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Determination and Comparison of *In Vitro* Radical Scavenging Activity of Both Garlic Oil and Aqueous Garlic Extracts and Their *In Vivo* Antioxidant Effect on Schistosomiasis Disease in Mice

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ABSTRACT

Garlic has long been utilizing as a folk remedy for various diseases. In this study, the effect of aqueous garlic extract (AGE) (125 mg kg^{-1}), garlic oil extract (GOE) (125 mg kg^{-1}), and the combination of both extracts (AGE+GOE) (62.5 from each) was studied over a parasitic disease namely schistosomiasis. Determination of some radical scavenging activities including Ferric Reducing Ability Power, Hydrogen peroxide, DPPH and nitric oxide radical scavenging activities were studied. The antioxidant properties of the mentioned extracts on both normal and *Schistosoma mansoni*-infected mice was also studied. The liver tissues were analyzed for the level of Malondialdehyde (MDA) and glutathione (GSH) known as biomarkers for oxidative and reducing status, respectively. The results showed that almost all tested extracts showed high effect of radical scavenging activity. Furthermore, remarkable decrease in worms and tissue eggs were analyzed in all tested extracts as AGE+ GOE (1015 ± 1.41) represented higher level of prohibition in total number of worms and eggs compared to infected untreated mice (1502 ± 2.13). The garlic extracts were potent antioxidants with slight differences.

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Introduction

Garlic (*Allium sativum* L.), one of the first cultivated crops, is commonly used for several purposes due to its therapeutic and medicinal properties both in traditional and modern medicine (FAO, 2013). Garlic has been consumed either as raw vegetable (fresh leaves or dried cloves), or processed in the form of oil, extract and even powder. It has some activities such as antidiabetic, anticancer, immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmolytic, renal protective, gastro-protective and antioxidant properties for a long time (Lanzotti et al., 2014). Garlic has been introduced as a potent vegetable containing remarkable antioxidant properties due to having two main classes of antioxidant components, called flavonoids and sulfur-containing compounds, such as diallyl sulfide and trisulfide, and allyl-cysteine (Kahkonen et al., 1999; Sattler and Griffin 2003). It has been reported that, Garlic has potential of anti-bacterial and anti-fungal activities and proved to play a significant role in over 100 diseases such as cancer, atherosclerosis and arthritis (Thomas, 1995; Benkeblia, 2005). Garlic supplements are classified into four different groups including commercially available in form of oil capsules, capsule and tablets, soft gel capsule and concentrated extract in powdered form (Bayan et al., 2014). Row garlic along with its oil extracts and powder extracts have shown numerous activities such as reduction of cholesterol and triglycerides of blood levels during the high fat meals intake in human studies (Bayan et al., 2014). Additionally, the most famous brand of garlic extract namely KYOLIC is obtained by keeping maintained at room temperature in the form of sliced and soaked into a water/ethanol mixture for more than 20 months (Amagase and Milner, 1993). Aged garlic extract (AGE), a commercial garlic product, which is manufactured soaking sliced fresh garlic in aqueous ethanol and maturing the extract for up to 10 or 20 months at room temperature was tested and various biological activities (Amagase and Milner, 1993; Kasuga et al., 2001; Morihara et al., 2011) including cardio protective (Rahman and Lowe, 2006), cancer-preventing (Tanaka et al., 2006) and scavenging free radicals (Morihara et al., 2011) have been reported. In another study according to the antioxidant activity of garlic essential oil (*Allium sativum*) grown in north Indian

plains, IC50 values for DPPH and nitric oxide scavenging assays were observed as 0.5 mg/mL and 50 µg/mL respectively (Lawrence and Lawrence, 2011). In another study, antioxidant properties of raw garlic (*Allium sativum*) extract was investigated and the results showed that raw garlic could be a source of antioxidant based on the results of the DPPH scavenging analysis (Rahman et al., 2012). Some *in vivo* studies referring to effects of garlic over different diseases have been recorded. Anthony et al., (2005) reported that garlic has displayed potentially new treatments for a range of parasitic infections. It was investigated that the antioxidant and anti-schistosomal effects of AGE alone and in the combination with *Nigella sativa* (NSO) on normal and *Schsitosoma mansoni*–infected mice and they represented that AGE was a promising agent in treatment of schistosomiasis disease (EL-Shenawy et al., 2008). Garlic has been used on Asian fish farms for a long time and it has been introduced as a useful anti-parasitic stimulant (Rodgers and Furones, 2009; Jegede, 2012; Schelkle et al., 2013). Garlic has been recorded to have an effect against monogenean parasites reducing *Anacanthorus penilabiatus* infections in cultivated pacu (*Piaractus mesopotamicus*) (Martins et al., 2002) *Neobenedenia* sp. in barramundi (Militz, 2013) and gyrodactylosis in *Oreochromis niloticus* fry (Abd El-Galil and Aboelhadid, 2012). In another study, immunomodulatory effect of GOE on *Schistosoma mansoni* infected mice was tested and the results displayed that the critical larval stage of the parasite (7 d post infection) and after 42 d PI was due to its action on the parasite viability, mobility and fecundity associated with an enhanced immune response of the host towards the parasites (Kamel and El-Shinnawy, 2015). The purpose of this study was to investigate *in vitro* scavenging activity of two garlic extracts including GOE and AGE extracts on some free radicals including determination of DPPH radical scavenging activity, hydrogen peroxide radical scavenging activity (H_2O_2), superoxide radical scavenging activity, ferric Reducing Ability Power (FRAP) and nitric oxide radical inhibition assay. Besides, the antioxidant effect of mentioned garlic extracts as alternative treatments against schistosomiasis disease was tested in mice determining some hematological parameters such as White blood cells (WBCs), Red blood cells (RBCs), Hemoglobin percentage (Hb%), hematocrit value (PCV) and Thrombocytes to analyze the existence of anemia, besides, tissue malondialdehyde (MDA) and glutathione (GSH) were also measured to figure out the tissue damage, the mentioned tested were done to introduce the most effective extract for containing antioxidant effect.

Materials and Methods

Preparation of Garlic Extracts

To obtain aqueous extract, peeled garlic (40 g), crushed with distilled water in a mortar, was carefully decanted by pressing and 60 mL of aqueous extract was extracted. As one milliliter of aqueous extract contained 660 mg of garlic Materials (Sener et al., 2006). To obtain GOE, distillation method was used according to method described by Kamel and El-Shinnawy, (2015).

In vivo Experimental Design

Forty male Swiss albino mice with weights between 21-23 g were obtained from experimental research center of Urmia University. They were put in polypropylene cages at 25 ± 2°C with 12 h/12 h light/dark cycle, and had free access to pelletal food with tap water ad libitum. The above-mentioned animals were spontaneously divided into eight groups including five in each, according to the experimental design, which is shown in Table 1. Four groups of mice were infected by exposing them to 50 *Schistosoma mansoni* (*S. mansoni*) cercariae with Iranian Strain. The animals were treated by intraperitoneal route (i.p.) with different regime (Table 1) for 28 days (three times per week) starting from 1st day post infection (pi).

Table 1 The experimental design

Groups of mice	Treatment Regime
Non-infected control	Group I received 0.1 mL of saline Group II received 125 mg kg-1 of AGE Group III received 125 mg kg-1 of GOE Group IV received a mixture of AGE (62.5 mg kg^{-1}) and GOE (62.5 mg kg^{-1}).
S. mansoni-infected	Group V received 0.1 mL of saline Group VI received 125 mg kg-1 of AGE Group VII received 125 mg kg-1 of GOE Group VIII received a mixture of AGE (62.5 mg kg^{-1}) and GOE (62.5 mg kg^{-1}).

Parasitological Study

Mice were killed by decapitation according the ethical rules and animal experimentation Committee of faculty of science, department of biology, Nalu University at day 49 post infection. After dissecting the animals, the whole liver was put into a 20×20 cm plastic folder and compressed between two 21×21 cm glass plates until the parenchyma was smoothly and evenly dispersed into a thin transparent layer then it was examined under a stereomicroscope (EL-Shenawy et al., 2008). The adult worms were sexed and counted referring to the method of reference (Wang et al., 2004). The ending part of small intestine, placed in a Petri dish, was put under stereomicroscope. The male and female worms in the mesenteric veins were removed and counted. Number of *Schistosoma* eggs per gram of liver was measured (Kloetzel, 1967). The proportion of eggs in various stages of maturity was estimated for oogram study (Pellegring et al., 1962). One hundred eggs per oogram were haphazardly chosen and the qualified by microscopic; they were categorized as dead, viable immature and mature in all infected and treated groups.

Determination of Hematological Parameters

Decapitation was used to kill mice at day of 49 post infection and blood was collected for hematological and biochemical parameters. Blood samples used for hematological analysis were gathered into polyethylene tubes containing an anticoagulant, ethylene diamine tetraacetic acid (EDTA). The cell counter (ADVIA 60\Cell Dyne counting, ABOTT1800, Ireland) was used to determine erythrocytes (RBCs), total leucocytes counts (WBCs) Hemoglobin (Hb) %, hematocrit value (PCV)

and absolute values of erythrocyte indices (EL-Shenawy et al., 2008).

Tissue Malondialdehyde (MDA) and Glutathione (GSH) Assays

The hepatic reduced glutathione (GSH) level was determined by the method of reference (Ellman, 1959). Briefly, after 0.2 g liver tissues were homogenized in 4 mL of 0.02 M EDTA Na2 (using an all glass Ten-Broeck homogenizer in an ice bath). 2.5 mL tissue homogenates (aliquots) were mixed with 2.0 mL of distilled water and 0.2 mL of 50 % TCA. All tubes were shaken intermittently for 10-15 min and centrifuged for 15 min at approximately 3000 ×g. 2.0 mL of 0.4 M Tris buffer (pH 8.9) and 0.1 mL of 0.01 M 5,5'-dithiobis- 2-nitrobenzoic acid (DTNB) were added to 2.0 mL of tissue supernatant, and the sample was shaken. The absorbance was read within five min of the addition of DTNB at 412 nm against a reagent blank with no homogenate. GSH levels were calculated using standard curve prepared by known amounts of GSH (Aldrich chemical Co. LTD-Germany). The concentration of GSH was expressed as mg/g tissue. Hepatic lipid peroxidation (LPOX) level was measured by a colorimetric reaction with thiobarbituric acid-positive reactant substances (TBARS) and was expressed in terms of the malondialdehyde (MDA) concentration using 1,1,3,3- tetraethoxy propane as a standard10. The liver samples were homogenized at the tissue concentration of 50 mg/mL in 0.1 M of ice-cold phosphate buffer (pH-7.4). The homogenates were centrifuged at 10,000×g at 4ºC for five min. 0.5 mL supernatant was mixed with 0.5 mL of normal saline and 2 mL of TBA-TCA mixture. The mixture was boiled at 100ºC for 10 min, and then cooled at room temperature. This mixture was centrifuged at 4000×g for 10 min. The whole supernatant was transferred in spectrophotometer cuvette and read at 535 nm. The levels of TBARS are expressed as micromoles of MDA per mg of tissue (mmol/mg).

Determination of Hydrogen Peroxide Radical Scavenging Activity (H_2O_2)

A method to determine the H_2O_2 scavenging ability previously mentioned was used with a little modification (Ruch et al., 1989). Garlic extracts (2 mg/mL) were dissolved in 3 mL of a 0.1 M phosphate buffer (pH = 7.4) solution and mixed with 600µL of a 43 mM solution of hydrogen peroxide previously prepared in the same buffer. A blank solution was prepared the same way without presence of H_2O_2. The absorbance of the solutions was measured for recognizing the concentration of hydrogen peroxide at 230 nm. Gallic acid was used as the reference compound. The hydrogen scavenging activity was calculated using the following equation:

$$H_2O_2 \text{ scavenging activity}\% = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 was the absorbance of control (blank, without extract) and A_1 was the absorbance of the solution in the presence of the extract and gallic acid.

Determination of Ferric Reducing Ability Power (FRAP)

The FRAP was determined using FRAP assay with some modifications (Benzie et al., 2002). The FRAP solvent included 5 mL of 10 mMol/L TPTZ (2, 4, 6-tripyridyl- s- triazine) solution in 40 mM HCl, 2.5 mL of 20 mM FeCl3, and 25 mL of 0.3 M acetate buffer (pH 3.6) (0.3 mol/L, pH = 3.6). 3 mL FRAP was added to 100µL of mixed sample and the final solution was measured at the absorbance of 593 nm after incubation at 37˚C for 10 min. Methanolic solutions of known Fe (II) concentration, in the range of 0.6–10 µmol/L ($FeSO_4$), were used for obtaining the calibration curve. The FRAP value shows the ratio between the slope of the linear scheme for reducing Fe^{3+} -TPTZ reagent by *Garlic* extract in comparison with the slope of the scheme for $FeSO_4$.

Determination of DPPH Radical Scavenging Activity

DPPH radical scavenging activity in Garlic extracts were determined using the method described by reference (Hatano, 1988). A volume of 40 µL of each extract in various concentrations (3-20 mg/mL) was added to 1 mL of DPPH (1-1-diphenyl 2-picryl hydrazyl) radical solution dissolved in ethanol with a 0.2 mM final concentration. Two different standards called ascorbic acid and gallic acid (0.125–20 mg/mL) were reference standards. The different tubes containing the solutions were placed in a dark room for 30 min to be incubated. The absorbance of mixtures was measured at 520 nm immediately. The DPPH radical scavenging activity was calculated using the following equation:

$$\text{DPPH scavenging activity}\% = \frac{A_0 - A_1}{A_0} \times 100$$

A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance of the whole materials including extract, ascorbic acid, gallic acid, DPPH.

Determination of Nitric Oxide Radical Inhibition Assay

Nitric oxide radical inhibition can be measured using Griess Ilosvay reaction reaction (Garrat, 1964). Naphthyl ethylene diamine dihydrochloride (0.1% w/v) was used instead of napthylamine (5%). The final combination of mixture (3 mL) containing phosphate buffer saline (0.5 mL), sodium nitroprusside (10 mM, 2 mL) and the alcoholic extract of *Garlic* (0.5 mL) was incubated at room temperature for 150 min. After incubation, 1mL of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) was added to 0.5 mL of the prepared mixture and allowed to stand for 5 min for complementation of diazotization. Then, 1 mL of naphthyl ethylene diamine dihydrochloride was added and allowed to stay at room temperature for 30 min. The final solution had a pinkish color in diffuse light. Ascorbic acid and gallic acid were utilized as the reference compounds. The absorbance of the solutions was measured at 540 nm in the comparison of blank. The nitric oxide radical scavenging activity was calculated using the following equation.

$$\text{Nitric oxide scavenging activity}\% = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance of the whole materials including extract, ascorbic acid and gallic acid.

Statistical Analysis

All the investigations were carried out in triplicate. Experimental information was revealed as mean ± standard deviation (SD). SPSS software, Version 19.0, was used to analyze the data. One way analysis of variance was provided by ANOVA procedures. Values of $P<0.05$ were organized as significant utilizing Duncan's multiple range tests to show the differences between means.

Results and Discussion

In vivo Studies

Plants have been used as nonconventional Anthelmintics with considerable attention in recent times due to world-wide development of resistance to chemical anthelmintics in worm populations (Kamel and El-Shinnawy, 2015; El-Kott et al., 2011). In the last years, plant extracts have been utilized in treatment of *S. mansoni* infection (Molgaard et al., 2001; Kamel and El-Shinnawy 2015). This work was aimed to assess the effect and potency of GOE and AGE secretly and as a mixture in treating *S. mansoni* and introducing more potent extract in treatment of *S. mansoni*. As it is represented in the current paper, garlic has the antiparasitic mode of action. It has been reported that this mechanism of action is made by enhancing the immunity of the host to attack the parasite (EL-Shenawy et al., 2008). It is also thought that the antioxidative properties of both AGE and GOE have strong effects in *Schistosoma* infected mice (EL-Shenawy et al., 2008; Kamel and El-Shinnawy, 2015; El-Kott et al., 2011). The development of eggs are continued in the tissue until maturation, then until the time of death, the mature ova are remained alive in the tissue. Accordingly, it has been reported that, garlic effects on the reproductive organs of the worms (El-Kott et al., 2011). As shown in Figure 2, groups VI treated with AGE (17.33 ± 2.69) contained more mature eggs than the groups treated with GOE (14.33 ± 1.69) and both extracts (AGE + GOE) (14.66 ± 1.24) while the number of mature eggs were decreased in all tested groups comparing to group V (40.33 ± 0.41). GOE could decrease the number of mature eggs much more than the other two groups treated by AGE or AGE + GOE. The number of immature eggs was higher in VI (73.66 ± 1.69) and VII (72.33 ± 1.61) compared to VIII (67.33 ± 2.44) while all the test samples were effective in increasing the number of immature eggs in comparison to V (54.31 ± 1.42), as a result, it can be obvious that AGE could increase the number of immature eggs higher than the other two groups with a slight difference. On the other hand, VIII (17.31 ± 1.76) could be able to increase the number of dead eggs much higher than VI (9.01 ± 0.34) and VII (13.21 ± 0.89) samples while all samples showed remarkable increase in the number of dead eggs in comparison to V (5.35 ± 2.16) surprisingly. As a result, it can be reported that the VIII group was able to cause high number of dead eggs compared to the other tested extracts, which are shown in Figure 2. It was exhibited that aqueous garlic extract disrupted the development and maturity of *Schistosoma* eggs as there was an increase in number of dead eggs (EL-Shenawy et al., 2008). It can be obtained that, there is a correlation between the number of worms with the reduction of the ova due to the possible linear relationship between the egg output and the worm burden (Riad et al., 2007). However, there should be numerous possibilities responsible for the reduction of eggs. The mentioned factors may be responsible for diminishing fecundity of the worm pairs and cause an increase in the rate of egg excretion according to the worm pairs (Mantawy et al., 2011). As displayed in Figure 1, the number of total worms including male and female worms were decreased in infected mice treated with AGE+ GOE (1015 ± 1.41) compared to infected untreated mice (1502 ± 2.13) notably.

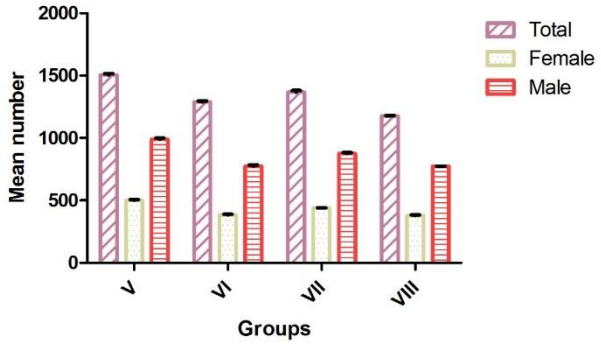

Figure 1 Mean number of adult worms recovered from *S. mansoni*-infected mice after administration of 125 mg kg^{-1} of GOE, 125 mg kg^{-1} of AGE separately or in combination (62.5 from each).

V-VIII: S. mansoni infected mice; VI: treated with AGE, VII: treated with GOE, VIII: treated with mixture of GOE and AGE.

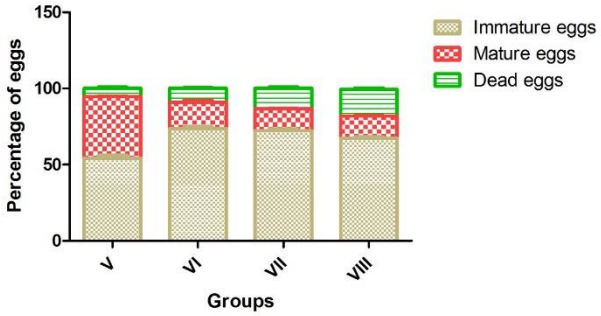

Figure 2 Percentage of eggs (immature, mature and dead) after administration of 125 mg kg^{-1} of GOE, 125 mg kg-1 of AGE separately or in combination.

V-VIII: *S. mansoni* infected mice, VI: treated with AGE, VII: treated with GOE, VIII: treated with mixture of GOE and AGE.

It was also reported that, the more immunoglobulins elevated there were, the more reactive oxygen was decreased in treatment of schistosomiasis, therefore, this mechanism can be happened through antioxidants defenses from infected hepatic cells increasing constant oxidative stress and oxidation of lipids, protein and other macromolecules such as DNA (Mantawy et al., 2011). Anemia was observed due to a strong decrease in the level of Hb percentage in infected untreated mice (7.6 ± 0.3, $P\leq0.05$) compared to non-infected untreated mice (11.2 ±

0.2, P≥0.05) therefore it decreased the number of RBCs, Group VI treated with 125 mg kg1 of AGE (8.5 ± 0.4, P<0.001) showed the highest amount of WBCs compared to non-infected group, besides, the number of PVC was also decreased in untreated infected mice (33.54 ± 0.1, P≤0.01) in comparison with non-infected untreated mice (41.36 ± 0.6, P≥0.05). As it is shown in Table 2, the number of Thrombocytes, components of blood to stop bleeding, was increased in infected untreated mice (685 ± 8.5, P≤0.05) in comparison to the non-infected untreated mice (344 ± 2.9, P≥0.05). Parallel to the present study, it was represented that anemia could be observed in the case of reduction in the level of three different parameters such as RBC count; PCV and Hb content (EL-Shenawy et al., 2008). According to the results, interaction of AGE + GOE in both infected (17.5 ± 0.5, P≤0.01) and non-infected (18.4 ± 0.4, P≤0.01) groups enhanced the Hb percentage more than the action of AGE (non-infected:14.1 ± 0.1, P≥0.05; infected: 9.1 ± 0.5, P≤0.01) and GOE (non-infected: 12.8 ± 0.2, P≥0.05; infected: 9.3 ± 0.2, P≤0.01), apart from this, all infected groups including untreated and treated groups showed a notable decrease in number of RBCs secretion, which are all shown in Table 2. Accordingly, it was represented that the mentioned decreases were caused by two ways including extrusion of egg through intestinal wall ending to the bleeding or consumption by adult schistosomes (Sturrock et al., 1996). A key antioxidant namely GSH is an essential constituent of intracellular protective mechanisms against oxidative stress (Ross, 1988). In experimental liver fibrosis, the more antioxidants are decreased, the freer radicals are secreted; thereby, cellular damage is increased (Yang et al., 2004). Our results showed that the administrations of both garlic extracts separately or in combination prevent the hepatic GSH depletion, so that, garlic extracts are stressed to contain strong potent of antioxidant capacity in protecting the hepatic tissue against oxidative stress. The results also displayed that there was a significant increase in the hepatic MDA levels of infected mice probably along with scavenging the very reactive hydroxyl and peroxyl radicals. The GOE extract could decrease the MDA levels of both non-infected (0.51 ± 0.45, P≥0.05) and infected (0.32 ± 0.23, P≤0.01) mice stronger than the other extracts while the AGE extract could decrease the level of GSH in both non-infected (0.96 ± 0.06, P≤0.05) and infected mice (0.31 ± 0.31, P≤0.05) much stronger than the other utilized extracts which are shown in Table 3. It can be emphasized that, AGE had the highest potential in GSH depletion and is introduced as a potent antioxidant in comparison to GOE and AGE+ GOE. In accordance to the present study, it was also reported that chronic garlic intake decreased lipid peroxidation and increased endogenous antioxidants, such as catalase and GSH significantly (Banerjee and Maulik, 2002).

Determination of Different Radical Scavenging Activities

One of the most derivations of Hydrogen peroxide called hydroxyl radical initiating lipid peroxidation can cause DNA damage (Nakayama et al., 1993). In the body, protein and DNA should be protected via removing hydrogen peroxide because of its high potential in hydroxyl radicals' secretion. Hydroxyl radicals are highly reactive and short-lived radicals with high potential of disruption (Zhao et al., 2008; Hayyan et al., 2016). The hydrogen peroxide scavenging activities of extracts which are represented in Table confirmed that, AGE, GOE and AGE + GOE extracts represented high (99.76 ± 2.34 %), moderate (70.85 ± 2.87%) and low (35.45 ± 1.22%) scavenging capacity against H_2O_2, respectively. AGE (99.76 ± 2.34) had the strongest potential of H_2O_2 radical Scavenging activity in comparsion to Galic acid (15.41 ± 1.24), therefore it can be suggested that AGE contains effective necessary compounds for hydrogen peroxide elimination.

Determination of Ferric Reducing Ability Power (FRAP)

The FRAP method called Ferric ion reducing antioxidant power is an antioxidant capacity assay that uses Trolox as a standard (Benzie et al., 1996). In this study, it was used to evaluate the reducing potential of the tested extracts. In the presence of antioxidants, it reduces a ferric 2,4,6-tripyridyl-s-triazine complex (Fe3+-TPTZ) to its ferrous colored form (Fe2+-TPTZ). The results of the present study displayed that, in the presence of 125 mg kg-1 of samples, FRAP activity of GOE (397.75 ± 3.47) was the highest compared to AGE (279.51 ± 3.40) and AGE + GOE (314.63± 5.65). As it is displayed in Table 4, the FRAP activity of all tested compounds were less than standards.

Table 2 Some blood parameters after administration of aqueous garlic extract (125 mg kg^{-1}) and GOE (125 mg kg^{-1}) or the mixture of both mentioned extracts.

Groups of mice		WBCs	RBCs	Hb%	PCV	Thrombocytes
Non-infected control	I	2.24 ± 0.2	9.76 ± 0.4	11.25 ± 0.2	41.36 ± 0.6	344 ± 2.9
	II	4.98 ± 0.3[a]	9.45 ± 0.2	14.11 ± 0.1	49.93 ± 0.4[a]	695 ± 4.0[a,b]
	III	2.12 ± 0.4	10.90 ± 0.4	12.82 ± 0.2	46.71 ± 0.5[a]	332 ± 7.9
	IV	4.23 ± 0.3[a]	10.65 ± 0.3	18.41 ± 0.4[d]	52.51 ± 0.3[a]	335 ± 8.4
S. mansoni- infected	V	7.13 ± 0.1	5.17 ± 0.4	7.63± 0.3[c]	28.32 ± 0.1[c]	685 ± 8.5[ab]
	VI	8.51 ± 0.4[c]	4.93 ± 0.1	9.15 ± 0.5[d]	33.54 ± 0.1[c,d]	882 ± 7.6[a]
	VII	5.43 ±0.3	5.42 ± 0.2	9.36 ± 0.2[d]	33.83 ± 0.4[c,d]	382 ± 11.0[c,d]
	VIII	4.65 ± 0.1	5.21 ± 0.1	17.52 ± 0.5[d]	30.83 ± 0.6[b,c,d]	623 ± 10.3[ab]

RBCs; Erythrocytes count, WBCs; total leucocytes count, Hb%; Hemoglobin, PCV; hematocrit value. I-IV non-infected control groups of mice treated with 0.2 mLsaline, 125 mg kg^{-1} of AGE, GOE 125 mg kg^{-1} and the mixture of both mentioned extracts (62.5 mg kg^{-1} from each). V-VIII *S. mansoni* infected mice treated as mentioned above. The data are presented as mean ± S.D, n = 6-8. The minimum and maximum values are shown in parentheses. The treatment regime as described in the Table 1, [a] Significant difference as compared with normal control group (P≤0.05), [b] Significant difference between GOE and AGE groups (P≤0.05), [c] Significant difference as compared with corresponding control group (P≤0.05), [d] Significant difference as compared with infected group (P≤0.01).

Table 3 GSH and MDA after administration of aqueous garlic extract (125 mg kg^{-1}) and GOE (125 mg kg^{-1}) or the mixture of both mentioned extracts (62.5 mg kg^{-1} from each).

Groups of mice		Malondialdehyde (MDA) level	glutathione (GSH) level
Non-infected control	I	0.56 ± 0.03	1.91 ± 0.04
	II	0.76 ± 0.43	0.96 ± 0.06^{ab}
	III	0.51 ± 0.45	1.02 ± 0.54^{ab}
	IV	0.66 ± 0.65	2.65 ± 0.43
S. mansoni- infected	V	2.33 ± 0.12^{c}	0.33 ± 0.12^{c}
	VI	0.52 ± 0.43^{d}	0.31 ± 0.31^{d}
	VII	0.32 ± 0.23^{d}	0.66 ± 0.08^{d}
	VIII	0.54 ± 0.36^{d}	0.68 ± 0.41^{d}

GSH and MDA after administration, the data are presented as mean ± S.E, n = 6-8. I-IV non-infected control groups of mice treated with 0.2 mL saline, 125 mg kg-1 of GOE, 125 mg kg-1 of AGE, mixture of GOE and AGE (62.5 mg kg-1 from each), respectively. V-VIII *S. mansoni* infected mice treated as mentioned above, $_{a}$ Significant difference as compared with normal control group (P≤0.05), $_{b}$ Significant difference between GOE and AGE groups (P≤0.05), $_{c}$ Significant difference as compared with corresponding control group (P≤0.05), $_{d}$ Significant difference as compared with infected group (P≤0.01), Table 4: Free radical scavenging activity of AGE, GOE and GOE + AGE (62.5 mg kg-1 from each).

Table 4 H_2O_2, FRAP, nitric oxide and DPPH radical scavenging activities values of AGE, GOE and GOE + AGE

Extract	H_2O_2 radical Scavenging activity (%)	FRAP value (μmol/100g)	DPPH scavenging activity%	Nitric oxide scavenging activity%
AGE	99.76 ± 2.34d	279.51 ± 3.40a	97.95 ± 1.03a	74.35 ± 3.45a
GOE	70.85 ± 2.87c	397.75 ± 3.47a	94.54 ± 0.83c	81.22 ± 4.52c
AGE + GOE	35.45 ± 1.22b	314.63± 5.65a	96.67 ± 2.47b	74.35 ± 3.61a
Gallic acid	15.41 ± 1.24a	812.56 ± 5.91c	94.66 ± 0.70c	63.25 ± 3.44b
Ascorbic acid	–	467.56 ± 3.14b	93.20 ± 0.56c	69.06 ± 2.77b

Each value is presented as the mean ± SD (n = 3)

Determination of DPPH Radical Scavenging Activity

An organic chemical compound, 2,2-diphenyl-1-picrylhydrazyl, is a dark-colored crystalline powder composed of stable free-radical molecules. It is a stable purple free radical and accepts an electron or hydrogen radical to become a stable yellow compound (diphenyl-picrylhydrazine) at room temperature. The reduction of DPPH induced by antioxidants are measured by the decrease in its absorbance at 520 nm (Brand-Williams et al., 1995; Sharma et al., 2009). As it is shown in Table 4, all extracts could overtake the standards. Accordingly, AGE (97.95 ± 1.03%) had the strongest potential of DPPH scavenging activity compared to AGE + GOE (96.67 ± 2.47%) and GOE (94.54 ± 0.83%). Therefore, it can be expressed that AGE had the highest DPPH free radical scavenging activity and reducing power.

Determination of Nitric oxide radical inhibition assay

Nitric oxide, which is a free radical with chemical formula of NO is one of several oxides of nitrogen. Interactions of NO with reactive oxygen species (ROS) such as H_2O_2 and O2- can be either protective or cytotoxic (Beligni et al., 2009; Lund et al., 2011). Although oxidative injury can be limited in physiological level of No, high concentration of NO cause the production of N_2O_3 and ONOO- which are strong reactive nitrogen oxide species and can be the reason for causing toxic reactions including lipid peroxidation, DNA modification and SH- oxidation Griess Ilosvay reaction can be used for measuring nitric oxide radical inhibition (Moncada et al., 1991). As it is displayed in table 4, all tested samples including GOE (81.22 ± 4.52 %) and AGE + GOE (74.35 ± 3.61) and AGE (74.35 ± 3.45) could overtake the tested standards including gallic acid (63.25 ± 3.44) and ascorbic acid (69.06 ± 2.77), therefore can be reported that GOE has the highest potential in terms of Nitric oxide scavenging activity.

Conclusion

In conclusion, it could be reported that all tested samples including AGE, GOE and AGE + GOE could decrease the total number of worms including male and female while AGE + GOE could affect the total number of eggs higher than the separately used extracts (AGE or GOE) with a slight difference. In terms of mature, immature and dead eggs, it can also be suggested that AGE + GOE extract was able to increase the number of dead eggs more than the other extracts while both AGE and GOE extracts used separately could increase the number of immature eggs, so it is being suggested that all tested samples had potential in prohibition of the unwanted parasites of schistosomiasis disease whereas AGE + GOE could be more effective in comparison to the other samples. The level of GSH was depleted with all tested samples while AGE showed higher level of depletion, which meant that AGE was a potent antioxidant. It can be obtained that almost all tested extracts were highly effective H_2O_2, nitric oxide, DPPH radical scavengers while any of the tested extracts could not overtake the standards in terms of FRAP value.

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