraction with ethyl alcohol (95%) using Soxhlet apparatus till complete extraction. The obtained extract was cooled filtered and evaporated under vacuum for concentration, then was dissolved in Dimethylsulfoxide (DMSO). The

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solution was mixed with petroleum ether (BP, 40-60°C), and centrifuged at 5000 rpm. (Christen and Veuthey, 2001).

Clinical examination

Fishes were grossly examined for the presence of any clinical abnormities and any external parasite according to (Noga, 2010).

Experimental design

A total of 180 common carps with apparent natural external parasitic infestation of *Argulus* and Lernaea (which were identified according to the morphological characters and microscopical examination), and another 30 apparent healthy with normal behavioral reactions, (with average body weight of 50±10 g), were collected from private farms at Kafr El-Sheikh Governorate, north Egypt, in a separate air blower plastic containers filled with water from the farm, and transferred to AHRI, Tanta Lab., Egypt. They were randomly distributed and stocked in glass aquaria (70 x 40 x 30 cm) filled with non-chlorinated water and supplied with compressed air via air-stones from air pumps and fed on balanced commercial pellets (with a rate of 3% of its body weight) twice daily at 9:00 am. and 3:00 pm.

Carps were divided into seven groups (7-G) in triplicate (10 carps were placed in each fish tank for each treatment trial, with three replicates were achieved for each group, at a rate of 30 fish / group) as follow: (G1): Negative-control, non-infested, apparently healthy carps without any treatment. (G2): Positive control, apparently infested carps with *Argulus* and Lernaea without any treatment. (G3): Infested carps were treated with dipping in sodium chloride solution at a concentration of 5g /L for 5 minutes daily, for 3 consecutive days. (G4 and G5): Infested carps were stopped feeding a day before the start of these treatment trials, then treated with dipping in solutions of *Artemisia* extract with concentrations of 50% and 60% respectively, for 5 minutes, then carps were accommodated directly to an aerated freshwater aquarium for recovery, changes in fish behaviour were observed during these treatment trials and even for 10 minutes after transferring to the recovery tank such as; irritation in fish swimming, increase in number of opercular movements, hitting the insides of the tanks and trying to jump out of the tank then carps returned to the normal behavior. (G6 and G7): Infested carps were placed in aquarium supplied with *Artemisia* extract with a concentration of 3mg/L and 6mg/L respectively for 15 consecutive days.

The aquaria were daily cleaned, and the fish excreta were siphoned, the dead fish was daily recorded and removed. After the end of the treatment trials fishes were inspected daily for 21 days for the presence of any moralities or appearance of any parasitic infestations again.

Survival and parasitic elimination rates calculations

At the end of the experiment the survival and parasitic elimination rates of each group was calculated to determine the best treatment.
Survival % = 100 × final number/initial number.
Parasitic elimination % = 100 × eliminated parasites number/initial number.

Sampling

Blood samples

Blood samples of all groups were taken twice; post treatment (PT) and at experiment end (three weeks post-treatment). Carps were not fed in the 24 hours immediately prior to sampling. Fishes were anaesthetized with 50 mg/L of benzocaine solution. Blood samples were drawn from the caudal vein (5 fish/group) and the samples were taken in clean dry centrifuge tube without anticoagulant. The collected blood was centrifuged at 5000 rpm for 5 minutes at room temperature for serum collection which was stored at -20°C for further assays.

Some serum biochemical investigations were measured by enzymatic methods using an automated analyzer which included hepatic and kidney health indicators: aspartate and alanine aminotransferases (AST and ALT) activities (Reitman and Frankel, 1957), creatinine (Houot, 1985), urea (Batton and Crouch, 1977), total protein (TP) (Doumas *et al*., 1981), and albumin (Reinhold, 1953). Globulin was calculated by subtraction of albumin value from TP, and albumin/ globulin ratio was also estimated by dividing both values.

All testes were determined using commercial kits (Spectrum, ELITech, BioSystems and Biomed Companies, Egypt) in accordance with the manufacturer's instructions.

Histopathological specimens and examination

At the end of the experiment, specimens from skin, gills, liver and intestine were collected and evaluated for any gross abnormalities before being fixed in 10% neutral buffered formalin. The fixed samples were cleaned, dehydrated, clarified, and paraffin embedded. The paraffin blocks were sectioned at a 5-micron thickness. Hematoxylin and eosin were used to stain the sections according to (Bancroft and Layton, 2012), and then they were examined under a light microscope (Olympus BX50, Japan).

Water samples

Water temperature, pH, dissolved oxygen, and salinity of each treatment tank were recorded, by using digital thermometer, pH indicator strips (MColorpHast-Germany), dissolved oxygen digital meter (HI 9142, HANNA, China) and salinity meter (YSI Eco Sense EC300 Salinity/Conductivity 151, China), respectively.

All water quality parameters were compared with the acceptable ranges according to the recommended standard guidelines (APHA, 1998).

Statistical analysis

The IBM SPSS22 (2012) software program (USA, Chicago, IL, IBM SPSS Inc.). was used to conduct statistical analyses of the data. Significance were determined at $P \leq 0.05$

Results

The clinical picture

Argulus and Lernaea were distributed along both sides of the body surface, on skin, fins and gills of all infested carps. *Argulus* appeared grossly as a translucent parasite with dorsoventrally flattened body, attached on the epidermis of the skin as shown in Fig.1A-B. However, Lernaea appeared grossly as grayish to greenish worm-like copepods as short threads embedded in the musculature of fish and protruded on the fish external body as shown in Fig.1C-D, and the surrounding sites of attachment and penetration of Lernaea appeared inflamed and swollen with severe lesions including hemorrhagic circumscribed ulcers as shown in Fig.1E.

The clinical picture of the infested carps was in the form of lethargy, poor appetite, reduction in body weight, restlessness, repeated rubbing of fins, abnormal swimming, erratic movements, nervous manifestation with easily detached scales and abraded areas, excessive mucus secretion on skin and gills, open wounds with or without haemorrhages on the surface of the body (Fig.1F-G). Gills and Internal organs (Liver, kidney, gills and heart) revealed diffuse mild to moderated paleness (Fig.1H-I).

At the end of the experiment heavy infestations of high parasitic load of both *Argulus* and Lernaea with high mortalities was observed at G2.

On other hand, there were a good parasitic elimination rates, clinical

improvement with fasten recovery and healing of injuries and ulcerations which caused by *Argulus* and Lernaea infestation in all groups treated with *Artemisia* extract in comparison with G2 and G3 especially at G5 and G7, as shown in Fig.1J-K-L, which showed a complete parasitic elimination and an improvement in the healing of injuries and ulcerations of skin and fins of carps from G5 and G7. A noticeable healing of ulcerations which even reached up to the normal appearance of skin and fins of a carp was observed in G7 (Fig.1M).

Fig.1. (A-B); A common carp fish from G2 naturally infested with *Argulus* species. (C-D); A common carp fish from G2 naturally infested with *Lernaea cyprinacea*. (E); Naturally infested carp with Lernaea from G2 showing ulceration, hemorrhage and inflammation in the surrounding site of attachment and penetration of the parasites around the mouth. (F-G); Naturally infested carps with *Argulus* and Lernaea from G2 showing severe ulcerations and inflammation of skin and fins. (H-I); Naturally infested carps with *Argulus* and Lernaea from G2 showing paleness of liver, kidney and other internal organs. (J-K); Common carps from G5 showing complete parasitic elimination with improvement in the healing of injuries and ulcerations. (L-M); common carps from G7 showing a noticeable improvement of the healing of ulcerations which even reached upto the normal appearance of skin and fins.

Survival and parasitic elimination rates

This study revealed that the treatment with *Artemisia* extract showed a significant dose-dependent increase in the survival rate of carps against *Argulus* and Lernaea infestations, where all the treated groups had an increase in survival rates and killing of both parasites compared with the G2 in the ascending manner (G3-G6-G4-G7-G5), as shown in Table 1.

Table 1. Survival and parasitic elimination percentage of infested Carp treated with NaCl and *Artemisia* extract against *Argulus* and Lernaea infestation.

	Survival percentage (%)	*Argulus* elimination (%)	Lernaea elimination (%)
G2	22	-	-
G3	40	30	35
G4	80	70	75
G5	100	100	100
G6	70	55	60
G7	95	84	90

Survival rates of carps and the parasitic elimination percentages were proportionally increased, with increasing the concentration of *Artemisia*, from 80% survival, 70% parasitic elimination of *Argulus* and 75% Lernaea elimination and in case of dipping in a solution with 50% *Artemisia* concentration (G4) to 100% both survival and parasitic elimination of *Argulus* and Lernaea in case of dipping in 60% concentration of *Artemisia* (G5). The results revealed 70% survival, 55% *Argulus* elimination and 60% Lernaea elimination, in case of the concentration 3 mg/L *Artemisia* (G6), to 95% survival, 84% *Argulus* elimination and 90% Lernaea elimination, in case of the concentration 6 mg /L *Artemisia* (G7). On other hand, the survival rate was 40% and the elimination of *Argulus* and Lernaea were 30% and 35% respectively, in case of G3. Whereas a significant high mortality rate (78 %) was recorded in G2 (positive control) as shown in Table 1. So, the best results of survival and elimination of *Argulus* and Lernaea rates were recorded at G5 followed by G7.

Serum biochemical parameters

All the measured serum biochemical parameters ($p<0.05$) were improved (higher total protein, albumin, globulin and reduced AST, ALT, creatinine and urea), in all treated groups in comparison with the positive control group as shown in Table 2. It is also important to mention that over time after treatment, these levels directed toward the normal levels compared to G1 especially at G7 followed by G6, G5, G4 then G3 respectively, as shown in Table 3. which revealed the levels of serum biochemical parameters at the end of the experiment. So, it was approved that most of serum biochemical parameters levels were improved proportionally with increasing both the concentration and the duration of exposure to *Artemisia* extract. Treatment with *Artemisia* extract insignificantly affected the albumin/globulin (A/G) ratio in all groups.

Gross and histopathological alterations

At the end of the experiment the gross and histopathological alterations were recorded in skin, gills, liver, and intestine as follow:

In Group 2, skin showed diffuse moderate to severe ulcerations with haemorrhages, diffuse moderate to severe hyperplasia and degeneration of the epidermis at the margins of the wound with hyperplasia of dense connective tissue in sub cutaneous areas. Lernaea were embedded and

Table 2. Effect of Nacl and *Artemisia* extract on some serum biochemical parameters of infested Carp with *Argulus* and Lernaea in all groups post treatment

G	AST (U/L)	ALT (U/L)	Creatinine (mg/dl)	Urea (mg/dl)	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
G1	45.3±2.02^{a}	44.3±4.84^{a}	0.11±0.02^{a}	7.76±0.23^{a}	3.7±0.25^{d}	1.36±0.16^{c}	2.34±0.08^{d}	0.58±0.05^{a}
G2	115.3±6.6^{d}	98±1.73^{c}	0.26±0.03^{d}	14.8±0.45^{d}	2.16±0.1^{a}	0.78±0.05^{a}	1.38±0.08^{a}	0.56±0.03^{a}
G3	99±4.17^{d}	92±1.16^{c}	0.22±0.02^{c}	11.76±0.75	2.46±0.08^{a}	0.93±0.08^{a}	1.53±0.01^{a}	0.6±0.046^{b}
G4	78±2.08^{c}	85±2.64^{c}	0.22±0.01^{c}	11.16±0.178^{c}	2.8±0.05^{b}	1.06±0.08^{b}	1.74±0.13^{b}	0.61±0.09^{a}
G5	69.66±0.881^{b}	79.33±1.76^{d}	0.21±0.00^{c}	10.46±0.145^{b}	2.93±0.088^{b}	1.08±0.04^{b}	1.85±0.10^{b}	0.58±0.05^{b}
G6	64.33±1.203^{b}	69.66±1.6^{b}	0.19±0.00^{b}	9.03±0.291^{b}	3.06±0.03^{b}	1.17±0.03^{b}	1.89±0.05^{b}	0.62±0.03^{c}
G7	55±1.73^{b}	58±1.52^{b}	0.17±0.00^{b}	8.76±0.176^{a}	3.26±0.08^{c}	1.23±0.03^{c}	2.03±0.15^{c}	0.6±0.005^{c}

Data are expressed as Mean±S.E. Different superscript letters in the same column statistically significant difference at P< 0.05.

circled with connective tissues. The liver section showed severe congestion and marked hepatic necrosis (Fig. 2A), the gills section showed hypertrophy and hyperplasia in many areas with severe adhesion of primary and secondary lamellae and sever chondrodysplastic lesions within the cartilage of gill lamellae associated with presence of the Lernaea parasites (Fig. 2B), the intestinal section showed marked necrotic and sloughing changes within the intestinal mucosa associated with presence of the *Argulus* parasites within the lumen of the intestine (Fig. 2C), also there were catarrhal, necrotic and sloughing changes within the intestinal mucosa associated with the presence of the *Argulus* parasites within the lumen of the intestine (Fig. 2D).

In Group 4, the intestinal section of fish from G4 showed marked necrotic and sloughing changes within the intestinal mucosa associated with the presence of the arugulas parasites within the lumen of the intestine (Fig. 2E). Also, there were desquamative changes associated with presence of the *Argulus* within the lumen of the intestine as shown in (Fig. 2F).

At group 5, there were a complete elimination of the parasites externally with an improvement in healing of injuries and ulcerations of skin and fins of fish. Whereas the hepatic section of fish showed mild vacuolation of hepatocytes and normal pancreatic cells (Fig. 2G). The gill section showed mild hyperplasia of the cartilage of the primary lamellae and mild adhesion between the secondary lamellae (Fig. 2H). The intestinal section showed normal mucosa associated with presence of dead parasitic section within the lumen of the intestine (Fig. 2I).

In Group 6, there were an improvement in healing of injuries and ulcerations of skin and fins of fish. The gill section showed mild adhesion between the secondary lamellae (Fig.2J).

In Group 7, a normal appearance of skin and fins of fish was grossly observed. The hepatic section showed mild vacuolation of hepatocytes and normal pancreatic cells (Fig. 2K). Gill section showed normal primary and secondary lamellae (Fig. 2L). Intestinal section showed normal mucosa with pseudostratified epithelium with goblet cells (Fig. 2M).

Water quality parameters

During the experiment the water quality parameter mean values±SE were kept at: temperature (26±1°C), pH (8±0.2), dissolved oxygen (8±0.5) mg/L, and water salinity 10-12 ‰.

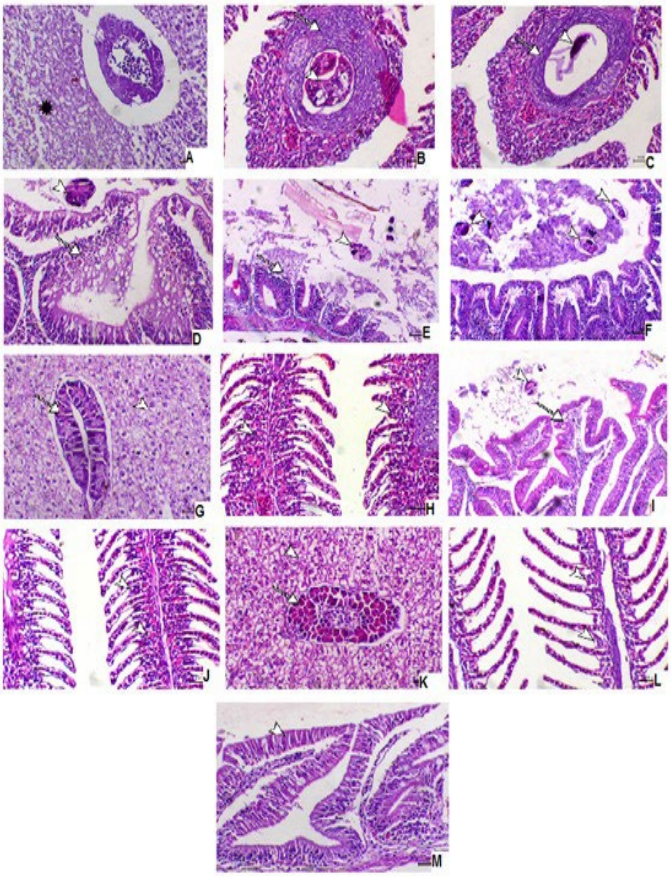

Fig. 2. (A)- Hepatic section of carp from G2 showing marked hepatic necrosis (asterisk), H&E, X200, bar= 100 µm. (B)- Gills section of carp from G2 showing severe chondrodysplastic lesions within the cartilage of gill lamellae (arrow) associated with presence of the parasites (arrowhead), H&E, X200, bar= 100 µm. (C)- Gills section of carp from G2 showing severe chondrodysplastic lesions within the cartilage of gill lamellae (arrow) associated with presence of the lernaea parasites (arrowhead), H&E, X200, bar= 100 µm. (D)- Intestinal section of carp from G2 showing catarrhal, necrotic and sloughing changes within the intestinal mucosa (arrow) associated with presence of the arugulas parasites within the lumen of the intestine (arrowhead), H&E, X200, bar= 100 µm. (E)- Intestinal section of carp from G4 showing marked necrotic and sloughing changes within the intestinal mucosa (arrow) associated with presence of the arugulas parasites within the lumen of the intestine (arrowhead), H&E, X200, bar= 100 µm. (F)- Intestinal section of carp from G4 showing desqumative changes associated with presence of the arugulas parasites within the lumen of the intestine (arrowheads), H&E, X200, bar= 100 µm. (G)- Hepatic section of carp from G5 showing mild vacuolation of hepatocytes (arrowhead) and normal pancreatic cells (arrow), H&E, X200, bar= 100 µm. (H)- Gill section of carp from G5 showing mild hyperplasia of the cartilage of the primary lamellae and mild adhesion between the secondary lamellae (arrowheads), H&E, X200, bar= 100 µm. (I)- Intestinal section of carp from G5 showing normal mucosa associated with presence of dead parasitic section within the lumen of the intestine (arrowhead) (arrow), H&E, X200, bar= 100 µm. (J)- Gill section of carp from G6 showing mild adhesion between the secondary lamellae. (K)- Hepatic section of carp from G7 showing mild vacuolation of hepatocytes (arrowhead) and normal pancreatic cells (arrow), H&E, X200, bar= 100 µm. (L)- Gill section of carp from G7 showing normal and primary secondary lamellae (arrowhead) (arrow), H&E, X200, bar= 100 µm. (M)- Intestinal section of carp from G7 showing normal mucosa with pseudostratified epithelium with goblet cells (arrowhead), H&E, X200, bar= 100 µm.

Table 3. Effect of Nacl and *Artemisia* extract on some serum biochemical parameters of infested Carps with *Argulus* and Lernaea in all groups at the end of the experiment.

G	AST (U/L)	ALT (U/L)	Creatinine (mg/dl)	Urea (mg/dl)	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
G1	46.33±0.66^{a}	44.66±1.45^{a}	0.13±0.0032^{a}	7.56±0.176^{a}	3.73±0.233^{d}	1.38±0.06^{d}	2.35±0.20^{d}	0.58±0.66^{d}
G2	80.33±1.20^{c}	70.67±3.48^{c}	0.21±0.026^{c}	12.09±0.277^{c}	2.67±0.08^{a}	1.006±0.058^{b}	1.664±0.045^{b}	0.6±0.043^{b}
G3	70.33±2.90^{c}	66.33±0.88^{b}	0.19±0.015^{b}	11.16±0.375^{c}	2.76±0.218^{a}	1.16±0.098^{b}	1.6±0.144^{a}	0.72±0.1587^{c}
G4	60.33±0.882^{b}	62±2.08^{b}	0.17±0.0067^{b}	10.53±0.0178^{b}	2.83±0.0333^{b}	1.11±0.0448^{b}	1.72±0.0625^{b}	0.64±0.1324^{a}
G5	50.33±1.76^{b}	55.66±1.20^{b}	0.16±0.0131^{a}	9.16±0.176^{b}	3.1±0.057^{c}	1.15±0.0203^{b}	1.95±0.0524^{c}	0.58±0.0133^{b}
G6	48.66±0.88^{a}	49±0.57^{b}	0.17±0.015^{b}	8.13±0.20^{a}	3.53±0.032^{c}	1.32±0.032^{c}	2.21±0.052^{c}	0.590.046^{b}
G7	44.33±1.20^{a}	46.33±0.33^{a}	0.12±0.003^{a}	7.83±0.06^{a}	3.76±0.08^{d}	1.4±0.011^{d}	2.36±0.085^{d}	0.59±0.040^{b}

Data are expressed as Mean±S.E. Different superscript letters in the same column statistically significant difference at P< 0.05.

Discussion

In the last few years, fish culture rapidly expanded its driving economic growth and shared in global food security (FAO, 2020). However, it faced many of emerging microbial hazards which threaten its sustainable production (Iqbal *et al*., 2020). Argulosis and Lernaeosis are considered the most common and economically important ectoparasitic diseases causing infection and even mortality in aquaculture especially in carp culture. Ecto-parasite attaches to skin, fins and gills of fish sucking blood, mucous and body fluids of the host resulting in anaemia, localized hyperplasia, injuries, and ulcerations.

Previous fish farmers have used conventional antiparasitic chemicals for many years for controlling fish parasites, however the previous research revealed an accumulation of these residues in fish tissues and their discharges into the aquatic environment which may affect and destruct the habitat (Wunderlich *et al*., 2017). Therefore, it is necessary to find alternative, cheap, effective and safe methods for controlling and treatment of parasites such as plant extracts and essential oils. The results obtained in the current investigation revealed that, the increased of the concentration of *Artemisia* extract had proportionally caused increases in the survival and parasitic elimination percentage of carps infested with these parasites than the positive control group, which were reached up to100 % for both, in case of dipping in a solution with 60% *Artemisia* concentration. Also, it is important to clarify that elongation of the duration of exposure to *Artemisia* extract resulted in faster recovery, healing and improvement of serum parameters of infested fish, perhaps this is due to the fact that the extract increase the immune efficiency of treated carps. Many of reported biological effects of this plant extract such as antioxidant, anthelmintic, antivenom, nematocidal, antibacterial, *in vitro* antileishmanial activities in addition to the pharmacology and toxicology also were approved in various reviews (Mohamed *et al*., 2010). The efficacy of *Artemisia* may be due to its medicinal properties, which have a several compounds such as alkaloids, glycosides such as artemisinin, santonin, absinthin. Thujone and allicin saponin, tannins and other nonpolar water-soluble substances (Al-Khazraji, 1991). Artemisinin has proven to be effective against multi-drug resistant Plasmodium strains and other parasitic species in humans, small ruminants and chickens (Jones-Brando *et al*., 2006; Mishina *et al*., 2007; Ma *et al*., 2004; Brisibe *et al*., 2008; Ferreira *et al*., 2006; Hart *et al*., 2007; Turner and Ferreira, 2005). As artemisinin destroys the parasitic cells through the generation of highly reactive oxygen-based free radicals or electrophilic intermediates, by alkylating and oxidizing lipids and proteins of the parasite membranes as well as inactivation of channel proteins (Ridley and Hudson, 1998).

The obtained results agreed with Al-Dulaimi *et al*. (2021) who showed that the application of aqueous extract of *Artemisia herba-alba* was effective against *Lernaea cyprinacea* infestation in common carp. As well as Mamadou *et al*. (2013) who mentioned that *Argulus* species can be controlled by some plant extracts such as the crude extract of the plant Ocimum gratissimum in Nile tilapia. Moreover, Ekanem and Brisibe (2010) who indicated that artemisinin, artesunate, and the ethanolic extract of *Artemisia annua* are also effective in elimination and killing of monogenean parasites of *H. longifilis*. Also, several reports recorded that the extract of *Artemisia herba-alba* was affective against protoscolices of *Echinococcus granulosus in vitro*, and *in vitro* inhibition of egg hatching of the nematode *Haemonchus contortus* (Al-Rubbie, 1999; Al-Quraishi *et al*., 2015; Ahmed *et al*., 2020).

The biochemical examination of serum of infested carps with *Argulus* and Lernaea of G2 showed hypoproteinaemia, hypoalbuminaemia and hypoglobulinaemia (possibly due to excess suction of blood and body fluid), and high levels of AST, ALT, creatinine and urea when compared with their levels in serum of normal healthy carps (G1), as *Argulus* and Lernaea infestation could be resulted in renal and hepatocellular damage and dysfunction or increased synthesis of their enzymes. Ahamad *et al*. (2016), also recorded that toxin produced by *Argulus* infestation in freshwater carp lead to damage of liver and kidney. Creatinine level is an important indicator of renal health, as it is an easily measured byproduct of muscle metabolism which excreted unchanged through kidneys; where if the filtration in the kidney is deficient, Creatinine blood level rise. Whereas the elevation in the Urea level in the infected carps may be due to gill dysfunctions as the Urea excreted mainly through the gills (Murray *et al*., 1990). This result agrees with Rastiannasab *et al*. (2016) who stated that the levels of AST, ALT enzymes activities as well as creatinine and urea were higher in the in common carp infested with *Dactylogyrus* spp. and *Gyrodactylus* spp. when compared with non-infected fish.

However there were improvement in the results of serum biochemical parameters (increased total protein, albumin and globulin with reduction in AST, ALT, creatinine and urea) in all treated groups compared to the control positive group, especially in the groups which treated with *Artemisia* extract, with the knowledge that this improvement in serum parameters occurred more faster to the normal with increasing both concentration and the duration of exposure to the extract as occurred in G7, this could be due to revealing the stress diminution by decreased protein catabolism and hepatocellular and renal damage which occurred as a result of the parasitic control and elimination by *Artemisia* extract. This result agreed with Soliman *et al*. (2017) who recorded that total protein, albumin, and globulin levels were increased, while AST, ALT, creatinine and urea levels decreased in all groups of *Oreochromis niloticus* treated with *Artemisia* extract. Also, Kumar *et al*. (2012) recorded that serum parameters showed reduction in AST and ALT and in goldfish treated with azadirachtin group than the positive control group.

In regarding gross and histopathological alterations of infested carps with *Argulus* and Lernaea, the results of the present study revealed severe histopathological alterations especially in the gills, liver and intestine of infested fish. These alterations characterized by diffuse moderated to sever ulceration with haemorrhages, diffuse moderated, hyperplasia of dense connective tissue in sub cutaneous areas, degenerated muscle fiber, hypertrophy and hyperplasia in many areas with severe adhesion of primary and secondary lamellae of gills leading to hypoxia. The parasites feds on blood and other bodily fluids and cause further harm to the fish by injecting digestive enzymes that can lead to systemic illness causing liver and kidney dysfunctions (Ahamad *et al*., 2016), severe congestion and marked hepatic necrosis was found. Marked necrotic and sloughing changes within the intestinal mucosa associated with presence of the *Argulus* parasites within the lumen of the intestine, these results agree with the results recorded by Taylor *et al*. (2005); AL-Darwesh *et al*. (2014); Ahamad *et al*. (2016) and Attia *et al*. (2022). It is important to mention that the treatment of Lernaea and *Argulus* infestations with *Artemisia* extract accelerate the healing of tissues, especially with increasing the duration of exposure to the extract, this result could be due to the positive effect of *Artemisia* extract on the immune efficiency and resistance of fish.

Conclusion

Application of *Artemisia* extract for treatment of *Argulus* and Lernaea parasitism in cultured carp has been approved to cause a reduction and even complete elimination of these parasites, noting that the treatment with higher *Artemisia* concentrations with short exposure time results in an increased survival and the parasitic elimination percentage, however increasing the duration of exposure with lower concentrations accelerate healing and recovery of fish. Consequently, further investigation is recommended on the use of the plant against other fish parasites responsible for fish mortalities under culture conditions.

Conflict of interest

The authors declare that they have no conflict of interest.

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