Tropical Biomedicine 41(4): 427-437 (2024)
https://doi.org/10.47665/tb.41.4.002

RESEARCH ARTICLE

Anthelmintic activity and pathophysiological effect of *Allium sativum* crude extract against carcinogenic liver fluke, *Opisthorchis viverrini*

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ARTICLE HISTORY

Received: 14 December 2023
Revised: 27 January 2024
Accepted: 29 January 2024
Published: 31 December 2024

ABSTRACT

Opisthorchis viverrini poses a substantial risk for cholangiocarcinoma (CCA) in Thailand. Despite praziquantel (PZQ) remains the primary treatment for opisthorchiasis, its association with adverse effects and potential CCA development during prolonged treatment, particularly in cases of reinfection and chronic infection, underscores the imperative for alternative herbal interventions with anthelmintic potential. In this context, a prior study suggested the inhibitory effects of *Allium sativum L.* (garlic) on various protozoa and helminths, prompting the investigation of its efficacy against *O. viverrini* in this study. Therefore, this study aimed to assess the efficacy of garlic against *O. viverrini*. *O. viverrini* adult worms were exposed to varying concentrations of garlic crude extract (20, 30, and 40 mg/ml). As comparators, another set of adult worms was treated with PZQ (20 mg/ml) and Roswell Park Memorial Institute 1640 medium, serving as the positive and negative control groups. The quantification of reactive oxygen species (ROS) as markers of oxidative stress was executed using 2',7'-Dichlorodihydrofluorescein diacetate staining. Morphological damage of *O. viverrini* adult worms were evaluated through scanning electron microscopy. Additionally, motility assessment was conducted at various exposure times (0, 30 minutes, 1, 2, 3, 6, 12, and 24 hours) by estimating relative motility values and survival index. The results revealed significantly elevated ROS levels and distinctive morphological damage, characterized by swelling of microvilli and papillae in the garlic-treated groups. In contrast, the positive control group exhibited minor morphological damage, while the negative control group did not display such alterations. The reduction in movement and increased mortality were observed in the groups treated with garlic, as evidenced by the RM and SI values, in comparison to both the positive and negative control groups. These findings suggest that garlic extract possesses potent anthelmintic properties against *O. viverrini* adult worms and holds promise as an alternative therapeutic avenue for Opisthorchiasis.

Keywords: *Allium sativum*; against-parasite; *Opisthorchis viverrine*; garlic crude extracts.

INTRODUCTION

Opisthorchis viverrini, a parasitic liver fluke, it cause of opisthorchiasis, prevalent in northern and northeastern Thailand, with an overall infection rate of 2.2%, escalating to 14.3% in high-risk areas (Wattanawong *et al*., 2021). The persistent inflammatory response induced by *O. viverrini* in the bile ducts poses a substantial long-term risk for cholangiocarcinoma (CCA) (Pinlaor *et al*., 2009; Hanpanich *et al*., 2017). Praziquantel (PZQ) is the drug for Opisthorchiasis treatment, inducing spastic spasms in parasites by facilitating the influx of calcium ions, ultimately leading to paralysis and the eventual demise of the parasite (Pax *et al*., 1978; Becker *et al*., 1980). However, recurrent PZQ treatment correlates with intrahepatic and papillary CCA, and emerging drug resistance poses concerns (Fallon & Doenhoff, 1994; Cioli & Pica-Mattoccia, 2003; Luvira *et al*., 2018). Persistent apprehensions regarding PZQ side effects (Erko *et al*., 2012) underscore the need to explore alternative herbal remedies. Bioactive substances like tannins, flavonoids, and alkaloids, known for their efficacy against various parasites (Mushtaq *et al*., 2018), it can be considered as a guidelines for exploring key bioactive compounds for the elimination of parasites in the future.

Since ancient times, garlic has been used extensively as a traditional treatment for a variety of illnesses. Garlic's primary bioactive ingredients include diallyl disulfide, s-allyl cysteine,

methyl cysteine, and allicin, which have all been shown to have strong antibacterial, anticancer, and antiparasitic effects (Corzo-Martínez *et al*., 2007). Especially allicin, extensively researched, shows therapeutic potential against cardiovascular diseases, offers antioxidant effects, and protects the liver from toxins and alcohol (Rabinkov *et al*., 1998; Corzo-Martínez *et al*., 2007). Garlic's utility is documented in *in vivo* and *in vitro* studies, inhibiting protozoa, helminths and influencing immune responses. It has been observed that garlic demonstrates inhibitory effects on the development of various protozoa, including *Trypanosoma brucei brucei*, *T. congolense*, *T. vivax*, *Trypanosoma* spp., and *Entamoeba histolytica* (Nok *et al*., 1996; Behnia *et al*., 2008). In infected mice, garlic has also shown enhance immune response against *Plasmodium yoelii* (Feng *et al*., 2012). Additionally, garlic has proven effective against *Haemonchus contortus*, a parasitic nematode in ruminant animals (Palacios-Landín *et al*., 2015), *Anisakis* sp., a nematode in marine fish (Morsy *et al*., 2021), and *Gyrodactylus turnbulli*, a monogenean parasite (Schelkle *et al*., 2013). Studies indicate that garlic influences *Schistosoma mansoni* at various stages, including adult worms, cercaria, schistosomula, and miracidia (Mantawy *et al*., 2012; Riad *et al*., 2013; Aly *et al*., 2017; Cortes *et al*., 2017). It has enhanced the immune response in mice infected with *S. mansoni* (El Shenawy *et al*., 2008; Mantawy *et al*., 2011, 2012; Metwally *et al*., 2018). Furthermore, garlic extract has been shown to affect the morphological damage caused by the liver fluke, *Fasciola gigantica* (Singh *et al*., 2009).

Therefore, this study investigates garlic extracts' anthelmintic effects on *O. viverrini* adult worms, comparing them with PZQ and a negative control. Evaluation parameters include relative motility (RM), survival index (SI), reactive oxygen species (ROS) generation, and morphological surface alterations. The review underscores garlic's potential as a naturally derived therapeutic agent against various parasitic diseases, supporting its role in effective and sustainable parasite management.

MATERIALS AND METHODS

Ethical approval

The Animal Ethics and Bio Ethics have been approved by Committee of the institute of research and development, Suranaree University of Technology, Thailand (Animal Ethics: Ethical Clearance No. SUT-IACUC-0013/2023 and Bio Ethics: Ethical Clearance No. SUT-IBC-008-2023).

Parasite preparation

O. viverrini metacercariae were obtained from naturally infected cyprinid fish in an endemic region spanning Nakhon Ratchasima and Chaiyaphum Province, northeastern Thailand. Fresh cyprinid fish underwent digestion in a 0.25% pepsin-hydrochloric acid solution, followed by incubation at 37°C for 1-2 hours. Subsequently, the solution underwent filtration and centrifugation using 0.85% normal saline solution in a sedimentation jar. Metacercariae identification was conducted based on their morphology under a stereomicroscope. Male Syrian golden hamsters aged 6-8 weeks were orally infected with 50 *O. viverrini* metacercariae through intragastric intubation. This facilitated the development of *O. viverrini* adult worms in the liver bile ducts over a period of 2-3 months. Subsequently, the *O. viverrini*-infected hamsters were sacrificed to collect adult worms from the liver bile ducts for experimental purposes.

Garlic crude extract preparation

Garlic cloves were meticulously peeled and washed with filtered water to remove any dust or particulate matter. One hundred and fifty grams of cloves were ground, and garlic water was extracted by pressing it through three layers of gauze. The garlic water underwent centrifugation at 5,000 RPM for 10 minutes to collect the supernatant, which was further filtered using Whatman filter No.1. The resulting liquid was then subjected to freeze-drying to yield a powdered extract (LABCONCO FRECZ DRY®, Kansas, USA) under conditions of 133x10-3 mBar for 48 hours. The powdered extract was stored at -20°C until used.

O. viverrini experimental allocation

Three months post-infection (p.i.), hamsters were euthanized with 1-3% isoflurane. A surgical incision was made to open the abdominal cavity. The liver hamster was removed and placed immediately in 0.85% NSS. Adult worms were pressed from the liver bile duct and incubated in culture media (Roswell Park Memorial Institute or RPMI-1640). Subsequently, the actively adult worms were selected for experimentation. Forty *O. viverrini* adult worms were allocated into five distinct groups (4 worms/group, duplicate in each group): group 1 received the RPMI-1640 culture medium, group 2 was subjected to treatment with a standard drug of 20 mg/ml PZQ, groups 3 to 5 involved treatment of *O. viverrini* adult worms with varied concentrations of garlic crude extract (20, 30, and 40 mg/ml), with each group supplemented with 100 µg/ml of streptomycin antibiotic. All groups exposed at different time intervals (0, 30 minutes, 1 hours, 2 hours, 3 hours, 6 hours, 12 hours, and 24 hours) under 37°C atmosphere.

Pathophysiological effect of *A. sativum* crude extract

Morphological study by scanning electron microscope (SEM)

The evaluation of morphological damage in *O. viverrini* adult worms, following 12 hours of incubation, was performed using SEM. Subsequently, the adult worms underwent multiple washes with distilled water (DW). The specimens were fixed overnight in a glutaraldehyde fixative solution at 4°C. Following fixation, the worms underwent three sequential 10-minute washes with DW. Post-fixation involved immersion in a 1% Osmium tetroxide fixative solution in 0.1 M phosphate buffer saline (PBS) with a pH of 7.2 for 1 hour, followed by three 10-minute DW washes. Subsequently, the samples were dehydrated through a series of graded acetone solutions (30%, 50%, 70%, 90%, 95%, and 100% alcohol) in two cycles. They were then desiccated using a critical point dryer (Leica CPD 300® Vienna, Austria), coated with a layer of gold using an Au ion sputtering device on conductive tape, and examined under scanning electron microscopy (FESEM/Carl Zeiss Auriga® Dresden, Germany) at an electric high tension 3.00 kV.

Measurement of stress generation due to reactive oxygen species

The generation of stress induced by garlic extract initiated a cellular response after 6 hours of incubation, establishing an equilibrium between antioxidant defenses and ROS or free radicals. Subsequently, the subjected *O. viverrini* adult worms underwent thorough rinsing with DW. Following the rinsing procedure, the worms were exposed to a 30 µM fluorogenic dye, 2',7'-Dichlorodihydrofluorescein diacetate (Med Chem Express®, New Jersey, USA), and incubated in darkness at 37°C for 30 minutes. After incubation, the samples underwent additional washing with DW to eliminate any surplus fluorogenic dye. Slides were prepared for fluorescent imaging utilizing an inverted fluorescence microscope (Ex/Em = 488/525 nm). The fluorescence levels emanating from ROS were quantified by analyzing fluorescence microscopy images through the ImageJ software (https://imagej.net/ij/download.html). The corrected total worm fluorescence (CTWF) was determined by subtracting the integrated density from the product of the selected worm's area and the mean fluorescence of the background readings (El-Sharkawey, 2016).

Anthelmintic activity on *O. viverrini* adult worm

The motility was assessed by examining adult worms under a stereomicroscope and scored based on the criteria established by Jiraungkoorskul *et al*. (2005) and Jeyathilakan *et al*. (2012) (3 = moving whole body, 2 = moving only parts of the body, 1 = immobile but alive, and 0 = died). The relative motility (RM) value was computed based on the motility scores across all experimental groups. Notably, the negative control group, where all parasites were scored 3, demonstrated an RM value of 100. Accordingly, a diminished RM value observed in the garlic-treated group suggested a more robust inhibition of motility due to the garlic extract. The RM values were determined employing the formula detailed below (Kiuchi *et al*., 1987; Lorsuwannarat *et al*., 2013).

$$\text{Motility index (MI)} = \frac{\sum nN}{\text{N}} \quad (1)$$

$$\text{Relative motility (RM) value} = \frac{\text{MI test} \times 100}{\text{MI control}} \quad (2)$$

n = motility score,
N = number of worms with the score of ''n''

The survival index (SI) was calculated to determine the percentage of live worms at a specific time after incubation. Worms that exhibited a motility score of 0 were classified as died, whereas those with other scores (3, 2, and 1) were regarded as still alive. The SI was calculated using the formula provided below (Kiuchi *et al*., 1987; Lorsuwannarat *et al*., 2013).

$$\%\ \text{Survival index (SI)} = \frac{\text{Number of live worm (each group)} \times 100}{\text{Total worm (each group)}} \quad (3)$$

Worm viability

After 24 hours of incubation, the worms were exposed to a 0.4% Trypan blue stain at room temperature for 2-3 minutes. Following this, the worms underwent three washes with 1x PBS, and their viability was assessed under a light microscope.

Data analysis

The RM values and SI were analyzed through the respective formulas. Mean scores and standard deviations of motility were calculated for each group. Data analysis was performed using IBM SPSS Statistics 26 (SPSS Inc., Chicago, USA) with a One-Way ANOVA conducted among five groups (Negative control, positive control, and three garlic-treated groups) to compare the mean motility scores. Statistical significance was determined by a p-value < 0.05.

RESULTS

Morphological surface study

In the negative control group, the worms exhibited a regular, smooth surface (Figure 1a), numerous short microvilli and the typical distribution of papillae around the oral and ventral suckers (Figure 1b and Figure 1c). The arrangement of papillae among the microvilli was also normal (Figure 1c). In the positive control group, the microvilli on the surface appeared swollen, and there was slight swelling of the papillae around the ventral sucker (Figure 1e and Figure 1f). Morphological surface alterations and damages resulting from treatment with garlic crude extract displayed a consistent pattern. The *O. viverrini* adult worm treated with garlic crude extract (20 mg/ml) exhibited an irregular and non-smooth surface (Figure 2a), significant morphological disruption around the oral sucker (Figure 2b), and considerable damage to the microvilli surface and papillae around the ventral sucker (Figure 2c and Figure 2d). Extensive swelling of the microvilli surface was evident throughout the entire body of the worm (Figure 2e and Figure 2f).

Measurement of stress generation

A substantial cellular stress response, leading to ROS generation, was evident in all groups after a 6-hour incubation period. ROS presence was determined using H2DCFDA fluorescence dye, enabling comprehensive ROS analysis. Minimal ROS generation was observed in the negative control group in the anterior, middle, and posterior regions of the *O. viverrini* adult worm, as depicted in Figure 3 (Figure 3a-c). The positive control group displayed partial ROS generation, primarily around the oral and ventral suckers in these regions (Figure 3d - f). In contrast, the garlic-treated groups exhibited significantly higher ROS levels throughout the entire *O. viverrini* adult worm body (Figure 3g-o). The median fluorescence intensity was lower in both control groups, whereas the garlic-treated group exhibited higher intensity, consistent with the fluorescence images presented in Figure 4.

Motility test and viability assay

Throughout the entire experimental duration, adult worms in the negative control group exhibited continuous active movement and sustained vitality (RM = 100 and SI = 100). In the garlic-treated groups with concentrations of 20, 30, and 40 mg/ml at the 0-minute mark, all worm sets displayed active motility and a healthy appearance, resembling the negative control group, with RM = 100 and SI = 100, respectively. However, after 1 hour of exposure, the garlic-treated groups at concentrations of 20, 30, and 40 mg/ml exhibited reduced motility, with RM values of 56.86, 47.06, and 47.06, respectively, while maintaining SI = 100 for all groups. These values were comparable to those of the positive control group, which showed an RM of 50.98 and SI of 100. By 3 hours, the garlic-treated groups displayed a substantial reduction in both RM and SI (RM = 23.52, 17.64, and 15.68, SI = 50.00, 37.50, and 25, respectively). Consequently, all worms in these groups ceased movement or perished by the 6-hour assessment (RM = 0 and SI = 0). In contrast, the positive control group exhibited a gradual reduction in RM and SI, culminating in complete cessation of movement at the 24-hour mark (RM = 0 and SI = 0) (Figure 5 and Figure 6). Viability confirmation through Trypan blue staining (Figure 7) revealed unstained worms in the negative control group (indicating vitality), stained worms in the positive control group (suggesting loss of viability), and extensive staining in garlic-treated groups, signifying a loss of viability. A comparative analysis utilizing One-Way ANOVA revealed significant differences between the RPMI culture media, negative control group, and the garlic-treated groups at concentrations of 20, 30, and 40 mg/ml. Conversely, no significant differences were observed between the 20 mg/ml PZQ positive control group and the 20, 30, and 40 mg/ml garlic-treated groups (p < 0.05) (Figure 8).

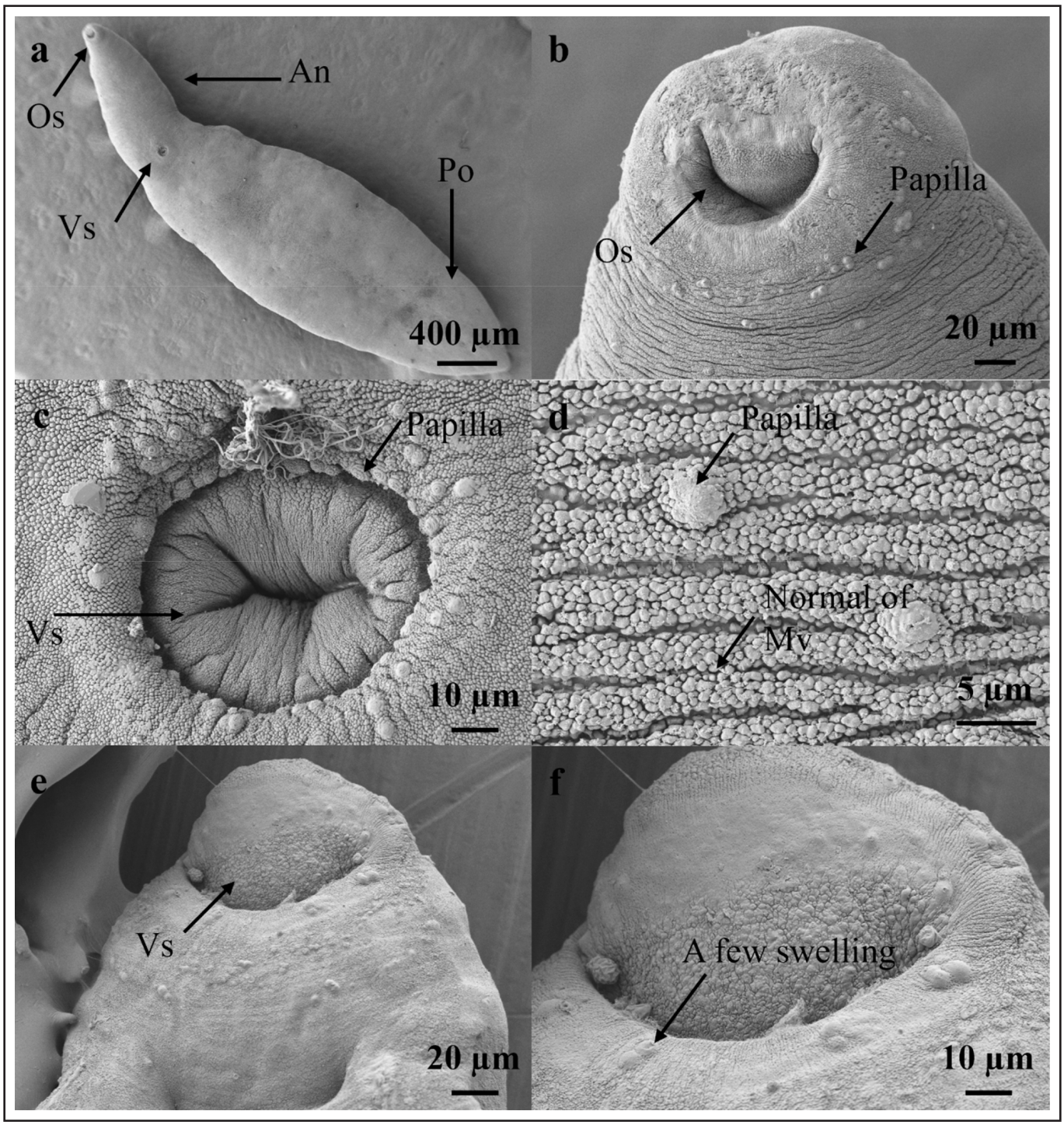

Figure 1. The morphological surfaces of adult *O. viverrini* adult worms were examined through SEM in both negative and positive control groups. In the negative control group: (a) a smooth body surface, (b) and (c) typical papillae around oral and ventral suckers, and (d) regular distribution of papillae among short microvilli. In the positive control group: (e) and (f) signs of swelling in papillae around the ventral sucker and microvilli. Abbreviations: An = Anterior region, Mv = Microvilli, Os = Oral sucker, Po = Posterior region, and Vs = Ventral sucker.

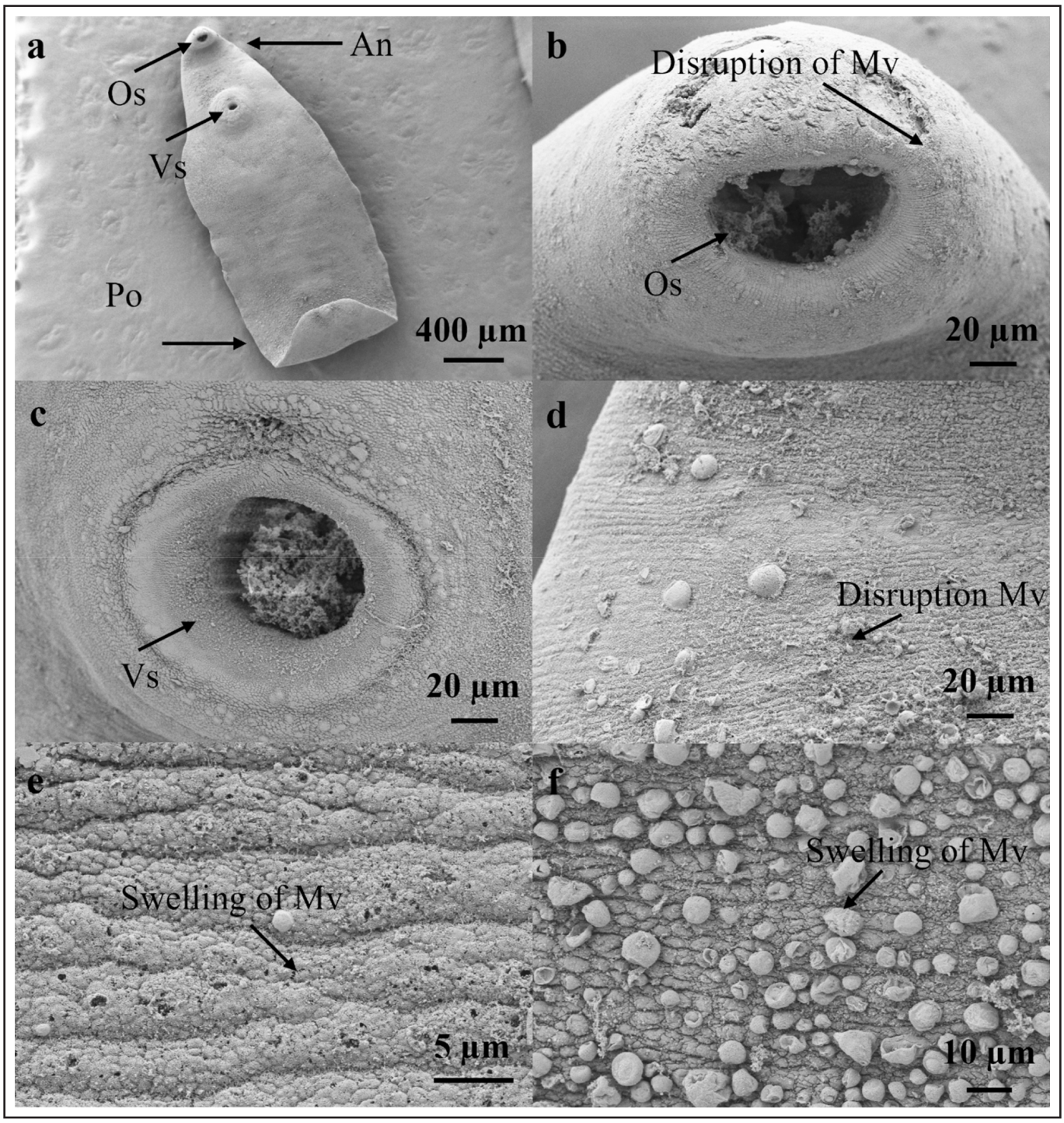

Figure 2. Morphological alterations on the surface of *O. viverrini* adult worms were investigated using SEM in the garlic-treated group with a concentration of 20 mg/ml. The SEM analysis revealed: (a) an irregular and non-smooth body surface of *O. viverrini* adult worms, (b) disrupted papillae around the oral sucker, and (c), (d), and (f) notable damage and swelling in both microvilli and papillae near the ventral sucker. Abbreviations: An = Anterior region, Mv = Microvilli, Os = Oral sucker, Po = Posterior region, and Vs = Ventral sucker.

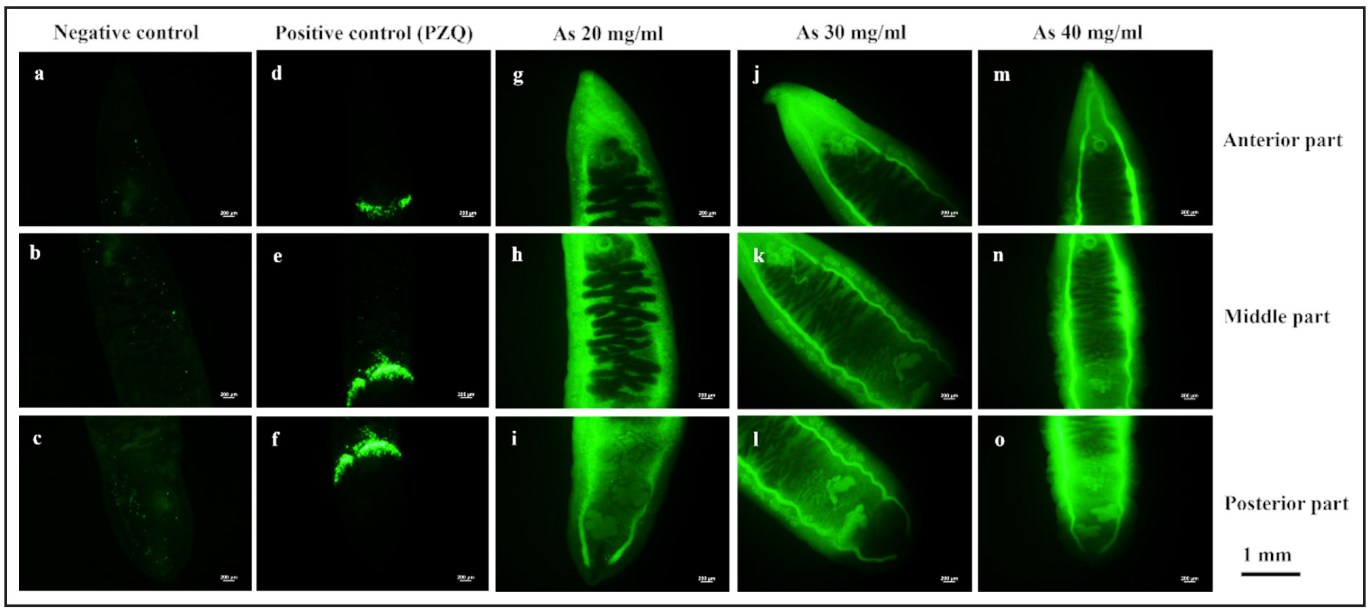

Figure 3. ROS generation in *O. viverrini* adult worms was observed following a 6-hour exposure. In the negative control group (a), (b), and (c), minimal ROS generation was observed in the anterior, middle, and posterior parts of the worms, respectively. In the positive control group (d), (e), and (f), partial ROS generation was observed in these regions. Conversely, the garlic-treated groups (g), (h), (i), (j), (k), (l), (m), (n), and (o) exhibited significantly higher ROS levels throughout the entire body of the worms, with specific representation for the 20 mg/ml, 30 mg/ml, and 40 mg/ml groups.

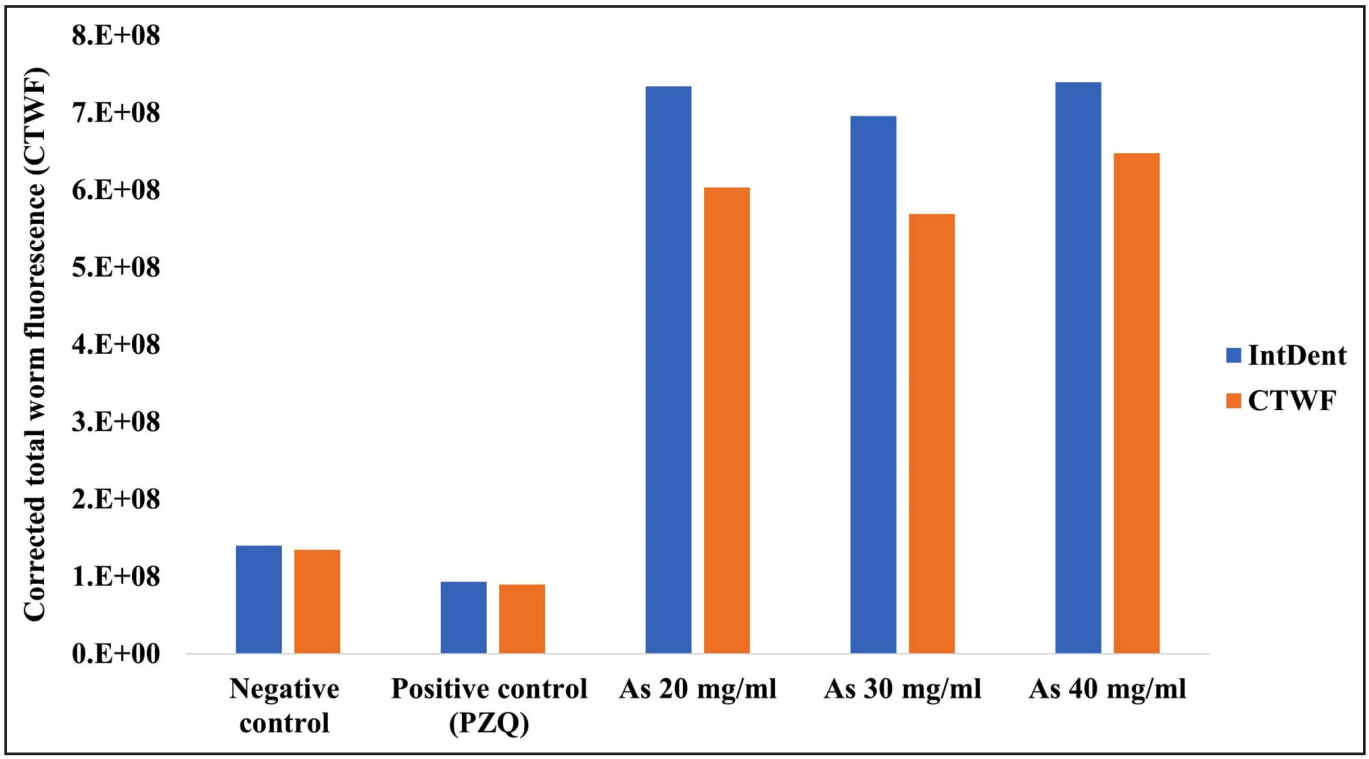

Figure 4. The CTWF was showed fluorescence intensity was lower in both the negative and positive control groups, whereas the garlic-treated group (20, 30, and 40 mg/ml) exhibited higher fluorescence intensity.

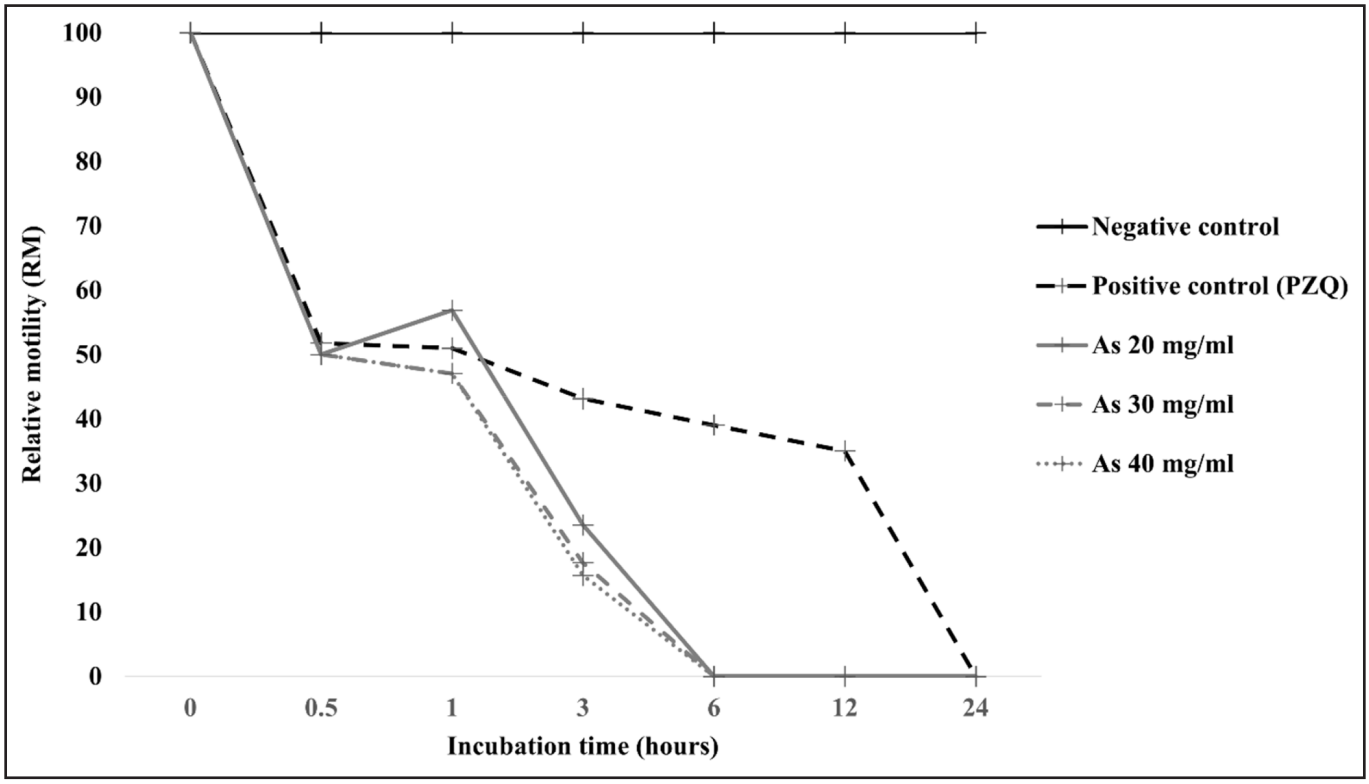

Figure 5. The RM values of *O. viverrini* adult worms were treated with garlic crude extract at various concentrations and times.

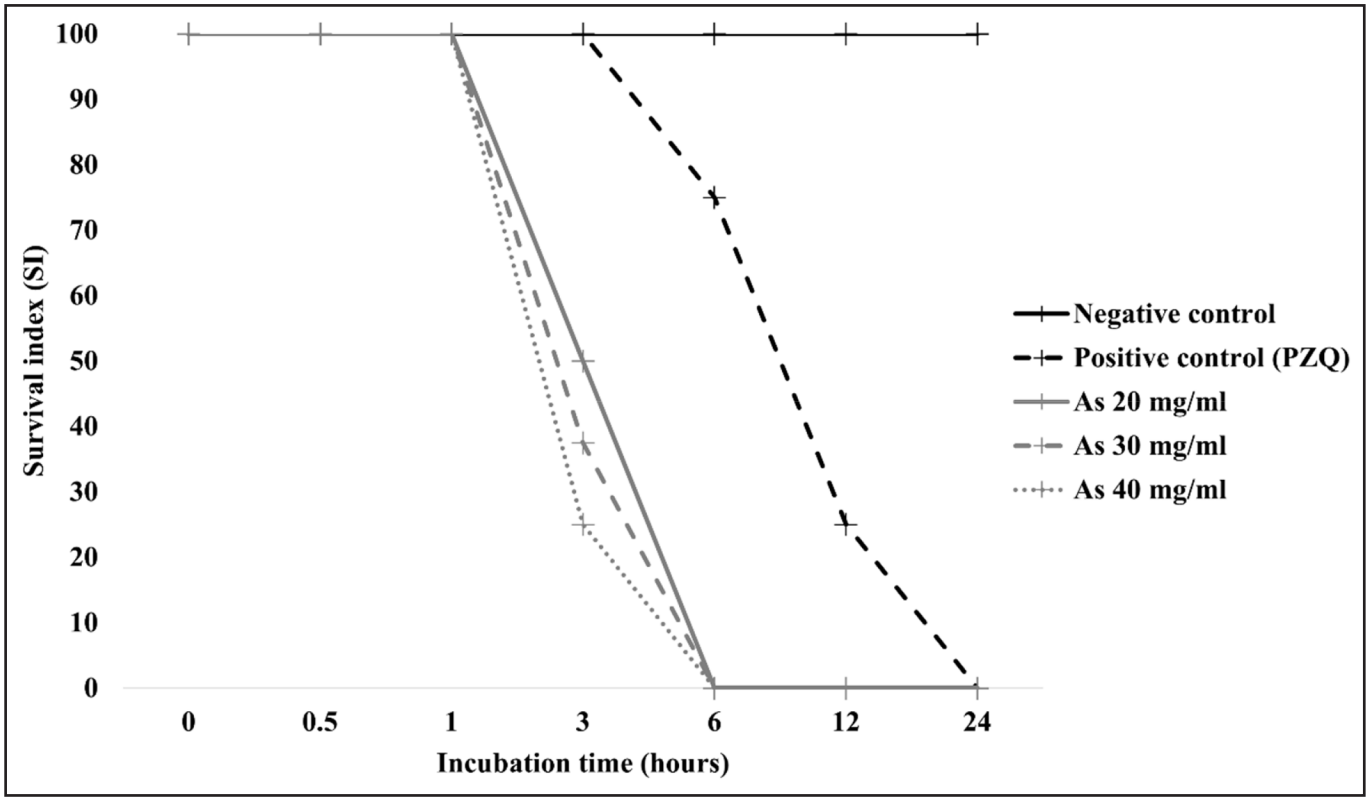

Figure 6. The SI of *O. viverrini* adult worms were treated with garlic crude extract at various concentrations and times.

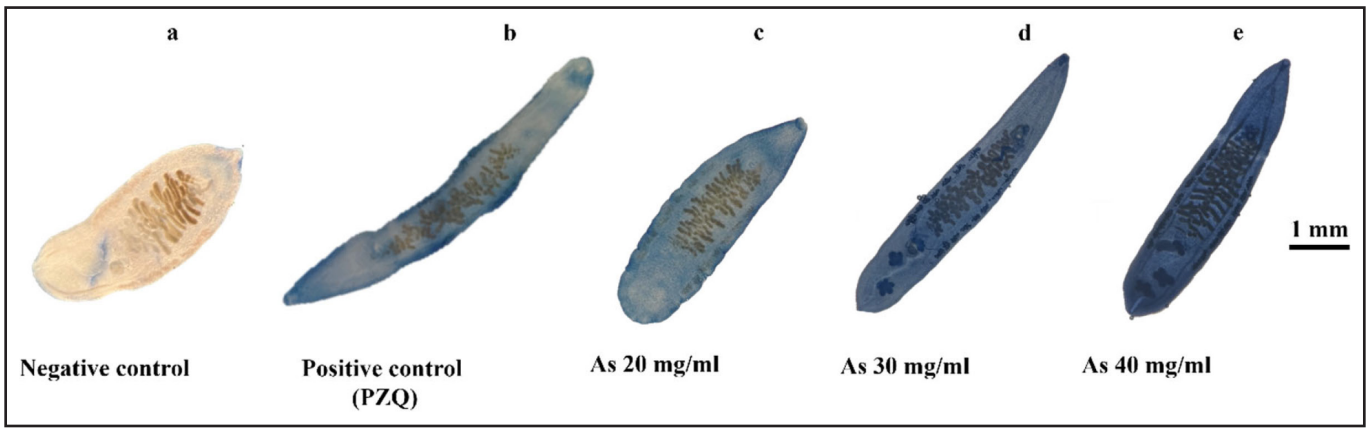

Figure 7. Viability of *O. viverrini* adult worms was evaluated via Trypan blue staining. (a) Negative control group worm remained unstained. (b) Positive control (PZQ) worm exhibited widespread Trypan blue staining, with darker areas on the body's side. (c), (d), and (e) Worms treated with garlic crude extract displayed extensive Trypan blue staining.

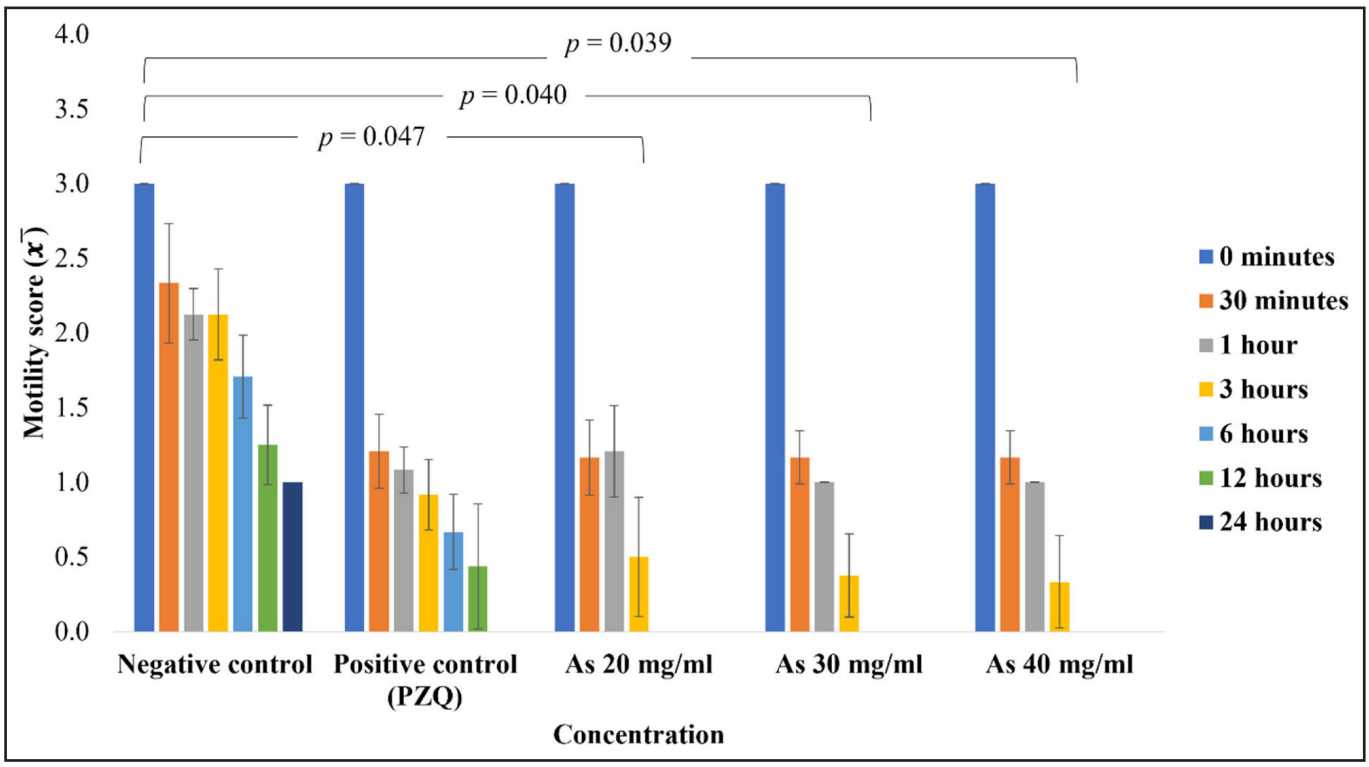

Figure 8. The mean motility scores were showed significantly different between the negative control and treated groups (PZQ and garlic crude extract), not significantly different was noted between the PZQ and garlic extract groups ($p < 0.05$).

DISCUSSION

This study represents the anthelmintic impact of garlic crude extract on a carcinogenic liver fluke, *O. viverrini* adult worms. In the negative control group, adult worms exhibited sustained movement and survival throughout the experiment. Conversely, all garlic extract-treated groups displayed a rapid reduction in RM and SI within the initial 1-3 hours, leading to cessation of movement or mortality by the 6-hour mark. In contrast, the positive control group exhibited a slower decline in RM and SI values between 3 to 6 hours, culminating in complete immobility by the 24-hour mark. These findings align with Lorsuwannarat *et al*. (2013) study on plumbagin *in vitro* anthelmintic effect on *S. mansoni*, where the plumbagin-treated group exhibited a more rapid decline in RM compared to the PZQ-treated group. Additionally, Jeyathilakan *et al*. (2012) demonstrated significant motility reduction in *Fasciola gigantica* adult worms treated with *A. sativum* and *L. inermis* extracts at a 5% concentration. Wannachat *et al.* (2020) reported analogous results using a crude extract from *Areca catechu* on *O. viverrini*, showing a rapid decrease in motility with increasing concentrations of the *A. catechu* extract. The outcomes of this report are in concordance with the findings of the present study. Previous report, Corral *et al*. (2016), they examined the efficacy of allicin a bioactive compound of garlic, their findings illustrated that allicin induces dysregulation of calcium homeostasis and oxidative stress, uncontrolled by the antioxidant defense of the cell, which leads to mitochondrial dysfunction and a bioenergetic catastrophe leading to cell necrosis and cell cycle arrest in the premitotic phase of the *Leishmania* sp.

parasite. Understanding the impact of garlic on parasites is crucial for developing effective treatments. Specifically, allicin, which disrupts essential cellular functions, holds potential in alternative anthelmintic drug development. Investigating the efficacy of garlic in disrupting essential biological functions holds promise for the development of targeted anti-parasitic medications, suggesting a novel therapeutic avenue for future anthelmintic treatments.

In this study The ROS generation were showed partial ROS generation in the negative control group and positive control group. While, in the garlic-treated groups exhibited significantly higher ROS levels throughout the entire *O. viverrini* adult worm body. This observation aligns with Goel *et al*. (2020) study on oxidative stress in *H. contortus* parasites treated with *Lansium parasiticum* aqueous extract-protected silver nanoparticles, indicating a metabolic shift in response to ROS-induced oxidative stress. Generally, the production of stress-induced ROS involves the generation of highly reactive molecules, such as superoxide anions, hydrogen peroxide, and hydroxyl radicals, within cells or organisms in response to various stressors. These stressors comprise physical, chemical, environmental, or biological factors capable of disrupting the normal ROS balance in cells. Under stress conditions, ROS overproduction can lead to oxidative stress, linked to protein, lipid, and DNA impairment, affecting cellular functionality (Finkel, 2011). The generation of stress-induced ROS is a complex process involving numerous cellular pathways and mechanisms, playing critical roles in cellular processes like signaling and immune responses, but can have both advantageous and detrimental effects (Schieber & Chandel, 2014). The present investigation constitutes a pioneering exploration into the morphological changes induced in *O. viverrini* by the administration of garlic crude extract. Descriptions of the typical morphology of *O. viverrini* adult worms are provided by Scholz *et al*. (1992) and Apinhasmit *et al*. (1993). These adult flukes exhibit a leaf-like body structure, featuring an oral sucker and a ventral sucker located at the tapered anterior end, with an average length and width of approximately 2.5 mm and 1 mm, respectively. The oral sucker and the ventral suckerare situated roughly one-quarter along the body length from the anterior end. The microvilli-covered surface of *O. viverrini* adult worms, with three papilla types dispersed throughout. Numerous gland cell openings are found in areas around the oral sucker and the excretory pore, with the ventral sucker positioned directly in front of the genital opening.

In the investigation of surface morphology, adult *O. viverrini* worms treated with garlic crude extract concentrations (20, 30, and 40 mg/ml) displayed irregular and non-smooth body surfaces. This alteration was characterized by significant damage to the microvilli surface, papillae surrounding the oral and ventral suckers, and extensive swelling of the entire body's microvilli surface. Conversely, worms in the negative control group exhibited normal morphological features, while those in the positive control group showed slight microvilli surface swelling. These observations align with previous studies by Riad *et al*. (2013) and Aly *et al*. (2017), demonstrating marked tegument changes in *S. mansoni* parasites treated with garlic extracts in mice. Our results clearly indicate that garlic crude extract has an impact on the morphological changes of the *O. viverrini* integument. This biological cascade involves cellular death, particularly when respiration halts, inducing hypoxia and metabolic shifts in tissues. Decreased ATP production prompts anaerobic metabolism, generating intracellular lactate and lowering pH. Diminished ATP triggers lysosome swelling and enzyme release. Tissue hypoxia escalates calcium influx, activating enzymes and damaging membranes and organelles. These events lead to cellular self-digestion (autolysis) and tissue decay (Madea *et al*., 2014). This study was indicated processes through observed alterations in *O. viverrini* adult worm morphological surfaces.

In contrast, although garlic has a historical reputation as a folk remedy for various ailments, its specific use in eliminating *O. viverrini* liver flukes lacks prior documentation. Garlic's bioactive compounds, including allicin, exhibit antimicrobial, anticancer, and antiparasitic properties, with extensive research probing allicin's therapeutic potential (Rabinkov *et al*., 1998; Corzo-Martínez *et al*., 2007). Therefore, it is intriguing for further investigation in the study of *O. viverrini* liver fluke. Garlic extract's efficacy is documented against *G. turnbulli* (Schelkle *et al*., 2013), schistosomicidal activity against various *S. mansoni* stages (Mantawy *et al*., 2012; Riad *et al*., 2013; Aly *et al*., 2017; Cortes *et al*., 2017), free radical inhibition, immune response stimulation in *S. mansoni*-infected mice (El Shenawy *et al*., 2008*;* Mantawy *et al*., 2011, 2012; Metwally *et al*., 2018), and morphological damage influence in *F. gigantica* liver fluke (Singh *et al*., 2009). Prior investigations into herbal remedies for against *O. viverrini in vitro* and *in vivo*. Wonkchalee *et al*. (2012, 2013) demonstrated the efficacy of *Thunbergia laurifolia* in reducing inflammation from pathological alterations in *O. viverrini*-infected hamsters. Aukkanimart *et al*. (2015) explored the antioxidant, anti-inflammatory, and anthelmintic effects of the traditional folk medicine *Garcinia mangostana* pericarp extract in hamsters with opisthorchiasis. Additionally, Intuyod *et al*. (2014) examined the activity of an anthocyanin complex containing cyanidin and delphinidin-rich extracts derived from turmeric against inflammation and periductal fibrosis in hamsters infected with *O. viverrini*. However, the specific use of garlic for eliminating *O. viverrini* liver flukes has not been previously reported.

This study unequivocally affirms garlic extract's anthelmintic effectiveness against *O. viverrini*, aligning with prior reports. The study emphasizes garlic extract's efficacy against *O. viverrini* adult worms. Elevated ROS expression induces worm motility reduction. Prolonged garlic extract exposure progressively immobilizes worms, culminating in their demise. These findings suggest that garlic crude extract exhibits efficacy as an anthelmintic treatment for the liver fluke, *O. viverrini*, in adult worms *in vitro*. This is evident in the rapid reduction of RM and SI compared to control groups. The generation of ROS was significantly higher in parasites treated with garlic, leading to morphological damage characterized by edema and disturbance. These findings imply that garlic extract harbors a more potent anthelmintic effect on *O. viverrini* adult worms, suggesting its potential as an alternative therapeutic approach for opisthorchiasis. However, it is important to note that the overall results demonstrate the preliminary potential of garlic crude extract on parasites. The reproducibility of these results may be influenced by various factors associated with garlic, including variations in garlic varieties, extraction methods, and environmental conditions (Pakakaew *et al*., 2021), it may distinctly profile of bioactive compounds in garlic may impact its efficacy against parasites. Subsequent research initiatives should encompass both *in vivo* and *in vitro* studies to investigate the mechanisms and effects of garlic crude extract on *O. viverrini* liver fluke. Additionally, there is a need to focus on identifying specific bioactive components within garlic crude extract that could potentially provide safe and effective anthelmintic activity. Despite the promising effects observed with garlic crude extract on *O. viverrini* liver fluke, it is imperative to conduct further investigations, particularly involving human subjects, to determine the feasibility, safety, and efficacy of employing garlic for parasite treatment and control.

ACKNOWLEDGMENTS

This study was supported by Suranaree University of Technology's grant "External Grants and Scholarships for Graduate Students, One Research One Graduate (OROG)", the Research and Development, Suranaree University of Technology (SUT), Thailand Science Research and Innovation (TSRI), and National Science, Research, and Innovation Fund (NSRF) (NRIIS number 179304). The authors express their gratitude to the Parasitic Diseases Research Center (PDRC) at the Institute of Medicine, SUT, as well as the Translational Medicine programs (TM) at the Institute of Medicine, SUT, and extended to the Center for Scientific and Technological Equipment for their provision of laboratory facilities essential to this study.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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