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Original Article

The oil of garlic, *Allium sativum* L. (Amaryllidaceae), as a potential protectant against *Anisakis* spp. Type II (L3) (Nematoda) infection in Wistar rats

O óleo de alho, *Allium sativum* L. (Amaryllidaceae), como potencial protetor contra *Anisakis* spp. Infecção tipo II (L3) (Nematoda) em ratos Wistar

Kareem Morsy[1,2]*; Ali AL-Ghamdi[3]; Saad Bin Dajem[1]; Mashael Bin-Meferij[4]*; Ali Alshehri[1]; Attalla El-kott[1,5]; Essam Ibrahim[1,6]; Atef Ali[2]; Hamida Hamdi[2,7]; Amin Al-Doaiss[1,8]; Sara Saber[9]

[1] Biology Department, College of Science, King Khalid University, Abha, Saudi Arabia
[2] Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt
[3] Biology Department, College of Science, Al Baha University, Al Baha, Saudi Arabia
[4] Biology Department, College of Science, Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia
[5] Zoology Department, Faculty of Science, Damanhour University, Damanhour, Egypt
[6] Blood Products Quality Control and Research Department, National Organization for Research and Control of Biologicals, Cairo, Egypt
[7] Biology Department, Faculty of Science, Taif University, Taif, Saudi Arabia
[8] Anatomy and Histology Department, Faculty of Medicine, Sana'a University, Sana'a, Republic of Yemen
[9] Histopathology Department, National Organization for Drug Control and Research – NODCAR, Cairo, Egypt

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Abstract

The consumption of inadequately thermally treated fish is a public health risk due to the possible propagation of *Anisakis* larvae. The present study demonstrated the physiological and histopathological changes that accompanied an oral inoculation of crude extracts from fresh and thermally treated *Anisakis* Type II (L3) in rats. Worms were isolated from a marine fish and examined and identified using light and scanning electron microscopy. The study was performed in 6 rat groups: control (I), garlic oil (GO) inoculated (II), fresh L3 inoculated (III), thermally treated L3 inoculated (IV), fresh L3 + GO inoculated (V), and a thermally treated L3 + GO inoculated (VI) groups. Rats inoculated with fresh and thermally treated L3 showed abnormal liver and kidney functions associated with the destruction of normal architecture. GO produced a protective effect in rat groups inoculated with L3 extracts + GO via the amelioration of liver and kidney functions, which was confirmed by the marked normal structure on histology. Cooking of L3-infected fish induced severe alterations compared to uncooked fish. The administration of garlic before and after fish eating is recommended to avoid the dangerous effect of anisakids, even if they are cooked.

Keywords: Anisakid larvae, histopathology, zoonotic parasites, morphology, physiology.

Resumo

O consumo de peixe inadequadamente tratado termicamente representa um risco para a saúde pública, com a possibilidade da propagação de larvas de *Anisakis*. O presente estudo demonstrou as alterações fisiológicas e histopatológicas acompanhadas de inoculação oral de extractos brutos de *Anisakis* tipo II (L3) frescos e termicamente tratados em ratos. Os vermes foram isolados de um peixe marinho, examinados e identificados por microscopia de luz e eletrônica de varredura. O estudo foi conduzido em 6 grupos de ratos: controle (I), óleo de alho (GO) inoculado (II), L3 fresco inoculado (III), L3 tratado termicamente inoculado (IV), L3 fresco + GO inoculado (V), e um grupo L3 + GO tratado termicamente inoculado (VI). Observou-se que ratos inoculados com L3 fresco e tratados termicamente mostraram funções hepáticas e renais anormais, associadas à destruição da sua arquitetura normal. GO produziu um efeito protector em grupos de ratos inoculados com extractos L3 + GO através da melhoria das funções do fígado e dos rins, o que foi confirmado pela estrutura normal marcada da sua

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*Corresponding author: Kareem Morsy. E-mail: kareemsaid156@yahoo.com. Mashael Bin-Meferij. E-mail: mmbinmufayrij@pnu.edu.sa

histologia. A cozedura de peixes infectados com L3 induziu alterações mais graves do que os peixes não cozidos. Recomenda-se a administração de alho antes e depois do consumo de peixe, para evitar o efeito perigoso dos anisakids, mesmo que sejam cozidos.

Palavras-chave: Anisakid larvas, histopatologia, parasitas zoonóticos, morfologia, fisiologia.

Introduction

Anisakidosis is a vital fish-borne zoonotic disease caused by third or, infrequently, fourth larval stages of the nematode *A. simplex*, which live as encapsulated larvae embedded in the edible muscle of marine fish (Morsy et al., 2013). The life cycle begins when eggs are passed in the feces of marine mammals into water, where they hatch to release free-living larvae (L2), which are consumed by crustaceans and further develop into L3. When infected crustaceans are ingested by fish, the third larvae enter the body cavity and muscles (Kassem & Bowashi, 2015; Nieuwenhuizen, 2016). Humans may be accidentally infected by the L3 larvae when they ingest raw, undercooked meat of parasitized fish (Mattiucci et al., 2013). The pathological effects appear when the third larvae pierce the mucosal layers of the host's gastrointestinal tract, which directly produces tissue injury and causes nausea, diarrhea, and abdominal pain (Kassem & Bowashi, 2015). Some patients develop an IgE–mediated "gastroallergic anisakiasis", which leads to clinical manifestations that range from urticaria to life-threating anaphylactic shock (Nieuwenhuizen, 2016). *Anisakis* larvae pose a health risk to humans even when the fish is thoroughly cooked because dead or thermally treated L3 produce a number of physiological and histopathological changes in humans after fish ingestion, which led to the concept of acute anisakiasis (Montalto et al., 2005). Traditional medicines worldwide identified the benefits of plants in human health and have taken advantage of the empirical treatment of common human diseases. Plant phytochemicals act at the molecular and cellular levels to protect human cells against oxidative damage, stimulate enzymes, interfere with DNA replication, and affect infection processes (Seckiner et al., 2014). Garlic (*Allium sativum* L., Alliiaceae) is one the oldest familiar plants used for health and the treatment of numerous diseases in folk medicine (Lee et al., 2012). Garlic was recently suggested as a promising candidate for maintaining homeostasis. Garlic has a large number of potentially active chemical components, such as amino acids, organosulfates, minerals, and vitamins (WHO, 2000). Substantial studies showed that garlic and its bioactive constituents exhibit antioxidant, anti-inflammatory, antibacterial, antifungal, immunomodulatory, cardiovascular protective, anticancer, hepatoprotective, digestive system protective, anti-diabetic, anti-obesity, neuroprotective, and renal protective properties (Yun et al., 2014). Garlic exhibits immunomodulatory actions, such as the modulation of cytokine secretion, phagocytosis promotion, macrophage activation, immunoglobulin production, allergic reactions, and lymphocyte proliferation (Liu et al., 2009; Mahima et al., 2012; Percival, 2016). Therefore, the present study demonstrated the liver and kidney functions and histopathological changes in Wistar albino rats after oral inoculation of fresh and thermally treated *Anisakis* spp. type (L3) larvae isolated from the Red Sea fish *Dicentrarchus labrax*. The present study also evaluated the protective and ameliorative effects of garlic oil (GO) in L3-inoculated rats.

Materials and Methods

Parasite collection and identification

Twenty specimens of the European seabass *Dicentrarchus labrax* (family: Moronidae) were collected alive from fishermen at boat landing sites along the Red Sea in Jizan, Saudi Arabia, and sometimes from local markets. Fish were transported to the laboratory of Parasitology and were identified according to Randall (1992). Worms were collected as encapsulated larvae from the surfaces of visceral organs, such as the stomach, intestines, and muscles, of the examined fish. The worms were rinsed in phosphate-buffered saline for further experiments. For the morphological study, some of the collected worms were fixed in hot 70% ethanol for light microscopy (Morsy et al., 2013). For scanning electron microscopy (SEM), 10 worms were fixed in 3% phosphate-buffered glutaraldehyde (pH 7.3) for 3 h, then washed thoroughly with the same buffer and post-fixed in aqueous osmium tetroxide for 4 h according to Madden & Tromba (1976), dehydrated through acetone, and dried in a critical point drier (BOMER-900, Leica Microsystems, Morrisville, USA) using liquid CO2, mounted on an aluminum stub, coated with gold palladium in a JEOL, JEC-3000FC, and then examined with JSM-6060LV microscope (JEOL, Tokyo, Japan) at 10kV.

Garlic Oil (GO) and FT-Raman spectroscopy

GO was purchased in the form of capsules. Each capsule contained 10 mg/kg pure GO equivalent to 1000 mg of fresh garlic bulb and other ingredients (Vitamin Shoppe Co., New Jersey, U.S.A.). Using the amount of garlic oil concentration printed on some of the commercial capsules labels and described in the literature (Iberl et al., 1990; Amagase et al., 2001), mixtures were prepared from standard garlic oil. The mass fractions of garlic oil were in the range of 0.00% to 1.75% (0.00%, 0.25%, 0.50%, 0.75%, 1.00%, 1.25%, 1.50%, 1.75%). Using an automatic pipette, the masses of the components of each mixture were weighed on a watch glass in a calibrated analytical balance, and then stirred for 3 minutes to ensure homogeneity. The instrument used for Infrared (IR) analysis was a NICOLET 6700 Fourier transform infrared spectroscopy (FT-IR) optical spectrometer from Thermo Scientific. The spectra were obtained in the medium infrared region, from 4000 to 650 cm^{-1}, as an average of 16 scans at a resolution of 4 cm-1, under a controlled temperature of 23 ± 2 °C.

Experimental design

Thirty-six adult male Wistar albino rats (*Rattus norvegicus*), 150–170 g in weight, were housed in a suitable cage under a 12 h light/12 h dark cycle. Rats were housed in metal cages under a controlled temperature and humidity with water and food available *ad libitum*. The experimental protocols for animal use were performed in accordance with the regulatory laws of experimental ethics of animal use and collecting permits and the NODCAR research ethics Committee for experimental and clinical studies, Egypt (Approval no. NODCAR/III/35/19).

The rats were divided into the following six groups (n=6) (Figure 1):

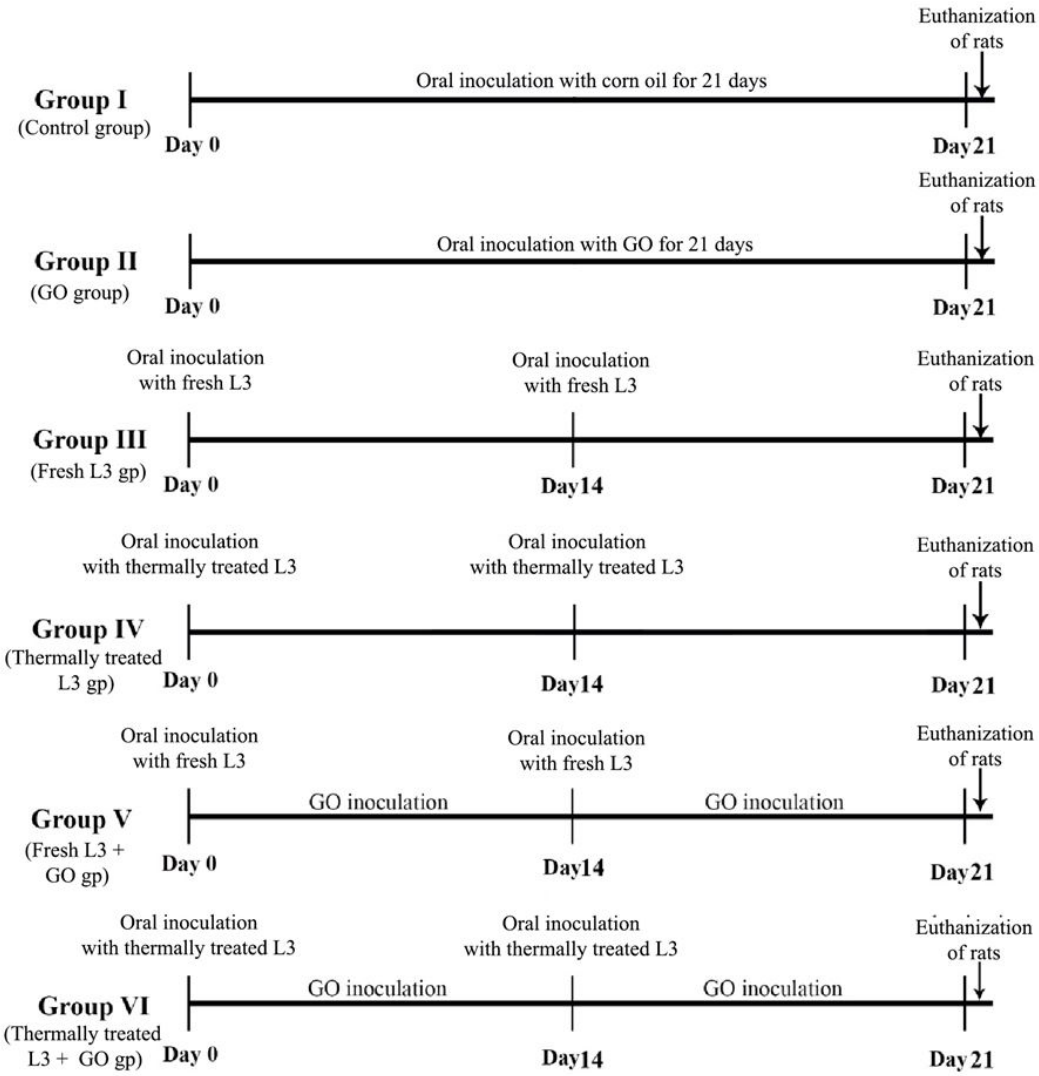

Figure 1. Timeline of experimental rat's inoculation and euthanization.

Group I: The control group, inoculated with corn oil only (2 ml/kg, p.o.) as a vehicle for 21 days (according to Wu et al. (2001)).

Group II: GO group, inoculated with GO only (100 mg/kg b.wt.) for 21 days (Mohamed et al., 2016).

Group III: The fresh L3 inoculated group. Rats were inoculated with corn oil for 21 days (2 ml/kg p.o.) and inoculated twice with fresh *Anisakis* spp. (L3) crude extract on the 0^{th} and 14^{th} days.

Group IV: The thermally treated L3 inoculated group. Rats were inoculated with corn oil for 21 days (2 ml/kg p.o.) and inoculated twice with thermally treated (100 °C water bath, 10 min) *Anisakis* spp. (L3) crude extract on the 0^{th} and 14^{th} days.

Group V: The fresh L3+ GO group. Rats were inoculated with GO for 21 days (100 mg/kg b.wt.) and inoculated twice with fresh *Anisakis* spp. (L3) crude extract on the 0^{th} and 14^{th} days.

Group VI: The thermally treated L3 + GO group rats were inoculated with GO for 21 days (100 mg/kg b.wt.) and inoculated twice with thermally treated (100 °C water bath, 10 min) *Anisakis* spp. (L3) crude extract on the 0^{th} and 14^{th} days.

Rats were orally inoculated with fresh or thermally treated L3 as crude extracts placed on the pharynx or directly into the stomach. GO extract was given to the rats at a dose of 100 mg/kg b.wt. according to Riad et al. (2007) using an esophageal tube. Crude extracts were prepared via the homogenization of L3 for each rat separately before inoculation, where 10 portions (about 5 worms) of fresh or thermally treated L3 of an equal size were ruptured using microtube pestles and sonicated on ice at 100 W five times for 30 s using an ultrasonic homogenizer (Abe & Teramoto, 2014).

Serum preparation for biochemical studies

Twenty-four hours after the last dose, rats were euthanized, and blood samples were collected via cardiac puncture and left to stand. The samples were centrifuged at 3000 rpm for 15 min. Some biochemical parameters were measured in the collected serum of the control and experimental rat groups using accessible assay kits (Sigma–Aldrich, *Missouri,* U.S.A.): aspartate aminotransferase (AST), alanine aminotransferase (ALT) (Reitman & Frankel, 1957), alkaline phosphatase (ALP) activities (Danikowski & Cheng, 2019), total protein (Henry, 1964), total bilirubin level (Wang et al., 2016), albumin level (Doumas et al., 1971), creatinine (Bartels et al., 1972), urea (Fawcett & Scott, 1960), and uric acid (Watts, 1974).

Histopathological study

A portion of the liver and kidney tissues was excised from the control and experimental rat groups at the end of the experiment and fixed in a 10% neutral buffered formalin solution for at least 12 h. A phosphate buffer was used to wash the samples 2–3 times for 10 min. The specimens were dehydrated in a graded ethanol series, cleared in butanol, and embedded in paraplast at 62 ^{o}C. Sections of 5-µm thickness were prepared using a rotary microtome. After wax removing, sections were hydrated in a descending series of ethanol and stained with Hematoxylin and eosin (H&E). The stained sections were examined and photographed using a BX53 microscope (Olympus Corporation, Tokyo, Japan).

Image preprocessing

The quantification of histopathological changes between the control and experimental rat groups was performed and analyzed in detail using ImageJ software (version k 1.45). Histopathological images of the rat liver and kidney tissues stained with hematoxylin and eosin were used for image morphometry, which is the best conventional staining for comparisons. Measurements were performed in 10 low-power fields of (X: 100)/rat, except for the nuclear/cytoplasmic ratio, which was performed in 10 high-power fields of (X: 400)/rat.

Statistical analysis

Statistical analysis was executed using Statistical Package for the Social Sciences (SPSS) version 23. Duncan's test was applied to determine similarities in all of the studied biochemical parameters among the studied experimental groups. Data was expressed as mean ± standard error. Data was considered significant if $P<0.05$.

Results

FT-Raman results

Figure 2 presents the FT-Raman spectra of garlic oil. Four major peaks were observed in the region between 3100 and 2900 cm^{-1}. The first peak at 3086-3083 cm^{-1} corresponds to the asymmetric stretch vibration of = CH2, the second peak at 3010 cm^{-1} shows C–H stretching, the third peak at 2075 cm^{-1} is the symmetric stretch vibration of = CH2, and the fourth peak at 2910 cm^{-1} corresponds to – CH2 – stretching. The region from 1600 to 1000 showed four peaks. An intense peak at 1630 cm^{-1} was assigned as the C = C stretching vibration of the allyl group. The peak at 1401 cm^{-1} was assigned to the stretching of a – CH2 – group, and the CH2 = CH – stretching shifted to 1300 cm^{-1}. The skeletal vibration of diallyl sulfide molecule caused a peak at 1201 cm^{-1}. The peak at 1000 cm^{-1} is due to the stretching of the disulfide bridge when S atoms are abundant.

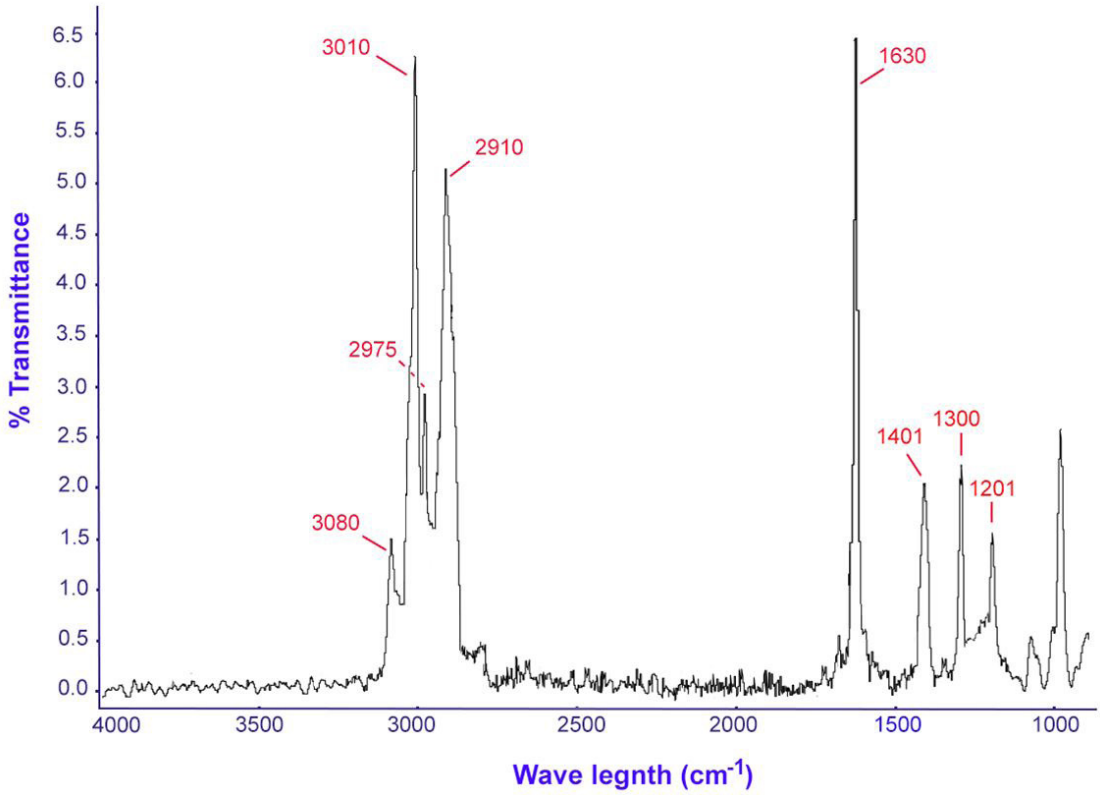

Figure 2. FT-Raman spectrum of garlic oil capsules.

Parasitological study

Nine European sea bass out of 20 (45%) were naturally infected by nematode worms, which were recovered from the peritoneal cavity as encapsulated larvae. The worm intensity ranged from 5 to 25 worms per fish (Figure 3a, b). Morphologically (Figure 3c-h), the recovered parasites were identified as *Anisakis* spp. The worms possessed slender bodies (0.67–2.8 cm long and 0.2–15 mm wide), with prominent boring teeth at the anterior extremity and four small papillae (two dorsolateral and two ventrolateral) surrounding their triangular mouths. Their cuticles had transverse striations that extended from the cephalic region prior to the anus. The postanal tail was rounded without a terminal mucron.

Biochemical study

The activities of AST, ALT, ALP as well as the levels of TP, TBil, Alb, urea and uric acid in serum of all of the experimental rat groups were estimated (Tables 1, 2 and Figures 4, 5). AST, ALT and ALP activities in serum of groups III and IV were similar but significantly greater than the rest of experimental groups. In serum of groups III, IV and V,

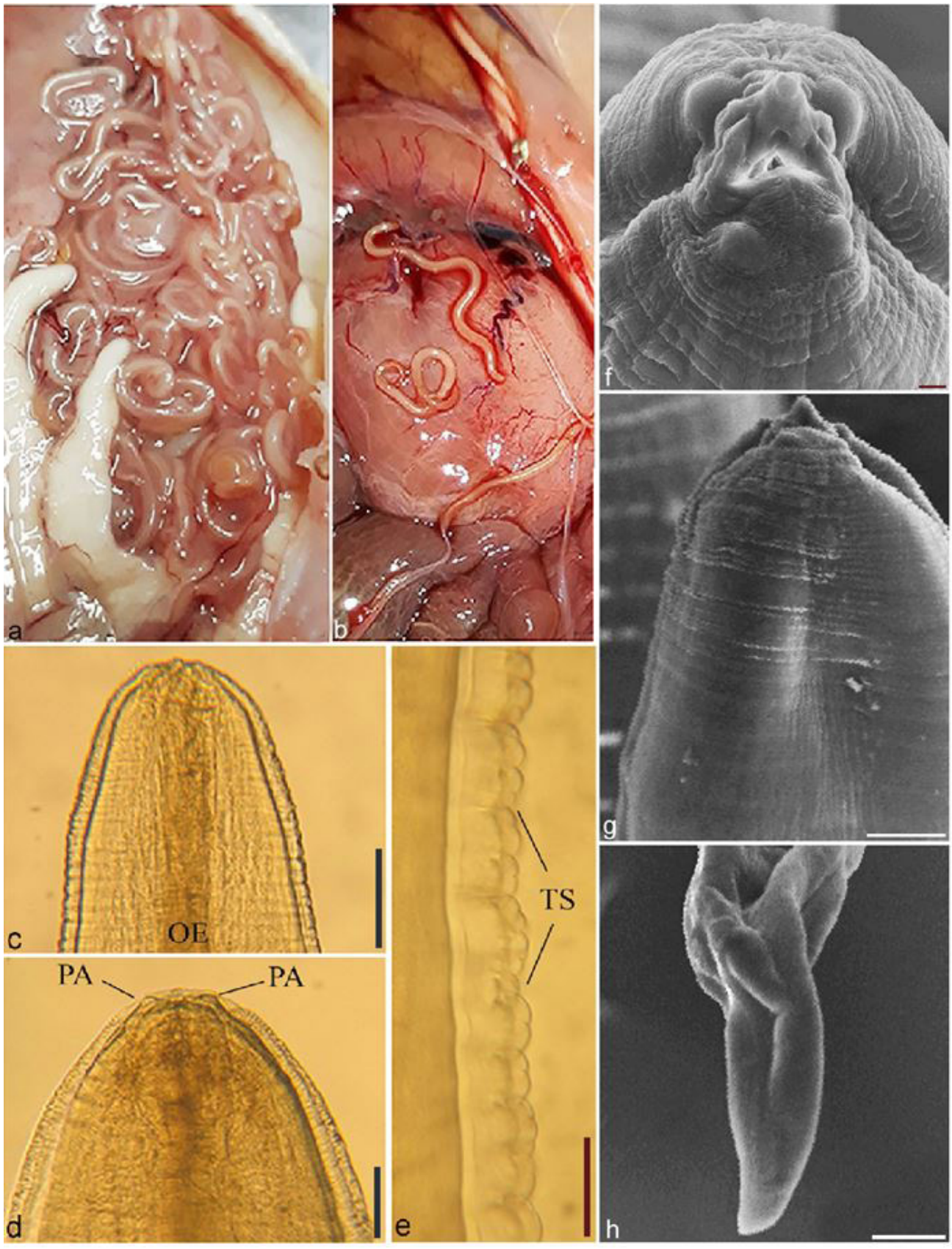

Figure 3. (a, b) Photographs showing *Anisakis* Type II (L3) worms infecting *Dicentrarchus labrax*. **(c-e)** *Anisakis* Type II (L3) light microscopy; (**c, d)** The anterior part, OE oesophagus, PA papillae, Bars, 40µm; 10µm; (**e)** Cuticle, transversely striated (TS), Bar, 20µm; (**f-h)** SEM showing; (**f, g)** The anterior part, triangular mouth (MO) with boring tooth (BT) surrounded by four papillae (PA), TS transverse striation of cuticle, bars, 10µm, 100 µm; (**h)** the posterior rounded end with no mucron, Bar, 100µm.

Table 1. The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein (TP), total bilirubin (TBil) and albumin (Alb) in serum of experimental rat groups.

Groups	AST (U/ml)	ALT (U/ml)	ALP (IU/L)	Bilirubin (mg/dl)	Total Protein (g/dl)	Albumin (g/dl)
I	238.67 ± 5.69 ^{A}	18.23 ± 1.22 ^{A}	73.77 ± 7.15 ^{A}	2.03 ± 0.15 ^{A}	7.36 ± 0.60 ^{C}	4.12 ± 0.18 ^{B}
II	272.92 ± 8.51 ^{A}	16.10 ± 0.79 ^{A}	70.58 ± 9.11 ^{A}	3.42 ± 0.12 ^{A}	6.31 ± 0.49 ^{BC}	3.87 ± 0.21 ^{B}
III	361.83 ± 6.88 ^{B}	30.67 ± 2.12 ^{B}	99.50 ± 1.65 ^{B}	6.18 ± 0.77 ^{B}	4.98 ± 0.26 ^{A}	2.78 ± 0.24 ^{A}
IV	385.31 ± 44.72 ^{B}	28.62 ± 0.47 ^{B}	98.92 ± 2.09 ^{B}	9.33 ± 1.82 ^{C}	5.11 ± 0.37 ^{A}	3.19 ± 0.05 ^{A}
V	269.75 ± 2.09 ^{A}	20.62 ± 2.30 ^{A}	73.07 ± 1.99 ^{A}	3.73 ± 0.65 ^{AB}	5.58 ± 0.27 ^{A}	3.97 ± 0.26 ^{B}
VI	254.83 ± 1.35 ^{A}	16.83 ± 3.48 ^{A}	67.28 ± 5.74 ^{A}	3.75 ± 0.43 ^{AB}	6.07 ± 0.26 ^{AB}	3.76 ± 0.16 ^{B}
ANOVA (Effect of treatment)	$F_{5,30}$= 10.29, P=0.000	$F_{5,30}$= 9.72, P=0.000	$F_{5,30}$= 7.23, P=0.000	$F_{5,30}$= 9.03, P=0.000	$F_{5,30}$= 4.91, P=0.002	$F_{5,30}$= 6.94, P=0.000

Data is displayed as mean ± standard error. Bars marked with different letters are significantly different (P<0.05), whereas those marked with similar ones are insignificantly different (P>0.05). **I**: Control group, **II**: Garlic oil-administered, **III**: Fresh third stage larvae of *Anisakis* spp. (L3) inoculated group, **IV**: Cooked L3 inoculated group, **V**: Fresh L3 inoculated + GO-treated group and **VI**: Cooked L3 inoculated + GO-treated group. F: $F_{calculated}$ whereas 5,30: represent degrees of freedom of treatment (between groups) and error (within groups), respectively.

Table 2. The levels of creatinine, urea and uric acid in the serum experimental rat groups.

Groups	Creatinine (mg/dl)	Urea (g/dl)	Uric acid (mg/dl)
I	4.50 ± 0.41^{A}	3.26 ± 0.16^{A}	5.35 ± 0.09^{AB}
II	4.45 ± 0.32^{A}	3.41 ± 0.02^{A}	5.21 ± 0.10 ^{AB}
III	6.45 ± 0.03^{B}	4.46 ± 0.05^{B}	6.13 ± 0.15 ^{C}
IV	6.54 ± 0.04^{B}	4.72 ± 0.12^{B}	5.93 ± 0.46 ^{BC}
V	4.08 ± 0.17^{A}	3.47 ± 0.03^{A}	5.03 ± 0.21 ^{A}
VI	4.21 ± 0.08^{A}	3.44 ± 0.07^{A}	5.77 ± 0.16 ^{BC}
ANOVA (Effect of treatment)	$F_{5,30}$= 27.85, P=0.000	$F_{5,30}$= 47.38, P=0.000	$F_{5,30}$= 3.50, P=0.013

Data is displayed as mean ± standard error. Bars marked with different letters are significantly different (P<0.05), whereas those marked with similar ones are insignificantly different (P>0.05). **I**: Control group, **II**: Garlic oil-administered, **III**: Fresh third stage larvae of *Anisakis* spp. (L3) inoculated group, **IV**: Cooked L3 inoculated group, **V**: Fresh L3 inoculated + GO-treated group and **VI**: Cooked L3 inoculated + GO-treated group. F: $F_{calculated}$ whereas 5,30: represent degrees of freedom of treatment (between groups) and error (within groups), respectively. P< 0.05: represent significant difference.

TP levels were similar to group VI whereas remarkably lower than in groups I and II. The levels of TBil in serum of group V and VI were significantly lower than in groups III and IV but remarkably greater than in group I and II. The serum levels of Alb of groups III and IV were similar but significantly lower than the rest of experimental groups. The serum levels of urea, creatinine and uric acid of groups III and IV were significantly greater than in groups V and VI.

Histopathological study

Histopathological examination of the liver and kidney tissues of the control and GO-administrated rats showed normal histological features without concerning pathological evidence.

Liver

The livers of the control and GO rats showed a normal organization of hepatocytes in the cords or plates radiating from the central vein with a hepatic sinusoid in between (Figure 6a, b). In contrast, the liver tissues from rats orally inoculated with fresh L3 crude extracts showed moderate to severe pathological alterations. Hepatocytes with vacuolated cytoplasm, focal inflammatory cell aggregates with perivascular edema and inflammation, dilated sinusoids with inflammatory cells, and focal necrotic areas were observed (Figure 6c–e). Extensive histological changes were observed in the liver tissues from rats orally inoculated with thermally treated L3. All of the pathological

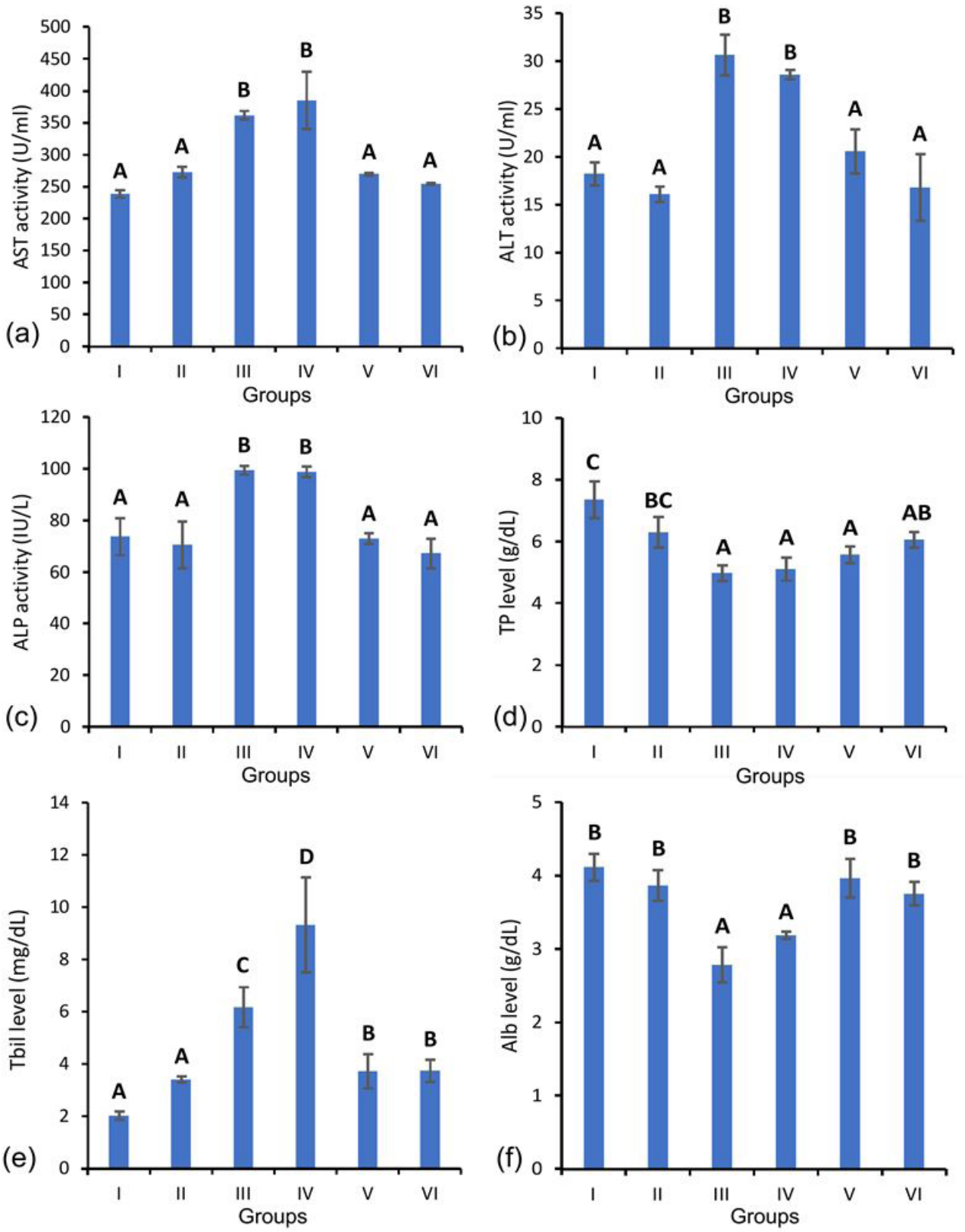

Figure 4. (a-f): The activities of (a) aspartate aminotransferase (AST); (b) alanine aminotransferase (ALT); (c) alkaline phosphatase (ALP) as well as the levels of (d) total protein (TP); (e) total bilirubin (TBil) and (f) albumin (Alb) in serum of all the experimental groups were estimated after 21 days of experiments. Data is displayed as mean ± standard error. Bars marked with different letters are significantly different (P<0.05), whereas those marked with similar ones are insignificantly different (P>0.05); **I**: Control group; **II**: Garlic oil-administered; **III**: Fresh third stage larvae of *Anisakis* spp. (L3) inoculated group; **IV**: Cooked L3 inoculated group; **V**: Fresh L3 inoculated + GO treated group and **VI**: Cooked L3 inoculated + GO treated group.

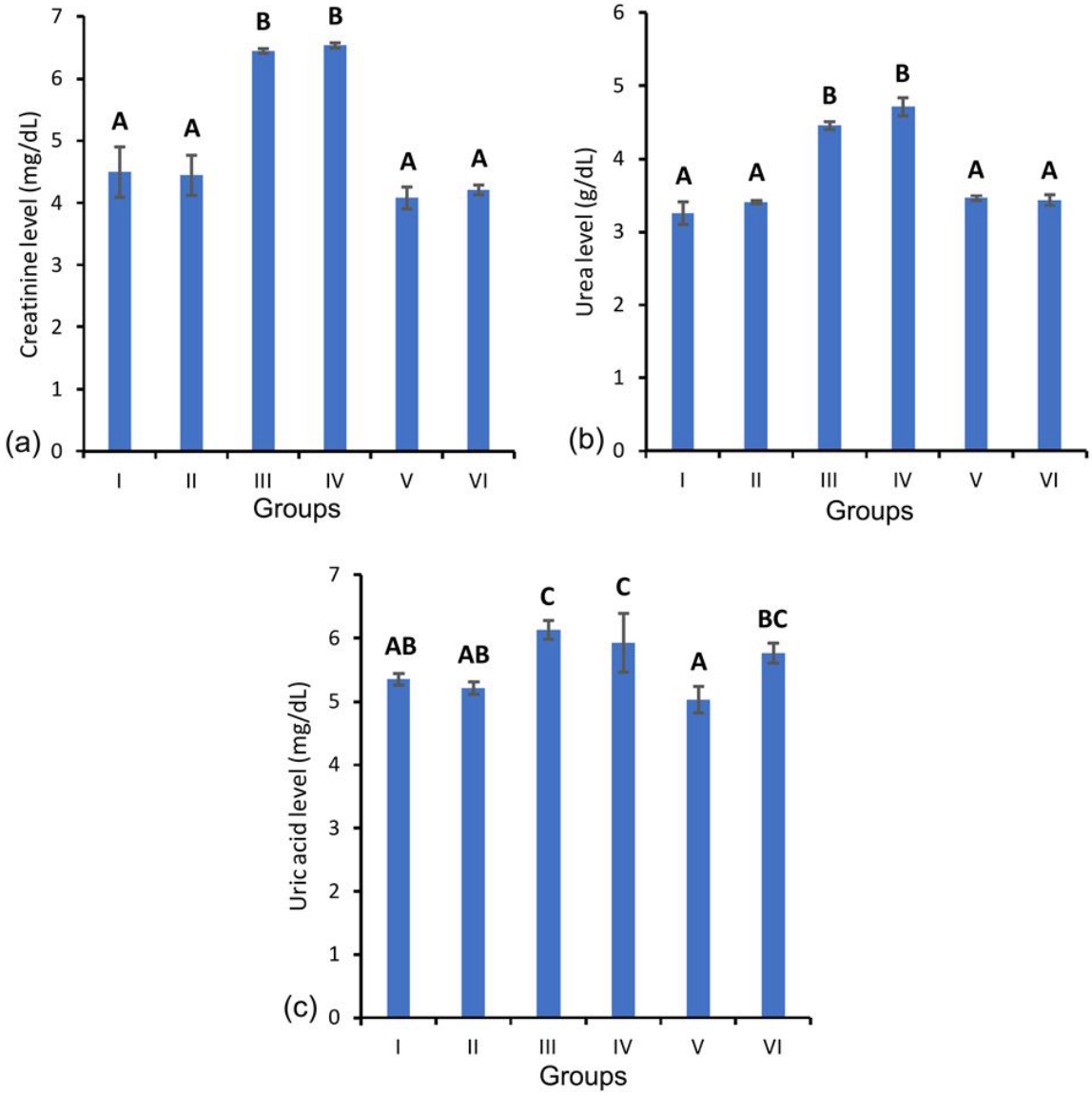

Figure 5. (a-c) The levels of (a) creatinine, (b) urea and (c) uric acid in the serum of all the experimental groups were estimated after 21 days of experiments. Data is displayed as mean ± standard error. Bars marked with different letters are significantly different (P<0.05), whereas those marked with similar ones are insignificantly different (P>0.05). **I**: Control group, **II**: Garlic oil administered, **III**: Fresh third stage larvae of *Anisakis* spp. (L3) inoculated group, **IV**: Cooked L3 inoculated group, **V**: Fresh L3 inoculated + GO treated group and **VI**: Cooked L3 inoculated + GO treated group.

alterations in the liver tissues observed in fresh inoculated L3 rats were pronounced in addition to a severe loss of liver architecture, ballooning and degenerated hepatocytes (swollen cytoplasm and pyknosis of the nucleus), apoptotic hepatocytes alternated with necrotic cells without any nuclear structure, and a portal tract that appeared with proliferated bile ducts with congested and dilated blood vessels, dilated sinusoids filled with inflammatory cells, and a prominent hyaline body with a granuloma structure (Figure 6f–i). In contrast, the liver tissues of GO and fresh L3 extract-inoculated rats showed a marked restoration of normal architectures (Figure 6j). Significant improvement in liver tissues was found in rats inoculated with GO and thermally treated L3 extract, with only mild focal necrotic areas (Figure 6k). Liver morphometry from an image analysis of the nuclear/cytoplasmic ratio and the diameter of central vein are shown in Table 3.

Kidney

The kidney tissues of the control and GO rats exhibited a normal architecture with renal tubules and renal corpuscles containing the glomerular tuft and Bowman's space (Figure 7a, b). In contrast, the kidney tissues from

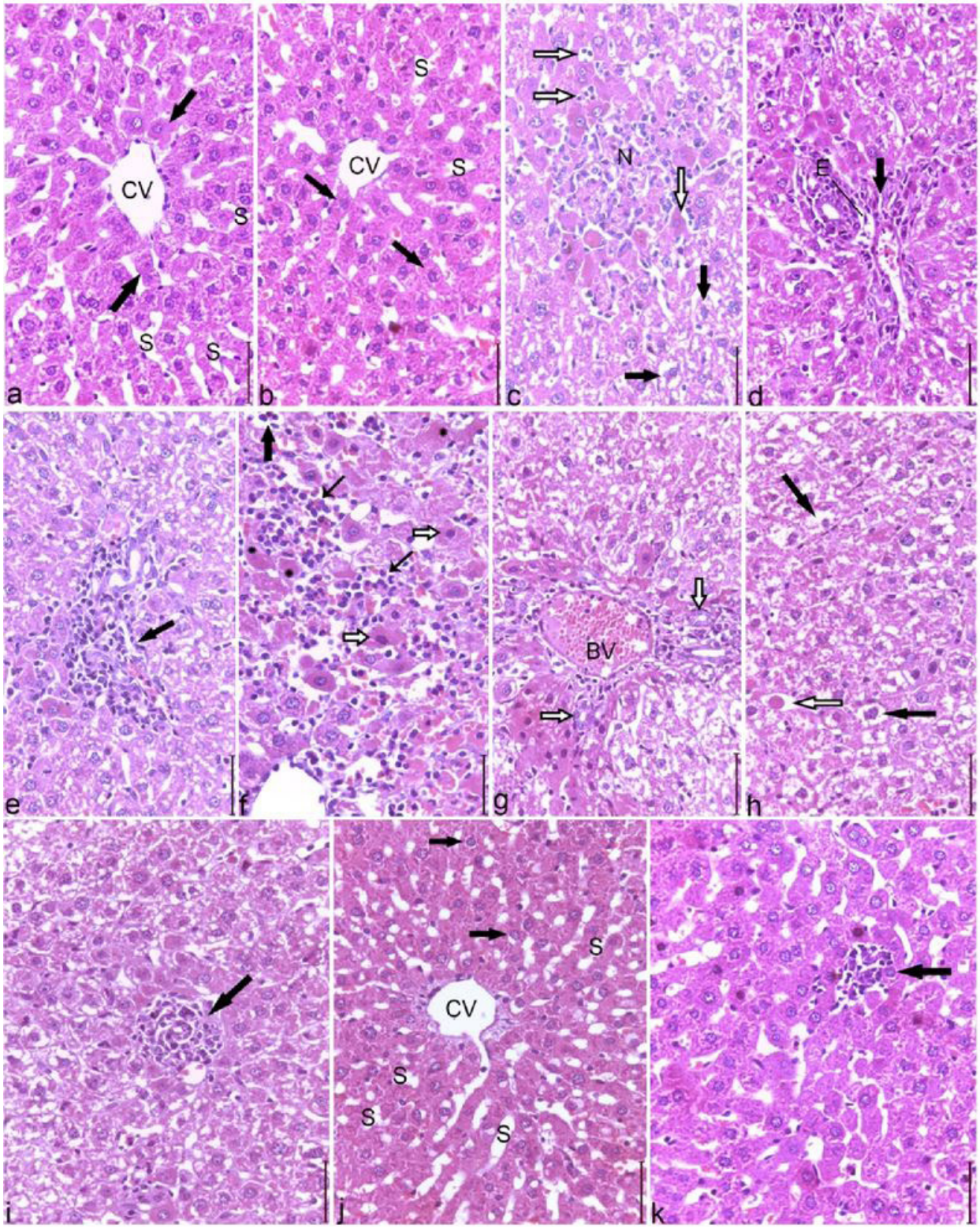

Figure 6. (a-k) Photomicrographs showing liver sections (H&E) of: (**a)** Control rats (group I); (**b)** GO rats (group II); CV central vein; S sinusoid, black arrows assigned for hepatocytes; **(c-e)**: Fresh L3 inoculated rats (groups III) showin; (**c)** Focal necrotic area (N), vacuolated hepatocytes (black arrow), dilated sinusoid with inflammatory cells (white arrow); (**d)** Perivascular edema (E), perivascular inflammation (arrow); (**e)** Focal inflammatory cell aggregates (arrow); **(f-i)** Thermally treated L3 inoculated rats (group IV) showing: (**f)** Apoptotic hepatocytes (white arrows), necrotic hepatocytes (*), sinusoid stuffed with inflammatory cells (black arrows), inflammation (small arrows); (**g)** Congested and dilated blood vessel (BV), proliferated bile ducts (white arrows); (**h)** Ballooning hepatocytes (black arrows), hyaline body (white arrow); (**i)** Granuloma (arrow); (**j)** GO + fresh L3 inoculated rats (group V) with normal liver architecture and normal hepatocytes (arrows); (**k)** GO + thermally treated L3 (group VI), almost normal with focal minute necrotic area (arrow). Bars, 50µm.

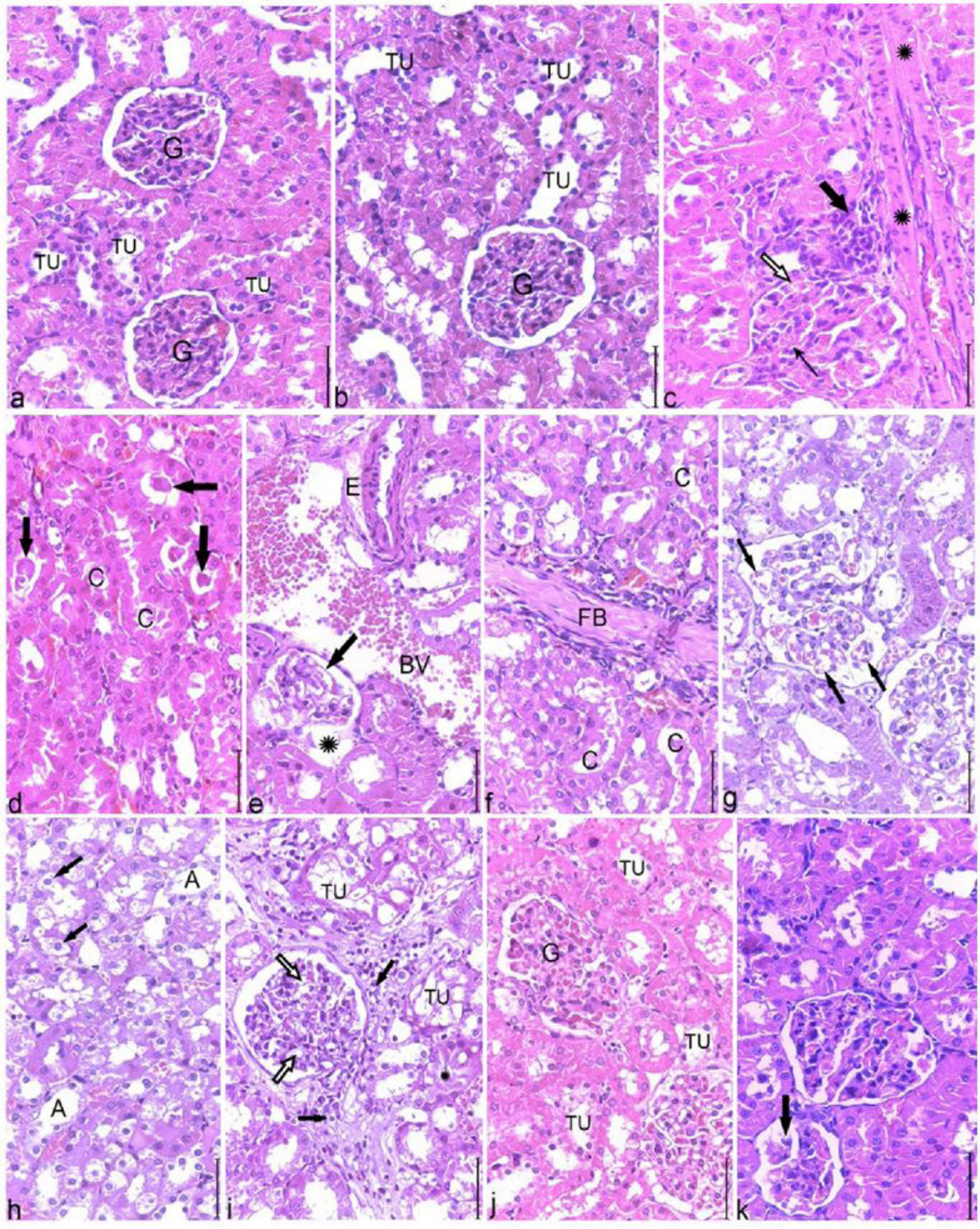

Figure 7. (a-k) Photomicrographs showing kidney sections (H&E) of: (**a)** Control rats (group I); (**b)** GO rats (group II); glomerulus (G), renal tubules (TU); **(c, d)** Fresh L3 inoculated rats (groups III) showing: (**c)** Thicken wall vessel (*), perivascular inflammation (black arrow), lobulated glomerulus (small arrow), narrowing in bowman's space (white arrow); (**d)** Cloudy swellings (C), hyaline cast (black arrows); **(e- i)** Thermally treated L3 inoculated rats (group IV) showing; (**e)** Congested and dilated blood vessel (BV), perivascular edema (E), destructed glomerulus (black arrow), increase in capsular space (*); (**f)** Cloudy swellings (C), interstitial fibrotic change (FB); (**g)** Dilated capillaries in glomerulus (black arrows); (**h)** Atrophied tubules (A), vacuolar degeneration in epithelial cell lining tubules (black arrows); (**i)** Coagulative necrosis in tubules (*), degenerative changes in mesangium cell (white arrows), interstitial inflammation (black arrow), degenerated tubules (TU); (**j)** GO + fresh L3 inoculated rats (group V) showing normal renal appearance; (**k)** GO + thermally treated L3 (group VI), almost normal with mild destructed glomerulus (black arrow). Bars, 50µm.

rats orally inoculated with fresh L3 crude extracts exhibited some renal tubules with cloudy swelling, and other tubules showed hyaline casts in their lumen, perivascular inflammation thickening the walls of blood vessels, and lobulated glomerular tufts with narrowed Bowman's spaces (Figure 7c, d). Histological changes in the renal tissues of rats orally inoculated with thermally treated L3 included a marked vacuolar degeneration of the epithelial cells lining tubules and atrophied renal tubules with cloudy swelling, severely congested dilated blood vessels, interstitial fibrosis with perivascular edema, coagulative necrosis in some tubules, destroyed glomerular tufts with an increase in capsular space and severely congested dilated blood capillaries in some glomerular tufts (Figure 7e–i). In contrast, the kidney tissues of rats inoculated with GO and fresh L3 extract showed normal structural patterns of glomerular tufts and renal tubules (Figure 7j). Although notable intact kidney tissues were found in rats inoculated with GO and thermally treated L3 extract (most of the renal tubules showed a nearly intact appearance), some glomeruli exhibited mild destruction (Figure 7k). The kidney morphometry using image analysis of the glomerular tuft diameter and the number of tubules with hyaline casts are shown in Table 3.

Table 3. Quantification of liver and kidney pathology for the six experimental groups.

Organ / Group		I	II	III	IV	V	VI
Liver	Nuclear/ cytoplasmic ratio	0.2624 ± 0.0743	0.2407± 0.067	0.1862 ± 0.056 ***	0.2002± 0.064***	0.2087±0.039	0.2506±0.063
	Diameter of central vein	1.886 ± 0.394	1.959 ±0.574	6.647 ±1.31***	8.880 ±1.034***	2.458 ±0.8964	3.196 ±1.225
Kidney	Diameter of glomerular tuft	4.61 ±0.832	3.465 ± 1.194	2.693 ± 0.755***	3.220 ± 0.411***	3.769±0.783w	3.439 ± 0.175
	Number of tubules with hyaline cast / section	2	3	15***	20***	3	5

Values are presented as mean ± SD; ***significant difference at $P < 0.05$ compared to control.

Discussion

The current study performed a morphological examination of worms recovered from examined host fish using light and scanning electron microscopy and revealed that the worms belonged to the genus *Anisakis* because they possessed all of the characteristic features of the genus according to the studies carried out by Valero et al. (2006), Casti et al. (2017) and Eissa et al. (2018): inconspicuous three lips with a prominent boring tooth on the anterior end around the triangular mouth; a straight anterior gut consisting of an esophagus, ventriculus, and intestines; the posterior end terminating with or without a mucron; and a transversely striated cuticle. There are two morphotypes of genus *Anisakis*: Type I and II. The recovered worms in the current study were identified as *Anisakis* Type II, according to Berland (1961), due to the presence of a triangular mouth surrounded by four papillae with no lips, and the rounded postanal tail without a terminal mucron. In contrast, Type I *Anisakis* spp. possess a rounded mouth opening with dorsal and ventrolateral lips equipped with papillae, and the postanal region terminates with a small mucron.

The present study observed that *Anisakis* spp. Type II (L3) inoculated into Wistar rats as fresh or thermally treated larvae induced hepatorenal toxicity. This result was evident based on the significant changes in the values of AST, ALT, ALP, total protein, creatinine, and urea compared to the control group. These values are consistent with Kliks (1983) and Mercado et al. (2001), who noted that the L3 proteins of anisakids are occasionally absorbed through the gastric tract to cause acute liver and kidney disorders. This hypothesis was supported by the increases in ALT and AST and consistent with Choudhary et al. (2003), who noted that an increase in liver enzyme activity may be due to liver weakness, with a consequent reduction in enzyme biosynthesis and bio-changes in membrane permeability, which allows the enzymes to leak into the blood and directly exposes the liver to toxic products. Changes in hepatic membrane permeability caused by the L3 crude antigen inoculation were also confirmed by a significant increase in ALP, which is consistent with Kaneko et al. (2008), who claimed that helminth protein inoculation produced hepatic insults (other enzymes were also elevated). The present study observed that albumin

levels decreased significantly in rat groups inoculated with fresh and thermally treated L3. This result is consistent with Olorunnisola et al. (2012), who claimed that a change in the normal metabolism of rat livers inoculated with larval proteins produced a marked decrease in albumin levels, which was associated with an elevated bilirubin level, compared to the control rats. Wang et al. (2016) explained this observation as the bilirubin that is released from the dysfunctional liver into blood cannot bind to the circulating albumin becomes the albumin level is insufficient to bind the elevated bilirubin from liver.

High serum creatinine concentrations may be attributed to lower glomerular filtration in the kidneys and reflects a defect in the kidney tubes in accordance with Eissa & Zidan (2010). The increase in the levels of urea and uric acid in rat groups inoculated with fresh and thermally treated L3 compared to the control rats is consistent with Larsen et al. (2014), who claimed that, due to toxicity, ammonia is generally rapidly excreted directly or is detoxified into other harmful nitrogenous molecules, such as urea and uric acid. Hammond et al. (1997) showed that the continued and inappropriate use of synthetic antihelmintics led to the development of antihelmintic resistance. This phenomenon compromised the efficiency of current and future nematode control programs and prompted the use of plant extracts to control nematode infestations in flocks (Hammond et al., 1997). *A. sativum* is a plant with well-documented anti-parasitic properties, which prompted this present investigation of its antihelmintic abilities.

The current results showed that GO significantly modulated liver and kidney function markers after inoculation in combination with fresh and thermally treated L3 crude extracts. The increased activity of ALT, ALP, and AST in fresh and thermally treated L3 rats decreased significantly in the rat groups inoculated with GO and L3 extracts. This result suggests that the hepato-protective activity may be due to the effects of GO against cellular leakage and its protection of the integrity of the cell membrane in rat liver. These findings are inconsistent with Hassan et al. (2009), Yin et al. (2002), and Zhang et al. (2012), in which GO scavenged free radicals ($O_2^{\cdot-}$, H_2O_2) via its polyphenolic compounds. GO also decreased hepatic and renal MDA and NO contents and replenished anti-oxidative enzymes (GRD, SOD, and CAT) in treated rat groups. This result reflects the antioxidant activity of GO, which has membrane-stabilizing activity that attenuates MDA and subsequently modulates membrane permeability (Tsuchiya, 2015). Organosulfur compounds, such as the diallyl disulfide of GO, have hepato-protective properties that suppress the inducible levels of CYP450 2E1, which is responsible for the generation of oxyradicals (Abdel-Naim et al., 2002). The organosulfur compounds of GO modulate the antioxidant defense system because these compounds regulate the GSH-related antioxidant system, which is the main intracellular antioxidant molecule against oxidative damage (Wu et al., 2001). Previous reports also showed that the organosulfur compounds present in GO may be responsible for the hepatorenal amelioration via their antimicrobial and antiparasitic activities (Yavuzcan Yildiz et al., 2019).

The present study observed severe pathological alterations in the liver and kidney of rats orally inoculated with *Anisakis* L3 crude extracts. This result is consistent with Arcos et al. (2014) and Fæste et al. (2014), who observed that *A. simplex* caused disease in experimental animals, even without exposure to live worms. The inoculation of rats with fresh and thermally treated L3 elicited inflammatory and necrotic changes, which are consistent with Audicana et al. (1997), who noted that the ingestion of fresh and processed fish products with heat- and frost-stable worm allergens elicited allergic reactions in consumers, and several of the antigens were stable under freezing, heating, pepsin exposure, and autoclaving conditions (Kobayashi et al., 2007; Caballero et al., 2008; Carballeda-Sangiao, et al., 2014). Anisakid infections also induce obstruction via inflammatory cells and enhance the immune response mediated by the Th17 lineage, which leads to the recruitment of neutrophils and proinflammatory reactions (Nieuwenhuizen, 2016; Bušelić et al., 2018). Necrosis may stimulate inflammation due to the leakage of intracellular constituents into adjacent tissues. The changes in the biochemical parameters observed herein were associated with liver and kidney injury, which is consistent with Prianti et al. (2007). These findings are also consistent with Rizvi et al. (2012), who showed that the kidneys of rats inoculated with parasite allergens had tubules filled with eosinophilic (hyaline) proteinaceous casts, which led to increased glomerular permeability, glomerular damage, reactive oxygen species, and proapoptotic signals that ultimately contributed to cell death. Lemos et al. (2013) also noted that mice acutely infected with parasitic worms demonstrated a significant increase in renal inflammatory infiltration, renal vascular permeability, and an increased production of nitric oxide and cytokines in renal tissues and a decrease in creatinine clearance, primarily at the highest parasite loads.

The ameliorative and protective effects of GO on the four tissues examined may be related to the design of GO inoculation before and after crude L3 extract administration. This observation is consistent with Kamel & El-Shinnawy (2015), who reported that GO administration before and after parasite inoculation induced mild pathological changes that correlated to the statistically significant liver and kidney functions of these groups.

Conclusion

To avoid nematode infestations and their implications, fish should be inspected well before eating using the candling process. This process involves examining fish fillets over lights to detect surface parasites that should be removed. Fish with a heavy infestation of worms in their muscles and internal viscera should not be eaten. Fish meals should be eaten with garlic, which has a history of tissue protection against parasites. Garlic also detoxifies and gently stimulates elimination, and it has antioxidant properties to protect against the oxidation caused by parasitic toxins. One should buy fish from a trusted store where they carefully choose healthy fish for consumers.

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References

Abdel-Naim AB, Khalifa AE, Ahmed SH. Protective effects of garlic oil against liver damage induced by combined administration of ethanol and carbon tetrachloride in rats. *Egypt J Hosp Med* 2002; 6(1): 27-36. http://dx.doi.org/10.12816/ejhm.2002.18854.

Abe N, Teramoto I. Oral inoculation of live or dead third-stage larvae of *Anisakis simplex* in rats suggests that only live larvae induce production of antibody specific to *A. simplex. Acta Parasitol* 2014; 59(1): 184-188. http://dx.doi.org/10.2478/s11686-014-0216-4. PMid:24570066.

Amagase H, Petesch BL, Matsuura H, Kasuga S, Itakura Y. Recent advances on the nutritional effects associated with the use of garlic as a supplement. Intake of garlic and its bioactive components. *J Nutr* 2001; 131: 955S-962S. http://dx.doi.org/10.1093/jn/131.3.955S. PMid:11238796.

Arcos SC, Ciordia S, Roberston L, Zapico I, Jiménez–Ruiz Y, Gonzalez–Muñoz M, et al. Proteomic profiling and characterization of differential allergens in the nematodes *Anisakis simplex sensu stricto* and *A. pegreffi. Proteomics* 2014; 14(12): 1547-1568. http://dx.doi.org/10.1002/pmic.201300529. PMid:24723494.

Audicana L, Audicana MT, Fernández de Corres L, Kennedy MW. Cooking and freezing may not protect against allergic reactions to ingested *Anisakis simplex* antigens in humans. *Vet Rec* 1997; 140(9): 235. http://dx.doi.org/10.1136/vr.140.9.235. PMid:9076923.

Bartels H, Böhmer M, Heierli C. Serum creatinine determination without protein precipitation. *Clin Chim Acta* 1972; 37: 193-197. http://dx.doi.org/10.1016/0009-8981(72)90432-9. PMid:5022083.

Berland B. Nematodes from some Norwegian marine fishes. *Sarsia* 1961; 2(1): 1-52. http://dx.doi.org/10.1080/00364827.1961.10410245.

Bušelić I, Trumbić Ž, Hrabar J, Vrbatović A, Bočina I, Mladineo I. Molecular and Cellular Response to Experimental *Anisakis pegreffii* (Nematoda, Anisakidae) Third–Stage Larval Infection in Rats. *Front Immunol* 2018; 9: 2055. http://dx.doi.org/10.3389/fimmu.2018.02055. PMid:30245697.

Caballero ML, Moneo I, Gómez–Aguado F, Corcuera MT, Casado I, Rodríguez–Pérez R. Isolation of Ani s5, an excretory–secretory and highly heat–resistant allergen useful for the diagnosis of *Anisakis* larvae sensitization. *Parasitol Res* 2008; 103(5): 1231-1233. http://dx.doi.org/10.1007/s00436-008-1105-2. PMid:18618144.

Carballeda–Sangiao N, Olivares F, Rodriguez–Mahillo AI, Careche M, Tejada M, Moneo I, et al. Identification of autoclave- resistant *Anisakis simplex* allergens. *J Food Prot* 2014; 77(4): 605-609. http://dx.doi.org/10.4315/0362-028X.JFP-13-278. PMid:24680072.

Casti D, Scarano C, Piras MC, Merella P, Muglia S, Piras F, et al. Occurrence of Nematodes of the Genus *Anisakis* in Mediterranean and Atlantic Fish Marketed in Sardinia. *Ital J Food Saf* 2017; 6(1): 6185. http://dx.doi.org/10.4081/ijfs.2017.6185. PMid:28299289.

Choudhary N, Sharma M, Verma P, Joshi SC. Hepato and nephrotoxicity in ratexposed to endosulfan. *J Environ Biol* 2003; 24(3): 305-308. PMid:15259607.

Danikowski KM, Cheng T. Colorimetric Analysis of Alkaline Phosphatase Activity in *S. aureus* Biofilm. *J Vis Exp* 2019; 12(146): 146. http://dx.doi.org/10.3791/59285. PMid:31033962.

Doumas BT, Ard Watson W, Biggs HG. Albumin standards and the measurement of serum albumin with bromcresol green. *Clin Chim Acta* 1971; 31(1): 87-96. http://dx.doi.org/10.1016/0009-8981(71)90365-2. PMid:5544065.

Eissa AE, Showehdi ML, Ismail MM, El-Naas AS, Abu Mhara AA, Abolghait SK. Identification and prevalence of *Anisakis pegreffii* and *A. pegreffii*; *A. simplex* (s.s.) hybrid genotype larvae in Atlantic horse Mackerel (*Trachurus trachurus*) from some North African Mediterranean coasts. *Egypt J Aquat Res* 2018; 44(1): 21-27. http://dx.doi.org/10.1016/j.ejar.2018.02.004.

Eissa FI, Zidan NA. Haematological, biochemical and histopathological alteration induced by abamectin and *Bacillus thuringiensis* in male albino rats. *Acta Biol Hung* 2010; 61(1): 33-44. https://doi.org/10.1556/abiol.61.2010.1.4.

Fæste CK, Jonscher KR, Dooper MM, Egge-Jacobsen W, Moen A, Daschner A, et al. Characterisation of potential novel allergens in the fish parasite *Anisakis simplex*. *EuPA Open Proteom* 2014; 4: 140-155. https://dx.doi.org/ 10.1016/j.euprot.2014.06.006.

Fawcett JK, Scott JE. A rapid and precise method for the determination of urea. *J Clin Pathol* 1960; 13(2): 156-159. http://dx.doi.org/10.1136/jcp.13.2.156. PMid:13821779.

Hammond JA, Fielding D, Bishop SC. Prospects for plant anthelmintics in tropical veterinary medicine. *Vet Res Commun* 1997; 21(3): 213-228. http://dx.doi.org/10.1023/A:1005884429253. PMid:9090048.

Hassan HA, El-Agmy SM, Gaur RL, Fernando A, Raj MH, Ouhtit A. *In vivo* evidence of hepato– and reno–protective effect of garlic oil against sodium nitrite- induced oxidative stress. *Int J Biol Sci* 2009; 5(3): 249-255. http://dx.doi.org/10.7150/ijbs.5.249. PMid:19305642.

Henry RJ. Colorimetric determination of total protein. In: RJ Henry: *Clinical Chemistry*. New York: Harper and Row; 1964.

Iberl B, Winkler G, Muller B, Knobloch K. Quantitative determination of allicin and alliin from Garlic by HPLC*. *Planta Med* 1990; 56(3): 320-326. https://doi.org/10.1055/s-2006-960969.

Kamel ROA, El-Shinnawy NA. Immunomodulatory effect of garlic oil extract on *Schistosoma mansoni* infected mice. *Asian Pac J Trop Med* 2015; 8(12): 999-1005. https://doi.org/10.1016/j.apjtm.2015.11.016.

Kaneko JJ, Harvey JW, Bruss ML. *Clinical biochemistry of domestic animals*. 6th ed. USA, Cambridge: Academic Press; 2008.

Kassem HH, Bowashi SM. Prevalence of Anisakid nematode larvae infecting some marine fishes from the Libyan Coast. *J Egypt Soc Parasitol* 2015; 45(3): 609-616. http://dx.doi.org/10.12816/0017928. PMid:26939239.

Kliks MM. Anisakiasis in the western United States: four new case reports from California. *Am J Trop Med Hyg* 1983; 32(3): 526-532. http://dx.doi.org/10.4269/ajtmh.1983.32.526. PMid:6683083.

Kobayashi Y, Shimakura K, Ishizaki S, Nagashima Y, Shiomi K. Purification and cDNA cloning of a new heat-stable allergen from *Anisakis simplex. Mol Biochem Parasitol* 2007; 155(2): 138-145. http://dx.doi.org/10.1016/j.molbiopara.2007.06.012. PMid:17689675.

Larsen EH, Deaton LE, Onken H, O'Donnell M, Grosell M, Dantzler WH, et al. Osmoregulation and excretion. *Compr Physiol* 2014; 4(2): 405-573. http://dx.doi.org/10.1002/cphy.c130004. PMid:24715560.

Lee DY, Li H, Lim HJ, Lee HJ, Jeon R, Ryu JH. Anti-inflammatory activity of sulfur-containing compounds from garlic. *J Med Food* 2012; 15(11): 992-999. http://dx.doi.org/10.1089/jmf.2012.2275. PMid:23057778.

Lemos JR, Rodrigues WF, Miguel CB, Parreira RC, Miguel RB, de Paula Rogerio A, et al. Influence of parasite load on renal function in mice acutely infected with *Trypanosoma cruzi. PLoS One* 2013; 8(8): e71772. http://dx.doi.org/10.1371/journal.pone.0071772. PMid:23951243.

Liu CT, Su HM, Lii CK, Sheen LY. Effect of supplementation with garlic oil on activity of Th1 and Th2 lymphocytes from rats. *Planta Med* 2009; 75(3): 205-210. http://dx.doi.org/10.1055/s-0028-1088396. PMid:19137499.

Madden PA, Tromba FG. Scanning electron microscopy of the lip denticiles of *Ascaris suum* adults of known ages. *J Parasitol* 1976; 62(2): 265-271. http://dx.doi.org/10.2307/3279282. PMid:1263037.

Mahima, Rahal A, Deb R, Latheef SK, Abdul Samad H, Tiwari R, et al. Immunomodulatory and therapeutic potentials of herbal, traditional/indigenous and ethnoveterinary medicines. *Pak J Biol Sci* 2012; 15(16): 754-774. http://dx.doi.org/10.3923/pjbs.2012.754.774. PMid:24175417.

Mattiucci S, Fazii P, De Rosa A, Paoletti M, Megna AS, Glielmo A, et al. Anisakiasis and gastroallergic reactions associated with *Anisakis pegreffii* infection, Italy. *Emerg Infect Dis* 2013; 19(3): 496-499. http://dx.doi.org/10.3201/eid1903.121017. PMid:23621984.

Mercado R, Torres P, Muñoz V, Apt W. Human infection by *Pseudoterranova decipiens* (Nematoda, Anisakidae) in Chile: report of seven cases. *Mem Inst Oswaldo Cruz* 2001; 96(5): 653-655. http://dx.doi.org/10.1590/S0074-02762001000500010. PMid:11500763.

Mohamed EH, Baiomy AA, Ibrahim ZS, Soliman MM. Modulatory effects of levamisole and garlic oil on the immune response of Wistar rats: Biochemical, immunohistochemical, molecular and immunological study. *Mol Med Rep* 2016; 14(3): 2755-2763. http://dx.doi.org/10.3892/mmr.2016.5551. PMid:27484629.

Montalto M, Miele L, Marcheggiano A, Santoro L, Curigliano V, Vastola M, et al. *Anisakis* infestation: a case of acute abdomen mimicking Crohn's disease and eosinophilic gastroenteritis. *Dig Liver Dis* 2005; 37(1): 62-64. http://dx.doi.org/10.1016/j.dld.2004.05.014. PMid:15702862.

Morsy K, Bashtar AR, Abdel–Ghaffar F, Mostafa N. New host and locality records of two nematode parasites *Dujardinnascaris mujibii* (Heterocheilidae) and *Hysterothylacium aduncum* (Anisakidae) from the common seabream *Pagrus pagrus*: a light and scanning electron microscopic study. *Parasitol Res* 2013; 112(2): 807-815. http://dx.doi.org/10.1007/s00436-012-3270-6. PMid:23315190.

Nieuwenhuizen NE. *Anisakis*–immunology of a foodborne parasitosis. *Parasite Immunol* 2016; 38(9): 548-557. http://dx.doi.org/10.1111/pim.12349. PMid:27428817.

Olorunnisola OS, Bradley G, Afolayan AJ. Protective effect of *T. violacea* rhizome extract against hypercholesterolemia–induced oxidative stress in Wistar rats. *Molecules* 2012; 17(5): 6033-6045. http://dx.doi.org/10.3390/molecules17056033. PMid:22614858.

Percival SS. Aged garlic extract modifies human immunity. *J Nutr* 2016; 146(2): 433S-436S. http://dx.doi.org/10.3945/jn.115.210427. PMid:26764332.

Prianti MG, Yokoo M, Saldanha LCB, Costa FAL, Goto H. *Leishmania* (*Leishmania*) *chagasi*- infected mice as a model for the study of glomerular lesions in visceral leishmaniasis. *Braz J Med Biol Res* 2007; 40(6): 819-823. http://dx.doi.org/10.1590/S0100-879X2007000600011. PMid:17581681.

Randall JE. *Red Sea reef fishes*. London, UK: Immel Publishing Limited; 1992.

Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am J Clin Pathol* 1957; 28(1): 56-63. http://dx.doi.org/10.1093/ajcp/28.1.56. PMid:13458125.

Riad NH, Fares NH, Mostafa OM, Mahmoud YI. The effect of garlic on some parasitological parameters and on hepatic tissue reactions in experimental *Schistosoma mansoni. J Appl Sci Res* 2007; 3(10): 949-960.

Rizvi A, Hasan S, Alam M, Zafar A, Fatima T, Shareef PA, et al. Levels of some antioxidant molecules and lipid peroxidation during *in vivo* transformation of the progenetic metacercaria of *Clinostomum complanatum* to ovigerous adult worms. *Vet Parasitol* 2012; 185(2-4): 164-167. http://dx.doi.org/10.1016/j.vetpar.2011.10.024. PMid:22079426.

Seckiner I, Bayrak O, Can M, Mungan AG, Mungan NA. Garlic supplemented diet attenuates gentamicin nephrotoxicity in rats. *Int Braz J Urol* 2014; 40(4): 562-567. https://doi.org/10.1590/S1677-5538.IBJU.2014.04.17.

Tsuchiya H. Membrane interactions of phytochemicals as their molecular mechanism applicable to the discovery of drug leads from plants. *Molecules* 2015; 20(10): 18923-18966. http://dx.doi.org/10.3390/molecules201018923. PMid:26501254.

Valero A, Mar López-Cuello M, Benítez R, Adroher F. *Anisakis* spp. in European hake, *Merluccius merluccius* (L.) from the Atlantic off north-west Africa and the Mediterranean off southern Spain. *Acta Parasitol* 2006; 51(3): 209-212. http://dx.doi.org/10.2478/s11686-006-0032-6.

Wang B, Zhu Y, Zhang H, Liu L, Li G, Song Y, et al. Effects of aluminum chloride on serum proteins, bilirubin, and hepatic trace elements in chickens. *Toxicol Ind Health* 2016; 32(9): 1693-1699. http://dx.doi.org/10.1177/0748233715578035. PMid:25896954.

Watts RWE. Determination of uric acid in blood and in urine. *Ann din Biochem* 1974; 11: 103-111.

World Health Organization – WHO. *The World health Report: health systems: improving performance* [online]. Geneva: WHO; 2000 [cited 2000 Jul 20]. Available from: https://apps.who.int/iris/handle/10665/42281

Wu CC, Sheen LY, Chen HW, Tsai SJ, Lii CK. Effects of organosulfur compounds from garlic oil on the antioxidation system in rat liver and red blood cells. *Food Chem Toxicol* 2001; 39(6): 563-569. http://dx.doi.org/10.1016/S0278-6915(00)00171-X. PMid:11346486.

Yavuzcan Yildiz H, Phan Van Q, Parisi G, Dam Sao M. Anti-parasitic activity of garlic (*Allium sativum*) and onion (*Allium cepa*) juice against crustacean parasite, *Lernantropus kroyeri*, found on European sea bass *(Dicentrarchus labrax). Ital J Anim Sci* 2019; 18(1): 833-837. http://dx.doi.org/10.1080/1828051X.2019.1593058.

Yin MC, Hwang SW, Chan KC. Nonenzymatic antioxidant activity of four organosulfur compounds derived from garlic. *J Agric Food Chem* 2002; 50(21): 6143-6147. http://dx.doi.org/10.1021/jf0204203. PMid:12358493.

Yun HM, Ban JO, Park KR, Lee CK, Jeong HS, Han SB, et al. Potential therapeutic effects of functionally active compounds isolated from garlic. *Pharmacol Ther* 2014; 142(2): 183-195. http://dx.doi.org/10.1016/j.pharmthera.2013.12.005. PMid:24333688.

Zhang CL, Zeng T, Zhao XL, Yu LH, Zhu ZP, Xie KQ. Protective effects of garlic oil on hepatocarcinoma induced by N–nitrosodiethylamine in rats. *Int J Biol Sci* 2012; 8(3): 363-374. http://dx.doi.org/10.7150/ijbs.3796. PMid:22393308.