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The Impact of Leek (*Allium ampeloprasum* L.) Extract on *Prohemistomum vivax* (Sonsino, 1892) Encysted Metacercariae in *Clarias gariepenus* Fish.

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ABSTRACT

The anti-parasitic properties of leek *(Allium ampeloprasum* L.) leaf extract (LLE) were evaluated. *Clarias gariepenus*, catfish were collected from fish farm at Kafr El-Shaik governorate, Egypt. Fish were examined for the presence of encysted metacercariae (EMC), and the prevalence rate of infection was 100 %. Prohemistomatid EMC were isolated from muscles and liver. Fish were assigned to 3 groups, the 1st two groups were fed with diet containing 2% and 4% LLE for 45 days, while the 3rd group was fed with basal diet only as a control group. Histopathological studies showed that the EMC were embedded in the affected tissues inducing pressure atrophy, degenerative changes and necrosis with some tissue reactions as formation of mononuclear cells and melanomacrophages. The experimental infection of mice with EMC revealed that *P. vivax* were the only recorded digenetic trematode. A significant decrease in number of adult worms recovered from mice inoculated with both *in vivo* and *in vitro* treated EMC in comparison with control group. The *in vivo* treatment with LLE 4% and 2% was more effective than *in vitro* treated EMC. To conclude, LLE have strong anthelmintic activity and can be used in killing or inactivation of metacercarial parasitic infection in fish.

Keywords: *Prohemistomum vivax, Allium ampeloprasum L.*, *Clarias gariepinus*

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INTRODUCTION

Fish was considered as a proteinaceous food since the beginning of mankind. Catfish, *Clarias gariepinus* is one of the most famous and cheapest fishes in Egypt. However fish is considered as a bi-edged weapon besides its benefits, it may act as an intermediate, paratenic (transport) or final host for several stages of parasites [1].

Infections by fish borne trematode affect health of more than 18 million people all over the world, mostly in Asian countries. These flukes create noticeable morbidity and cause significant damage to aquaculture in developing countries [2]. While fish zoonotic trematodes are well-known causes of liver and intestinal trematode diseases in humans [2], they are among the most neglected tropical disease agents [3].

Digenetic trematodes and their metacercariae were be considered as one of the most prevalent parasites infecting fish giving rise to low weight gain, high mortality, unmarketability and some of these parasites may possess zoonotic importance [4].

Prohemistomum vivax is transmissible to man and cause acute enteritis in case of heavy infestation and rarely may cause death [4]. Marzouk *et al.* [5] and Alghabban [6] recorded the same parasite from rats experimentally fed on encysted metacercariae (EMC) from *Clarias gariepinus*.

The limitation of chemotherapy in aquaculture and using of natural treatments could promote the consumption of aquaculture products. Also, their use could reduce costs of treatment and be more environmentally friendly as they tend to be more biodegradable than artificial molecules and they are less likely to produce drug resistance in parasites due to the high variety of plant extract molecules [7].

Elephant garlic or Leek (*Allium ampeloprasum* L.) is one of the daily edible green vegetables for Egyptians; it is cheap and widely cultivated. It is belonging to the Allium genus [8] and a species related closely to garlic (*Allium sativum* L.) [9].

Some recent researches on Allium reported that *Allium ampeloprasum* L. have more therapeutic property than other Alliums and conventionally used as treatments for over 5000 years, but only a scarce researches have been carried out on it. It is considered that its medicinal benefit is much greater than garlic (*Allium sativam* L.) and can play more effective role in diet therapy [10].

It was found that elephant garlic extracts, as other Allium species, had antimicrobial factors named allicin containing diallylsulphide and thiosulfinate which are extremely effective against main foodborne pathogens [11], actively and directly kills parasites [12].

In aquaculture, garlic has been demonstrated to exhibit antibacterial activity against a number of pathogenic bacteria of freshwater fish, but only a few reports describe the use of garlic extracts for management of parasitic diseases in fish [13]. Garlic extracts have previously been reported to eradicate ectoparasitic trichodinids [14] and has demonstrated *in vitro* toxicity towards aquaculture significant protozoan parasites (*Ichthyophthirius multifiliis* [15], *Neoparamoeba pemaquidensis* [16], *Spironucleus vortens* [17]). Furthermore, allicin-containing garlic extracts offer prospect for development as a remedy for infections with monogenea in intensive aquaculture [14].

There were scant studies proved the effect of Allium species on EMC of digenetic trematodes in fish. Wei Ping [18] concluded that garlic juice are capable of *in vitro* killing all tested *Clonorchis sinensis* metacercariae isolated from fish fillets after 36 hours. Also, *in vitro* treatment of unidentified EMC isolated from catfish muscles by *Allium cepa* bulb (onion) converted it to opaque immobile mass, 3 days after treatment [19].

As there were no available researches reported the effect of *Allium ampeloprasum* L. on EMC in fish, our study is performed to evaluate the effect of leek leaf (*Allium ampeloprasum* L.) extract on the infectivity of *Prohemistomum vivax* EMC infecting catfish (*C. gariepenus*). Histopathological changes resulted from EMC infections were also recorded after and before treatment with of *Allium ampeloprasum* L.

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MATERIALS AND METHODS

Fish:

Around 165 catfish, *C. gariepinus* were obtained from private fish farm at Kafr El-Shaik governorate, Egypt and transported alive to laboratory of Hydrobiology Department at National Research Centre for acclimatization in aerated free-flowing freshwater for 2 weeks in glass aquaria. The clinical examination of fish was performed according to Conroy & Hermann [20]. During acclimatization, fish were fed twice daily with commercial diets. A random sample of 30 fish were examined for the presence of EMC macro and microscopically and the prevalence rate of it. The total length and weight of the all inspected catfishes (*C. gariepinus*) ranged from 22 - 28 cm total length and 65 - 130 g weight.

Preparation of plant extract:

Leaves of leek (*Allium ampeloprasum* L.) were obtained from local market, Cairo, Egypt and allowed to dry in fresh air. One kg of dried leaves was extracted using 95% methyl alcohol by percolation until it was exhausted and filtered off. Then the collected filtrates were evaporated under reduced pressure and low temperature using a rotator evaporator. The obtained residue, leek leaf extract (LLE) was kept at 8˚C until use [21].

Diet preparation:

A commercial diet was divided into two parts one mixed with LLE 2% (2g/ 100g diet) and the other with 4% (4g/ 100g diet) and little amount of water to make it into small pellets and the two diets were allowed to dry then stored at 4˚C until use.

Fish feeding:

One hundred and thirty five fish (*C. gariepinus*) were randomly distributed into 3 groups, each group containing 45 fish (15 x three replicates) and fed for 45 days. The first 2 groups were fed with commercial diet containing 2% and 4% LLE while the 3rd group was fed with basal commercial diet without LLE as control.

Isolation of EMC from infected muscles of *C. gariepinus*:

After 45 days (end of the feeding experiment), fish (*C. gariepinus*) from each group were inspected for the presence of EMC. This was done by squeezing a piece (1g) of muscle between two glass slides and the examination under a dissecting microscope. EMC were examined, mechanically removed (minced in a blender) and isolated, counted according to Khalil [22]. With the aid of a dissecting microscope, they were then withdrawn by a Pasteur pipette and kept in 0.75% saline solution for the infection of laboratory mice. For preparing permanent mounts of EMC, the technique described by Kruse & Pritchard [23] was applied. The collected EMC was identified according to Mahdy [24].

Experimental design and infection:

The experiments were performed on 36 albino mice weighing 20-25±5g and of 6-8 week's age. They were obtained from small breeding house animal in the National Research Centre. The mice were kept in the laboratory for one week before the experimental study and maintained on a standard rodent diet and water available adlibitum. The experimental protocol was approved by the Local Ethics Committee and Animals Research.

The isolated EMC were orally inoculated to mice by using rubber catheter and a mouth gag in accordance with protocols approved by the Cairo University institutional animal care and use committee (stomach tube).

Experimental design:

The 36 mice were assigned into six groups (Gps), each Gp. contains 6 mice (Table1). All mice were inoculated with about 200±50 EMC/ mouse except the mice in Gp.6 left as negative control Gp. (without inoculation), while the mice in Gp.5 were inoculated with (200 EMC/mouse) without treatment with LLE as a positive control group. The mice in Gps.1 and 2 were inoculated with EMC (200EMC/mouse) collected from *C. gariepinus* after *in vivo* treatment with LLE 4% and 2% respectively.

Table (1): Experimental design

Gps	Mice no.	Method of treatment of EMC with LLE before experimental infection
Gp.1	6	*In vivo* treated with LLE 4%
Gp.2	6	*In vivo* treated with LLE 2%
Gp.3	6	*In vitro* treated with LLE 4%
Gp.4	6	*In vitro* treated with LLE 2%
Gp.5	6	EMC without treatment (control +ve)
Gp.6	6	- (control –ve)

LLE: Leek leaf extract.

The mice in Gps.3 and 4 were inoculated with *in vitro* treated EMC (200EMC/mouse) with 20 ml of LLE 4% and 2% respectively [EMC were exposed to each extract at room temperature (30 °C) for 1 hour (till the movement of EMC stopped)]. The movement of EMC was examined under a light microscope according to Buddhachat *et al*. [25]. Each inoculated mouse was sacrificed after detection of eggs in faeces (nearly on 5th day post infection)**.**

Parasitological examination:

Egg output:

Microscopic examination of the fecal samples of all the experimental mice was daily carried out post-infection by both the direct and sedimentation techniques [26] for the early detection of fluke's eggs.

Worm burden:

Recovery of adult worms from the experimentally infected mice was done post eggs detection. The small intestines of the sacrificed inoculated and controlled mice were removed and separately left in a Petri dish containing saline for half hour, then were dissected and the adult flukes were washed with 0.85 % NaCl solution and then counted. Some of these worms were kept in a refrigerator at 4°C and then fixed in 10% formalin, carmine stained and permanently mounted according to Kruse & Pritchard [23].

Statistical analysis:

Results are presented as means ± SE. Significant differences in the measured values between the control and experimental groups were determined by one-way ANOVA test followed by Duncan's multiple range test (MRT) [27]. All statistical analyses were performed using a computer program of SPSS Inc. (version 17.0 for Windows) at the 0.05 level of significance.

Histopathological examination:

Fresh tissue specimens were collected from skin and liver for histological examination. These specimens were rapidly fixed in Davidson's fixative for 24 hours then transferred to 70% ethanol till processing proceeds. The fixed specimens were processed through the conventional paraffin embedding technique (dehydration through ascending grades of ethanol, clearing in xylene and embedding in paraffin wax at 60 °C). Paraffin blocks were prepared and cutting 3 µm-thick tissue sections by using microtome (Leica 2155), then the slides were stained with H&E stain and examined by light microscopy according to Bancroft & Gamble [28].

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RESULTS

Clinical examination of fish:

Macroscopical examination:

The clinical examination of *C. gariepenus* fish revealed the presence of excessive mucous secretion, as well as general emaciation of naturally infected fishes was manifested by the thinning of the body muscles in some of fish samples. The investigations of the internal organs of the naturally infected fishes showed gray whitish sand like dots were detected in muscles and liver of naturally infected *C. gariepenus* fish.

Microscopical examination:

The isolated EMC from the examined *C. gariepinus* fish were identified using dissecting binuclear microscope as Prohemistomatid EMC (Fig.1b). The prevalence rate of EMC infection was found 100% and there was high intensity of EMC in different organs and tissues (Fig.1a). The number of EMC/gram of muscles ranged from 120-200 EMC/g.

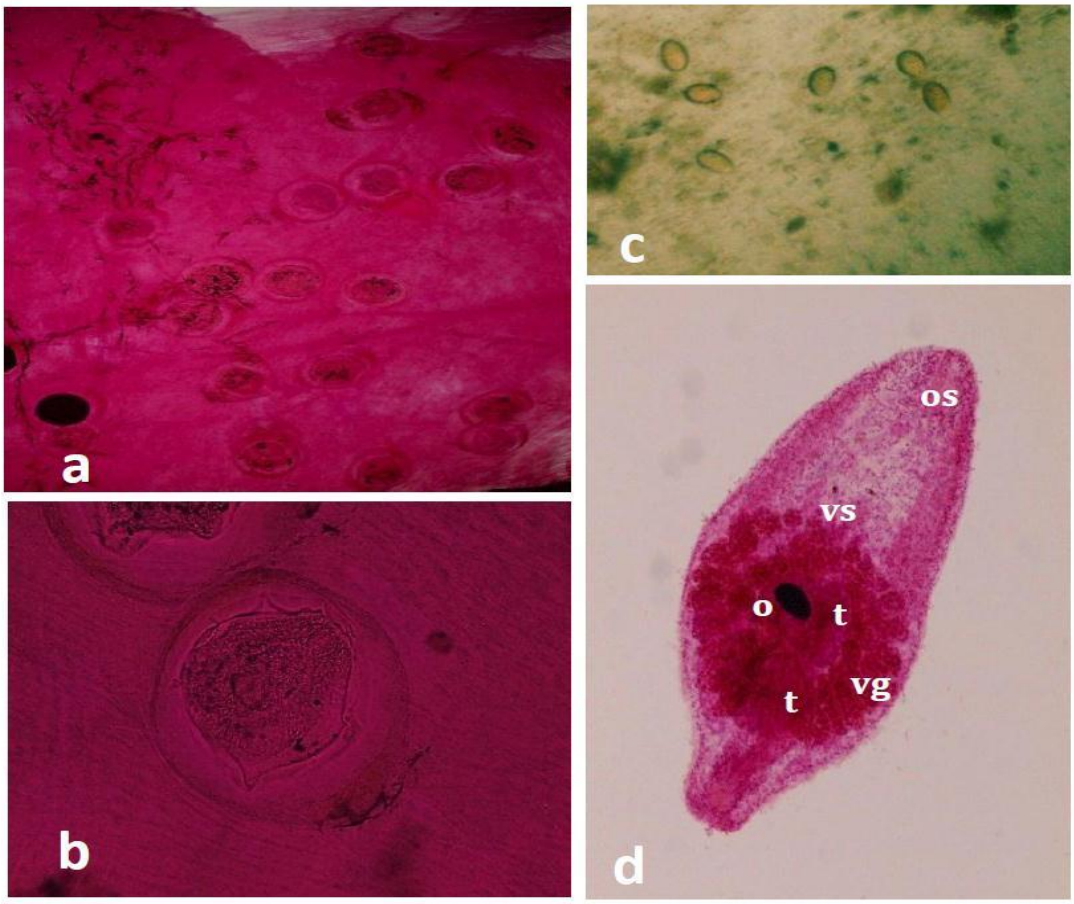

Fig. (1): (a-b) *Prohemistomatidae* EMC in the muscles of *C. gariepinus* (aX40- bX100). (c) Eggs of *P. vivax* isolated from faeces of experimentally infected mice (X100). (d) *Prohemistomum vivax* isolated from experimentally infected mouse (X100): (os)oral sucker, (vs)ventral sucker, (vg) vitelline glands, (t) testis, (o) ovary.

Determination of the egg output:

Prohemistomatidae eggs were collected from fecal samples of experimentally infected mice Gps.1, 2, 3, 4 and positive control Gp.5 (Fig.1c). Examination was done by using the sedimentation technique before mice sacrifice. The difference in egg output between experimentally inoculated mice with LLE (4% and 2%) *in*

vivo, *in vitro* and the control positive group were presented in Table (2). *In vivo* (Gp.1 and Gp.2), mice inoculated with EMC showed a significant reduction in the egg output starting from the 4th day after infection more than *in vitro* treated Gps (Gp.3 and Gp.4) in comparison with the control positive group(Table 2).

Table (2): Eggs and adult *P. vivax* recovered from the experimentally infected mice with EMC isolated from catfish *in vivo* and *in vitro* treated with LLE.

Gps	No. of EMC/ mouse	No. of mice	No. of eggs/MF			No. of adult flukes			Recovery rate (%)		
			mean	±	SE	mean	±	SE	mean	±	SE
Gp.1	200	6	2.17	±	1.45^{a}	6.17	±	3.56^{a}	3.08	±	1.78^{a}
Gp.2	200	6	4.50	±	2.43^{a}	18.83	±	11.69^{ab}	9.46	±	5.85^{ab}
Gp.3	200	6	11.83	±	0.79^{b}	39.50	±	5.68^{b}	19.75	±	2.84^{b}
Gp.4	200	6	18.17	±	1.47^{c}	78.67	±	6.97^{c}	39.33	±	3.49^{c}
Gp.5	200	6	22.67	±	1.86^{d}	112.00	±	15.28^{d}	56.00	±	7.64^{d}
Gp.6	0	6	0.00	±	0.00^{a}	0.00	±	0.00^{a}	0.00	±	0.00^{a}

Means with the same letter within the same column are not significantly different (P>0.05).
SE= standard error. No.: Number. MF: Microscopic field.

Worm burden:

The present study showed that only one species of adult digenetic; *Prohemistomum vivax* was obtained from experimentally infected mice with EMC that isolated from the skeletal muscles of naturally infected *C. gariepinus* after 5 days of experimental infection (Fig.1d).

The average percentage of recovered adult fluke from positive controlled Gp (Gp.5) was 56% after 5 days of inoculation with 200 EMC. The recovery rate was significantly decreased in Gp.1 and Gp.2 in mice inoculated with EMC collected from *C. gariepinus* after *in vivo* treatment with LLE 4% and 2%, respectively which was significantly more effective than that in Gp.3 and Gp.4 of mice which were inoculated with *in vitro* treated EMC with LLE 4% and 2%, respectively (Table 2). It is also noticed that LLE 4% was significantly more effective than LLE 2% in both *in vivo* and *in vitro* treatment.

Statistical analysis:

There are highly significant differences (Tables 2 and 3) between the values of the number of adult flukes (F = 25.46, P<0.01), recovery rate (%) (F = 25.44, P<0.01) and number of eggs/MF (F = 35.55, P<0.01) among different experimental groups. The highest mean values of the number of adult flukes, recovery rate (%) and number of eggs/MF were obtained at groups Gp.5 and Gp.4 (112.00, 56.00 & 22.67 and 78.67, 39.33 & 18.17, respectively). The lowest value (0.00) for the number of adult flukes, recovery rate (%) and number of eggs/MF was obtained at Gp.6.

Table (3): One-way ANOVA test of Eggs and adult *P. vivax* recovered from the experimentally infected mice with EMC isolated from catfish *in vivo* and *in vitro* treated with LLE.

	Source of variation	Sum of Squares	*df*	Mean Square	*F*-value	Sig.
No. of eggs/MF	Between Groups	2532.222	5	506.44	35.55	0.000**
	Within Groups	427.3333	30	14.24		
	Total	2959.556	35			
Adult flukes	Between Groups	59002.47	5	11800.49	25.46	0.000**
	Within Groups	13906.50	30	463.55		
	Total	72908.97	35			
Recovery rate (%)	Between Groups	14744.70	5	2948.94	25.44	0.000**
	Within Groups	3477.469	30	115.92		
	Total	18222.17	35			

***F*-value = ANOVA *F*-test.** **Sig. = significance level. **ANOVA (highly significant difference, P<0.01).**

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The present results clearly indicate that the experimental groups are in the following order: Gp.6<Gp.1<Gp.2<Gp.3< Gp.4< Gp.5.

Histopathological investigations:

The histopathological examination of the control non treated fish skin revealed hyperplasia and hypertrophy of the mucous secreting cells in the epidermal layer associated with accumulation of edematous fluid subepidermal leading to splitting of the subepidermal connective tissue (Fig.2a). Encysted metacercariae with marked edema and dispersion of muscle bundles were also detected (Fig.2b). The muscle fibers undergo coagulative necrosis (Fig.2c).

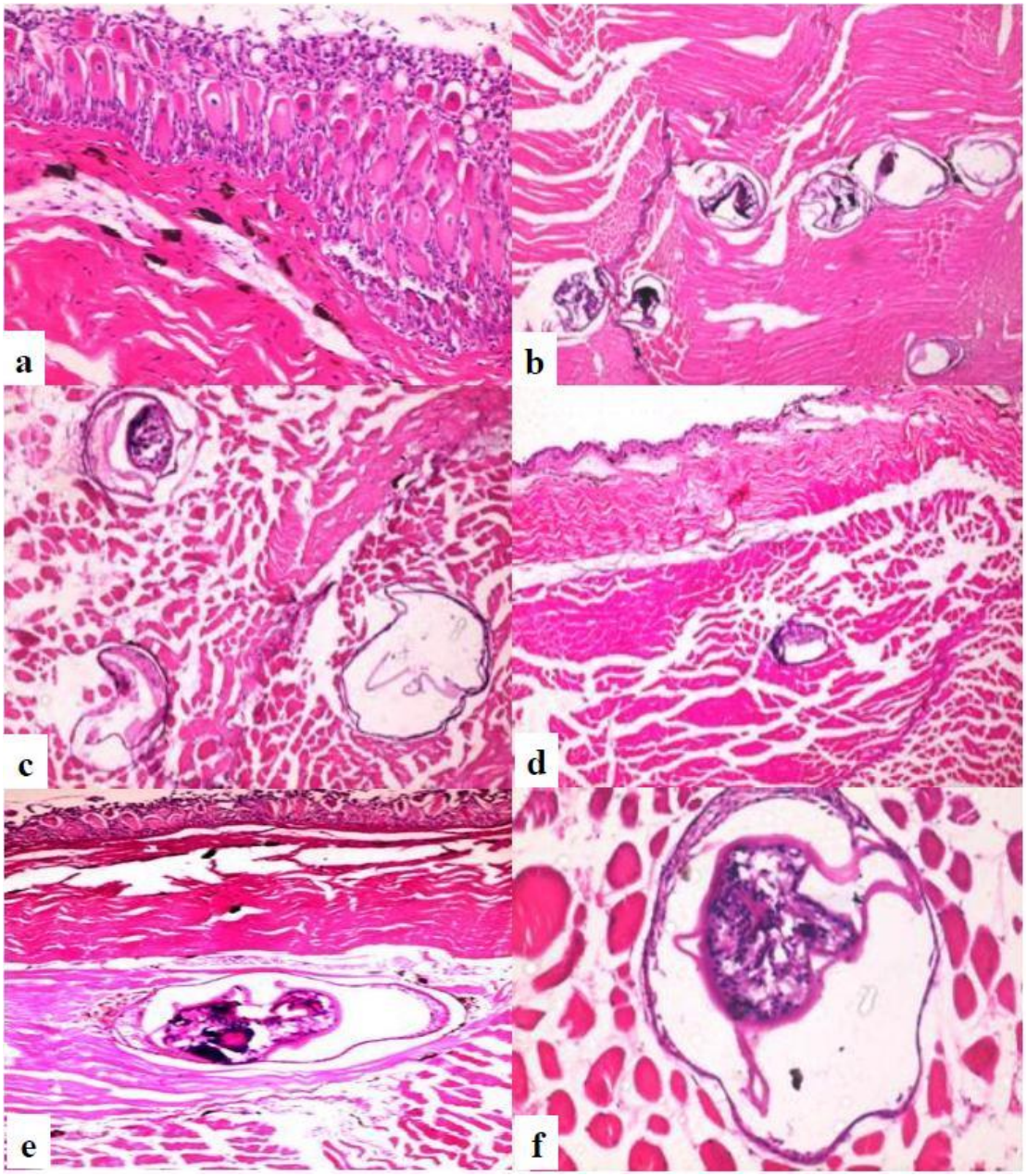

Fig. (2): Skin of *C. gariepinus* fish (stained with H&E): (a) Control Gp (not treated with LLE) showing hyperplasia and hypertrophy in the mucous secreting cells, increase the melanophores and accumulation of edematous fluid subepidermal (X400). (b) Control Gp showing heavy infestation of EMC in between the muscle layer, edema and dispersion of muscle bundles (X200). (c) Control Gp showing EMC in between the muscle layer with coagulative necrosis in the muscle fibers (X200). (d, e, f) Treated Gps with LLE showing the same changes in a, b, c with increase numbers of melanophores in the epidermal and subepidermal layers (d X100, e X200, f X400).

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Fish groups treated with LLE 2% and 4%, showed the same accumulation of melanophores in between the subepidermal connective tissue more than in the control group (Fig.2d, e &f).

Also, the liver of control group showed degenerative and necrotic changes in the hepatocytes with congestion of the hepatic blood vessels. Encysted metacercariae were detected embedded in between the hepatic parenchyma surrounded with connective tissue capsule (Fig.3a&b). Infiltration of chronic inflammatory cells and melanomacrophages in between the hepatic parynchyma especially around the hepatic blood vessels also detected (Fig.3c). there was excessive infiltration of melanomacrophages in between the hepatocytes (Fig.3d) in both LLE treated groups (2% & 4%) which become large circumscribed area of aggregated melanomacrophage cells present in between the hepatic parenchyma in the group treated with LLE 4% (Fig. 3e &f).

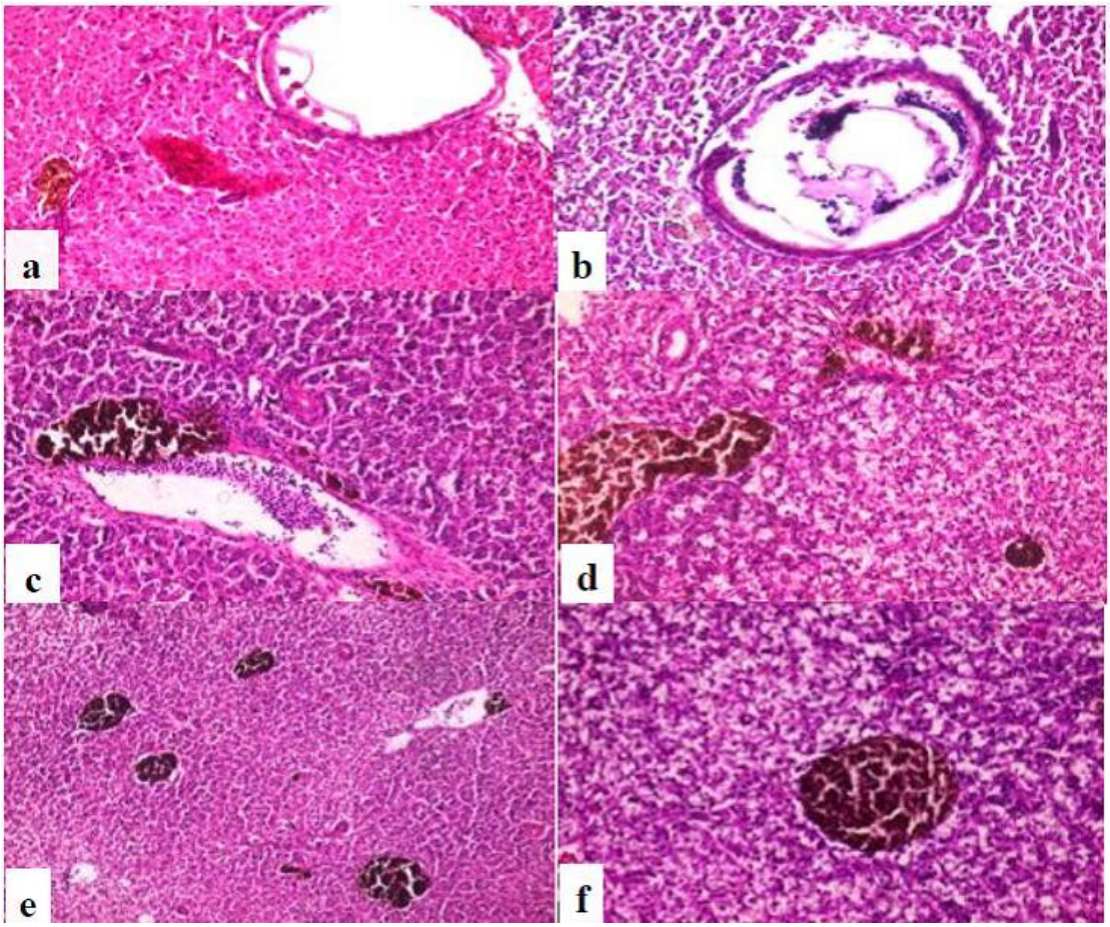

Fig. (3): Liver of *C. gariepinus* fish (stained with H&E): (a,b) Control Gp showing degenerative and necrotic changes in the hepatocytes, congestion of the hepatic blood vessels and EMC embedded in between the hepatic parenchyma surrounded with connective tissue capsule (X400). (c) Control Gp showing infiltration of chronic inflammatory cells and melanomacrophages in between the hepatic parenchyma especially around the hepatic blood vessels (X400). (d) Treated Gp with LLE 2% showing excessive infiltration of melanomacrophages in between the hepatocytes (X400). (e,f) Treated Gp with LLE 4% showing large circumscribed area of aggregated melanomacrophage cells in between the hepatic parenchyma (eX200& f X400).

DISCUSSION

Metacercarial infections are the main larval digenean causing severe economic loss among fishes in both open water resources and fish culture. Also, many of metacercariae in fish have public health importance [29].

In the present study, the detected clinical changes of *C. gariepenus* fish as the presence of excessive mucous secretion, as well as general emaciation of naturally infected fish which manifested by the thinning of

the body muscles in some of fish samples. And also the presence of gray whitish sand like dots in the muscles and liver of the naturally infected fish were in accordance with the aforementioned clinical signs and lesions which were previously reported by Marzouk *et al.* [5].

The inspected catfishes (*C. gariepinus*) total length and weight were ranged from 22-28 cm and 65-130 g, respectively and revealed 100% EMC infection rate. This result nearly agreed with Marzouk *et al.* [5], who recorded that the highest prevalence of EMC (83.6%) in *C. gariepenus* was observed in fish length 31-35 cm and weighed from 101-150 g.

The high prevalence rate of EMC (100%) detected in the different organs and tissues of examined *C. gariepinus* in the present study was corroborative with that reported by Shaapan [30] who recorded an incidence rate of 95.07% and nearly agree with El- Gayar & Aly [31] who reported 87% incidence rate. While, it was higher than that reported by Atwa [32] and Marzouk *et al.* [5], who recorded the incidence rate of EMC in African catfish in Egypt, 27.85% and 52.89% respectively . The number of EMC/g of muscles was ranged from 120-200 EMC/g.

The high incidence of EMC in *C. gariepinus* may be due to the thin skin and soft muscles of catfish which probably help in penetration of cercariae, also the fish habitat (bottom feeder) helped their infection, as the feeding on vegetation, where the first intermediate host snail is found giving a suitable chance to liberated cercariae to penetrate their skin. The high incidence of EMC infection in the examined *C. gariepinus* mainly related to the prevalence of the first intermediate host snails (*Cleopatra bulimoides*) of *P. vivax* [33] and the failure of their precise control in the natural water resources in Egypt. In addition, the presence of final host (birds) of this digenetic trematode in the cultivated Nile Delta lands with the escape of eggs and miracidia of these trematodes through agricultural drainage water to the water resources [34].

In the present study one group of EMC was identified belonging to prohemistomatid and only one adult digenetic trematode was recovered from the small intestine of experimentally infected mice with EMC from muscles of *C. gariepinus* including *P. vivax.* The morphological features of obtained *P. vivax* are similar to that were described in several studies [5, 6, 31]. The detected *P. vivax* has a public health importance due to its ability of transmission to man [4].

Egg count is an indicator showing the intensity of infection and as a parameter for cure. It can be applied both experimentally and clinically to determine the validation of the treatment by egg counting before and after drugs have been given [35].

In the present study, *in vivo* (Gp.1 and Gp.2), mice inoculated with EMC showed a significant reduction in the egg output starting from the 4^{th} day after experimental infection more than *in vitro* treated ones (Gp.3 and Gp.4) in comparison with the control +ve group (Gp.5).

Concern worm count recovered from the small intestine and worm recovery rate (WRR) there were significantly lower WRR in groups *in vivo* treated with LLE (Gp.1 and Gp.2) than that in control +ve one. Also, WRR in Gp.1 and Gp.2 (*in vivo* treated with LLE 4% and 2%, respectively) was significantly lower than that in Gp.3 and Gp.4 (*in vitro* treated EMC with LLE 4% and 2%, respectively). Furthermore, it is noticed that LLE 4% significantly has higher efficacy than LLE 2% in both *in vivo* and *in vitro* treatment.

While, there were no available researches reported the effect of *Allium ampeloprasum* L. on EMC in fish, scant studies proved the effect of Allium species on EMC of digenetic trematodes in fish. Wei Ping [18] recorded that garlic (*Allium sativum* L.) juice are capable of killing all tested *Clonorchis sinensis* metacercariae isolated from fish fillets (*in vitro*) after 36 hours. Also, El-Mansy [19] showed that *in vitro* treatment of unidentified EMC isolated from catfish muscles by *Allium cepa* bulb (onion) converted it to opaque immobile mass, 3 days after treatment.

In the present study *in vivo* treatment was more significantly effective than *in vitro* one, it may return to the longtime feeding of LLE in fish diet (45 days), while the *in vitro* application of LLE was done for short period (one hour) that may be not sufficient to kill or inactivate all the EMC.

Leek (*Allium ampeloprasum* L.) is belonging to the Allium genus [8] and a closely related species of garlic (*Allium sativum* L.) [9]. It was found that *Allium ampeloprasum* extracts, as other Allium species had antimicrobial factors named allicin containing diallylsulphide and thiosulfinate which are extremely effective against main foodborne pathogens [11], actively and directly kills parasites [12]. Allicin has a remarkable ability to permeate and digs in living tissue and this has implications on its potency and prolonged effect [13]. Moreover, Millet *et al.* [17] reported that whole, freeze-dried garlic and other allium-derived components had an inhibitory action on gas metabolism, rapid growth rate and definitive growth yield of *Spironucleus* vortens of fish. The inhibitory effects of garlic extract on another important fish parasites, including Ichthyophthirius and Gyrodactylus have been also reported [14, 15].

Concerning the recorded histopathological alterations in fish infected with metacercariae could be due to embedding of the EMC in the affected muscle fibers that resulted in pressure atrophy, activation of mucous secreting cells and proliferation of the melanomacrophages. These findings were parallel with that described by El- Gayar & Aly [31].

The degenerative and necrotic changes in the hepatocytes may be resulted from the pressure atrophy of embedded EMC in between the hepatocytes and the toxic substances produced by the parasitic cysts. Similar observations were previously recorded by Ramadan *et al.* [36].

The aggregation of melanomacrophages indicated the activated body defense. They also play an important role in the response of fish to foreign materials, including infectious agents. Many studies recorded that the general function of melanomacrophages are detoxification, focalization, destruction, or recycling of exogenous and endogenous materials [37].

Finally, there is an increasing interest in consuming organic and environmentally friendly food. Thereafter, the limitation of chemotherapy in aquaculture and the use of natural treatments could enhance the consumption of aquaculture products. Moreover, their use could reduce costs of treatment and be more environmentally sound as they tend to be more biodegradable than artificial molecules and they are less likely to produce drug resistance in parasites due to the high diversity of plant extract molecules [7].

From the present study it is indicated that Leek leaf extract is effective, safe and cheap for treatment of parasites in *C. gariepinus* and its parasitic activity due to its contents of allicine. Due to the presence of medicated feed in water for longtime, it may have not only internal but also external direct effect on EMC present in fish skin and superficial muscles especially in catfish because of its relative thin scalless skin.

The current study boosts and validates anthelmintic activity of leek leaf extract that was investigated through an experimental study.

CONCLUSION

This study highlights the anti-parasitic effect of leek (*Allium ampeloprasum* L.) leaf extract and its potential to be used as an alternative to chemical treatments. Further studies are required for *in vitro* application of LLE on EMC for longer period (more than one hour) that will be sufficient to kill or inactivate all the EMC. Also, studies are needed to evaluate its effect on another species of fish parasites and analyze the long-term effects on fish physiology.

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