academicJournals

Vol. 7(20), pp. 1481-1493, 25 May, 2013
DOI: 10.5897/JMPR12.1085
ISSN 1996-0875 ©2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Effect of *Zingiber officinal* (ginger) and *Glycyrrhiza uralensis* (licorice) on experimental *S. mansoni* life cycle and investing the composition (metabolites) changes in different tissues

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Accepted 31 October, 2012

The present study was undertaken to evaluate the role of both ginger (*Zingiber officinal)* and licorice *(Glycyrrhiza uralensis)* on different *S. mansoni* life cycle; miracidia, cercariae, schistosomula and adult worm *in vitro*. In addition, this study aimed at separation and quantification of pyruvic, lactic, succnic, fumaric, malic, acetic, propionic and acetoacetic acids in the tissue of each parasite life cycle using ion-suppression reversed -phase high performance liquid chromatography (HPLC) after treatments of the different *S. mansoni* parasites with the lethal doses of plant extracts. Using such data, we can then evaluate in a broader manner, the physiological processes that cannot permit the parasites to survive under these treatment, and to test the potentiality of these extracts to produce disturbances in these metabolites, as they refer to different metabolic pathways to be used as diagnostic and therapeutic biomarkers.

Key words: *Zingiber officinal*, *Glycyrrhiza uralensis*, metabolites, life cycle.

INTRODUCTION

Schistosomiasis is a public health problem in many developing countries. An estimated 80% of all infected people are now concentrated in Africa (WHO, 2009). Water resource schemes for power generation and irrigation have resulted in a tremendous increase in the transmission and out breaks of schistosomiasis in several African countries (Yapi et al., 2005; Sarkinfade et al., 2009).

Study of cercarial population in a natural water body may provide a useful means for locating schistosoma transmission foci and help in evaluating the success of bilharziasis control programs. There are limited options availableforthechemotherapeutictreatmentofschistosoma infection, with drug of choice being praziquantel (WHO, 1993, 1999). Unfortunately, the long-term, world wide application of drug, coupled with the recent discovery of praziquantel-tolerant schistosoma, has generated concern over the development of drug-resistance schistosoma strains (Shengliang et al., 2001; Appiah and DeValas, 2002). So, for combating schistosomiasis, there is an urgent need to develop new drug alternative to praziquantel. Traditional medicinal plants were applied for treatment of schistosomiasis (Molgaard et al., 2001). It was found that plant extracts with molluscicidal and cercaricidal properties may provide cheap, locally produced, biodegradable and effective control agents in rural areas of

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developing countries where schistosomiasis is endemic (Brachenbury, 1998). The attenuation of *S. mansoni* cercariae with a molluscicide was previously achieved *in vitro* (Perrett et al., 1994). It was noticed that most of the plants screened against schistosomiasis cercariae and miracidia were generally effective at levels less than that of their molluscicidal ones. So, *S. mansoni* worms were not detected from mice exposed to cercariae previously treated for one hour with 100 ppm of the plant *Anagalli arvensis* dry powder (Mahmoud et al., 2005; Kamel et al., 2010). In addition, *Solanum nigrum* has a suppressive effect on the infectivity of *S. mansoni* cercariae to albino mice (Helmy et al., 2007). However, *S. mansoni* miracidia and cercariae were killed by 100 ppm dry powder of *Calendula micrantha* within 2 and 24 h of exposure, respectively (El-Emam et al., 1986).

In this concern, De-Almedia et al. (2007) reported that, *Chenopodium ambrosioides* plant has a potential activity against ascariasis, and reduces more than 95% of the infective larvae of goats gastrointestinal nematodes at 110.6 mg/ml. Also, it was found that, ethanol extract of *Sesbania sesban* displayed a weak larvicidal activity against both *S. mansoni* miracidia and cercariae (Rizk, 1998)

Several studies had examined the influence of parasites on the host organisms, the mechanisms of host location and the molluskcs resistance to the parasites, that is incompatibility of the host (Haas, 1985; Boehmler et al., 1996). In case of *S. mansoni* the larvae obtained their energy and growth substrates from the host, and released intermediate product of their metabolism into host's body.

Organic acids are important component of parasite metabolism and participate in both catabolic (glycolysis) and anabolic (gluconeogenesis) pathways. Pyruvate and lactate are indicators of glycolytic processes under aerobic conditions, while fumarate, succinate and malate are indicators of the tricarboxylic acid cycle. The presence of ketone bodies, such as β-hydroxybutrate and acetoacetate, as well as fatty acids, such as acetate and propionate, are indicative for lipid metabolism (Boehmler et al., 1996). Organic acids play a central role in the parasite of *S. mansoni* metabolism, as they serve as indicators of various metabolic reactions representing important components of energy and parasites metabolism. Thus, they may indicate the use of carbohydrates as an energy source in the flow of aerobic and anaerobic transition, the replacement of glucose through gluconeogenesis, and of protein via glucogenic amino acids or metabolism of lipids on a smaller scale via fatty acids and ketone bodies (Bezerra and Becker, 1999).

Ginger (*Z. officinal L.,* Zingiberaceae) is widely used in traditional Chinese medicine (Goto et al., 1990). The medicines are purported to be effective treatment for inflammation, oxidant stress, helminthiasis and schistosomiasis (Iqbal et al., 2006; Islam and Choi, 2008). It has also antischistosomal effect against *S. mansoni* miracidia and cercariae (Adewunni et al., 1990). Phytochemical reports have shown that the main constituents of ginger are zingerone, paradol, gingerols and shogoals. These agents are known to have the ability to suppress the inflammatory and transformative processes of carcinogenesis. Some agents have been found to have antibacterial and antiprotozoae activities (White, 2007; Ali et al., 2008). Another study has suggested that ginger free radical scavenging activity may reduce larvae survival (Lopes et al., 1998; Hierro et al., 2004).

Licorice or liquorice (*G. uralensis*) and its principle component, glycyrrhizin, has extensive use in foods, tobacco and in both traditional and herbal medicine. Biochemical studies have indicated that glycyrrhizinates inhibit 11-β-hydroxysteroid dehydrogenase, the enzyme responsible for inactivating cortisol. As a result, the continuous, high level exposure to glycyrrhizin compounds can produce hypermineralocorticoid -like effects in both animals and humans. These effects are reversible upon withdrawal of licorice or glycyrrizin. Other *in vivo* and clinical studies have reported beneficial effects of both licorice and glycrrizin consumption including anti-ulcer, anti-viral and hepatoprotective responses. Various genotoxic studies have indicated that glycyrrhizin is neither teratogenic nor mutagenic and may possess anti-genotoxic properties under certain conditions (Isbrucker and Burdock, 2006).

Licorice contains glycyrrhizin, oleane triterpenoids, glucose, flavonoids, and licorice flavonoids (LF) are known for a variety of biological activities, including inhibition of enzyme activity, antioxidants, and anti-inflammatory properties (Armanini et al., 2002). Thus, the present study is designed to estimate the possible effects of total ethanol extract of ginger, ginger tablets and licorice on *S. mansoni* different stages. The viability of miracidia, cercariae, shistosomula and adult worms after 24 h incubation at different concentrations of ginger extract, tablets and licorice were recorded. In addition, it is possible to determine simultaneously, the concentration of nine organic acids in the tissue homogenates of the parasites, using high performance liquid chromatography of different *S. mansoni* life cycle; miracidia, cercariae, schistosomula and worms. Using such data, we can then evaluate in a broader manner, the physiological processes that cannot permit the parasites to survive under previously mentioned treatments.

MATERIALS AND METHODS

The present work examines the profile of organic acids present in different life cycle of *S. mansoni*; miracidia, cercariae schistosomula and worm, in order to determine whether the type of organic acid presents or changed post treatment of ginger, ginger tablets and licorice extracts. The organic acids were extracted from the different life cycle of schistosoma parasite homogenates. The parasites were

fined in liquid nitrogen and sonicated by ultrasound for 7 min, at 4℃ for 30 min. Centrifugation at 4,000 rpm for 20 min at 4℃, followed by filtration through 50 µm syringe was done. The organic acids were extracted immediately using Bond-Elut columns (SAX-anion exchange-quartenary amine, manufactured by Analytichem International, Habor City, USA). Under vacuum, the columns were activated by consecutive washes with 1 ml of 0.5 M HCl, 1 ml of methanol and 2 ml of HPLC-grade water. They were loaded with 200 µl of filtrate and 2 ml water. The columns were disconnected from the vacuum pump and 250 µl of 0.5 M sulphuric acid were applied to elute the organic acids retained on the matrix. The elute was centrifuged at 1200 × g for 5 min and the supernatant stored at -70℃ until analysis by high performance liquid chromatography. The liquid chromatography (HPLC-system Milton Roy-Analyst 7800) was performed at room temperature using a BIORAD–Aminex ion exclusion HPX-85 column (300 × 7.8 mm) designed specifically for the separation of organic acids. The separation column was protected by a BIORAD-Aminex HPX-85 gurad column. The mobile phase was sulphuric acid (0.5) delivered at a flow rate of 0.8 ml/min. The elution profile was determined at 210 nm. The injection volume of each sample was 100 µl (Rumsby et al., 1987).

Licorice (*Glycyrrhiza* sp.) root extract

The roots Licorice were obtained from Mohamed Abdel Rahman Haraz international company market (Cairo, Egypt). The roots are dug up, washed and transported to warehouses for bailing, sorting and drying. The dried roots were crushed by millstones and the pulp is boiled with 500 ml of ethanol to make the extract. After removal of the solids, the extract was vacuum dried to a dark paste, which is cast into blocks or short sticks (Isbrucker and Burdock, 2006).

Sample preparation of ginger extract

The rhizome of ginger (gingerol) was authenticated at the National Research Centre, Egypt where a voucher specimen was deposited (NRC-0234) by Dr Mohamed E. Ibrahim, from the Cultivation and Production of Medicinal and Aromatic Plants Department. In order to prepare the ethanolic (EthOH) total extract, it was ground into a fine powder using a pestle and mortar, and the powder of ginger (30 g) was refluxed in ethanol (600 ml) in a Soxhlet apparatus for 2 days. EthOH was evaporated under reduced pressure to give a brown extract (yield: 11%). The material was subsequently reconstituted in a known volume of sunflower oil (Ahui et al., 2008).

Parasites and infection

Laboratory Swiss albino mice weighing 20 to 25 g were obtained from Theodor Bilharz Research Institute (SBSP/TBRI). They were maintained on standard diet 24% protein content. Mice were sacrificed and perfused to detect adult worms, eight weeks post infection. The mean number of worms/mouse was considered as an indicator on the infectivity of cercariae to mice in each experiment (Smithers, 1965). Cercariae of an Egyptian strain of *S. mansoni* were obtained from Schistosome Biological Materials Supply Program, Theodor Bilharz Research Institute (SBSP/TBRI). Miracedia was obtained immediately after shedding from *Biomphalaria alexandrina* snails.

Infection of mice was carried out by intraperitoneal (ip) injection of 100 ± 5 cercariae/mouse (Smithers, 1965). Schistsomula was obtained from infected mice, 4 days post infection before its transformation to adult worm.

Efficacy of ginger and licorice on different stages of *S. manosni* parasite

The miracidia

Miracidia tests were carried out using the technique described by Techounwou et al. (1991). Laboratory tissue culture plates were used as test chambers to observe the viability and death of miracidia under the dissecting microscope, 20 miracidia were placed in 1 ml dechlorinated water in each well of the test chamber. Serial double concentrations (0.5 to 5 ppm) of ginger and licorice (dissolved in dimethyl sulfoxide; DMSO) were then added, giving a total of 2 ml in each experimental well. Three replicates were made for each concentration. Mortality of miracidia was recorded after one min. Twenty freshly hatched miracidia were maintained in 2 ml dechlorinated water as controls.

Cercaricidal activity

Ethanol extracts of the two experimental plant species were used in the toxicity tests as aqueous solutions against *S. mansoni* cercariae. A series of concentrations were prepared on the basis of weight/volume, using dechlorinated water. 25 ml of dechlorinated water containing 100 fresh shed cercariae were mixed with another 25 ml of double concentration from the plants' methanol extract (using different gradual concentrations).

During exposure period, stereomicroscopic observations on the cercarial movement and mortality were recorded at successive intervals 15, 30, 45 and 60 min. 50 ml of dechlorinated water containing 100 fresh cercariae were used as control. Series of 1 ml samples of water containing 20 freshly shed cercariae were with 1 ml of double serial concentrations (0.5 to 5 pmm) of ginger and licorice dissolved in DMSO. Three replicates were made for each tested concentration. Viability of the cercariae was determined by removing the tested material after 5 min according to Ritchie et al. (1974).

S. manosni adult worms or schistosomula

Twenty worms were cultured in 24 well Falcon plates at 37℃ in ml of RPMI-1640 media supplemented with penicillin (100 U/ml), streptomycin (100 mg/ml), and 10% fatal calf serum (Gibco), 2 g/l glucose, 0.39 g/l glutamate, and 20 g/l $NaHCO_3$. Ginger or licorice were dissolved in 10% DMSO and then diluted with sterilized distilled water to the desired concentrations (range from 1 to 10 ppm) or 10 to 50 ppm of licorice. Control worms or schistosomula were treated with 10% DMSO in sterile distilled water. The movement and viability of adult worms or schistosomula were monitored for 24 h. All procedures were carried out using aseptic techniques in a laminar flow cabinet (John Bass Ltd) (Ritchie et al., 1974).

Statistical analysis

The data are presented as mean ± standard deviation (Mean ± SD). The mean groups were compared by analysis of variance. Comparison of means was done by 2-tailed unpaired t-test (Sokal et al., 1981). The sub lethal concentrations were calculated through a computer Statistical Package for the Social Sciences (SPSS), employing the probit analysis (Finney, 1971). In addition , statistical analysis of organic acids is carried out using one way analysis of variance (ANOVA), using Co-stat computer program coupled with least significance difference; LSD at $P \leq 0.05$.

Table 1. *In vitro* effect of ginger extract on miracidia.

Concentration (ppm)	1 min (% mortality)	2 min (% mortality)	3 min (% mortality)	4 min (% mortality)	5 min (% mortality)	10 min (% mortality)	LC_{50}	LC_{90}
50	5	10	30	60	75	100	3.8	5.84
100	10	20	40	80	85	100	3.17	5.15
200	20	30	50	85	95	100	2.69	4.64
300	25	50	70	90	100	100	2.05	3.89
400	30	55	80	100	100	100	1.76	3.28
500	75	85	100	100	100	100	0.26	1.94
LC_{50}	4.26	3.15	1.74	0.13	0.35			
LC_{90}	7.21	6.01	4.43	1.71	1.33			

The LC_{90} was 4.43 at the concentration of 500 ppm after 3 min.

Table 2. *In vitro* effect of ginger tablets on miracidia.

Concentration (ppm)	1 min (% mortality)	2 min (% mortality)	3 min (% mortality)	4 min (% mortality)	5 min (% mortality)	10 min (% mortality)	LC_{50}	LC_{90}
50	5	12	30	65	80	100	3.64	5.55
100	10	20	40	80	85	100	3.17	5.15
200	23	30	50	85	95	100	2.60	4.68
300	25	50	70	92	100	100	2.03	3.82
400	31	55	85	100	100	100	1.71	3.15
500	76	87	100	100	100	100	2.11	1.86
LC_{50}	4.19	3.10	1.71	0.36	-.62			
LC_{90}	7.15	5.97	4.24	2.24	1.23			

The LC_{90} was 4.24 reached to 100% at the concentration 500 ppm after 3 min.

RESULTS

Table 1 shows the effect of ginger (*Z. officinal*) on miracidia, it reveals the most toxic effect on *S. mansoni* miracidiam, the LC_{90} was 4.43 at the concentration of 500 ppm after 3 min. Thereafter, there was very low activity as their LC_{90} values (5.84) at 50 ppm after 10 min of exposure. Table 2 shows the effect of ginger tablets on miracidia and reveals the most toxic effect on *S. mansoni* miracidia. At LC_{90}, 4.24 reached 100% at the concentration of 500 ppm after 3min. Table 3 recorded licorice as the most toxic one to *S. mansoni* miracidia, LC_{90} was 3.16 at 500 ppm after 4 min. Thereafter, very low activity was seen, as their LC_{90} values reached 50.39 at 50 ppm, after 30 min of exposure. On the other hand, the effect of ginger on cercariae (Table 4), demonstrate that, ginger is the most toxic effect at LC_{50} (3.06) after 3 min at 100 ppm, while LC_{90} was 7.69 at 100 ppm after 3 min and 2.43 after 10 min. Table 5 shows the most toxic effect of ginger tablets on cercariae. The LC_{90} was 3.14, reaching 100% at the concentration of 100 ppm after 3 min,

Table 3. *In vitro* effect of licorice on miracidia.

Concentration (ppm)	1 min (% mortality)	2 min (% mortality)	3 min (% mortality)	4 min (% mortality)	5 min (% mortality)	10 min (% mortality)	20 min (% mortality)	30 min (% mortality)	LC_{50}	LC_{90}
50	0	0	10	20	30	40	45	50	24.74	50.39
100	0	5	20	50	70	75	95	100	6.43	13.32
200	10	20	40	60	85	90	100	100	3.40	5.63
300	30	40	50	70	90	100	100	100	2.57	5.58
400	50	55	70	80	100	100	100	100	1.44	4.47
500	60	70	80	100	100	100	100	100	0.84	3.16
LC_{50}	4.22	3.78	2.93	1.64	0.78	0.61	0.54	0.47		
LC_{90}	6.34	6.09	5.79	4.28	2.49	1.79	0.90	1.87		

Licorice was the most toxic one to *S. mansoni* miracidia LC_{90} was 4.28 at 500 ppm after 4 min.

Table 4. *In vitro* effect of ginger extract on cercariae.

Concentration (pmm)	1 min (% dead cer)	2 min (% dead cer)	3 min (% dead cer)	4 min (% dead cer)	5 min (% dead cer)	10 min (% dead cer)	LC_{50}	LC_{90}
20	20	30	45	60	80	100	3.19	6.23
5 0	30	50	60	80	98	100	2.13	4.46
100	70	80	100	100	100	100	0.51	2.14
LC_{50}	7.29	5.04	3.06	1.32	1.17			
LC_{90}	14.55	12.56	7.69	6.08	2.43			

The more toxic effect was noticed at LC_{50} was 3.06 after3 min at 100 ppm, while LC90 was7.69 at 100 ppm after 3 min and 2.43 after 10 min, cer = cercariae

3 min, while licorice shows the most toxic effect on *S. mansoni* cercariae at LC90 equivalent to 28.06 at 500 ppm after 15 min, and the very low cercaricidal activity as their LC90 values were 18.16, at 200 ppm after 30 min of exposure (Table 6). Table 7 shows the effect of ginger on schistosomula, the LC_{90} was 4.62 and reached 100% at the concentration of 40 ppm after 1 min. The effect of ginger tablets on schistosomula (Table 8) declared that, the LC_{90} was 3.35 and reached 100% at the concentration of 40 ppm after 1min, while licorice shows the LC_{90} as 7.54 and reached 100% at the concentration of 100 pmm after 45min (Table 9). Table 10 shows the effect of ginger extract on the mortality rate of adults worm. It reveals that the strongest lethal effect of LC_{90} was 3.77 and reached 100% at the concentration of 100 ppm after 3 min. Ginger tablets exhibited more effect on adult worm than ginger extract .The LC_{90} recorded was 7.99 and reached 100% at the concentration of 100 ppm after 15 min. Table 11 shows the effect of ginger tablets on the worm, the LC_{90} was 3.35 and reached 100% at the of concentration of 100 ppm after 3 min. In addition, the effect of licorice extract on the mortality rates of adults worm recorded the strongest lethal effect of LC_{90} which was 7.99 and reached to 100% at the concentration of 100 ppm after 15 min. The worm shrunk at the first 5 min (Table 12).Table 13 declared the effect of ginger extract, tablets and licorice on miracidia and cercariae of *S. mansoni* life cycle. Significant increase was observed in post treatment

Table 5. *In vitro* effect of ginger tablets on cercariae.

Concentration (µl)	1 min (% dead cer)	2 min (% dead cer)	3 min (% dead cer)	4 min (% dead cer)	5 min (% dead cer)	10 min (% dead cer)	LC_{50}	LC_{90}
10	10	15	30	40	60	80	5.42	10.85
20	25	30	45	60	85	100	3.05	6.1
50	34	50	63	80	98	100	2.02	4.58
80	60	70	80	90	100	100	0.66	3.68
100	75	85	100	100	100	100	0.26	1.94
LC_{50}	6.65	5.17	3.14	1.58	0.24			
LC_{90}	13.30	11.29	8.66	6.88	2.92			

The LC_{90} was 8.66 and reached 100% at the of concentration of 100 ppm after 3 min. cer = cercariae.

Table 6. *In vitro* effect of licorice on cercariae.

Concentration	1 min (% dead cer)	2 min (% dead cer)	3 min (% dead cer)	4 min (% dead cer)	5 min (% dead cer)	10 min (% dead cer)	15 min (% dead cer)	20 min (% dead cer)	30 min (% dead cer)	LC_{50}	LC_{90}
200	30	40	45	50	60	75	80	90	100	4.42	18.16
300	40	45	60	65	75	80	90	98	100	1.60	13.34
500	50	60	65	75	80	95	100	100	100	0.83	7.42
LC_{50}	48.82	35.78	22.38	16.94	1.61	0.71	7.06	3.78			
LC_{90}	125.59	110.34	104.31	76.29	70.69	40.73	28.06	20.03			

It is seen from Table 4 that licorice is the most toxic one to *S. mansoni* cercariae, LC_{90} was 28.06 after 15 min at concentration of 500 ppm and the very low cercaricidal activity as their LC_{90} values were 18.16 at 200 ppm after 30 min of exposure. cer = cercariae.

Table 7. *In vitro* effect of ginger extract on schistosomula.

Concentration (ppm)	1 min (% dead schistosomula)	2 min (% dead schistosomula)	3 min (% dead schistosomula)	4 min (% dead schistosomula)	5 min (% dead schistosomula)	10 min (% dead schistosomula)	LC_{50}	LC_{90}
10	5	20	40	80	90	100	3.16	4.84
20	50	70	90	100	100	100	1.13	2.84
30	70	80	100	100	100	100	0.51	2.14
40	100	100	100	100	100	100		
LC_{50}	2.84	2.15	1.21	0.54	0.1			
LC_{90}	4.62	4.18	3.0	1.24	0.99			

The LC_{90} was 4.62 reached to 100% at the concentration 40 ppm after 1 min.

Table 8. *In vitro* effect of ginger tablets on schistosomula.

Concentration (pmm)	1 min (% dead schistosomula)	2 min (% dead schistosomula)	3 min (% dead schistosomula)	4 min (% dead schistosomula)	5 min (% dead schistosomula)	10 min (% dead schistosomula)	LC_{50}	LC_{90}
10	8	25	40	80	92	100	3.04	4.82
20	50	71	93	100	100	100	1.12	2.72
30	73	80	100	100	100	100	0.38	2.11
40	100	100	100	100	100	100		
LC_{50}	2.18	1.66	1.14	0.66	0.36			
LC_{90}	3.35	3.12	1.88	1.18	0.94			

The LC_{90} was 3.35 and reached 100% at the concentration of 40 ppm after 1 min.

Table 9. *In vitro* effect of licorice on schistosomula.

Concentration (ppm)	5 min (% dead schistosomula)	10 min (% dead schistosomula)	15 min (% dead schistosomula)	30 min (% dead schistosomula)	45 min (% dead schistosomula)	1 h (% dead schistosomula)	LC_{50}	LC_{90}
20	0	0	0	20	30	50	57.13	84.83
40	0	0	0	50	60	65	43.78	69.16
60	0	5	10	55	75	100	31.87	48.76
80	8	20	30	70	90	100	23.32	42.21
100	10	30	50	80	100	100	17.29	32.60
LC_{50}	14.05	11.30	9.74	5.26	3.56	2.38		
LC_{90}	18.56	15.36	13.17	11.94	7.54	5.07		

The LC_{90} was 7.54 and reached 100% at the concentration of 100 ppm after 45 min.

Table 10. *In vitro* effect of ginger extract on worm.

Concentration (ppm)	1 min (% dead worm)	2 min (% dead worm)	3 min (% dead worm)	4 min (% dead worm)	5 min (% dead worm)	LC_{50}	LC_{90}
20	50	75	85	90	100	0.91	3.46
40	55	80	90	100	100	0.85	2.67
60	70	85	95	100	100	0.01	2.06
80	75	88	96	100	100	0.001	1.69
100	80	90	100	100	100		
LC_{50}	2.14	2.01	3.93	0.79			
LC_{90}	13.55	6.92	3.77	1.99			

The LC_{90} was 3.77 and reached 100% at the concentration of 100 ppm after 3 min.

Table 11. *In vitro* effect of ginger tablets on worm.

Concentration (ppm)	1 min (% dead worm)	2 min (% dead worm)	3 min (% dead worm)	4 min (% dead worm)	5 min (% dead worm)	LC_{50}	LC_{90}
10	40	50	75	80	100	1.76	4.29
20	55	80	90	95	100	0.69	2.97
40	60	85	100	100	100	0.81	2.06
60	73	82	95	100	100	0.01	1.69
80	76	88	100	100	100	0.16	2.29
100	80	90	100	100	100	0.04	1.64
LC_{50}	1.86	-2.14	0.09	0.003			
LC_{90}	12.92	8.03	1.89	1.54			

The LC_{90} was 1.89 and reached 100% at the concentration of 100 ppm after 3 min.

Table 12. *In vitro* effect of licorice on worm.

Concentration (ppm)	5 min (% dead worm)	10 min (% dead worm)	15 min (% dead worm)	30 min (% dead worm)	45 min (% dead worm)	1 h (% dead worm)	LC_{50}	LC_{90}
20	0	0	0	20	30	50 all died after 2 h	57.13	84.83
40	0	0	0	50	60	65	43.78	69.16
60	0	5	10	50	75	100	30.73	47.48
80	70	80	90	100	100	100	0.38	14.39
100	75 shrinking	90	100	100	100	100	1.29	8.93
LC_{50}	10.31	7.32	7.00	4.68	3.65	2.39		
LC_{90}	8.2	8.34	7.99	9.19	8.29	5.07		

The LC_{90} was 7.99 and reached 100% at the concentration of 100 ppm after 15 min, the worm become shrinking at the first 5 min.

post treatment of miracidia with licorice, while insignificant change was detected in its concentration as the result of both ginger extract and ginger tablets. The same results were obtained for lactate, fumarate, malate and acetate. On the other hand, there was significant increase in succinate and acetoacetate of miracidia post licorice and ginger treatments. However, propionate showed insignificant change in miracida post licorice treatment and significant decrease with ginger extract and tablets. Concerning the effect of licorice on cercariae, insignificant change was observed in pyruvate, lactate, succinate and fumarate after licorice treatment, while significant decrease in their concentrations was observed in post ginger extract and tablets. Although malate and propionate metabolites showed significant decrease after different treatments, acetate exhibited significant increase post licorice treatment and significant decrease post ginger extract and tablets. In contrast, acetoacetate showed significant increase post various treatments.

Table 14 demonstrated the effect of ginger extract, tablets and licorice on schistosomula and adult worms of *S. mansoni* life cycle, and showed significant increase in organic acid; pyruvate, lactate, succinate and aceto-acetate in schistosomula treated either with ginger or ginger tablets, while significant decrease in fumarate malate, acetate and propionate as compared to the normal control group. In addition, significant increase was recorded in pyruvate, lactate, succinate, malate, acetate, and acetoacetate as a result of schistosomula treatment with licorice, while significant decrease in fumarate and propionate was recorded as compared to normal untreated schistosomula. On the other hand, insignificant change was observed in pyruvate, succinate and acetate in worm treated by both ginger extract and ginger tablets; although significant decrease was observed in lactate, fumarate and propionate in worm treated with ginger extract and tablets. Concerning, the effect of licorice on worm, significant increase was noticed in pyruvate, succinate and acetate. However, significant decrease in lactate, fumarate, malate and propionate was noticed.

DISCUSSION

This study was performed to evaluate *in vitro* the antischistosomal effect of ginger and licorice for controlling schistosomiasis.

The main constituents of ginger are zingerone, paradol, gingerols and shogoals. These agents have been found to have antibacterial and antiprotozoae activities, though they were not sure to what extent this affected larvicidial activity (Lopes et al., 1998; Ahmed et al., 2005). However, Adewunmi et al. (1990) found that gingeriol and shogaol had potent molluscidal activity on *B. glabrata* and on different satge of *S. mansoni in vitro*. Gengerol was found to completely abolish the infectivity of *S. mansoni*

Table 13. *In vivo* effect of ginger and licorice on organic acids of *S. mansoni* life cycle; miracidia and cercariae.

Parameter / Group	Control miracidia	Control cercariae	Licarice + cercariae	Licarice + miracedia	Ginger + cercariae	Ginger tablets + cercariae	Ginger + miracedia	Ginger tablets + miracedia
Pyruvate	33.17±0.15^{c}	36.37±0.32^{c}	36.0±0.3^{c}	38.66±0.57^{g}	33.04±0.05^{d}	33.93±0.06^{ef}	32.55±0.48^{c}	32.88±0.02^{c}
Lactate	797.07±2.61^{a}	877.03±2.6^{d}	817.37±2.51^{f}	837.52±2.50i	767.69±2.53^{e}	782.92±2.60^{g}	767.95±0.05^{a}	787.41±2.51^{a}
Succinate	870.68±0.47^{d}	860.44±0.58^{e}	859.37±0.56^{e}	925.30±2.04^{c}	820.69±0.45^{f}	824.44±1.0^{g}	945.33±0.58^{b}	922.10±1.01^{h}
Fumarate	98.63±0.54^{e}	106.81±0.25^{c}	109.16±1.26^{c}	110.73±0.30^{g}	98.51±0.50^{e}	99.22±0.69^{e}	97.59±0.179^{e}	99.33±0.58^{e}
Malate	37.0±0.50^{b}	43.19±0.17^{a}	40.67±0.30^{d}	40.66±0.29^{g}	35.46±0.41^{d}	36.87±0.025^{e}	39.91±0.09^{b}	38.39±0.34^{b}
Acetate	1911.67±1.53^{f}	2090.33±1.59^{c}	2205.37±4.50^{e}	2210.0±1.000^{a}	1935.52±0.51^{d}	1942.8±2.241^{e}	1925.9±3.6^{f}	1982.55±4.80^{f}
Propionate	338.99±0.94^{b}	345.98±0.47^{a}	333.43±1.91^{e}	334.53±2.93^{b}	283.140±3.56^{d}	294.67±0.57^{f}	234.24±0.67^{g}	259.13±0.81^{h}
Acetoactate	86.37±1.5^{h}	92.30±0.45^{g}	142.68±0.51^{e}	128.93±0.11^{b}	147.95±0.05^{f}	103.45±0.47^{f}	146.7±0.25^{a}	149.56±0.51i

*Mean ± SD. Statistical analysis is carried out using one way analysis of variance (ANOVA) using Costat computer program coupled with post-hoc (least significance difference LSD at P ≤ 0.05). Unshared letters indicate significant correlation at P ≤ 0.05. The concentration of each acid is expressed as nmole/g wet weight.

Table 14. *In vivo* effect of ginger and licorice on organic acids of *S. mansoni* life cycle; schistosomula and adult worms.

Parameter / Groups	Control Schistosomula	Control worm	Schistosomula + ginger extract	Schistosomula + ginger tablets	Schistosomula + licorice	Worm + ginger	Worm + ginger tablets	Worm + Licorice
Pyruvate	28.77±0.3^{e}	30.29±0.04^{d}	33.36±0.60^{a}	31.45±0.51^{a}	35.47±0.45^{c}	30.69±0.28^{d}	30.23±0.19^{d}	31.53±0.08^{c}
Lactate	748.82±2.25^{f}	817.87±1.85^{a}	792.78±2.80^{c}	790.45±1.36^{d}	796.29±0.61^{d}	756.99±1.92^{b}	800.26±2.6^{b}	774.37±2.02^{d}
Succinat	806.80±1.9^{d}	795.86±2.8 ^{e}	907.89±2.2^{a}	993.26±1.81^{c}	947.39±2.44^{f}	795.18±3.73^{e}	725.70±2.9 ^{e}	860.2±0.97^{b}
Fumarat	82.17±0.75^{d}	90.40±0.52 ^{b}	80.98±0.015^{a}	75.71±0.41^{d}	75.63±0.33^{f}	75.70±0.24^{d}	82.69±0.28^{F}	82.65±0.22^{c}
Malate	32.88±0.13^{e}	34.69±0.38^{d}	30.61±0.3^{a}	31.70±0.37^{f}	40.57±0.42^{g}	35.72±0.39^{b}	36.81±0.24^{C}	28.63±0.23^{h}
Acetate	1707.66±6.80^{d}	1713.70±1.13^{cd}	1666.86±4.6^{a}	1621.54±2.74^{h}	2212.13±2.50^{e}	1708.09±2.48^{c}	1717.71±2.0^{D}	1822.62±0.34^{b}
Propionate	255.93±0.95^{d}	261.13±1.03^{d}	185.75±0.23^{e}	150.94±0.37^{b}	245.61±0.52^{h}	235.76±0.33^{a}	232.65±0.33^{G}	257.27±0.63^{c}
Acetoactate	101.67±7.6^{d}	97.33±2.08^{d}	140.82±0.23^{a}	154.73±0.23^{f}	131.46±0.51^{c}	133.86±0.16^{e}	129.57±0.49 ^{h}	101.75±0.35^{d}

Mean ± SD. Statistical analysis is carried out using one way analysis of variance (ANOVA) using Costat computer program coupled with post-hoc(least significance difference LSD at P ≤ 0.05). Unshared letters indicate significant correlation at P ≤ 0.05. The concentration of each acid is expressed as nmole/g wet weight .

miracidia and cercariae in *B. glabrata*. Whereas, pervious investigations on ginger components showed larvicidal activity against *S. mansoni* (Adewunmi et al., 1990; Sanderson et al., 2002). In addition, Rong-Jyh et al. (2010) fond that ginger's constituents exhibited larvicidal activity and caused loss of spontaneous movement.

In the present work, examination of miracidia, cercariae, schistosmula and adult worm viability using stereo-microscope revealed its significant mortality rates. Treatment of ginger extract on meracidia showed very low miracidia activity as their LC_{90} was 4.43 at the concentration 500 ppm after 3min. While the effect of ginger on cercariae was the most toxic one, LC_{90} was 7.69 at 100 ppm

after 3 min and 2.43 after 10 min. The current study demonstrated that ginger extract possesses strong schistosomicidal activity against different *S. mansoni* life cycle. The exposure of miracidia and cercariae to the ginger extract or tablets caused death, and the lethal effect was dependent on the concentration and the time of incubation (after 10 min for miracidia and 3 min for cercariae). Regarding the effect of ginger extract or tablets on the mortality rates of adults worm after 24 h, the strongest lethal effect at 100 ppm after 3 min was observed where 100% of parasite was dead.

The viability of schistosomula was affected following addition of ascending concentrations of ginger extract (1, 2, 3 and 4 ppm). The death rate of schistosomula reached 100% at all concentrations after 24 h. In agreement with the present results, Adewunmi et al. (1990) found that ginger had antischistosomal effect against *S. mansoni* miracidia and cercariae .In *in vitro* bio-assay screening of medicinal plants for antischistosomal activity, Sanderson et al. (2002) found that ginger affected the viability of schistosome adult worms. On the other hand, the potential effect of licorice on *S. mansoni* life cycle was related to the anti-inflammatory flavonoid compounds (Cheng Xie et al., 2009). Several studies have reported that licorice exerts anti-inflammatory and anti-carcinogenic effects due to the presence of glycyrrhizin which inhibit inflammation, cell cycle progression in MCF-7 human breast cancer cells and tumour growth (Jo et al., 2005; Kim et al., 2006; Choi et al., 2008). Glycyrrhizin is considered as the major compound in the licorice root, evidences profound anti-inflammatory activities, such as the inhibition of LPS/Dgalactosamine-induced liver injury (Yoshida et al., 2007), the prevention of free fatty acid-induced hepatic lipotoxicity (Wu et al., 2008), and the attenuation of carrageenan-induced lung injury (Menegazzi et al., 2008).

Regarding the concentrations of organic acids in the tissue of different schistosoma parasites post different treatments, these acids represent metabolites directly linked to energy production and facultative metabolic ways. Under the conditions employed, the four *S. mansoni* parasites studied showed different concentrations of organic acids, the profiles of which appeared to be specific for each one (Ishak et at., 1975; Patience et at., 1983; Hardewig et at., 1994). An interesting finding in the present work was the high concentration of pyruvate in the different cycle of schistosoma parasites. Pyruvate is the end product of the glycolytic process and serves as a substrate for the formation of acetyl-CoA, which will either enter the Krebs' cycle to form acetate or lactate. The high concentration of pyruvate in different life cycle of schistosoma parasites after treatment either by ginger or licorice may therefore be explained as a high intensive glycolytic rate as compared to control untreated, or by its low usage as a substrate and hence lower removal from the hemolymph of parasites (Bezerra et al., 1999).

The increase in pyruvate concentration observed in different treated parasites may reflect an increase in anaerobic metabolism via intensified glycolytic processes. However, an increase in pyruvate may also result from an enhanced protein catabolism, leading to an increase in glucogenic amino acids (Hochachka, 1983), and finally to an increase of this organic acids (Ellersiek, 1976). The success of both the parasite's penetration of the snail's skin (Loker and Bayne, 1982; Bayne and Loker, 1987), and the establishing of an infection (Frandsen, 1979) are generally correlated with the hemolymph composition. In this context, it is possible that the parasite is able to absorb host substances which will provide either energy or structural substrates to enable the parasite to become established (Wright, 1974). The presence of parasite-repelling substances may indicate either a host metabolic state not conductive to the parasite's settling or the presence of important concentration differences for certain substrates.

The concentration of lactate showed significant fluctuated levels in different *S. mansoni* life cycle after treatment either by ginger or licorice, as compared to control untreated one. The control untreated parasites cycle had a high level of this acid. Flechter and LoVerde (1981) suggested a correlation between *S. mansani* lactate dehydrogenase values and the parasite's infectivity in snails. However, the results shown here indicate significant difference in the tissue lactate levels of different treated life cycle parasites.

For fumarate, we detected a consistent significant difference between different treated parasites life cycle. It is interesting to note that the fumarate reductase system is gaining increasing attention in invertebrate biochemistry.

Particularly, the presence of this system has a valuable physiological meaning in the adaptation of invertebrates with facultative anaerobic metabolism under adverse ecological conditions, and is very important in the studies of helminthes parasites metabolism to elucidate the characteristics of the fumarate-reductase system in relation to energy generation and to the effects of antihelmintics on the energy metabolisms (Saz, 1990; Kita, 1992; Takamiya et al., 1984, 1993; Tielens, 1994).

In conclusion, the results of this work show a variable concentration of organic acids in the tissue of *S. mansoni* life cycle parasites after treatment, either with licorice or ginger. These differences may be correlated with the parasite's ability to stand or develop during host parasites association or within snails.

The concentrations of the components of the tricarboxylic acid cycle analyzed here, succinate, fumarate, and malate showed fluctuated levels. Thus, the significant difference in tissue succinate concentration in different treated parasites life cycle may indicate a gain disturbance in energy via anaerobic pathways. Although, the increase in succinate level in miracidia and

schistosomula post treatment with ginger and licorice may be explained on the basis of enhancement in anaerobic glycolysis and inhibition of Kreb's cycle attributed to the enhancement in energy expenditure, as a result of a high in metabolic activities. This agrees with the low oxygen consumption reported by von Brand and Mehlman (1953) for aestivating *B. glabrata*. Thus, with the reduction in basal metabolism, the aerobic pathways were still able to maintain an appropriate level of metabolism.

One of the most interesting results was the significant increase in acetate in the miracidia post ginger and licorice treatments and in schistosomula and worms of *S. mansoni* post licorice treatment only as compared to control untreated. This may reflect the oxidative decarboxylation of pyruvate to acetyl-CoA which can lead to the generation of free acetate and Adenosine-5'-triphosphate (ATP) via acetyl phosphate, thereby inducing partial anaerobic metabolism. The formation of acetate probably resulted not only from a decrease in oxygen consumption, but also from an imbalance in the redox system. However, if this were the case, we would expect an accumulation of other final products of such "anaerobic metabolism".

The end products of anaerobic pathways, for example succinate, lactate and propionate, are well known in molluscs (Wieser, 1980; Wolmarans, 1987). Therefore, there is expectation to an increase in organic acid levels. However, this was not the case. Thus, it is still unclear which metabolic pathways account for the accumulation of acetate. An increase in the level of acetate (a volatile acid) is not believed to be very toxic for cells (Mchlman and Von Brand, 1951), since this compound may easily be excreted across the body surface (Bryant, 1993).

Regarding acetoacetate, the significant fluctuation in their concentrations as compared to the normal control reflected disturbances in lipid synthesis which in turn affected parasite activity (Bezerra et al., 1999). The significant change in the acid concentrations as a result of ginger and licorice treatments is explained by their possible use as metabolites by developing parasites, which may cause mechanical and lytic damage to the parasites. The damage probably results in a leakage of the acids. Moreover, the increased metabolic activity associated with the presence of larval trematodes may accelerate the use of the acids by host cells in the digestive gland. The change in metabolite levels may be due to different causes; activities of membrane transport proteins may have been altered, and rates of metabolic reactions may have shifted, or ranch point enzyme activities might have changed. Even if metabolite levels are found unchanged between different experiment situation, the underlying flux differences and enzymatic activities might still have changed (Abou Elseoud et al., 2010).

In a good agreement with the present finding, Sanderson et al. (2003) found that, *in vi*tro study, 200 mg l^{-1} of ginger produced a significant effect on worm survival. Al-Sharkawi et al. (2007) showed that ginger administration caused a highly significant reduction in ova and egg load in liver tissue, and these effects might be due the effects of ginger administration on worms' fecundity.

The results obtained in the present study is in agreement with those of Sanderson et al. (2002) who reported *in vitro* bioassay a significant reduction in the mean egg output of surviving females following exposure to sub-lethal concentrations of ginger extract. It is not known if these anti-fecundity effects were the result of one or more of the compounds present. On the other hand, most research into the biochemistry and pharmacology of glycyrrhizate compounds has focused on the possible mechanism which may cause the death of different *S. mansoni* life cycle *in vitro*. The glycyrrhetic acid component has been reported to alter several enzymatic process in animals and cell culture systems and also the Inhibition of mitochondria oxidative phosphorlytation, cytochrome P450 monoxygenase systems and N-acetyl tranferase activity (Isrucker and urdock, 2006). The authors added that the *in vivo* hepatoprotective mechanisms of glycyrrhizic acid is due to its aglycone, 18 β-glycyrrhetic acid, which inhibits both free radical generation lipid peroxidation, as well as anti-carcinogenic effects.

Conclusion

In vitro study revealed that both ginger and licorice had lethal effect on different *S. mansoni* life cycle. The consistent differences in carbohydrate metabolites profiles of miracidia, cercariae, schistosomula and worms after treatment with the lethal dose of both ginger, ginger tablets and licorice were matched by untreated control and identified with HPLC strategy, which allowed the prediction of effective drug and possibly determination of the more damaged or toxic one.

These findings highlighted the potential of metabolomics as a novel approach for fundamental investigations of host-pathogen interactions, as well as disease surveillance and control.

ACKNOWLEDGMENTS

We are really grateful to Dr. Soheir Mahmoud in parasitology laboratory in Theodor Billarz Research Institute for help in this experiment.

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