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Protection Against Oxidative Damage Induced by *Schistosoma mansoni* using Susceptible/Resistant Nucleoproteins from *Biomphalaria alexandrina* Snails

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ABSTRACT

The aim of the present study was to investigate the action of susceptible and resistant nucleoprotein vaccine of *Biomphalaria alexandrina* on lipid peroxide and certain antioxidant defense system in the liver and serum of mice after *Schistosoma mansoni* infection. The vaccination schedule consisted of a subcutaneous injection of 50 μg protein of each antigen followed by another inoculation 15 days later. The data showed that lipid peroxide, Xanthin Oxidase (OX), fructoseamine, serum alfa-fetoprotein (AFP), total serum immunoglobin E (IgE) and tumor necrosis factor-alfa (TNF-α) were significantly increased while glutathione (GSH), Vitamin C, E and the other antioxidant enzymes (glutathione reductase, superoxide dismutase and catalase) showed significant reduction after infection. Both susceptible and resistant vaccine treatment ameliorate oxidative stress of *Schistosoma mansoni* effect through improvement of all the previous parameters. The results indicated that oxidative stress due to *Schistosoma mansoni* infection was ameliorated by susceptible and resistant nucleotide vaccines of *Biomphalaria alexandrina* snail with more regards to the vaccine of susceptible snails.

Key words: Bilharziasis, vaccine, free radicals, snails, enzymes

INTRODUCTION

Schistosomiasis is one of the most widespread parasitic infections. Hepatic fibrosis, resulting from *S. mansoni* infection is of primary importance among chronic liver diseases worldwide (Allam, 2009). *Schistosoma mansoni*, a helminthic parasite that causes bilharziasis, settles in the mesenteric veins of the gut. Its eggs migrate to the liver where they induce a delayed hypersensitivity response. In schistosomiasis, morbidity and mortality are due to a unique form of liver fibrosis followed by portal hypertension as the main complication (Hamed, 2010). The egg cuticle, composed of cross-linked proteins, encloses a larva that releases enzymes and antigens through multiple pores. The host reaction presumably involves Reactive Oxygen Species (ROS). Oxidative stress is seen either as a large increase in the cellular reduction potential or a large decrease in the reducing capacity of the cellular redox couples, such as glutathione (Kazura *et al*., 1981, 1985; Caulfield *et al*., 1985; Schafer and Buettner, 2001; El-Rigal *et al*., 2006; El-Shenawy *et al*., 2008). Moreover, the ultimate pathophysiological effects of ROS production associated with inflammation depend upon a balance between opposing mechanisms

which can either terminate the oxidative process or in contrast lead to the generation of potentially harmful long-lived oxidants (Kazura *et al*., 1981; Alger and Williams, 2002). Eosinophils which concentrate in Schistosoma-induced hepatic granulomas, generate the superoxide anion (O^-) and the hydroxyl radical (OH^-) (McCormick *et al*., 1996). Using a murine model of the disease, it has been shown that the parasite induces hepatic oxidative stress due to production of ROS (Abdallahi *et al*., 1999) and it reduces the antioxidant defense processes of the organ (Gharib *et al*., 1999). The ultimate result of ROS generation may be the killing of the parasite eggs however, the processes are potentially harmful for the host. The production of ROS may also initiate a fibrogenesis cascade in the liver (Casini *et al*., 1997; Houglum *et al*., 1997; Poli and Parola, 1997). Moreover, the ultimate pathophysiological effects of ROS production associated with the inflammatory response depend on a balance between opposing mechanisms which can either terminate the oxidative process or lead to the increase generation of potentially harmful and long-lived oxidants.

The aim of the present study was to evaluate the immunogenic and biochemical effects of the nucleoprotein from either susceptible or resistant *B. alexandrina* snails on *S. mansoni* infection in mice. This was achieved by measuring certain antioxidant parameters and inflammatory mediators; alfa-fetoprotein (AFP), fructoseamine, tumor necrosis factor-alfa (TNF-α) and immunoglobin E (IgE).

MATERIALS AND METHODS

The study was conducted on March 2009 at Therapeutic Chemistry Depart., National Research Center, Cairo, Egypt.

Chemicals: All chemicals used were of high analytical grade, product of Sigma (US), Merk (Germany) and BDH (England).

Animals: Thirty six Swiss albino mice CDI strain weighing 18-22 g were raised and maintained throughout the expriment in the Schistosome Biological Materials Supply Program, Theodor Bilharz Research Institute (SBSP/TBRI), Giza, Eygpt. Mice were provided with balanced commercial pellet diet and water *ad libitum* during the duration of the experiment.

Snails selection: *Biomphalaria alexandrina* snails were obtained from Egyptian laboratory stock at Medicinal Chemistry Department, National Research Center (NRC), Egypt. Snails were maintained, as stock cultures, in a well-prepared snail room, under suitable environmental conditions, in glass aquaria containing Snail-Conditioned Water (SCW) in a density of 10 snails L^{-1}. The snails were fed on fresh lettuce leaves, supplemented with tetramine (fish food) and chalk. After careful selection on the basis of size and age, method of separating schistosome-resistant and-susceptible strains from *B. alexandrina* (Egyptian strain) was performed as descriped below.

Ethics handling: Anesthetic and sacrifice procedures were followed with the legal ethical guidelines approved by the Ethical Committee of World Health Organization (WHO, USA) that was approved by the Medical Ethical Committee of the National Research Centre in Egypt.

Antigen preparation: Nucleoproteins from susceptible and resistant snails were prepared according to the method of Nabih *et al*. (1992).

Antigen administration protocol: The protein content of each extraction was determined by the method of Bradford (1976). Antigen administration protocol was performed according to Maghraby *et al.* (2007). Each mouse was sensitized with a single subcutaneous injection of the selected antigen in a dose of 50 μg protein. After 15 days, a second inoculation with the same antigen concentration was done, i.e., each mouse received a total dose of 100 μg protein.

Parasites and infection: Cercaria of an Egyptian strain of *S. mansoni* were obtained from SBSP/TBRI and used for infection immediately after shedding from *Biomphalaria alexandrina* snails. After 15 day of the last antigen injection, all vaccinated mice were infected with 80 cercaria by tail-immersion technique (Oliver and Stirewalt, 1952) for two months.

Experimental design: The animals were divided into six groups, each of 6 mice. Group 1: native control, treated with one dose of 50 μL 5M phosphate buffer saline/week for two weeks. Group 2 is control mice vaccinated with antigen of resistant snails, Group 3: control vaccinated with antigen of susceptible snails. Groups 4-6 are *S. mansoni* infected mice and classified as follow: Group 4 is *S. mansoni*-infected animals (positive control), Group 5 infected mice pre-vaccinated with 100 μg with antigen of resistant snails and Group 6 infected mice pre vaccinated with 100 μg of susceptible nucleotide by the same immunization regimens. All mice were scarified after two months of the last injection.

Tissue homogenate: Liver tissue was homogenized in 0.9 N NaCl by a ratio 1:10 w/v for estimation of lipid peroxide, glutathione, Vitamin C and E, glutathione reductase, superoxide dismutase, catalase and xanthin oxidase. The other parameter APF, fructoseamine, TNF and IgE were assayed in the serum.

Parameters assay: Protein was estimated using the method of Bradford (1976). Lipid peroxide was determined as malondialdehyd by the method of Buege and Aust (1978). Glutathione was estimated by the colorimetric assay according to the method of Moron *et al.* (1979). Vitamin C was carried out by the method of Jagota and Dani (1982). Vitamin E was measured by the colorimetric assay using the method of Angustin *et al.* (1985). The activity of glutathione reductase was determined spectrophotometrically by the method of Zanetti (1979). Superoxide dismutase activity was estimated by method of Nishikimi *et al.* (1972). Catalase was assayed according to Lubinsky and Bewley (1979). Xanthine Oxidase (XO) activity was determined by the method of Fried and Fried (1974). Tumor necrosis factor-alfa (TNF-α) was quantified using a commercial ELISA kit (Endoge Woburn, MA). Fructosamine (glycated serum protein) was determined using reagents, calibrators and controls from Sigma Diagnostics (St. Louis, MO) and application parameters for the Cobas Mira automated chemistry analyzer. AFP was assayed by ELISA using commercial kit. The level of total IgE was measured by ELISA and compared with known mouse IgE standard (BD PharMingen).

Statistical analysis: Analysis of date was carried out using one way Analysis of Variance (ANOVA) by Co Stat computer program. Significance value between groups was at $p<0.0001$.

RESULTS

Table 1 showed nucleotide effect of susceptible and resistant *Biomphlaria alexandrina* snails on lipid peroxide and some non-enzymatic antioxidants. Lipid peroxide recorded a significant

Table 1: Effect of vaccination with nucleoprotein of resistant and susceptible snails on some antioxidant levels

Parameters	Control	Control vaccinated (A)	Control vaccinated (B)	Infected	Vaccinated infected (A)	Vaccinated Infected (B)
Lipid peroxide	0.46 ± 0.05^{e}	0.52 ± 0.04^{d}	0.49 ± 0.03^{de}	1.39 ± 0.02^{a}	1.01 ± 0.05^{b}	0.66 ± 0.04^{c}
Glutathione	34.13 ± 2.29^{ab}	33.32 ± 2.63^{b}	35.46 ± 1.47^{a}	15.42 ± 0.50^{e}	17.87 ± 1.08^{d}	26.81 ± 1.84^{c}
Vitamin C	7.01 ± 0.79^{a}	6.15 ± 0.36^{bc}	6.33 ± 0.47^{b}	4.50 ± 0.43^{e}	5.07 ± 0.74^{de}	5.64 ± 0.45^{cd}
Vitamin E	2.68 ± 0.17^{a}	2.08 ± 0.22^{bc}	2.31 ± 0.35^{b}	1.05 ± 0.09^{e}	1.59 ± 0.13^{d}	1.96 ± 0.12^{c}

Data are Mean±SD of six mice in each group. Data are expressed as nmoL mg^{-1} protein for lipid peroxide, µg mg^{-1} protein for glutathione and Vitamin (C and E). (A) and (B) are nucleoprotein from resistant and susceptible snails. Unshared superscript letters between groups are the significance values at $p<0.0001$

Table 2: Effect of vaccination with nucleoprotein of resistant and susceptible snails on some antioxidant enzymes

Parameters	Control	Control vaccinated (A)	Control vaccinated (B)	Infected	Vaccinated infected (A)	Vaccinated Infected (B)
Glutathione reductase	12.09 ± 0.53^{b}	15.80 ± 2.30^{a}	11.80 ± 0.68^{b}	8.02 ± 0.49^{c}	11.20 ± 0.76^{b}	15.15 ± 1.70^{a}
Super oxidismutase	2.50 ± 0.27^{d}	3.50 ± 0.47^{ab}	3.80 ± 0.59^{bc}	0.79 ± 0.04^{e}	2.30 ± 0.14^{cd}	4.50 ± 1.30^{a}
Catalase	16.00 ± 0.88^{ab}	15.20 ± 0.58^{b}	17.13 ± 1.80^{a}	6.50 ± 1.30^{c}	9.20 ± 1.10^{c}	16.70 ± 1.90^{ab}
Xanthine oxidase	1.23 ± 0.42^{b}	1.53 ± 0.14^{b}	1.47 ± 0.21^{b}	5.04 ± 0.66^{a}	1.20 ± 0.08^{b}	1.30 ± 0.39^{b}

Data are Mean±SD of six mice in each group. Data are expressed as umol/min/g tissue for GR,, SOD, CAT, XO. (A) and (B) are nucleoprotein from resistant and susceptible snails. Unshared superscript letters between groups are the significance values at $p<0.0001$

elevation in infected liver (202.17%) and infected liver treated with the nucleotide of susceptible and resistant snails (43.48 and 119.56%, respectively). Improvement levels reached to 158.52 and 82.44%, respectively after vaccination with the two antigens were recorded. Control mice treated with the same vaccines showed insignificant change. Glutathione, in the present results recorded a significant reduction in the infected liver (54.80%) and the infected liver treated with the same above vaccines (21.44 and 47.64%, respectively) with an improvement equal to 33.38 and 7.18%, respectively. Vitamin C and E recorded significant decrease in infected mice by 35.80 and 60.82%. Vaccinated mice with susceptible and resistant snails antigen showed significant decrease by 19.54, 27.67 for Vitamin C and 26.86, 40.67% for Vitamin. E, respectively. Improvement equal to 16.26, 8.13% and 33.95, 20.15% for Vitamin C and E, respectively were recorded after vaccination with both vaccines. The same parameters showed an insignificant change in the control liver treated with vaccines. Table 2 showed the nucleotide effect of susceptible and resistant *Biomphlaria alexandrina* on some enzymatic antioxidants; glutathione reductase, superoxide dismutase and catalase enzymes. All enzymes showed reduction in the infected liver (33.66, 68.40 and 59.38%, respectively) and infected liver treated with the nucleotides of susceptible (25.31, 80.00 and 4.38%) and resistant (%) snails (7.36, 8.00 and 42.50%). An improvement equal to 58.98 and 26.31% in case of gutathione reductase, 148.4 and 60.4% for superoxide dismutase activity and 63.8 and 16.9% for catalase enzyme activity in both susceptible and resistant nucleotide treated liver were observed. Xanthin oxidase enzyme activity recorded a significant elevation in infected mice (309.76%) with an improvement equal to 304.01 and 312.14% of the infected liver treated with susceptible and resistant nucleotide, respectively. Control liver treated with the previous vaccines showed insignificant changes in the above antioxidant enzyme activities.

Table 3 showed the nucleotide effect of both susceptible and resistant *Biomphlaria alexandrina* snails on serum alfa-fetoprotein (AFP), fructoseamine, tumor necrosis factor-alfa (TNF-α) and total

Table 3: Effect of vaccination with nucleoprotein of resistant and susceptible snails on some serum parameters levels

Parameters	Control	Control vaccinated (A)	Control vaccinated (B)	Infected	Vaccinated infected (A)	Vaccinated infected (B)
Alfa-fetoprotein (AFP)	8.32±0.92^{e}	11.73±0.92^{e}	10.99±1.51^{e}	60.56±10.02^{a}	30.66±1.63^{b}	26.14±1.64^{b}
Fructoseamine	0.26±0.08^{d}	0.27±0.09^{d}	0.28±0.06^{cd}	0.96±0.17^{a}	0.60±0.09^{b}	0.48±0.13^{bc}
Tumor necrosis factor-alfa (TNF-a)	14.48±1.24^{e}	14.93±1.36^{e}	16.78±1.81^{e}	74.43±5.77^{a}	36.51±2.56^{b}	31.87±3.19^{b}
Immunoglobin E (IgE)	36.98±2.47^{e}	38.56±2.52^{e}	41.42±2.25^{e}	112.94±7.02^{a}	53.12±3.67^{b}	52.16±3.38^{b}

Data are Mean±SD of six mice in each group. Data are expressed as ng mL^{-1} for AFP, m mol L^{-1} for fructoseamine ,pg mL^{-1} for TNF and Iu (mL) for IgE. (A) and (B) are nucleoprotein from resistant and susceptible snails. Unshared superscript letters between groups are the significance values at $p<0.0001$

serum immunoglobin E (IgE). All parameters showed a significant increase in both infected (627.88, 269.23, 414.02 and 205.41%) and vaccinated mice with susceptible (214.18, 84.62, 120.09 and 41.05%) and resistant nucleotide (268.50, 130.76, 152.14 and 43.64%). An improvement equal to 359.38 and 413.70% in case of AFP, 138.47 and 184.62% for fructoseamine, 261.94 and 293.99% in case of TNF and 161.76 and 164.35% for IgE after treatment with resistant and susceptible vaccine, respectively. Control mice treated with the previous vaccines showed insignificant changes in the above parameters.

DISCUSSION

Hepatosplenic schistosomiasis is a serious manifestation o f *S. mansoni* infection that may lead to irreversible sequelae (Sayed *et al*., 2004; Bashtar *et al*., 2006). Recent research stresses the role of free radicals and oxidative stress in progression of liver injury in various chronic liver diseases such as viral hepatitis, alcoholic hepatitis and cirrhosis (Parola and Robino, 2001). Schistosomiasis is no exception: oxidative stress occurs in the liver at the site of inflamation in the vicinity of eggs of *S. mansoni*. This state of oxidative stress is attributed to increased generation of ROS and exhaustion of endogenous antioxidant enzymes (El-Rigal *et al*., 2006; El-Rigal and Heeta, 2006). Oxidative processes occurred at the site of granulomatous inflammation and on the other hand the antioxidant capacity of the liver decreased, leading to the generation of lipid peroxides which may play a central role in the pathology associated with schistosomiasis (Mahmoud *et al*., 2002).

The data obtained in the present study showed that lipid peroxides was elevated in the liver infected with *S. mansoni*. Since the complex mechanism of lipid per oxidation is known to require the participation of highly reactive oxygen and other reactive metabolites in the chain of biochemical reaction, thus, in any part of the body where these free radicals are produced, lipid peroxides are in turn increased. Such phenomenon was previously reported by Shaheen *et al*. (1994). Moreover, several authors reported that oxidative stress due to schistosomiasis causes an elevation in lipid peroxides (Soliman *et al*., 2000; Mahmoud *et al*., 2002; El-Rigal *et al*., 2006; Botros *et al*., 2007).

Elevation of MDA as a result of infection with *S. mansoni*, Poli (2000) and Mahmoud *et al*. (2002) has been suggested to be due to release of significant amount of O^{-} from macrophages of hepatic granulomas. At the same time, liver GSH was drastically depleted in infected mice. 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 (Feldman *et al*., 1990; Yousif and El-Rigal, 2004; El-Rigal *et al*., 2006). There are other examples of an infectious

disease-associated decrease of hepatic catalase and GSH levels (Hayashi and Mita, 1997; Xiao *et al*., 1998) leading to a greater sensitivity to inflammation-derived products (Utzinger *et al*., 2001).

The activity of anti-oxidant enzyme, catalase, in the liver tissue of infected mice with *S. mansoni* also decreases where catalase detoxifies hydrogen peroxide produced by inflammatory cells to water (Inoue, 1994). Therefore, treatment with nucleotids may protect hepatocytes from damage, demise and dysfunction that caused by oxidative stress at the sites of inflammation (Allam, 2009).

Also, the present study shows a reduction in hepatic Vitamin E and C levels in *S. mansoni*-infected mice. This is likely related to the increased free radical generation which depletes the cell of these antioxidants as they are used as scavengers. SOD activity is possibly diminished since, as a protein, this enzyme can be readily damaged by the abundance of free radicals being generated (El-sokkary *et al*., 2002; El-Rigal *et al*., 2006). Treatment with nucleotides of Susceptible and resistant snails of *Biomphlaria alexandrina* ameliorate all the above antioxidants parameters with more improvement in case of susceptible vaccine.

Also, infection induces the production of inflammatory mediators which are indicated by significant increase, XO activity in tissue of different organs (liver, spleen and kidney), the highest levels of these mediators was observed in liver as it is the target organ affected by *S. mansoni* infection (Adewusi *et al*., 1996; Haseeb *et al*., 2001; DeJesus *et al*., 2002; Davies *et al*., 2004; Metwally, 2006). Xanthine oxidase is an enzyme catalyzes the oxidation of hypoxanthine to xanthine and the later to uric acid. It was reported that XO is an endogenous source of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) as Nitric Oxide (NO) that can induce oxidative stress and inflect tissue injury (Winterbourn and Sutton, 1986; Mohamed *et al*., 2001; Harrison, 2002).

Alfa-fetoprotein (AFP) is a glycoprotein, of unknown function, normally produced during neonatal development by the liver and in small concentrations by the gastrointestinal tract (Abelev *et al*., 1963). Abnormal elevated serum level of AFP has been reported in patients with liver cirrhosis and hepatocellular carcinoma (Gupta *et al*., 2003). Elevated level of AFP in sera of *S. mansoni* infected mice presented in the current study may be considered as an index for liver fibrosis related to schistosomiasis.

In line with previous authors, the present study showed that *S mansoni* parasitic infection induces the production of inflammatory mediators which are indicated by significant increase of fructosamine in serum, some publications described *in vitro* evidence of the direct role of glucose and its abnormal metabolism in the development of tissue fibrosis (Huang *et al*., 1999). Serum fructosamine, one of the markers of abnormal glucose metabolism, is a glycated protein resulting from spontaneous non enzymatic condensation of glucose and proteins such as plasma protein which is generally referred to fructosamine due to its structural similarities to fructose (Huang *et al*., 1999; Misciagna *et al*., 2004; Mohamed *et al*., 2008). As albumin is the most abundant protein in serum and contains multiple lysine residues, measurement of fructosamine is mainly the determination of glycated albumin (Lapolla *et al*., 2005). Fructosamine has been recognized as a major cause of tissue fibrosis, as it has a main role in increasing the expression of ECMPs and activating of protein kinase C which has a central role in tissue fibrosis (Hattori *et al*., 2001). This nonenzymatic modification of protein is considered by several authors as a possible common mechanism involved in the progression of many pathological conditions (Aly *et al*., 2006; El-Rigal and Hetta, 2006; Vijayan *et al*., 2007) including myocardial and renal fibrosis (Chowdhury and Lasker, 1998), colorectal adenoma and chronic renal failure (Sabater *et al*.,

1991; Misciagna *et al*., 2004). Depending on the above information and from the present study, it can be suggest that increased serum fructosamine, may be used as a useful marker for liver fibrosis and associated complications of *S. mansoni* parasitic infection.

Besides, TNF-α level with concomitant increase in immunoglobulin E (Ig E) in serum of the present study in comparison to control healthy mice and this in agreement with (Adewusi *et al*., 1996; Haseeb *et al*., 2001; DeJesus *et al*., 2002; Davies *et al*., 2004).

It was reported that TNF-α essentially functions as a atrophic factor for maintaining adult schistosome viability, it is expressed during egg deposition and has a crucial role in the modulation of granulomatous reaction induced by the eggs (Joseph and Boros, 1993; Haseeb *et al*., 2001). Torben and Hailu (2007) stated that increased level of this inflammatory cytokine after egg excretion may be an indication of its effect in complications of schistosomiasis, it capable of inducing tissue injury and fibrosis through inducing ROS production, lipid per oxidation (Poli, 2000), collagen synthesis, other fibrogenic risk factors (Booth *et al*., 2004a) and inhibiting matrix metallo-proteinases (ECMPs) production, the key enzyme in the degradation of collagens (Pender *et al*., 1998). Chronic exposure to TNF-α was found to be associated with high risk of periportal fibrosis (Henri *et al*., 2002; Booth *et al*., 2004b), ascites accumulation and splenomegaly (Vassalli, 1992). As parasite eggs induce a strong type-2 response, Th2 cytokine, IL-4, has been demonstrated to play an important role in promoting β-cells proliferation and the isotype class switch to IgE (Finkelman *et al*., 1998). In consistent with this finding, increasing serum level of inflammatory antibody, total IgE in group of infected mice was observed in the present study. This is supported by previous studies stated that increasing circulating IgE level is a humoral response to egg and adult worm antigens suggesting that this mechanism might be involved in hepatic pathological patterns (Silva *et al*., 2003). IgE was reported to have the major role in mast cells stimulation which has a central role in the induction of chronic inflammation (Jayapal *et al*., 2006) and the progression of hepatic fibrosis by producing fibrogenic inflammatory mediators as well as the components of the ECMPs (Gruber, 2003; Shen, 2008). Accordingly, modulation of the inflammatory risk factors and reducing oxidative stress may be considered as targets for pharmacological or molecular interventions for the treatment of liver fibrosis and consequence complications in murine schistosomiasis (Lucey *et al*., 1996). All the previous parameters ameliorated after treatment with nucleotide vaccine of both susceptible and resistant *Biomphlaria alexandrina* with mention that vaccine of susceptible snails shows more improvement thane the resistant snail nucleotides.

The ameliorating effect of all parameters under investigations was confirmed by our previous results (Aly and Hamed, 2006; Hamed *et al*., 2010) through reduction in worm burden, ova count in liver and intestine as well as the histopathological picture of the liver.

CONCLUSION

Nucleoproteins appear to play a role in the repair and regeneration of liver in mice infected with *S. mansoni*. Susceptible and resistant *Biomphlaria alexandrina* snails recorded significant improving levels of all parameters under investigation with more regard to the vaccine of the susceptible snails. More detailed studies are needed to ascertain whether dietary nucleotides might have a therapeutic effect on human liver cirrhosis or not. Therefore, the combination of susceptible snails nucleotide with one of anti-schistosome drugs may be the aim for the future work in this field to evaluate the therapeutic possibility of such combination.

REFERENCES

Abdallahi, O.M.S., S. Hanna, M. de Reggi and B. Gharib, 1999. Visualization of oxygen radical production in mouse liver in response to infection with *Schistosoma mansoni*. Liver, 19: 495-500.

Abelev, G.I., S.D. Perova, N.I. Khramkova, Z.A. Postnikova and I.S. Irlin, 1963. Production of embryonal á-globulin by transplantable mouse hepatomas. Transplantation, 1: 174-180.

Adewusi, O.L., N.A. Nix,.X. Lu, D.G. Colley and W.E. Secor, 1996. *Schistosoma mansoni*: Relationship of tumor necrosis factor-á to morbidity and collagen deposition in chronic experimental infection. Exp. Parasitol., 84: 115-123.

Alger, H.M. and D.L. Williams, 2002. The disulfide redox system of *Schistosoma mansoni* and the importance of a multifunctional enzyme, thioredoxin glutathione reductase. Mol. Biochem. Parasitol., 121: 129-139.

Allam, G., 2009. Immunomodulatory effects of curcumin treatment on murine *Schistosomiasis mansoni*. Immunobiology, 214: 712-727.

Aly, S.A. and M.H. Hamed, 2006. Effect of Ailanthus altissima and *Zizyphus spina christi* on bilharzial infestation in mice: Histological and histopathological studies. J. Applied Sci., 6: 1437-1446.

Aly, S.A., N.S. El-Rigal and M.Z. Rizk, 2006. Nutritional supplementation with *Ailanthus altissima* and *Ziziphus spina christi* to compensate for some metabolic disorders in *Schistosoma mansoni* infected mice. Pak. J. Biol. Sci., 9: 1700-1706.

Angustin, J., B.P. Klevn, J.B. Barker and P.B. Venagepa, 1985. Vitamin E in Methods of Vitamin Assay. 4th Edn., Academic Press, Inc., New York, pp: 266.

Bashtar, A., S.A. Ahmed, A.M. Soliman and M.A. Hamed, 2006. Biochemical studies on hepatocytes after immunization of mice with schistosomal worm and egg antigens. Asian J. Biochem., 1: 224-235.

Booth, M., J.K. Mwatha, S. Joseph, F.M. Jones and H. Kadzo *et al*., 2004a. Periportal fibrosis in human *Schistosoma mansoni* infection is associated with low IL-10, low IFN-γ, high TNF-α, or low RANTES, depending on age and gender. J. Immunol., 172: 1295-1303.

Booth, M., B.J. Vennervald, A.E. Butterworth, H.C. Kariuki and C. Amaganga *et al*., 2004b. Exposure to malaria affects the regression of hepatospleenomegaly after treatment for *Schistosoma mansoni* infection in Kenyan. BMC Med., 2: 36-45.

Botros, S.S., M.R. Mahmoud, M.M. Moussa and M.M. Nosseir, 2007. Immunohistopathological and biochemical changes in *Schistosoma mansoni*-infected mice treated with artemether. J. Infect., 55: 470-477.

Bradford, M.M., 1976. A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Buege, J.A. and S.D. Aust, 1978. Microsomal lipid peroxidation. Methods Enzymol., 52: 302-310.

Casini, A., E. Ceni, R. Salzano, P. Biondi and M. Parola *et al*., 1997. Neutrophil-derived superoxide anion induces lipid peroxidation and stimulates collagen synthesis in human hepatic stellate cells: Role of nitric oxide. Hepatology, 25: 361-367.

Caulfield, J.P., H.L. Lenzi, P. Elsas and A.J. Dessein, 1985. Ultrastructure of the attack eosinophils stimulated by blood mononuclear cell products on schistosomula of *Schistosoma mansoni*. Am. J. Pathol., 120: 380-390.

Chowdhury, T.A. and S.S. Lasker, 1998. Elevated glycated heamoglobin in non-diabetic patients is associated with an increased mortality in myocardial infarction. Postgrad. Med. J., 74: 480-481.

Davies, S.J., K.C. Lim, R.B. Blank, J.H. Kim and K.D. Lucas *et al.*, 2004. Involvement of TNF in limiting liver pathology and promoting parasite survival during schistosome infection. Int. J. Parasitol., 34: 27-36.

De Jesus, A.R., A. Silva, L.B. Santana, A. Magalhaes and A.A. de Jesus *et al.*, 2002. Clinical and immunologic evaluation of 31 patients with acute *Schistosomiasis mansoni*. J. Infect. Dis., 185: 98-105.

El-Rigal, N.S. and M.H. Hetta, 2006. Effect of *Citrus reticulata* on some serum protein fractions of mice after *Schistosoma mansoni* infection. J. Applied Sci., 7: 1447-1455.

El-Rigal, N.S., S.A. Aly, M. Rizk and A. Said, 2006. Effect of *Ailanthus altissima* and *Ziziphus spina* christi extracts on some hepatic marker enzymes and antioxidants in *Schistosoma mansoni* infected mice. Polish J. Food Nutr. Sci., 15: 199-206.

El-Shenawy, N.S., M.F. Soliman and S.I. Reyad, 2008. The effect of antioxidant properties of aqueous garlic extract and *Nigella sativa* as anti-schistosomiasis agents in mice. Rev. Inst. Med. Trop. Sao Paulo, 50: 29-36.

El-Sokkary, G.H., H.M. Omar, A.F. Hassanein, S. Cuzzocrea and R.J. Reiter, 2002. Melatonin reduces oxidative damage and increases survival of mice infected with *Schistosoma mansoni*. Free Radic. Biol. Med., 32: 319-332.

Feldman, G.M., A.M. Dannenberg and T.L. Seed, 1990. Physiologic oxygen tensions limit oxidant-mediated killing of schistosome eggs by inflammatory cells and isolated granulomas. J. Leukoc. Biol., 47: 344-354.

Finkelman, F.D., I.M. Katona, J.F. Urban Jr., J. Holmes and J. Ohara *et al.*, 1998. IL-4 is required to generate and sustain *in vivo* IgE response. J. Immunol., 141: 2335-2341.

Fried, R. and L.W. Fried, 1974. Xanthine Oxidase (Xanthine Dehydrogenase). In: Methods of Enzymatic Analysis, Bergmeyer, H.U. (Ed.). 2nd Edn. Academic Press, London, pp: 644-649.

Gharib, B., O.M.S. Abdallahi, H. Dessein and M. de Reggi, 1999. Development of eosinophil peroxidase activity and concomitant alteration of the antioxidant defenses in the liver of mice infected with *Schistosoma mansoni*. J. Hepatol., 30: 594-602.

Gruber, B.L., 2003. Mast cells in the pathogenesis of fibrosis. Curr. Rheumatol. Rep., 5: 147-153.

Gupta, S., S. Bent and J. Kohlwes, 2003. Test characteristics of á-fetoprotein for detecting hepatocellular carcinoma in patients with hepatitis C: A systematic review and critical analysis. Ann. Internal Med., 139: 46-50.

Hamed, M.A., 2010. Strategic control of schistosome intermediate host. Asian J. Epidemiol., 3: 123-140.

Hamed, M.A., S.A. Ali, H.F. Aly, N.S. El-Rigal and M.Z. Rizk, 2010. *Biomphalaria alexandrina* snails as immunogens against *Schistosoma mansoni* infection in mice. Mem. Inst. Oswaldo Cruz., 105: 879-888.

Harrison, R., 2002. Structure and function of xanthine oxidoreductase: Where are we now. Free Radic. Biol. Med., 33: 774-797.

Haseeb, M.A., D.J. Shirazian and J. Preis, 2001. Elevated serum levels of TNF-α, sTNF-RI and sTNF-RII in murine schistosomiasis correlate with schistosome oviposition and circumoval granuloma formation. Cytokine, 15: 266-269.

Hattori, Y., H. Kakishita, K. Akimoto, M. Matsumura and K. Kasai, 2001. Glycated serum albumin-induced vascular smooth muscle cell proliferation through activation of the mitogen-activated protein kinase/extracellular signal regulated kinase pathway by protein kinase C. Biochem. Biophys. Res. Commun., 281: 891-896.

Hayashi, N. and E. Mita, 1997. Fas system and apoptosis in viral hepatitis. J. Gastroenterol. Hepatol., 12: S223-S226.

Henri, S., C. Chevillard, A. Mergani, P. Paris and J. Gaudart *et al.*, 2002. Cytokine regulation of periportal fibrosis in humans infected with *Schistosoma mansoni*: IFN-α is associated with protection against fibrosis and TNF-α with aggravation of disease. J. Immunol., 169: 929-936.

Houglum, K., A. Venkataramani, K. Lyche and M. Chojkier, 1997. A pilot study of the effects of d-α-tocopherol on hepatic stellate cells activation in chronic hepatitis C. Gastroenterology, 113: 1069-1073.

Huang, Z., W.C. Willett, G.A. Colditz, D.J. Hunter and J.E. Manson *et al.*, 1999. Wais circumference, waist: Hip ratio and risk of breast cancer in the Nurses' Health Study. Am. J. Epidemiol., 150: 1316-1324.

Inoue, M., 1994. Protective Mechanisms against Reactive Oxygen Species. In: The Liver: Biology and Pathobiology, Arias, I.M., J.L. Boyer, N. Fausto, W.B. Jakoby, D.A. Schachter and D.A. Shafritz (Eds.). Raven Press Ltd., New York, USA., pp: 443-459.

Jagota, S.K. and H.M. Dani, 1982. A new colorimetric technique for the estimation of Vitamin C using folin phenol reagent. Anal. Biochem., 127: 178-182.

Jayapal, M., H.K. Tay, R. Reghunathan, L. Zhi, K.K. Chow, M. Rauff and A.J. Melendez, 2006. Genome-wide gene expression profiling of human mast cells stimulated by IgE or FcεRI-aggregation reveals a complex network of genes involved in inflammatory responses. BMC Genomics, 7: 210-210.

Joseph, A.L. and D.L. Boros, 1993. Tumor necrosis factor plays a role in *Schistosoma mansoni* egg-induced granulomatous inflammation. J. Immunol., 151: 5461-5471.

Kazura, J.W., M.M. Fanning, J.L. Blumer and A.A. Mahmoud, 1981. Role of cell-generated hydrogen peroxide in granulocyte-mediated killing of schistosomula of *Schistosoma mansoni in vitro*. J. Clin. Invest., 67: 93-102.

Kazura, J.W., P. de Brito, J. Rabbege and M. Aikawa, 1985. Role of granulocyte oxygen products in damage of *Schistosoma mansoni* eggs *in vitro*. J. Clin. Invest., 75: 1297-1307.

Lapolla, A., P. Traldi and D. Fedele, 2005. Importance of measuring product of non-enzymatic glycation of proteins. Clin. Biochem., 38: 103-115.

Lubinsky, S. and G.C. Bewley, 1979. Genetics of catalase in *Drosophila melangaster* rates of synthesis and degradation of the enzyme in flies aneuploid and cuploid for the structural gene. Genetics, 19: 723-749.

Lucey, D.R., M. Clerici and G.M. Shearer, 1996. Type 1 and type 2 cytokine dysregulation in human infectious, neoplastic and inflammatory diseases. Clin. Microbiol. Rev., 9: 532-562.

Maghraby, S.A., K.H. Shker, H.G. Zahran and M. El-Sherbiny, 2007. *In vivo* the immunological effects of *Fasciola gigantica* worms homogenate mixed with saponin on mice infected with *Schistosoma mansoni*. J. Med. Sci., 7: 724-731.

Mahmoud, M.R., H.S. El-Abhar and S. Saleh, 2002. The effect of *Nigella sativa* oil against the liver damage induced by *Schistosoma mansoni* infection in mice. J. Ethnopharmacol., 79: 1-11.

McCormick, M.L., A. Metwalli, M.A. Railsback, J.V. Weinstock and B.E. Britigan, 1996. Eosinophils from schistosome-induced hepatic granulomas produce superoxide and hydroxyl radical. J. Immunol., 157: 5009-5015.

Metwally, N.S., 2006. Potency of *Allium sativum* and *Allium cepa* oils against *Schistosoma mansoni* infection in mice. Egypt. J. Hospital Med., 23: 319-332.

Misciagna, G., G. de Michele, V. Guerra, A.M. Cisternino, A. di Leo and J.L. Freudenheim, 2004. Serum fructosamine and colorectal adenoma. Eur. J. Epidemiol., 19: 425-432.

Mohamed, A.A., D.E. Abo-Amou, M.A. Shehata and N.E. El-Ashery, 2001. Glutathione peroxidase and nitric oxide in patients with chronic liver diseases. Egypt. J. Schistosomiasis Infect. Endem. Dis., 23: 27-46.

Mohamed, A.M., S.S. Mahmoud and A.A. Farrag, 2008. Influence of sativa seeds against liver fibrosis and consequence complications in murine *Schistosomiasis*. Int. J. Biotechnol. Biochem., 4: 325-346.

Moron, M.S., J.W. Depierre and B. Mannervik, 1979. Level of glutathione, glutathione reductase and glutathione S-transferase activity in rat lung and liver. Biochem. Biophys. Acta, 582: 67-78.

Nabih, I., M. Rizk and A.M. Soliman, 1992. Studies on carbohydrates extracted from native and chemically treated *Biomphalaria alexandrina* snails. Comp. Biochem. Physiol., 102: 397-402.

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

Oliver, L. and M.A. Stirewalt, 1952. Anefficient method for the exposure of mice to cercaria of *Schistosoma mansoni*. J. Parasitol., 38: 19-23.

Parola, M. and G. Robino, 2001. Oxidative stress-related molecules and liver fibrosis. J. Hepatol., 35: 297-306.

Pender, S.L.F., J.M.E. Fell, S.M. Chamow, A. Ashkenazi and T.T. MacDonald, 1998. A p55 TNF receptor immunoadhesin prevents T cell-mediated intestinal injury by inhibiting matrix metalloproteinase production. J. Immunol., 160: 4098-4103.

Poli, G. and M. Parola, 1997. Oxidative damage and fibrogenesis. Free Radic. Biol. Med., 22: 287-305.

Poli, G., 2000. Pathogenesis of liver fibrosis: Role of oxidative stress. Mol. Aspects Med., 21: 49-98.

Sabater, J., C. Quereda, I. Herrera, J. Pascual, J.J. Villafruela and J. Ortuno, 1991. Nonenzymatic glycosylation of hemoglobin and total plasmatic proteins in end stage renal disease. Am. J. Nephrol., 11: 37-43.

Sayed, H.A., A. El-Ayyat, A.A. Kader, H.Y. Sabry and N.M. Amer, 2004. Epidemiology of *Schistosoma mansoni* infection and its relationship to snail distribution in a village at the Nile bank south to Cairo. J. Egypt. Public Health Assoc., 79: 95-113.

Schafer, F.Q. and G.R. Buettner, 2001. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic. Biol. Med., 30: 1191-1212.

Shaheen, A.A., A.A. Abd-El-Fattah and F.A. Ebeid, 1994. Effects of praziquantel treatment on lipid peroxide levels and superoxide dismutase activity in tissues of healthy and *Schistosoma mansoni* infected mice. Arzneimittel Forschung, 44: 94-96.

Shen, D.Z., 2008. A target role for mast cell in the prevention and therapy of hepatic fibrosis. Med. Hypotheses, 70: 760-764.

Silva, L.M., R.M. Menezes, S.A. de Oliveira and Z.A. Andrade, 2003. Comparison of immune responses of *Schistosoma mansoni*-infected mice with distinct chronic forms of the disease. Rev. Soc. Bras. Med. Trop., 36: 335-341.

Soliman, K.M., A.K. El-Ansary and A.M. Mohamed, 2000. Effect of carnosine administration on certain metabolic parameters in bilharzial infected hamsters. J. Egypt. Soc. Parasitol., 30: 455-468.

Torben, W. and A. Hailu, 2007. Serum cytokinese of the 20 Krad-irradiated *S. mansoni* cercariae vaccinated, primary and superinfected *Cercopethicus aethiops* aethiops. Exp. Parasitol., 115: 121-126.

Utzinger, J., S. Xiao, J. Keiser, M. Chen, J. Zheng and M. Tanner, 2001. Current progress in the development and use of artemether for chemoprophylaxis of major human schistosome parasites. Curr. Med. Chem., 8: 1841-1860.

Vassalli, P., 1992. The pathophysiology of tumor necrosis factors. Annu. Rev. Immunol., 10: 411-452.

Vijayan, G., R.C. Sundaram, Z. Bobby, A. Hamide, N. Selvaraj and N.R. Dasse, 2007. Increased plasma malondialdehyde and fructosamine in anemic H pylori infected patients: Effect of treatment. World J. Gastroenterol., 13: 796-800.

Winterbourn, C.C. and H.C. Sutton, 1986. Iron and xanthine oxidase catalyze formation of an oxidant species distinguishable from OH: Comparison with the Haber-Weiss reaction. Arch. Biochem. Biophys., 244: 27-34.

Xiao, S.H., J.Q. You, H.F. Guo, P.Y. Jiao, J.Y. Mei, M.Y. Yao and Z. Feng, 1998. Effect of artemether on glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase and pyruvate kinase of *Schistosoma japonicum* harbored in mice. Zhongguo Yao Li Xue Bao., 19: 279-281.

Yousif, M.F. and N.S. El-Rigal, 2004. *C-glycolsyl flavones* O-glycosides of *Clerodendrum splendens G. Don.* and antioxidants activity in schistosome-infected mice. Egypt J. Biomed. Sci., 14: 128-137.

Zanetti, G., 1979. Rabbit liver glutathione reductase. Purification and properties. Arch. Biochem. Biophys., 198: 241-246.