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Original paper

Ganoderma lucidum extract inhibits *Schistosoma mansoni* survival *in silico* and *in vitro* study

Enas Abdelhameed Mahmoud HUSEEIN[1], Mohamed SAMIR[2], Ahmed A. AL-KARMALAWY[3], Shimaa S. HASSAN[4], Mona EMBAREK MOHAMED[5], Fatma Abdel Aziz Mohamed MOSTAFA[6], Ahmed M. MOHARRAM[7], Samia S. ALKHALIL[8], Haiam Mohamed Mahmoud FARRAG[1,8]

[1]Department of Medical Parasitology, Faculty of Medicine, Assiut University, Assiut 71516, Egypt
[2]Department of Medical Zoonoses, Faculty of Veterinary Medicine, Zagazig University, Egypt
[3]Department of Pharmaceutical Medicinal Chemistry, Faculty of Pharmacy, Horus University-Egypt, New Damietta 34518, Egypt
[4]Zoology Department, Faculty of Science, Zagazig University, Egypt
[5]Department of Medical Microbiology and Immunology, Faculty of Medicine, Assiut University, Assiut 71515, Egypt
[6]Department of Botany and Microbiology, Faculty of Science, Cairo University, Cairo 12613, Egypt
[7]Department of Botany, Faculty of Science, Assiut University, Assiut 71515, Egypt
[8] Department of Clinical Laboratory Sciences, Faculty of Applied Medical Sciences, Shaqra University, Kingdom of Saudi Arabia

Corresponding Author: Haiam Mohamed Mahmoud Farrag: e-mail: hayammahmoud@aun.edu.eg

ABSTRACT. Schistosomosis is a worldwide tropical disease primarily caused by *Schistosoma mansoni*. Praziquantel is the only available drug for controlling schistosomosis, with many challenges. This study aims to evaluate the *in vitro* anti-*Schistosoma* effect of *Ganoderma lucidum (G. lucidum)* against adult and larval stages of *Schistosoma* based on the prediction of the binding activity of *G. lucidum* protein with proteins of various stages of *S. mansoni* by molecular docking to confirm its inhibitory potential through an insilico study. Results showed that Leu143, Ser165, Met214, and Asn213 were the primary crucial amino acids involved in the binding, with a promising large area of interactions between the two studied proteins. The *in vitro* study evaluated the motility and survival of adult and larval stages, compared to praziquantel and niclosamide, respectively. There was a significant reduction in the motility of adults after the two-hour incubation, with all concentrations and 100% death of all parasites with the minimal concentration (10 µg/ml) within 4 and 6 h of incubation (P<0.01). Regarding the cercariae, at a concentration of 10 µg/ml, all the cercariae (100%) died (P<0.01) after 15 min, and the miracidial complete mortality rate (100%) (P<0.01) occurred at a concentration of 10 µg/ml after 8 min. This study first predicted the binding activity of *G. lucidum* protein with proteins of *S. mansoni* at various stages and proved the anti-*Schistosoma* effect of *G. lucidum in vitro*, considered a promising treatment for schistosomosis.

Keywords: *Schistosoma mansoni*, *Ganoderma lucidum*, *in silico*, molecular docking, *in vitro*

Introduction

Schistosomosis is a worldwide neglected tropical disease caused by *Schistosoma* parasites, mainly *S. mansoni*. It affects approximately 190 million people worldwide and results in more than 70 million new cases and thousands of deaths every year with high costs in public health and economic productivity, especially in developing countries [1]. Schistosomes have complex life cycles; they infect humans by penetrating cercariae through the skin in contaminated freshwater. Following penetration, the maturing schistosomules require about 5–7 weeks to become adults and produce eggs. The eggs that reach freshwater (passing through urine or faeces of infected individuals or animals) hatch and release ciliated miracidia, which infect a suitable snail intermediate host. There, the parasite undergoes

asexual replication, releasing tens of thousands of cercariae into the water, re-infect humans again [2]. Schistosomosis has been associated with disabling manifestations like; long-term under-nutrition, anemia, organ scarring, and fibrosis [3]. The detailed pathology results from an immunologic response that includes both the T helper-1 (Th1) type immune response, which is associated with the severe forms of the disease, and the Th2 type cells, which is correlated with reduction of the pathology with more benign disease course including the production of specific cytokines like interleukin 2 (IL-2), IL-4, IL-5, IL-10, and interferon γ (IFN-γ) that have been implicated in the regulation of the granulomatous changes in schistosomosis [4]. Currently, praziquantel is the only available and recommended drug for treating and controlling schistosomosis in humans. Although it is safe and well-tolerated, it is inefficient against the earlier stages of schistosomes, and its effectiveness needs booster doses to kill those immature parasites. Ultimately, the massive and exclusive use of a single drug for many decades raises possibilities of drug resistance [5]. Having only one drug that treats a disease affecting millions of people in wide geographical areas is challenging. Therefore, there is a growing need to develop new effective and safe antischistosomal drugs. For thousands of years, various mushrooms have been used traditionally in folk medicine to cure different health problems. Increasing interest arises for using these alternative therapies instead of the current drugs for pharmaceutical industries. One of these mushrooms is the *Ganoderma* genus [6]. *Ganoderma lucidum* (*G. lucidum*) is a fungal species of the genus *Ganoderma* that Karsten first described in 1881 and distributed on a limited range in China (named as *Lingzhiu* and means the herb of spiritual potency) and Japan (named as *Reishi*). Since its discovery, *G. lucidum* has been deemed a medicinal mushroom because of its pharmaceutical and nutritive properties. Different mushroom forms are available commercially, such as tea, powders, and dietary supplements. *G. lucidum* grows readily in hot and humid conditions, and the mushroom can be artificially cultivated by using substrates such as wood logs, grains, and sawdust [7]. The health benefits of *G. lucidum* are found in bioactive compounds present in different parts of the mushroom (e.g., fruiting body, mycelium, and spores). These compounds include polysaccharides, triterpenes, phenols, proteins, vitamins, steroids, nucleotides, and other components. Polysaccharides and triterpenes are the two major compounds found in *G. lucidum* that were reported to have powerful immunomodulating and antioxidant properties [6]. A great variety of high molecular weight polysaccharides and polyglycans have been frequently extracted from the fruit body of *G. lucidum,* with the main five sugars of medical importance being identified (i.e., glucose, galactose, fructose, xylose, and mannose) [8]. *G. lucidum* polysaccharides (GL-PSs) exhibit a wide range of bioactivities, including anti-inflammatory, anti-diabetic, anticancerogenic, and immune-enhancing functions [7]. GL-PSs were tested active against various bacterial and viral pathogens [8]. The anti-tumorigenic activity of GL-PSs was also reported in a group of cancer patients where GL-PSs enhanced IL-2, IL-6, IFN-γ and decreased IL-1 and tumor necrosis factor α (TNF-α) levels in the plasma of patients. GL-PSs were also reported to enhance natural killer (NK) cells, macrophages, and T lymphocytes activities. Triterpenes in *G. lucidum* composed mainly of ganoderic acids (GAs), have several biological activities, including anti-inflammatory, anti-tumorigenic, anti-viral, and hypolipidemic activities [8]. Traditionally, *G. lucidum* was used to treat some infectious diseases like bronchitis and hepatitis, and was believed for long time to decrease the risk of cancer, liver and heart diseases [6]. The anti-protozoal effect of *G. lucidum* was reported previously in a study done by Lovy and others [9], where its extract inhibits *Plasmodium falciparum* (the malaria parasite). In the current study, we first predicted the binding activity of *G. lucidium* protein with proteins of various stages of *S. mansoni* parasites and then determined its anti-*Schistosoma* effects *in vitro.*

Materials and Methods

Ethical statement

Animal studies reported complying with the ARRIVE guidelines. The experimental protocol was approved by the Institutional Review Board of Faculty of Medicine, Assiut University (IRB number 23112718). Thiopental sodium was used for anesthesia, and all efforts were made to minimize animal suffering, and animal care was according to the NIH Guide for care and use of laboratory animals [10,11].

Bioinformatics prediction of Ganoderma lucidum – Schistosoma interaction

Protein-protein docking studies

The protein-docking process for *G. lucidum* fungus and *S. mansoni* parasite was carried out. The molecular operating environment (MOE) 2019 drug design suite [12] was used to perform the docking studies to evaluate the binding affinity and strength of *G. lucidum* towards *S. mansoni* and confirm its inhibitory potential.

Preparation of target proteins

The X-ray structures of both *G. lucidum* fungus and *S. mansoni* parasite were downloaded from the Protein Data Bank (https://www.rcsb.org/, PDB codes: 5D8Z [13] and 3E0Q [14], respectively). Quickprep protocol was used for the preparation of the two proteins mentioned above, where automatic correction for errors in the connections and types of atoms was applied, hydrogen atoms were added with their standard 3D geometry to each system, all atoms were adjusted to be free during the energy minimization, and finally, energy minimization for each system was applied [15].

Protein-protein docking process

The protein-protein docking protocol was applied: the prepared *S. mansoni* protein (3E0Q) was selected to be the receptor during the process, the *G. lucidum* protein was adjusted to be in place of the ligand, and the docking process was initiated using the hydrophobic patch potential and complementary determining regions (CDRs) restraints for ligand site (*G. lucidum* protein). The scoring tools were adjusted to default values. The number of pre-placements poses and placement poses was 1000, and the refinement poses were 100. At the end of the docking process, the resulting 100 poses were studied, and the best one had the best protein-protein interactions, and the score value was selected and explained in detail.

Experimental validation of the effect of G. lucidum on S. mansoni

All chemicals used in the present study were purchased from Sigma-Aldrich (Sigma, St. Louis, MO 68178, United States) unless mentioned otherwise.

G. lucidum fungus extract

Powder of *G. lucidum* was obtained from DXN pharmaceutical (DXN Marketing Sdn, Selangor, Malaysia). Preparation of *G. lucidum* extract was performed as reported previously [16]. According to the company protocol, *G. lucidum* powder was subjected to hot water at 70°C for two hours. The extract was filtered and kept at −20°C until usage. Before usage, samples were diluted with RPMI-1640 culture medium to a final volume of 3 ml test solution of 100 µg/ml final concentration in a 1 ml vial for screening.

Preparation of parasite stages

The extract was determined for its effect against materials from different stages of *S. mansoni,* namely, adult worms, miracidia, and cercariae. All materials were procured and obtained from the *Schistosoma* Biological Supply Center (SBSC), Theodor Bilharz Research Institute (TBRI), Giza Governorate, Egypt. The bioassays were performed at SBSP/TBRI. Laboratory-bred hamsters (*Mescori - cetus aurautus*) were experimentally infected with 350–400 *S. mansoni* cercariae per hamster seven weeks prior to the euthanasia. Then, mature worms were obtained from these hamsters by percutaneous perfusion. According to Yousif et al. [17], the adult worms were rapidly placed in RPMI culture medium. The *Schistosoma* eggs were obtained from experimentally infected hamsters in physiological saline (0.9%). The miracidia were obtained from cleaned eggs by allowing egg hatching in small amounts of de-chlorinated tap water. Cercariae that were earlier shed from experimentally infected *Biomphalaria alexandrina* snails exposed to light for two hours at 25°C ± 2°C were immediately used.

In vitro studies

Schistosomicidal bioassay

According to Yousif et al. [17], *G. lucidum* was tested for its effect *in vitro* against *S. mansoni* adult worms. The freshly obtained worms (n=290–300) were cleaned from blood in small sieves of 20 µm mesh size using phosphate buffer saline (PBS). Two replicates were set up for each extract, and three pairs of *Schistosoma* worms (males and females equally represented) were placed in each well of 24-well culture plate using sterilized forceps. The worms were washed in RPMI 1640 culture medium (5 µg/ml) under a sterilized laminar flow. The RPMI media contained 20% fetal calf serum, 300 µg streptomycin, 300 IU penicillin, and 160 µg gentamicin/100 ml RPMI medium. Positive (praziquantel) and negative (1% dimethylsulfoxide; DMSO) controls were concurrently used.

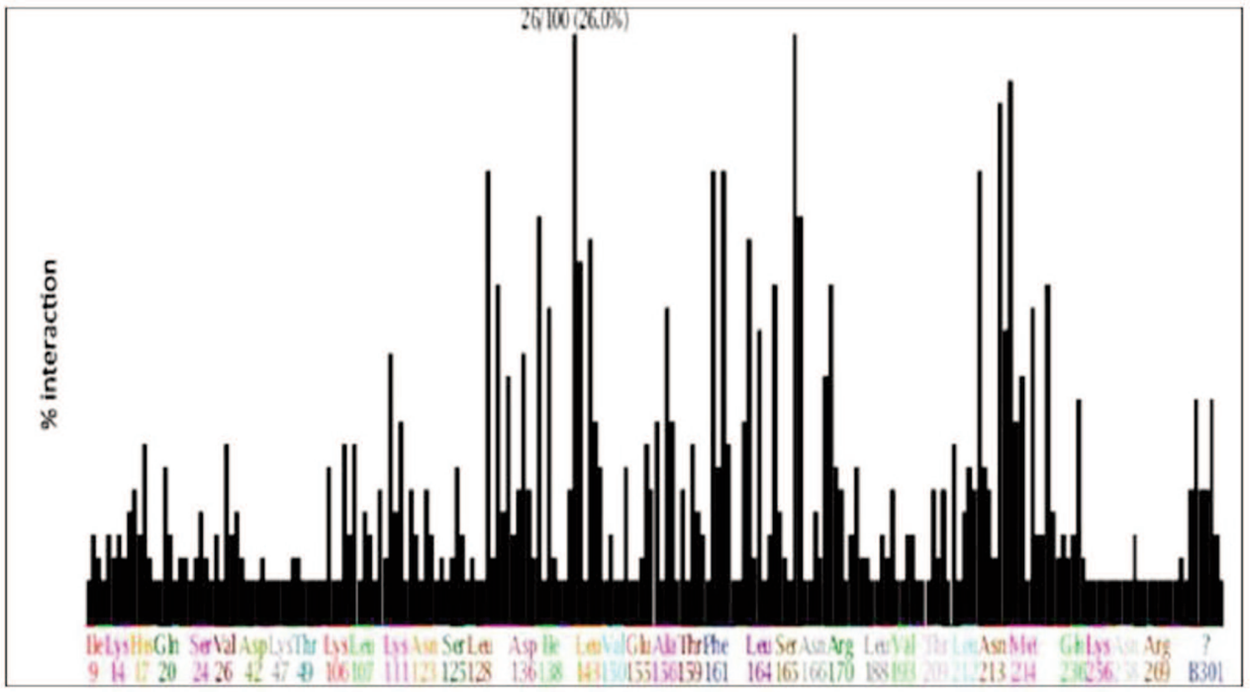

Figure 1. Protein-ligand interaction fingerprints (PLIF) for *Ganoderma-Schistosoma* docking (in population form). The amino acid at which the interaction occurs is shown below the figure. The peaks show the degree of interaction

Praziquantel 0.2 µg/ml was used as a reference drug. Two ml of the tested doses (10, 20, and 30 µg/ml) from the extract were added to each well. The final volume in each well was 2 ml. Test and control wells were incubated at 37°C, kept for 12 h, monitored every two hours, and examined daily for three days for worm viability using a stereo-microscope. Worms, which did not show any signs of motility for one minute, were considered dead. The schistosomicidal effect was measured by calculating the number of dead worms relative to the total number of worms compared to the negative and positive controls.

To determine lethal concentrations 50 (LC_{50}) and 90 (LC_{90}) of the tested extract, the same experiment was repeated several times using various concentrations of the compound, and the viability of worms was followed up for 3-days. The worm mortality was recorded in each case.

Larvicidal (*miracidicidal and cercaricidal*) *tests*

Ten ml of water containing hatched miracidia or freshly shed cercariae were put in a small, graduated Petri dish. The concentrations of molluscicidal LC_{50} of the extract were tested. The exact numbers of miracidia or cercariae were placed in a well containing 1% DMSO as a negative control. The survival of *Schistosoma* larval stages (miracidium and cercaria) was assessed *in vitro* by incubation with different concentrations of *G. lucida* extract (0.625–10 µg/ml) compared to the drug control (niclosamide) (0.02 mg/l to 0.2 mg/l). Survival and mortality at a successive intervals of 15, 30, 60, 90, 120, and 150 minutes were recorded. Two replicates were run in each case. Microscopical observation of the viability of these stages, as indicated by hatching or motility, was performed after five minutes of exposure. Dead or immobile miracidia and cercariae were enumerated and recorded. Constant motion signified that the miracidia and cercariae were alive, while no motion with a detachment of the tail in the latter case signified death [18].

Statistical analysis

The dead stage in all replicates of each bioassay was combined and expressed as the percentage mortality of each concentration. Probit analysis of concentration mortality data was conducted to estimate the LC_{50} and LC_{90} values using the statistical package for social science (SPSS) version 7.5 software employing the probit analysis [11].

Results

Docking results

By studying the protein-ligand interaction fingerprints (PLIF) that resulted from the docking between *G. lucidum* fungus and *S. mansoni* parasite, it was observed that Leu143, Ser165, Met214,

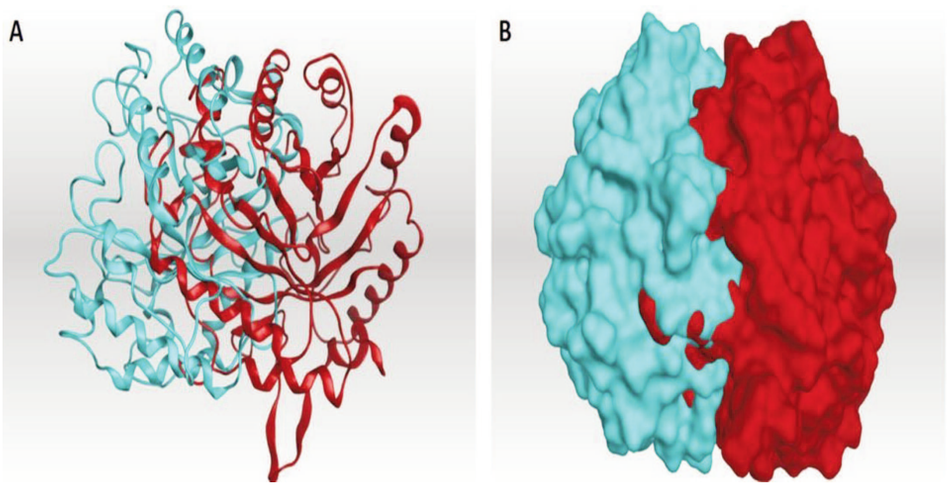

Figure 2.**A**. The large area of binding interactions between *G. lucidum* and *S. mansoni* protein ribbons (described in red and turquoise, respectively); **B**. The large area of binding interactions between *G. lucidum* and *S. mansoni* proteins in 3 D filling (described in red and turquoise, respectively)

Asn213 were the main crucial amino acids involved in the binding interactions between the two studied proteins (Fig. 1). Moreover, the most observed interactions between *G. lucidum* fungus and *S. mansoni* were recorded for pose number 10 with a binding score value of -60.00 kcal/mol and rmsd_refine value of 0.70. It showed a promising large area of interactions between proteins of both fungus and the parasite (Fig. 2 A, B).

In vitro schistosomicidal activity of G. lucidum

The tested extract affected the coupling process at different concentrations, causing the separation of a couple of schistosomes based on the concentrations used and the exposure time. Nearly all the adult worms had been separated within the first two hours using 10 µg/ml concentration. This was nearly similar to the results observed with the reference drug, praziquantel, compared to the negative control groups, which showed couple separation nearly at 10 h after incubation. Concerning the motility, a significant reduction in the worm movements was observed after two-hour incubation, with all concentrations, in a dose-dependent manner concerning the time till 6 h interval. The motor activity was decreased at 6 h intervals and completely lost at 10 h intervals in the negative control groups. On the other hand, praziquantel decreased motor activity starting from the first 2 h of incubation and ultimately lost in all adults at 4 h intervals.

As regards the survival of adult *S. mansoni*, the tested extract caused the death of 100% of parasites with the minimal concentration (10 µg/ml) within 4 and 6 h of incubation ($P<0.01$), with no difference between male and female adult worms in response to either the motility affection or the survival rates. The positive control group (praziquantel treated) showed total death of the adults (100%) after 4 h interval with LC_{50} and the LC_{90} values 3.7 and 22.4 µg/ml, respectively. The negative control group showed the death of the parasites at 12 h of incubation which was considered the endpoint of the experiment.

In vitro larvicidal activity of G. lucidum

The survival of *Schistosoma* larval stages (miracidium and cercaria) was in vitro assessed by incubation with different concentrations of *G. lucidum* extract (0.625–10 µg/ml). As regards the cercariae, at a concentration of 10 µg/ml, all the cercariae (100%) died ($P<0.01$) after 15 min, and after 25 min at 5 µg/ml concentration, after 40 min at 2.5 µg/ml, after 65 min at 1.25 µg/ml and after 120 min at 0.625 µg/ml concentration. The LC_{50} and the LC_{90} values were 2 and 6.48 µg/ml, respectively.

Regarding the miracidia, the complete mortality rate (100%) ($P<0.01$) occurred at a concentration of 10 µg/ml after 8 min and after 12, 15, 20, and 30 min at 5, 2.5, 1.25, and 0.625 µg/ml concentration, respectively. The LC_{50} and the LC_{90} values were 6.5 and 18.65 µg/ml, respectively. In contrast, the worms in the negative control group survived throughout the incubation period. Likewise, cercariae and miracidia exposed to Niclosamide showed normal viability up to 40 and 15 min, respectively, with the average concentration of the

Table 1. Effect of *Ganoderma lucidium* extract on larval and adult stages of *Schistosoma mansoni*

	Miracidiae	Cercariae	*S. mansoni* adult worms	
Concentrations of *G. lucidum* extract (μg/ml)	time to die (min)		Concentrations of *G. lucidum* extract (μg/ml)	time to die (hours)
0.625	30	120	10	4–6
1.25	20	65	20	2–4
2.5	15	40	30	2–4
5	12	25		
10	8	15		
Negative control*			~ 12 h	
Positive control**			2–4 h	

Explanations: time for 100% death of the larval stages of *S. mansoni* shortens with increasing concentrations of *G. lucidium* extract. For *S. mansoni* adult worms, 100% death of the worms occured within 2 h at the least used concentration (20 μg/ml). *negative control:1% dimethylsulfoxide (DMSO), **positive control: praziquantel (PZQ)

reference drug. The LC_{50} and the LC_{90} values were 0.07 and 0.17, respectively.

Interestingly, the lethal effect was obtained in a dose-dependent manner and depended on the parasite's stage, as shown in table 1.

Discussion

Until now, pharmacotherapy has been the most effective method for treating schistosomosis. In the last four decades, access to more effective drugs has reduced the prevalence and morbidity of this disease in various countries. There are many reported side effects with the use of schistosomicides (e.g., metallic taste of the mouth, diarrhea, abdominal pain, headache, dizziness, asthenia, decreased therapeutic efficacy, and resistance) [19]. Thus, searching for new effective drugs with fewer side effects is necessary. In this regard, many plant-derived natural products have been tested previously as new developmental therapies [20], yet the use of fungi in treating schistosomosis has not been tried before. Indeed, approximately 30 to 40% of the known fungi are capable of producing mycotoxins which are naturally toxic secondary metabolites with varying degrees of gravity, such as the toxin produced by fungi like *A. fumigatus*, *Aspergillus clavatus*, *A. terreus*, *A. giganteus*, *P. terreus*, *P. urticae*, *P. griseofulvum*, *Penicillium expansum*, *Egriseofulvum* and others [21].

G. lucidum is a traditional Chinese medicinal mushroom used for centuries to treat various diseases such as inflammatory, immunological disorders, and cancer [22]. The methanolic extracts of *Ganoderma lucidum* have been used as an effective and competent antimicrobial agent [23]. Its antiprotozoal effect has also been reported [9]. However, its antihelmenthic activity has not been tested yet. The present study aimed to evaluate, for the first time, the anti-parasitic activity of *G. lucidum* on different stages of *S. mansoni* parasites.

Molecular docking is one of the most important and fast tools for proposing and/or predicting the expected interactions [24]. Applying molecular docking here showed promising binding interactions between *G. lucidum* fungus and *S. mansoni*, indicating an expected intrinsic activity. In agreement with the docking data, our experimental data showed that the tested extract exhibited marked schistosomicidal activity, causing 100% mortality and slugged the motor activity of all adult male and female schistosomes. It showed a noticeable schistosomicidal effect against cercaria and miracidium, with a higher effect on miracidium.

The toxic effect of the tested fungal extract is probably due to the toxins produced by the fungus, as many fungi have a direct schistosomicidal effect. This is in addition to the potent anti-oxidant and radical scavenging effects of *G. lucidum* extracts [23].

The current study results revealed that the miracidial mortalities were greater than that of cercariae after incubation with tested extract for the same time intervals. This was expected and in line with the results of many studies that had demonstrated that some plants of different families

could exhibit an *in vitro* schistosomicidal activity [19]. However, it is not logical to compare our results with those of the previous reports due to the different nature of the tested agent. Few studies have evaluated the schistosomicidal effect of some fungi, such as that of Osman and others [25] that aimed to detect the effect of the fungal extract of *Aspergillus fumigatus* against the free larval stages of *S. mansoni* and revealed, to a large extent, close results to the current study. Such a study also evaluated the schistosomicidal effect of this extract against *S. mansoni*-infected *B. alexandrina* snails. It revealed a significant reduction in the growth rate, survival rate, and egg-laying capacity of both adult non-infected and infected snails. They referred their results back to the action of the evaluated fungal extract upon the steroid hormones and the harmful effect on the male and female genital tracts, affecting their fecundity by reducing or stopping their oviposition.

Lastly, this study first predicted the binding activity of *G. lucidum* protein with proteins of *S. mansoni* various stages and proved the anti-*Schistosoma* effect of *G. lucidum in vitro*. In the future, other studies are needed to clarify the exact mechanism involved in the schistosomicidal effect of *G. lucidum*, which is considered a promising treatment for the troublesome schistosomosis.

References

[1] Hotez P.J., Molyneux D.H., Fenwick A., Kumaresan J., Sachs S.E., Sachs J.D., Savioli L. 2007. Control of neglected tropical diseases. *The New England Journal of Medicine* 357(10): 1018–1027. doi:10.1056/NEJMra064142

[2] Gryseels B., Polman K., Clerinx J., Kestens L. 2006. Human schistosomiasis. *Lancet* 368(9541): 1106–1118. doi:10.1016/s0140-6736(06)69440-3

[3] de Carvalho L.S.A., Queiroz L.S., Alves Junior I.J., Almeida A.D.C., Coimbra E.S., de Faria Pinto P., da Silva M.P.N., De Moraes J., Da Silva Filho A.A. 2019. *In vitro* schistosomicidal activity of the alkaloid-rich fraction from *Ruta graveolens* L. (Rutaceae) and its characterization by UPLC-QTOF-MS. *Evidence-based Complementary and Alternative Medicine* 2019: article number 7909137. doi:10.1155/2019/7909137

[4] Zaia M.G., Cagnazzo T., Feitosa K.A., Soares E.G., Faccioli L.H., Allegretti S.M., Afonso A., Anibal F.F. 2016. Anti-inflammatory properties of menthol and menthone in *Schistosoma mansoni* infection. *Frontiers in Pharmacology* 7: article number 170. doi:10.3389/fphar.2016.00170

[5] Wang W., Wang L.., Liang Y.S. 2012. Susceptibility or resistance of praziquantel in human schistosomiasis: a review. *Parasitology Research* 11(5): 1871–1877. doi:10.1007/s00436-012-3151-z.

[6] Leskosek-Cukalovic I., Despotovic S., Lakic N., Niksic M., Nedovic V., Tesevic V. 2010. *Ganoderma lucidum* – medical mushroom as a raw material for beer with enhanced functional properties. *Food Research International* 43(9): 2262–2269. doi:10.1016/j.foodres.2010.07.014

[7] Wachtel-Galor S., Yuen J., Buswell J.A., Benzie I.F. 2011. *Ganoderma lucidum* (Lingzhi or Reishi). In: Herbal medicine: biomolecular and clinical aspects. (Eds. I.F. Benzie, S. Wachtel-Galor). 2nd ed. Boca Raton (FL): CRC Press/Taylor and Francis.

[8] Cör D., Knez Ž., Knez Hrnčič M. 2018. Antitumour, antimicrobial, antioxidant and antiacetylcholinesterase effect of *Ganoderma lucidum* terpenoids and polysaccharides: review. *Molecules* 23(3): article number 649. doi:10.3390/molecules23030649

[9] Lovy A., Knowles B., Labbe R., Nolan L. 2000. Activity of edible mushrooms against the growth of human T4 leukemic cancer cells, HeLa cervical cancer cells, and *Plasmodium falciparum. Journal of Herbs, Spices and Medicinal Plants* 6(4): 49–57. doi:10.1300/J044v06n04_06

[10] Finney D.J. 1952. Probit analysis: a statistical treatment of the sigmoid response curve. 2nd ed. Cambridge University Press, Cambridge, UK.

[11] Akçay A. 2013. The calculation of LD50 using probit analysis. *The FASEB Journal* 27(S1 1217): 28–28. doi:10.1096/fasebj.27.1_supplement.1217.28

[12] Inc C.C.G. 2016. Molecular operating environment (MOE). Chemical Computing Group Inc. 1010 Sherbooke St. West, Suite# 910, Montreal.

[13] Liu G., Li Q., Shang N., Huang J.W., Ko T.P., Liu W., Zheng Y., Han X., Chen Y., Chen C.C. 2016. Functional and structural analyses of a 1, 4-β-endoglucanase from *Ganoderma lucidum. Enzyme and Microbial Technology* 86: 67–74. doi:10.1016/j.enzmictec.2016.01.013

[14] Pereira H.M., Berdini V., Ferri M.R., Cleasby A., Garratt R.C. 2010. Crystal structure of *Schistosoma* purine nucleoside phosphorylase complexed with a novel monocyclic inhibitor. *Acta Tropica* 114(2): 97–102. doi:10.1016/j.actatropica.2010.01.010

[15] Elshal M.F., Eid N.M., El-Sayed I., El-Sayed W., Al-Karmalawy A.A. 2022. Concanavalin – a shows synergistic cytotoxicity with tamoxifen via inducing apoptosis in estrogen receptor-positive breast cancer: *in vitro* and molecular docking studies. *Pharmaceutical Sciences* 28(1): 76–85. doi:10.34172/PS.2021.22

[16] Abu-Serie M.M., Habashy N.H., Attia W.E. 2018. *In vitro* evaluation of the synergistic antioxidant and anti-inflammatory activities of the combined extracts from Malaysian *Ganoderma lucidum* and Egyptian

Chlorella vulgaris. *BMC Complementary and Alternative Medicine* 18(1): article number 154. doi:10.1186/s12906-018-2218-5

[17] Yousif F., Hifnawy M.S., Soliman G., Boulos L., Labib T., Mahmoud S., Ramzy F., Yousif M., Hassan I., Mahmoud K. 2007. Large-scale *in vitro* screening of Egyptian native and cultivated plants for schistosomicidal activity. *Pharmaceutical Biology* 45(6): 501–510.

[18] Tadros M.M., Ghaly N.S., Moharib M.N. 2008. Molluscicidal and schistosomicidal activities of a steroidal saponin containing fraction from *Dracaena fragrans* (L.). *Journal of the Egyptian Society of Parasitology* 38(2): 585–598.

[19] de Albuquerque D.G.R.D., Mahomoodally M.F., Lobine D., Suroowan S., Rengasamy K.R. 2020. Botanical products in the treatment and control of schistosomiasis: recent studies and distribution of active plant resources according to affected regions. *Biology* 9(8): article number 223. doi:10.3390/biology9080223

[20] Cheuka P.M., Mayoka G., Mutai P., Chibale K. 2016. The role of natural products in drug discovery and development against neglected tropical diseases. *Molecules* 22(1): article number 58. doi:10.3390/molecules22010058

[21] Bayman P., Baker J.L. 2006. Ochratoxins: a global perspective. *Mycopathologia* 162(3): 215–223. doi:10.1007/s11046-006-0055-4

[22] Chen X., Hu Z.P., Yang X.X., Huang M., Gao Y., Tang W., Chan S.Y., Dai X., Ye J., Ho P.C., Duan W., Yang H.Y., Zhu Y.Z., Zhou S.F. 2006. Monitoring of immune responses to a herbal immuno-modulator in patients with advanced colorectal cancer. *International Immunopharmacology* 6(3): 499–508. doi:10.1016/j.intimp.2005.08.026

[23] Fathima A.T., Reenaa M. 2016. Anticancer and antibacterial activity of *Ganoderma lucidum*. *International Journal of Current Microbiology and Applied Sciences* 5(10): 891–909. doi:10.20546/ijcmas.2016. 510.097

[24] Abo Elmaaty A., Hamed M.I.A., Ismail M.I., E B.E., H S.A., Khattab M., Al-Karmalawy A.A. 2021. Computational insights on the potential of some NSAIDs for treating COVID-19: priority set and lead optimization. *Molecules* 26(12): article number 3772. doi:10.3390/molecules26123772

[25] Osman G.Y., Mohamed A.M., Abdel Kader A., Mohamed A.A. 2013. Biological and biochemical impacts of the fungal extract of *Aspergillus fumigatus* on *Biomphalaria alexandrina* snails infected with *Schistosoma mansoni*. *Biosciences* 7(12): 473–485.

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