Antioxidant and schistosomicidal effect of *Allium sativum* and *Allium cepa* against *Schistosoma mansoni* different stages

M.M. MANTAWY[1], H.F. ALY[1], N. ZAYED[1], Z.H. FAHMY[2]

Therapeutic Chemistry Department[1], National Research Center, El Behooth Street, Dokki, Giza (Egypt); Parasitology Department[2], Theodor Bilharz Research Institute, Imbaba, Giza (Egypt)

Abstract. – OBJECTIVE, The schistosomicidal properties of garlic (*Allium sativum*) and onion (*Allium cepa*) powder were tested *in vitro* against *Schistosoma mansoni* miracidia, schistosomula, cercaria and adult worms. Results indicate their strong biocidal effects against all stages of the parasite and also show scavenging inhibitory effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide (NO).

MATERIALS AND METHODS, In the present work, the *in vivo* effects of *A. sativum* and *A. cepa* on lipid peroxide and some antioxidant enzymes; thioredoxin reductase (TrxR), sorbitol dehydrogenase (SDH), superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) (as they have a crucial role in host protection against invading parasite) were also studied.

RESULTS, The data demonstrate that, there was a significant inhibition in SOD, CAT, GR, TrxR and SDH in infected liver while, significant elevation was detected in lipid peroxide as compared to the normal control. The current results clearly revealed that, the used both edible plants enhance the host antioxidant system indicated by lowering in lipid peroxide and stimulation of SOD, CAT, GR, TrxR and SDH enzyme levels.

CONCLUSIONS, Enhancement of such enzymes using *A. sativum* and *A. cepa* could in turn render the parasite vulnerable to damage by the host and may play a role in the antischistosomal potency of the used food ingredients.

Key Words:

Allium sativum, Allium cepa, Schistosoma mansoni.

Introduction

Tremadode worms of the genus Schistosoma cause the disease schistosomiasis in man and animals. *Schistosoma mansoni (S. mansoni)* eggs hatch into the miracidia which live inside their intermediate host *Biomphalaria alexandrina* snails and are transformed into mother sporocyts, daughter sporocyts and these in turn give rise to the mature cercariae. Infection is caused by cercariae which penetrates the skin of the final host. The head of the cercariae is transformed into an endoparasitic larva, the schistosomula[1]. Adult *S. mansoni* in the mesenteric vein are resistant to the host immune response which at the same time is capable of overcoming challenge infection[2,3]. The major component of the immune response is cell mediated and as part of that response during early *S. mansoni* infection, the parasite is exposed to reactive oxygen species (ROS) generated by the host effectors' cells as macrophages, eosinophils, neutrophils and platelets[4]. To defend themselves against oxygen-mediated killing mechanisms of host, parasites have developed antioxidant enzyme systems. In *S. mansoni* superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPX), catalase (CAT) and glutathione-s-transferase (GST) are major antioxidant enzymes that are involved in detoxification processes[5]. These schistosoma oxidant-detoxicating systems play a role in protecting the parasite from damage as a result of ROS[3]. Therefore, the antioxidant defense mechanisms of adult worms may represent potentially good target chemotherapy. On the other hand, the host's response to *S. mansoni* infection involves the production of ROS where the antioxidant enzymes represent a target for immune elimination of adult worms[5]. In this concern, Sheweita et al[6] pointed out that levels of reduced glutathione and glutathione reductase increased, while the activity of glutathione-s-transferase decreased in human and mice infected with *S. mansoni*. In this respect *S. mansoni* infection alters the hepatic levels of glutathione and activities of glutathione metabolizing enzymes

and these alterations may affect the capacity of the liver to detoxify or neutralize the toxic effect of endogenous and exogenous compounds. Furthermore, Gharib et al[7] mentioned that superoxide dismutase, catalase and glutathione peroxidase, sorbitol dehydrogenase and thioredoxin reductase enzyme activities were decreased in livers of mice infected with *S. mansoni*. Similarly, Pascal et al[8] and Soliman et al[9] postulated that lipid peroxidation was elevated in both serum and liver of man and mice infected with *S. mansoni*.

There are limited options available for the chemotherapeutic treatment of *Schistosoma* infection with the drug of choice being praziquntel[10,11]. Unfortunately, the long term world wide application of the drug coupled with the recent discovery of praziquntel-tolerant schistosomes has generated concern over the development of drug resistant *Schistosoma* strains[12]. So, for combating schistosomiasis there is an urgent need to develop new drugs alternative to praziquntel. Traditional medicinal plants were previously applied by some Authors for the treatment of schistosomiasis[13-15].

Garlic (*Allium sativum*) and onion (*Allium cepa*) are two food ingredients widely used in our gastronomy. Moreover, *Allium sativum (A. sativum)* and *Allium cepa (A. cepa)* extracts have been recently reported to be effective in cardiovascular disease, because of their hypocholesterolemic, hypolipidemic, anti-hypertensive and anti-diabetic, anti-thrombotic and anti-hyperhomocysteinemia effects, and to possess many other biological activities including antimicrobial, antioxidant, anticarnogenic, antimutagenic, antiasthmatic, immunomodulatory and prebiotic activities. *A. sativum* is still being employed in traditional medicine all over the world for the treatment of a variety of diseases[16]. Evidence from several investigations suggests that the biological and medical functions of *A. sativum* and *A. cepa* are mainly due to their high organosulphur compounds[17-19]. Flavonoids are abundant in *A. cepa* but practically absent in *A. sativum*. A small amount of non-volatile water-soluble sulphur compounds found in *A. sativum*, as S-allyl cysteine (SAC), are also responsible for a great part of the health benefits of both vegetables[20]. In view of these findings, this study was undertaken to evaluate, *in vitro* the antischistosomal effect of *A. sativum* and *A. cepa* against *S. mansoni* miracidiae, schistosomula, cercariae (free living stages), and adult worms. Also, the scavenging effect of both *A. sativum* and *A. cepa* on DPPH and NO was evaluated. In addition, the study was extended to demonstrate the potential role of both vegetables against lipid peroxide and some antioxidant protecting enzymes i.e. SOD, CAT, GR, SDH, and TrxR. These agents may strength the antioxidant system of the host and may play a crucial role in the survival of adult worms inside the host.

Materials and Methods

Chemical
Chemicals were of analar quality, products of Merck, Darmstadt, Germany and Sigma (St Louis, MO, USA).

Preparation of A. cepa and A. satvium
Sliced onion and garlic pulps (3 mm thick) were dried at 50°C overnight and pulverized in a mortar and pestle, the powder was kept dry and stored at 4°C. *A. cepa* and *A. sativum* were administered in doses of 2 g/100 g body weight daily for 45 days in a standard pellet diet containing 24% protein, 4% fat and about 4-5% fiber, according to Mantawy and Mahmoud[21]. Praziquantel was administered in a dose of 500 mg/kg body wt on two successive days 45 days post infection[22].

The parasite – S. mansoni NMRI strain life cycle
(1) Schistosome eggs and miracidia are obtained from livers of experimentally infected mice according to the method described by Hira and Webbe[23] and used directly in the bioassays; (2) *S. mansoni* cercariae are obtained from experimentally infected snails by exposing them to artificial 28°C where the released cercariae were used directly after shedding[24]. Snails are obtained from Schistosomiasis Biological Supply Program, SB-SP, Theodor Bilharz Research Institute, Imbaba, Giza, Egypt; (3) Shistosomula and adult worms: 10 male hamsters (*Mesocricetus auratus*) provided by a laboratory-bred colony of similar age and mass (100-140 g) were obtained from SBSP. Each hamster was exposed separately to 150 *S. mansoni* cercariae for 1 h using the partial immersion technique[25]. Two and eight weeks post infection (PI) schistosomula and adult worms respectively were recovered from hepatic portal system and liver by the perfusion technique previously described by Smithers and Terry[2].

Efficacy of A. sativum and A. cepa on Different Stages of S. mansoni Parasite

(1) The miracidia: miracidia tests were carried out using the technique described by Techoun-wou et al[26]. Laboratory tissue 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 concentrations (0.5-5 ppm) of *A. sativum and A. cepa* (dissolved in 10% dimethyl sulfoxide: DMSO) were then added giving a total of 2 ml in each experiment well. Three replicates were made for each tested concentration. Mortality of miracidia was recorded after one min. Twenty freshly hatched miracidia were maintained in 2 ml dechlorinated water as control; (2) The cercariae: series of 1 ml samples of water containing 20 freshly shed cercariae were mixed with 1 ml of serial concentrations (0.5-5 ppm) of *A. sativum* and *A. cepa* 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 of exposure period and replacing it with fresh water for a recovery period of 24 h. Two ml dechlorinated water contain 20 freshly shed cercariae were used as control[27]; (3) Shistosomula, male, female and coupled adult worms, each of 20, were cultured in 24 well Falcon plates at 37°C in 1 ml of RPMI 1640 media supplemented with penicillin (100 U/ml), streptomycin (100 mg/ml), and 10% fetal calf serum (Gibco, Carlsbad, CA, USA), 2 g/L glucose, 0.39 g/L glutamate and 20 g/L NaH-CO_3. *A. sativum* and *A. cepa* crude powders *were* dissolved in 10% DMSO and then diluted with sterilized distilled water to the desired concentrations (1-3, 2-10 ppm for schistosomula and 10-110 ppm for adult worm, *A. sativum* and *A. cepa* respectively). Control shistosomula and worms were treated with 10% DMSO in sterile distilled water. The movement and viability of the shistosomula was monitored for 1-5 min, 5-30 min, 24 h for adult worms and for *A. sativum* and *A. cepa* respectively. All procedures were carried out using aseptic techniques in a laminar flow cabinet[28] (John Bass Ltd., Guildford, United Kingdom).

DPPH Scavenging Activity of A. sativum and A. cepa Assay Method

The antioxidant activity of serial concentrations of both plants (10:1000 μg/ml) was performed according to the method of Chen et al[29]. Control sample was run simultaneously with test using 2,2-diphenyl-1-picrylhydrazyl (DPPH) without sample. The decrease in optical density of DPPH$^-$ was calculated in relation to control as follows:

$$\% \text{ Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Determination of NO-free Radical Scavenging Activity

The NO-scavenging activity of plant powders was determined according to the method of Sreejayan and Rao[30]. Control test was run parallel with sample contains sodium nitroprusside and Griess reagent. The absorbance of the chromophore formed was read against blank at 546 nm (LKB 4050 Ultrospectrophotometer II UV/Vis, Kent City, MI, USA).

In vivo Experimental Design

Duration of experiment was three months. Ninety mice were divided into nine groups each of ten animals. Group 1: normal healthy control group. Group 2: mice were infected with NMRI strain *S. mansoni* by direct skin contact through exposure to 80 ± 10 cercariae/mouse according to the method of Olivier and Stirewalt[25] and sacrificed 45 days after the infection. Groups 3-9 were infected and treatment started 45 days post infection and continued for two successive days for praziquntel (group 3, where each mouse was given orally 500 mg/kg body weight for two successive days. Group 4; mice were treated with *A. cepa* (2 g/100 g body weight for 45 days. Group 5; mice were treated orally with *A. cepa* and paraziquantel. Goup 6; mice were treated orally with *A. sativum* (2 g/100 g body weight for 45 days Group 7; mice were treated with *A. sativum* and praziquantel. Group 8; mice were given orally *A. sativum* and *A. cepa* mixture (2 g/100 g body weight for 45 days). Group 9; mice were given orally *A. sativum* and *A. cepa* and praziquantel. Then mice were anaesthetized using diethyl ether and the blood collected from the subtongual vein and the animals were then dissected and liver separated.

Appropriate anaesthetic and sacrifice procedures were followed ensuring that animals did not suffer at any stage of the experiments and are complied according to legal ethical guidelines of the Ethical Committee of the Federal Legislation and National Institute of Health Guidelines in USA and approved by the Ethics Committee of the National Research Centre in Egypt.

Preparation of Tissue Homogenates

Liver tissue was homogenized in normal physiological saline solution (0.9% NaCl) by a ratio 1:9 w/v. The homogenate was centrifuged for 5 minutes at 3000 × g at 4°C and the supernatant was used for estimation of different enzymes.

Determination of Antioxidant Enzymes

Glutathione Reductase (GR)

GR was assayed according to the method of Erden and Bor[31]. The oxidation of NADPH was followed at 340 nm and one unit of activity is defined as the oxidation of 1 nmole NADPH/min/protein.

Catalase (CAT)

The method applied was according to Monhanty et al[32] H_2O_2 produced was measured at extinction 530-571 nm.

Superoxide Dismutase (SOD)

SOD was measured according to Nishikimi et al[33]. The inhibition in SOD activity is quantified by measuring the decrease in the colour development at 440 nm.

Lipid Peroxides

Lipid peroxide was determined as malondialdehyd spectrophotometrically at 535 nm. Malondialdehyde concentration was calculated using the extinction coefficient according to the method of Buege and Aust[34].

Sorbitol Dehydrogenase (SDH)

Liver tissue SDH was determined by using fructose as substrate spectrophotometrically at 430 nm[35].

Thioredoxin Reductase (TrxR)

Liver tissue TrxR was assayed according to the method of Arnér and Holmgren36. TrxR uses NADPH to reduce 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to 5-thio-2-nitrobenzoic acid (TNB) which is absorbed strongly at 405-414 nm.

Statistical Analysis

Data were analyzed by comparing values for different treatment groups with the values for individual controls. Results are expressed as means ± SD. The significant differences among values were analyzed using analysis of variance (one-way Anova) coupled with post-hoc and least significance difference (LSD) Anova at $p \leq 0.05$ using Co-stat program.

Results

The viability of *S. mansoni* different stages (miracidia, schistosomula cercariae and adult worms) was observed during the *in vitro* incubation with various concentrations of *A. cepa* and *A. sativum* powders.

Figures 1 to 6 show that, the exposure of miracidia, schistosomula and cercariae to the both edible plants powders resulted in the death of the parasites and lethal effect was dependent on the concentrations and time of the incubation (1 and 1 minutes for miracidia, 5 and 1 minutes for schistosomula, 4 and 4 minutes for cercariae and for *A. cepa* and *A. sativum* respectively). LC_{50} values of these plant powders *A. cepa* and *A. sativum* were recorded 50 ppm and 100 ppm respectively for miracidiae, where the concentrations of *A. cepa* and *A. sativum* reached to 6 and 2 ppm respectively for schistosomula. On the other hand, LC_{50} of *A. cepa* and *A. sativum* were corded 50 and 10 ppm for cercariae respectively. Figures 7 and 8 show the effect of the current plant powders on mortality rates of male and female adult worms after 24 h.
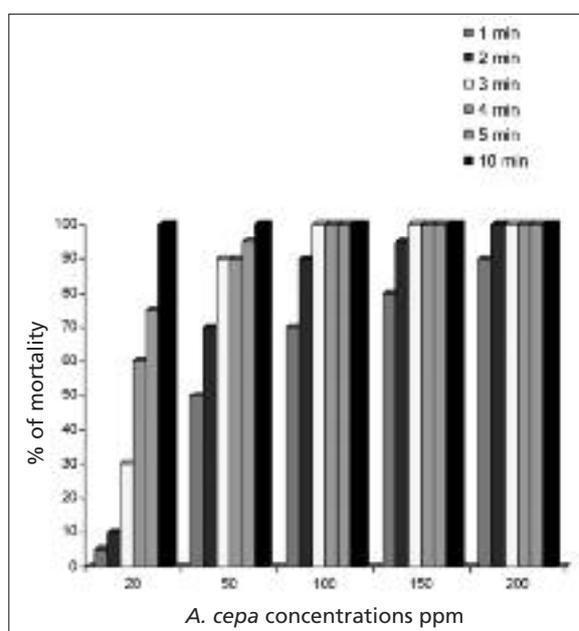

Figure 1. Effect of *A. cepa* on mortality rate of *Schistosoma mansoni* miracidia after 1-10 min of exposure respectively.
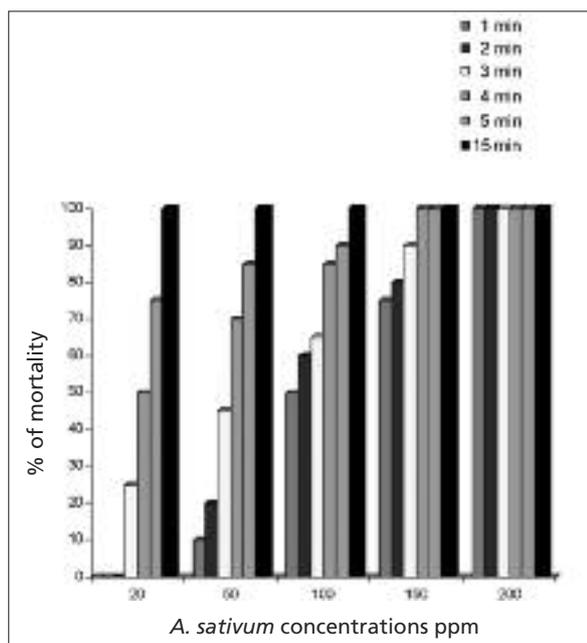
Figure 2. Effect of *A. sativum* on mortality rate of *Schistosoma mansoni* miracidia after 1-15 min of exposure respectively.
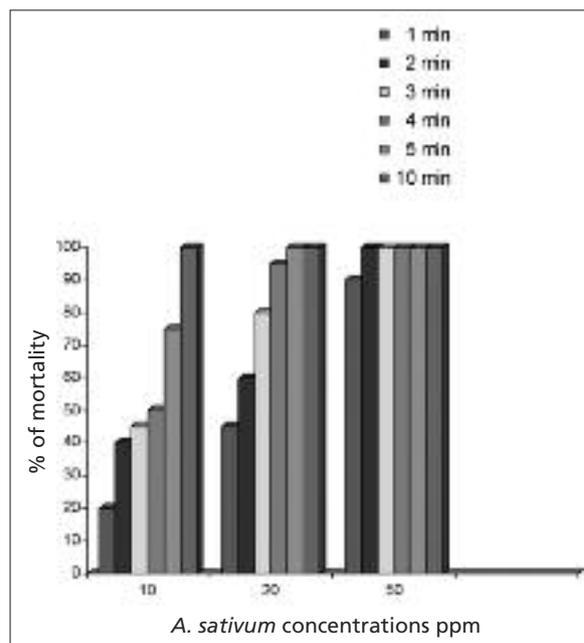

Figure 4. Effect of *A. sativum* on mortality rate of *Schistosoma mansoni* cercariae after 1-10 min of exposure respectively.

It was observed that, the plants powders have a strong lethal effect against both male and female worms showing an LC_{50} of 50 ppm for *A. cepa* and *A. sativum*. Hundred percent of the parasite were dead at concentrations of 100 and 110 ppm and for male and female worms. Coupled worms become separated into male and female worms under the effect of the drugs.

The DPPH free radical scavenging effect of *A. cepa* and *A. sativum* (Figure 9) shows appreciable free radical scavenging activities with a dose dependent relationship. *A. sativum* has the strongest radical scavenging activity at different concentrations compared to *A. sativum* since it recorded significant inhibitory percent of 45.90 ± 0.50, 60.20 ± 1.50, 66.52 ± 0.52, 69.32 ± 0.97
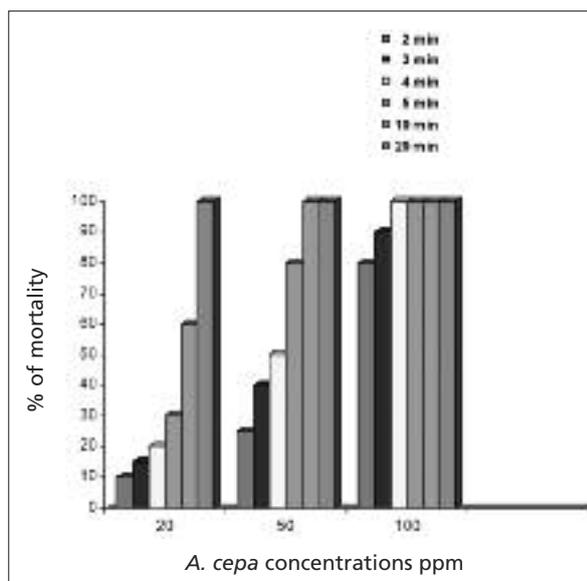

Figure 3. Effect of *A. cepa* on mortality rate of *Schistosoma mansoni* cercariae after 2-20 min of exposure respectively.
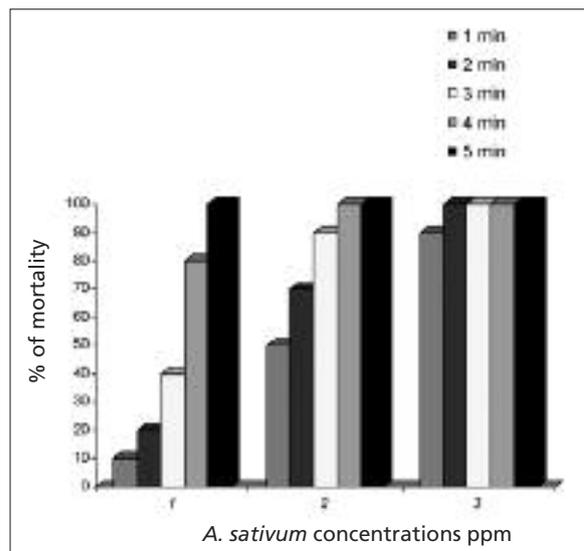

Figure 5. Effect of *A. sativum* on mortality rate of *Schistosoma mansoni* schistosomula after 1-5 min of exposure respectively.
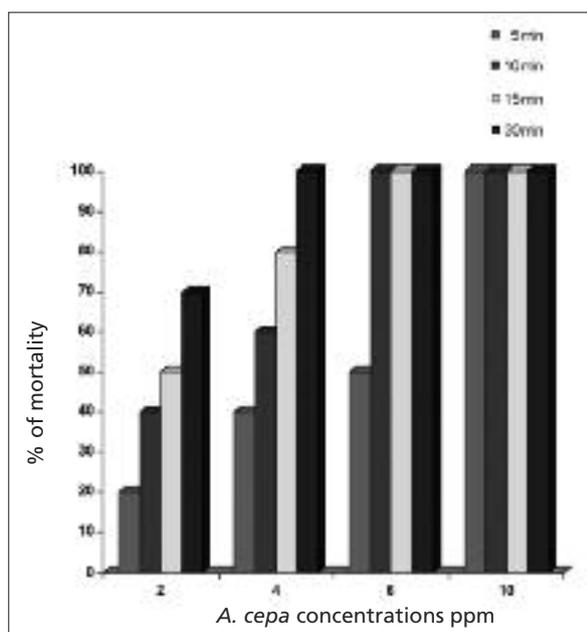
Figure 6. Effect of *A. cepa* on mortality rate of *Schistosoma mansoni* schistosomula after 5-30 min of exposure respectively.

and 72.19 ± 0.39 % ($p \leq 0.01$) at concentrations of inhibitors 10-1000 μg/ml respectively. The same results are achieved for NO scavenging effect (Figure 10), where *A. sativum* shows the most powerful reducing capacity (compared to *A. cepa*) at different concentrations with linear relationship in a dose dependent manner and recorded significant maximum reducing percent of 36.60 ± 2.22, 39.45 ± 2.90, 53.90 ± 2.55, 56.90 ±
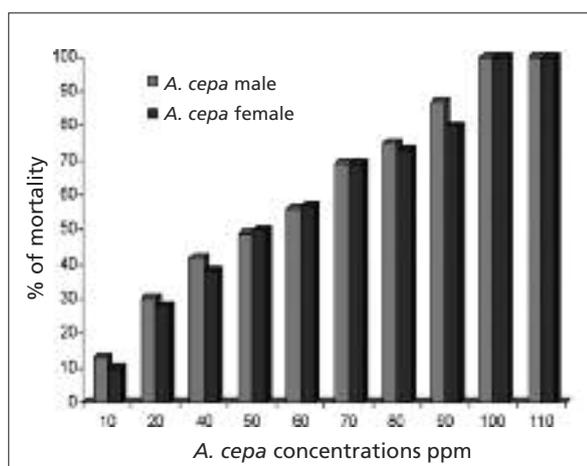

Figure 7. Effect of *A. cepa* on mortality rate of male and female *Schistosoma mansoni* adult worms after 24 h of exposure.
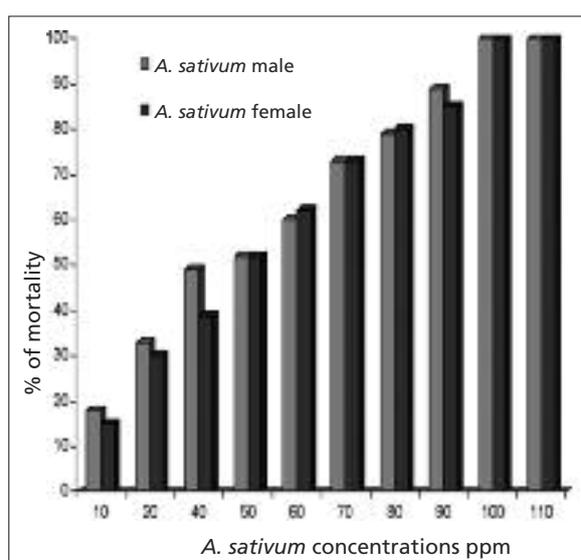

Figure 8. Effect of *A. sativum* on mortality rate of male and female *Schistosoma mansoni* adult worms of after 24 h of exposure.

1.60 and 69.59 ± 2.39% at the same concentrations of inhibitors (10-1000 μg/ml).

Resulted listed in the Table I show the effect of *A. cepa* and *A. sativum* on some antioxidant enzymes SOD, CAT, GR, lipid peroxides, in addition to TrxR and SDH. The data demonstrate that, there was a significant inhibition in SOD, CAT, GR, TrxR, SDH while significant elevation in
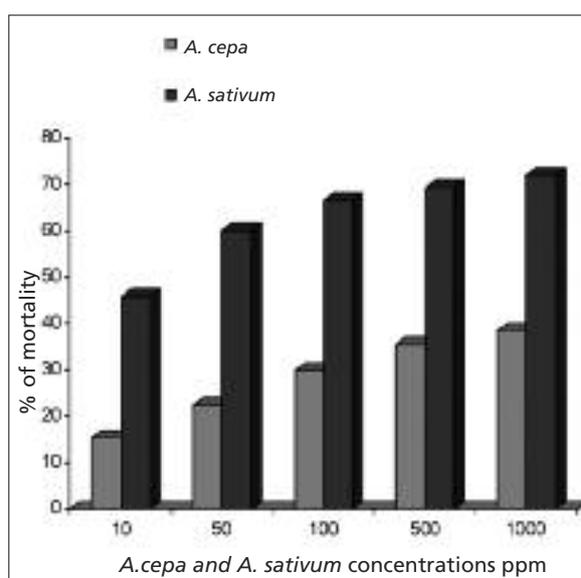

Figure 9. DPPH scavenging effect of *A. cepa* and *A. sativum* at different concentrations (10-1000 ug/ml). Data are means of 3 replicates.
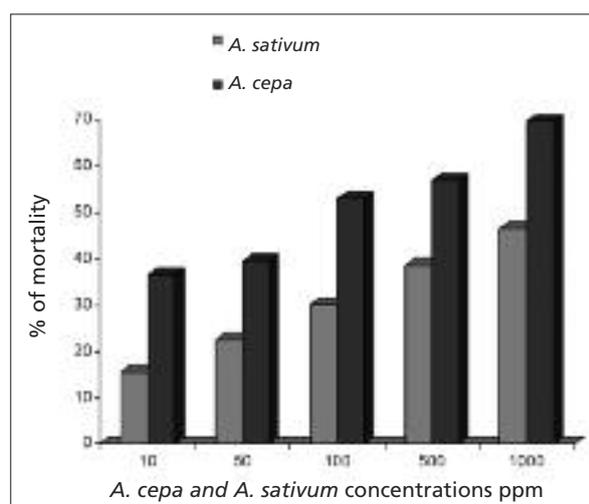
Figure 10. Effect of *A. sativum* on mortality rate of male and female *Schistosoma mansoni* adult worms of after 24 h of exposure.

lipid peroxide in infected liver as compared to the normal one. Significant amelioration was detected in all infected-treated groups with fluctuated percent, since, SOD, CAT and GR enzyme activities recorded percent of improvement amounting to 280.36, 112.40 and 86.29% for *A. cepa* mixed with *A. sativum* and PZQ respectively. Moreover, lipid peroxide exhibited insignificant change (as compared to the normal control group), upon treatment of infected mice with this combined mixture, recording percent of improvement amounting to 401.54%. On the other hand, SDH show insignificant change (as compared to the normal control group) post treatment of infected mice either with PZQ (62.07%) or *A. sativum* (62.07%) separately, while TrxR enzyme activity shows insignificant change as compared to the normal control group upon treatment of the infected mice with PZQ, recording percent of improvement reached to 91.43%.

Discussion

This study was performed to evaluate *in vitro* and *in vivo* the antischistosomal effect of both *A. sativum* and *A. cepa* for controlling schistosomiasis. In the last decades, plant extracts were widely used for the treatment of schistosomiasis[14]. *A. sativum* and *A. cepa* were recently proved to have anti-helminthic activity[37].

The current study demonstrated that *A. sativum* and *A. cepa* possess strong schistosomicidal ac-

Table I. Effect of *A. sativum* and *A. cepa* on some antioxidant enzymes in liver homogenates of *Schistosoma mansoni* infected and different treated mice.

Parameters	Control	Infected	Infected + PZQ	A. cepa	A. cepa + PZQ	A. sativ.	A. sativ. + PZQ	A. cepa + A. sativ	A. cepa + A. sativ + PZQ
Superoxide dismutase	0.056 ± 0.01a	0.013 ± 0.002b	0.038 ± 0.004c	0.100 ± 0.066a	0.060 ± 0.014a	0.163 ± 0.05e	0.017 ± 0.005g	0.083 ± 0.029h	0.17 ± 0.02g
Catalase	128.6 ± 12.6a	21.45 ± 0.198b	131.4 ± 16.3c	162.9 ± 22.5d	134.4 ± 60.9c	177.7 ± 30.5e	138.9 ± 32.1f	143.35 ± 10.4g	166.0 ± 19.5d
Lipid peroxides	6.50 ± 0.57a	32.61 ± 0.50b	12.14 ± 0.22c	14.83 ± 1.28d	10.44 ± 0.83e	13.25 ± 0.58f	8.29 ± 1.24g	13.08 ± 0.83F	6.51 ± 0.69a
Glutathione reductase	0.270 ± 0.03a	0.117 ± 0.02b	0.229 ± 0.046a	0.308 ± 0.08c	0.22 ± 0.03a	0.226 ± 0.043a	0.294 ± 0.043a	0.274 ± 0.01a	0.35 ± 0.01a
Thioredoxin reductase	0.35 ± 0.06a	0.08 ± 0.002b	0.40 ± 0.07a	0.21 ± 0.09d	0.45 ± 0.09e	0.11 ± 0.01d	0.47 ± 0.04e	0.29 ± 0.01f	0.46 ± 0.01e
Sorbitol dehydrogenase	0.029 ± 0.01a	0.011 ± 0.002b	0.29 ± 0.004a	0.034 ± 0.006c	0.057 ± 0.001d	0.029 ± 0.009a	0.032 ± 0.001c	0.033 ± 0.001c	0.059 ± 0.001d

Data are means ± SD of ten mice in each group. All enzyme activities are expressed in umole/mg protein, while lipid peroxide is expressed in $\mu g/g$ tissue. Statistical analysis is carried out by one way analysis of variance (ANOVA), using Co-state computer program. Unshared superscript letters between groups are significant values at p † 0.001.

tivity against *S. mansoni* miracidia, schistosomula, cercariae and adult worms (female and male worms).

The results agree with Singh et al[38] as they revealed the miracidial, schistosomulicidal and cercaricidal potency of *A. sativum* essential oil which produced complete paralysis of whole and strip preparations of the flukes after 15 min using 3 mg/ml concentration of the essential oil. It has been observed that, the essential oil of *A. sativum* at 1 and 3 mg/ml concentration caused complete inhibition of the gross visual mortality of the *Fasciola gigantica* and *S. mansoni* worms at 4 h of incubation. Following paralysis, the whole fluke and the strip preparations did not recover from paralysis even after 2-3 washes. These Authors added that, most commonly these antihelminthic agents act mainly through three different biochemical/physiological mechanisms, viz., affecting functioning of ion channels, microtubules and bioenergetics of helminth parasites[39]. Many of the antihelminthics cause paralysis of parasites by disrupting one or the other aspect of their neuromuscular system[40]. In addition, *A. sativum* and *A. cepa* compounds, inhibited protein prenylation and arterial smooth muscle cell proliferation [41]. Although the bioactive effects of garlic are attributed to the sulphur-containing molecules, other smaller metabolic breakdown products of these molecules have received increasing attention for their antimicrobial efficacy[42]. Essential oil of *A. sativum and A. cepa* had been shown to possess preventive and curative activity against *Tetratrichomonas gallinarum, Histomanas meleagridis*[43], *Ascaris lumbricoides* and *Enterobius vermicularis in vitro*[44].

Similar results have been recorded on the effect of *A. sativum*, *A. cepa* and other plants against schistosome parasite at different stages miracidia, schistosomula, cercariae and adult worms[14,45,46].

The inflammatory response of the liver is directly affected by the parasite. Our previous study showed that *A. sativum* does not only target schistosome parasites in hosts, but also exhibits antiinflammatory activity, thus, protecting host tissues. Indeed, infections cause an inflammatory response in the liver of mice. This response manifests itself as a perturbed liver structure and as a significant increase in iNOS and production of NO[47]. Raso et al[48] have viewed that the oxidative stress is mainly due to NO produced by the stimulated iNOS. This finding also fits our data showing that *A. sativum* and *A. cepa* have a scavenging property towards nitric oxide *in vitro*. The inhibitory effect of *A. sativum* and *A. cepa* is mainly attributed to the impaired action of iNOS[47].

The present investigation indicated also that the different concentrations (10-1000 mg/ml) of *A. sativum* and *A. cepa* were highly effective in DPPH and NO scavenging in a dose-dependent relationship *in vitro*. This was supported by Corzo-Martınez et al[49] and Singh et al[38] as they found dose dependent relationship of both plants and declared that red onion peel had markedly high antioxidant capacity that prevents or scavenging capacity against DPPH, NO and $FeCl_3$-induced lipid peroxidation, protein fragmentation and superoxide anion due to the presence of large amounts of phenolics and flavonoids. In addition, analysis of ethyl acetate (EA) fraction (EA) showed the presence of ferulic, gallic, protocatechuic acids, quercetin and kaempferol. The large amount of polyphenols contained in EA fraction may cause its strong antioxidant and antimutagenic properties. So, EA fraction of red onion peel can be used as natural antioxidant in nutraceutical preparations. Moreover, the non-volatile water-soluble sulphur compounds found in garlic, as S-allyl cysteine (SAC), (coming from enzymatic transformation of g-glutamyl cysteines when *A. sativum* is extracted with an aqueous solution), are also responsible for a great part of the DPPH and NO scavenging capacity of garlic and hence health benefits of both vegetables[20].

The present work was extended to study the *in vivo* effect of *A. sativum* and *A. cepa* on some antioxidant enzymes in infected and different infected-treated mice. The increased hepatocyte liberation of ROS as a result of infection with *S. mansoni* caused increased lipid peroxidation and oxidative inactivation of both membranous and soluble proteins[50], therefore, the structural and functional integrity of hepatic organelles, in general[51]. The data obtained in the present study show that significant reduction in SOD, CAT, GR with significant increase in lipid peroxide was noticed in the liver tissue post *S. mansoni* infection. Since the complex mechanism of lipid peroxidation is known to require the participation of highly ROS and other reactive metabolites in the chain of biochemical reaction, thus, in any part of the body where these free radicals are produced, lipid peroxide are in turn increased. Such phenomenon was previously reported by El-Rigal et al[52] and Botros et al[53]. At the same time, liver GSH is drastically depleted in the liver.

Such depletion is critical, as shown by the increased cytotoxicity of H_2O_2 in endothelial cells, as a result of inhibition of glutathione reductase, which keeps glutathione in its reduced state[52]. In agreement with the present data Mittelstaedt et al[54] suggested that nuclei and mitochondria act as major targets of toxic action, probably by increasing the generation of free radicals, lipid peroxidation and DNA adducts formation. The present results are in accordance to several Authors who found significant reduction in SOD, GPX and GR while in contrast significant inhibition of CAT enzyme activity in liver tissue of infected mice is reported. This is referred to increased activity of the enzymes in serum and to the number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. These dramatic changes in infectious state can be also explained on the basis of *S. mansoni* eggs trapped in the host liver which elicit a chain of oxidative processes that may be, at least in part, responsible for the pathology and progression of fibrosis associated with schistosomal infection[55,56].

Thioredoxin systems, involving redox active thioredoxins and thioredoxin reductases, sustain a number of important thioredoxin-dependent pathways. These redox active proteins support several processes crucial for cell function, cell proliferation, antioxidant defense and redox-regulated signaling cascades[57]. Adult schistosomes and their eggs are exposed to reactive oxygen compounds and must possess adequate mechanisms of detoxification. Eggs in particular are vulnerable to oxidative damage. Eggs seem to require the granuloma to facilitate passage across host tissue but must be resistant to damage from immune cells in the granuloma. Schistosomes, especially their eggs, possess significant amounts of thioredoxin peroxidase, an enzyme that can neutralize hydrogen peroxide. Thioredoxin peroxidase-1 requires reducing equivalents from thioredoxin. Thioredoxin is in turn maintained in the reduced state by thioredoxin reductase activity and NADPH. Schistosomes have a unique redox pathway controlling the multifunctional enzyme thioredoxin glutathione reductase. Thioredoxin glutathione reductase replaces both thioredoxin reductase and glutathione reductase. Thus, thioredoxin glutathione reductase maintains high levels of reduced glutathione either by direct reduction of glutathione disulfide by thioredoxin glutathione reductase or through reduction of glutathione disulfide by thioredoxin. So, thioredoxin system

plays a significant role in redox balance and in the antioxidant defenses of *S. mansoni*[58]. Our data showed a significant statistical inhibition of liver tissue enzyme TrxR in *S. mansoni* infected mice as compared to the normal control group, indicating cellular liver damage mainly due to leakage of this enzyme from liver cytosol into the blood stream as a result of liberation of free radical and oxidative process collaborated with *S. mansoni* infection[59]. The present finding demonstrates also, a significant inhibition in SDH enzyme activity in liver of infected mice. In a parallel results, Rutkowski and Bruce[60] found a significant, transient increase in the serum levels of both SDH and TrxR enzyme activities during the third or fourth week after cercarial exposure, the time at which immature parasites were migrating through the lung, heart and liver.

Significant improvement in the previously mentioned parameters was detected after treatment of infected mice with praziquantel. This may be due to the significant reduction in worm burdens accompanied with a significant increase in the percentage of dead ova and a decrease in the percentage of mature ova stages, reduction in hepatic and intestinal oogram as well as liver granuloma size compared with the *S. mansoni* infected group[37]. In addition, praziquantel causes worm tegument damage (that is accompanied by a large influx of calcium into worms leading to muscular contraction, surface disruption and eventual death of the parasite) that consequently limit or enhance significantly immune response of patients and generate a reversion of the level of fibrosis[53,61]. Thereby, as evidenced by several studies, the significant reduction in oxidative stress initiate a positive impact on the preservation of liver integrity and function, antioxidant enzymes, immunoglobulin profile (IgG and IgM), interleukins and tumor necrosis factors[61,62].

The mechanisms whereby the consumption of certain plants and plant extract can affect parasite viability, mobility and fecundity both *in vivo* and *in vitro* could be associated with an enhanced immune response of the host towards the parasites, as result of nutrient supplementation and, thus, improved nutrition. However, it appears that many plants that have been reported to have anthelmintic properties actually contain compounds that are directly active against parasites. In many cases these active compounds are secondary metabolites, i.e. plant products that have been associated with defensive mechanisms. Saponins, alkaloids, non-protein amino acids, tannins and

other polyphenols, lignin, glycolides are all secondary metabolites and some of them have been considered responsible for the anti- parasitic effect of plants[63]. In this concern, *A. sativum* and *A. cepa* can modify cytochrome P-450 activities and consequently influence toxicity and carcinogenicity of environmental carcinogens[64]. For example, *A. sativum* contains a wide range of allyl sulfides. Diallyl sulfide (DAS) could inhibit 1,2-dimethylhydrazine-induced colon tumor and esophageal cancer in mice[65]. Inhibition of cytochrome P450 after repeated dose treatments of rats with *A. sativum* could protect the liver against infection and the toxicity of CCl_4. The mechanism of cytochrome P450 inhibition caused by *A. sativum* might be due to interaction of its active component, DAS with one or more of the seven cysteinyl residues of the cytochrome P450 hemoprotein[66].

One of the hepatoprotective roles of *A. sativum* is that it decreases hepatic injury due to S*chistosoma mansoni* infections which is indicated by the lowered activities of ALT, AST, ALP and GT[67]. In addition, Gedik et al[68] have reported that one of the major protective functions of *A. sativum* is to decrease the oxidative damage in liver. Indeed, *A. sativum* prevents the infection induced loss of GSH and decrease of activities of CAT and SOD. These components are normally lowered during oxidative damage induced by infection as it has been also described by Georgieva et al[69] as they showed an increase in MDA levels of liver infected with *S. mansoni*. In accordance to the present results, Kiruthiga et al[70] found that *A. sativum* significantly decreased lipid peroxidation and increased GSH, CAT, GR and SOD. The antioxidative property of *A. sativum* has been previously ascribed mainly to its four major chemical components, i.e. allinin, allyl cysteine, allyl disulfide, and allicin[71].

Thus, all these infection-induced parameters were significantly less altered during *A. sativum* and/or *A. cepa* treatment. In particular, *A. sativum* and/or *A. cepa* counteracted the *S. mansoni*-induced loss of glutathione and the activities of catalase and superoxide dismutase. In parallel results, Dkhil et al[72] indicated that *A. sativum* treatments significantly attenuated inflammation and injury of the liver induced by murine *Eimeria papillata* infections. So, *A. sativum* exhibits antischistosomal activity, evidenced as a significant lowering in the eggs count of the infected mice. This diminished output reflects that garlic impairs the development of parasites in the host before the relatively inert eggs are formed and finally released.

In conclusion, the use of *A. sativa* and *A. cepa* as antischistosomal drug may affect the adaptive capability of adult worms against the oxidative killing by the host effector cells and this may help in the elimination of the parasite. Thus, both plants could be used as supplements against schistosomaisis for 45 days with the usual dose of the anti-schistosomal drug praziquantel.

References

1) HUSSEIN MF, KASEM EA, ABDWLWAHAB A, HAMID AR, BISHAI M, MAHMOUD MF. Priciples of Animal Biology. DarEl-Marref: Cairo 1976; pp. 145-150.

2) SMITHER SR, TERRY RJ. Immunity in schistosomiasis. Ann NY Acad Sci 1969; 160: 826-940.

3) JAMES SL. Experimental models of immunization against schistosomes. Lessons for vaccine development. Immun Invest 1992; 21: 477-493.

4) MAIZELS RM, BUNDY DA, SELKIRK ME, SMITH D, ANDERSON RM. Immunological modulation and evasion by helminth parasites in human populations. Nature 1993; 365: 797-805.

5) MEI H, LO VERDE PT. *Schistosoma mansoni*. Cloning the gene encoding glutathione peroxidase. Exp Parasitol 1995; 80: 319-322.

6) SHEWEITA SA, HABIB SL, MOSTAFA MH. Schistosomiasis induced changes in gluthione levels and glutathione reductase/glutathione-S-transferase activity in human liver. Biomed Lett 1997; 56: 119-127.

7) GHARIB B, ABD-ALLAH OM, DESSEIN H, DE-REGGI M. Development of eosinophil peroxidase activity and concomitant alteration of antioxidant defenses in the liver of mice infected with *Schistosoma mansoni*. J Hepatol 1999; 30: 594-602.

8) PASCAL M, ABD-ALLAH OM, EL-WALI NE, MERGANI A, QURASHI MA, MAGZOUB M, DE-REGGI M, GHARIB B. Hyaluronate levels and markers of oxidative stress in the serum Sudanese subjects at risk of infection with *Schistosoma mansoni*. Trans R Soc Med Hyg 2000; 94; 66-70.

9) SOLIMAN KM, EL-ANSARY AK, MOHAMED AM. Effect of carnosine administration on certain metabolic parameters in bilharzial infected hamsters. J Egypt Soc Parasitol 2000; 30: 455-468.

10) WHO. The control of schistosomiasis: Second report of the WHO expert committee. WHO technical report Series 830, Geneva 1993.

11) WHO. Report of the WHO information consulation on schistosomiasis control. WHO technical report Series 2, Geneva 1999.

12) APPIAH AD, DE VLAS SJ. Interpreting low praziquantel cure rates of *Schistosoma mansoni* infections in Senegal. Trends Parasitol 2002; 18: 125-129.

13) Ndamba J, Nyazema N, Makaza N, Anderson C, Kaondera KC. Traditional herbal remedies usedfor the treatment of urinary schistosomiasis in Zimbabwe. J Ethnopharmacol 1994; 42: 125-132.

14) Molgaard P, Nielsen SB, Rasmussen DE; Drummond RB, Makaza N, Andreassen J. Anthelminitic screening of Zimbabwean plants traditionally used against schistosomiais. J Ethnopharmacol 2001; 74: 257-264.

15) Abebe F. Novel antistosomal drugs from medicinal plants. Bot Med Clin Pract 2008; 175-183.

16) Ali M, Thomson M, Afzal M. Garlic and onions: their effect on eicosanoid metabolism and its clinical relevance. Prostag Leukot Ess Fatty Acids 2000; 62: 55e-73e.

17) Augusti KT, Mathew PT. Lipid lowering effect of allicin (diallyl disulfide oxide) on long-term feeding in normal rats. Experientia 1974; 30: 468e-470e.

18) Abu-El-Ezz NM. Effect of *Nigella sativa* and *Allium cepa* oils on Trichinella spiralis in experimentally infected mice. J Egypt Soc Parasitol 2005; 35: 511-523.

19) Githiori J B, Athanasiadou S, Thamsborg SM. Use of plants in novel approach for controle of gastrointestinal helminthes in livestock with emphasis on small ruminants. Vet Parasitol 2006; 139: 308-320.

20) Amagase H, Petesch BL, Matsuura H, Kasuga S, Itakura Y. Intake of garlic and its bioactive components. J Nutr 2001; 131: 955Se-962Se.

21) Mantawy MM, Mahmoud AH. Effect of *Allium cepa* and *Allium sativum* feeding on glucose, glycogen, and protein bands profile and phenol oxidase activity in Biomophalaria alexandrina. J Egypt Soc Parasitol 2002; 32: 271-283.

22) Piper KP, Mott RF, Mclaren DJ. *Schistosoma mansoni*: Histological analysis of the synergetic interaction between vaccine immunity and praziquntel therapy in the lungs of mice. Parasite Immunol 1990; 12: 367-373.

23) Hira PR, Webbe J. The effect of sublethal concentrations of themolluscicide triphenyllead acetateon Biomphlaria glabrata and on the development of *Schistosoma mansoni* in the snail. Helminthologia 1972; 45: 11-23.

24) Pellegrino J, DeMaria M. *In vitro* cercaricidal activity of schistosomiasis. J Parasitol 1996; 52: 612-625.

25) Olivier L, Stirewalt MA. An efficient method for exposure of mice to cercariae of *S. mansoni*. J Parasitol 1952; 38: 19-23.

26) Techounwou PB, Englande AJ, Malek EA, Anderson AC, Abdel-Ghany AA. The effects of Bayluscide Malathion on miracidial survival in schistosomiasis control. J Environ Sci Health 1991; 26: 69-85.

27) Ritchie LS, Lopez VA, Cola JM. Prolonged application of an organation against Biomphlaria glabrata and *Schistosoma mansoni*. In: C Tom, Molluscicidesin Schistosomiasis Control. Academic Press, New York, London 1974: pp. 77-88.

28) Mohamed AM, Metwally NM, Mahmoud SS. Sativa seeds against *Schistosoma mansoni* different stages. Mem I Oswaldo Cruz 2005; 100: 205-211.

29) Chen HY, Lin YC, Hsiech CL. Evaluation of antioxidants activity of aqueous extract of some selected nutraceutical herbs. Food Chem 2007; 104: 1418-1424.

30) Sreejayan A, Rao MNA. Nitric oxide scavenging by curcuminoids. J Pharm Pharmacol 1997; 49: 105-107.

31) Erden M, Bor NM. Changes in reduced glutathione, glutathione reductase and glutathione peroxidase after radiation in guinea pigs. Biochem Med 1984; 31: 217-227.

32) Monhanty JG, Jonathan S, Jaffe E, Edaward S, Donald GR. Ahighly sensitive fluorescent microassay of H_2O_2 release from activated human leukocytes using a dihydroxphenoxazine derivative. J Immunol Methods 1997; 202: 133-137.

33) Nishikimi M, Appaji N, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Bioph Res Comm 1972; 46: 849-854.

34) Buege JA, Aust SD. Microsomal lipid per oxidation. Method Enzymol 1978; 52: 302-310.

35) Bergmeryer HU. Sorbitol dehydrogenase. In: Methods of Enzymatic Analysis. 3rd Edn., Verly Chemie, Weinheim, Academic Press, London 1974; pp. 569-573.

36) Arnér E, Holmgren A. Physiological functions of thioredoxin and thioredoxin reductase. Eur J Biochem 2000; 267: 6102-6109.

37) Mantawy MM, Aly HF, Rizk M. Therapeutic effects of *Allium sativum* and A. cepa in *Schistosoma mansoni* experimental infection. Rev Inst Med Trop São Paulo 2011; in press.

38) Singh TU, Kumar D, Kumar S, Tandan S. Inhibitory effect of essential oils of *Allium sativum* and Piper longum on spontaneous muscular activity of liver fluke, Fasciola gigantica. Exp Parasitol 2009; 123: 302-308.

39) Kohler P. The biochemical basis of anthelmintic action and resistance. Int J Parasitol 2001; 31: 336-345.

40) Loukas A, Hotez PJ. Chemotherapy of helminth infections. In: The Pharmacological Basis of Therapeutics. Brunton LL, Lazo JS, Parker KL. (Eds.), Goodman & Gilman's, 11th ed. McGraw-Hill Companies, USA 2005; pp. 1073-1093.

41) Ferri N, Yokoyama K, Sadilek M, Paoletti R, Apitz-Castro R, Gelb MH, Corsini AA. Joene, a garlic compound, inhibits protein prenylation and arterial smooth muscle cell proliferation. Br J Pharmacol 2003; 138: 811-818.

42) Banerjee SK, Mukherjee PK, Maulik SK. Garlic as an antioxidant: the good, the bad and the ugly. Phytother Res 2003; 17: 97-106.

43) Zenner L, Callait MP, Granier C, Chauve C. *In vitro* effect of essential oils from *Cinnamomum aromaticum*, *Citrus limon* and *Allium sativum* on two intestinal flagellates of poultry, Tetratrichomonas gallinarum and Histomanas meleagridis. Parasite 2003; 10: 153-157.

44) Altug AM, Kaptan C. Parasitic control in organic stock breeding: possible medicinal plants. Turktarm 2002; 148: 71-85.

45) Naples JM, Shiff CJJ, Rosler KH. *Schistosoma mansoni*: cercaricidal effects of cedarwood oil and various of its components. Am J Trop Med Hyg 1992; 95: 390-396.

46) Ahmed AH, Ramzy PMR. Laboratory assessment of the molluscidial and cercaricidal activities of the Egyptian Weed, Solonium nigrum L. Ann Trop Med Parasitol 1997; 91: 931-937.

47) Chang HP, Chen YH. Differential effects of organosulfur compounds from garlic oil on nitric oxide and prostaglandin E2 in stimulated macrophages. Nutrition 2005; 21: 530-536.

48) Raso GM, Meli R, Di Carlo G, Pacilio M, Di Carlo R. Inhibition of inducible nitric oxide synthase and cyclooxygenase-2 expression by flavonoids in macrophage. Life Sci 2001; 68: 921-931.

49) Corzo-Martinez M, Nieves C, Mar V. Biological properties of onions and garlic. Trends Food Sci Technol 2007; 18: 609-625.

50) Metwally NS. Potency of *Allium sativum* and *Allium cepa* oils against *Schistosoma mansoni* infection in mice. Egypt J Hosp Med 2006; 23: 319-332.

51) Van Noorden CJ, Frederiks WM. Enzyme Histochemical Method. Oxford University Press. Amsterdam 1992; pp. 445-460.

52) El-Rigal NS, Aly SA, Rizk MZ, Said AA. Effect of *Ailanthus altissima* and *Ziziphus spina Christi* extracts on some hepatic marker enzymes and antioxidants in *Shistosoma mansoni* infected mice. Pol J Food Nutr Sci 2006; 15/56: 199-206.

53) Botros SS, Mahmoud MR, Moussa MM, Nosseir MM. Immunohistopathological and biochemical changes in *Schistosoma mansoni*-infected mice treated with artemether. J Infection 2007; 55: 47-477.

54) Mittelstaedt RA, Mei N, Webb PJ, Shaddock JG, Dobrovolsky VN, MacGarrity LJ, Morris SM, Chen T, Beland FA, Greenlees KJ, HeXich RH. Genotoxicity of malachite green and leucomalachite green in female Big Blue B6C3F1 mice. Mutat Res 2004; 561: 127-138.

55) Pedraza-Chaverri J. Post-transcripitional control of catalase expression in garlic-treated rats. J Ethnopharmacol 2001; 10: 123-128.

56) Kuntz AN. Davioud-Charvet E. Sayed AA, Califf LL, Dessoline J, Arner ES, Williams DL. Thioredoxin glutathione reductase from *Schistosoma mansoni*, an essential parasite enzymes and a key drug target. PLOS Medicine 2007; 4: e206-e215.

57) Elias SJ A. Focus on mammalian thioredoxin reductases–Important seleno proteins with versatile functions. Biochim Biophys Acta 2009; 1790: 495-526

58) Algera HM, Sayed AA, Stadecker MJ, Williams DL. Molecular and enzymatic characterisation of *Schistosoma mansoni* thioredoxin. Int J Parasitol 2002; 32: 1285-1292.

59) Soliman KM, Mohamed AM, Metwally NS. Attenuation of some metabolic deteriotions induced by diabetes mellitus using carnosine. J Appl Sci 2007; 7: 2252-2260.

60) Rutkowski RB, Bruce JI. Serum enzyme alteration of mice exposed to schistosomiasis. Int J Biochem 2007; 2: 137-145.

61) Pica-Mattoccia L, Orsini T, Basso A, Festucci A, Liberti P. *Schistosomal mansoni*, lack of correlation between praziquantel-induced intra worm calcium influx and parasite death. Exp Parasitol 2008; 119: 332-335.

62) Martins-Leite P, Gazzinelli G, Alves-Oliveira LF, Gazzinelli A. Effect of chemotherapy with praziquantel on the production of cytokines and morbidity associated with schistosomiasis mansoni. Antimicrob Agents Chemother 2008; 52: 2780-2786.

63) Stoll V, Seebeck E. Allium compounds. I. Alliin, the true mother compounds of garlic oil. Helv Chim Acta 1984; 31: 108-210.

64) Guengerich FP. Influence of nutrients and other dietary materials on cytochrome P-450 enzymes. Am J Clin Nutr 1995; 61: 651S-658S.

65) Wargovich M, Lmada O, Stephens L. Initiation and post-initiation chemopreventive effects of diallyl sulfide in esophageal carcinogenesis. Cancer Lett 1992; 64: 39-42.

66) Kwak M, Kim S, Kwak J, Novak R, Kim N. Inhibition of P450 2E1 expression by organosulfur compounds allylsulfide, allylmercaptan and allylmethyl sulfide in rats. Biochem Pharmacol 1994; 47: 531-539.

67) San Martin-Nunez BV, Ordonez-Escudero D, Alunda JM. Preventive treatment of rabbit coccidiosis with a-difluoromethyl ornithine. Vet Parasitol 1988; 30: 1-10.

68) Gedik N, Kabasakal L, Sehirli O, Ercan F, Sirvanci S, Keyer-Uysal M, Sener G. Long-term administration of aqueous garlic extract (AGE) alleviates liver fibrosis and oxidative damage induced by biliary obstruction in rats. Life Sci 2005; 76: 2593-2606.

69) Georgieva N, Koinarski V, Gadjeva V. Antioxidant status during the course of *Eimeria tenella* infection in broiler chickens. Vet J 2006; 172: 488-492.

70) Kiruthiga PV, Shafreen RB, Pandian SK, Devi KP. Silymarinprotection against major reactive oxygen species released by environmental toxins: exogenous H_2O_2 exposure in erythrocytes. Basic Clin Pharmacol Toxicol 2007; 100: 414-419.

71) Chung LY. The antioxidant properties of garlic compounds: allyl cysteine, alliin, allicin, and allyl disulfide. J Med Food 2006; 9: 205-213.

72) Dkhil MA, Abdel-Bakia AS, Wunderlicha F, Siesa H, Al-Quraishy S. Anticoccidial and antiinflammatory activity of garlic in murine *Eimeria papillata* infections. Vet Parasitol 2011; 175: 66-72.