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EFFECT OF *AILANTHUS ALTISSIMA* AND *ZIZIPHUS SPINA CHRISTI* EXTRACTS ON SOME HEPATIC MARKER ENZYMES AND ANTIOXIDANTS IN *SCHISTOSOMA MANSONI* INFECTED MICE

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Key words: *Ailanthus altissima, Ziziphus spina christi,* hepatoprotective agent, hepatic enzymes, antioxidant, mice, schistosomiasis

The chloroform extract of *Ailanthus altissima* stem bark and the alcoholic extract of *Ziziphus spina christi* roots were tested for their antischistosomal and hepatoprotective effects. The effect of schistosomal infection and treatment with both extracts on the activities of aspartate and alanine aminotransferases, acid phosphatase, 5'nucleotidase, glucose-6-phosphatase, lactate dehydrogenase, alkaline phosphatase and succinate dehydrogenase were estimated as measures of hepatic metabolic function, on free radical production in the form of lipid peroxides and on the levels of certain antioxidants namely, catalase, glutathione, vitamins C and E. In addition, the efficiency of the tested extracts on reducing the worm burden and ova counts in the infected mice was evaluated.

The obtained data revealed that infection with *S.mansoni* increased lipid peroxides and decreased all antioxidant levels. On the other hand, the activities of acid phosphatase and 5` nucleotidase were higher while those of glucose-6-phosphatase, lactate dehydrogenase, alkaline phosphatase and succinate dehydrogenase were lower with respect to control. However, treatment with both *A.altissima* and *Z.spina christi* ameliorated the disturbed lipid peroxides, antioxidants and enzymes' levels to nearly the control values, the chloroform extract of *A.altissima* showing a more pronounced improving effect against liver damage caused by parasitic infection.

This study confirms the recent approach of many researchers for the use of plants in the treatment of liver diseases as alternatives to the classical chemotherapeutic drugs which may provide a low incidence of side effects.

INTRODUCTION

Schistosomiasis continues to rank-following malaria, at the second position of the world's parasitic diseases in terms of the extent of endemic areas and the number of infected people. In Egypt, the disease remains a public health problem despite the continuous control efforts [El Baz *et al*., 2003]. The disease causes tremendous disorders in liver tissue resulting in fibrosis due to spontaneous modulation of the egg granuloma [Farah *et al*., 2000]. In addition, hepatic marker enzymes exhibit marked changes by schistosomal infections. There is yet no vaccine available and most of the antischistosomal drugs have deleterious side effects or low efficacy. However, the current main control is chemotherapy with the drug of choice, praziquantel. In view of concern about the development of tolerance and/or resistance to PZQ, there is a need for research and development of novel drugs for the prevention and cure of schistosomiasis [Sheirz *et al*., 2001]. This approach, however, necessitates a search for new safe and effective drugs [Abo-Madyan *et al*., 2004].

In recent years, researchers have examined the effects of plants used traditionally by indigenous healers and herbalists to support liver function and treat diseases of the liver. In most cases, research has borne out the traditional experience by discovering the mechanisms and modes of action of these plants, as well as confirming the therapeutic effectiveness of certain plants or plant extracts in clinical studies.

Previously, the schistosomicidal properties of the ethanol and acetone extracts of *Pavetta owariensis* and *Harrisonia abyssinieae* were assessed in *S.mansoni* infected mice and showed promising effects on the spleen weight, number of adult worms and eggs and on the size of granulomas [Balde *et al*., 1989]. In addition, 23 plant species used popularly against schistosomiasis in Zimbabwe were screened for their antihelmintic effect [Molgaard *et al*., 2001]. Later, Mahmoud *et al*. [2002] reported that *Nigella sativa* oil may play a role against the alterations caused by *S.mansoni* infection by improving the immunological and biochemical parameters in the host liver. Recently, Yousif & El-Rigal [2004] showed that two C-glycosyl flavone O-glycosides isolated from *Clerodendrum splendens* G. Don leaves possessed strong hepatoprotective effects by improving the reduced levels of hepatic antioxidants in *S.mansoni* infected mice.

In the present study, the chloroform extract of *Ailanthus altissima(Mill)* stem bark and the alcoholic extract of *Zizyphus spina* roots were screened for their hepatoprotective effect since both these extracts were previously reported to possess a variety of biological activities. Thus, Buzina *et al.* [2001] showed that *A.altissima* possessed an *in vitro* antituberculosis activity. Moreover the methanolic extract of the stem barks of *A.excelsa* revealed fungistatic and fungicidal activities [Joshi *et al*., 2003]. Also, extracts and compounds isolated from seedlings of *A.altissima* showed activity against chloroquine-resistant and chloroquine-sensitive strains of

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Plasmodium falciparum [Okunade *et al*., 2003]. In Taiwan, three new quassinoids (highly oxygenated triterpenes) were isolated from *A.altimissa* and were evaluated for their antitumour effects and displayed activity without any cytotoxicity [Tamura *et al*., 2003].

On the other hand, *Zizyphus spina christi* is one of the plants commonly used in Egyptian folk medicine for the treatment of different diseases. Thus, the butanol extract of *Z.spina-christi* leaves showed strong hypoglycemic and antihyperglycemic effects in diabetic rats [Glombitza *et al*., 2002]. Also Adzu *et al*. [2001] revealed an analgesic effect for the *Z.spina-christi* root bark extract in mice and rats. In a related study, Shahat *et al*. [2001] investigated different extracts and fractions of the leaves, fruits and seeds of the plant grown in Egypt and reported that they possessed antiviral, antifungal and antibacterial activities. In a more recent study by Adzu *et al.* [2003], the methanol extract of *Z.spina-christi* stem bark showed an antidiarrhoeal effect in rats.

Based on the previous information on the wide range of biological activities of both *A.altissima* and *Z.spina christi,* the present work was aimed at evaluating new biological activities for both these plants in a trial to open new areas of application of extracts of these plants as novel antischistosomal, antioxidant and hepatoprotective agents in folk medicine.

MATERIAL AND METHODS

Animals. Forty-eight male albino mice weight range (20–25 g) were caged with a free supply of food and water. After acclimatization, they were randomly assigned into six groups of eight mice each and were treated as follows: The 1st group was left uninfected untreated and served as control. The 2nd and 3rd groups were treated with a total dose of ¼ LD_{50} of both the chloroform extract of *A.altissima* stem bark (500 mg/kg b.w., using serial concentrations ranging from 100–4000 mg/kg b.w) and 70% ethanolic root bark extract of *Z.spina christi* (560 mg/kg b.w. according to Adzu *et al*. [2001]) respectively five times weekly for one month respectively, starting three months from the beginning of the experiment. The 4th group was infected with cercariae of the Egyptian strain *S.mansoni* by the tail immersion method and were kept to develop liver granuloma for four months. The 5th and 6th groups were infected with *S.mansoni*, left for three months, then treated with a total dose of ¼ LD_{50} of both the chloroform extract of *A.altissima* stem bark (500 mg/kg b.w, using serial concentrations ranging from 100–4000 mg/kg b.w) and 70% ethanolic root bark extract of *Z.spina christi* (560 mg/kg b.w. according to Adzu *et al.* [2001]) respectively five times weekly for one month.

Mice of all groups were sacrificed after four months. Appropriate anesthetics and sacrifice procedures were followed ensuring that animals did not suffer at any stage of the experiments. Anaesthetic procedures were complied according to legal ethical guidelines approved by the Ethical Committee of the Federal Legislation and National Institute of Health Guidelines in USA. An overdose of ether was given gradually to mice and then the abdomen was opened by a mid-line incision and livers were separated. The livers of infected groups were perfused for worm [Smithers & Terry, 1965] and ova counts [Cheever & Anderson, 1971], and then livers of all groups were subjected to the different antioxidant and hepatoprotective assays.

Antioxidant experiments were performed on fresh liver tissues whereas samples of liver tissues from the different mice groups were stored frozen at -80°C throughout a one-week period for the hepatic marker enzyme activities.

Extraction of plant material. One kg of *A.altissima* stem bark and *Z.spina christi* root bark were extracted with chloroform and 70% alcohol, respectively, in a continuous extraction Soxhlet apparatus till exhaustion, and the extracts were concentrated under reduced pressure. The extracts were phytochemically screened and the results showed the presence of alkaloids, sterols and/or triterpenes in both extracts, while saponins were also present in *Z.spina christi* extract.

BIOCHEMICAL ASSAYS

Antioxidant evaluation

Lipid peroxides. One volume of saturated thiobarbituric acid in 10% perchloric acid was added to three volumes of 20% trichloroacetic acid (working solution). Malondialdehyde (MDA), the end product of lipid peroxidation, is formed by the reaction of lipid peroxides present in 20% tissue homogenate with thiobarbituric acid and boiling to give a pink-coloured product that is read at 535 nm [Okhawa *et al*., 1979]. Malondialdehyde concentration was calculated using the extinction coefficient value of $1.56 < 10^5$ mol/L^{1}cm^{-1} [Buege & Aust, 1978].

Glutathione. GSH was estimated in 20% tissue homogenate by the method of Moron *et al*. [1979] using 0.6 mmol dithiobisnitrobenzoic acid (DTNB) dissolved in 0.2 mol/L phosphate buffer (pH 8). The colour developed in the supernatant after centrifugation was read against blank at 412 nm within 5 min and glutathione was calculated as mg/g tissue from a standard curve plotted for serial concentrations of glutathione (5–100 μg).

Estimation of vitamin C. Vitamin C was estimated by the method of Jagota & Dani [1982] by mixing 2 mol/L Folin-ciocalteu reagent and 10% TCA with 20% tissue homogenate. The blue colour developed in the supernatant after centrifugation was read at 760 nm after 10 min. The amount of ascorbic acid was calculated from a standard curve of vitamin C using 5–70 μg serial concentrations of the vitamin.

Estimation of vitamin E. Vitamin E was measured by the colorimetric assay using the method of Augustin *et al.* [1985]. The method is based on the oxidation of xylene-extracted tocopherols of the liver homogenate by ferric chloride and the pink complex of ferrous ions with bathophenanthoroline is measured colorimetrically at 536 nm.

Estimation of catalase. Catalase activity was assayed according to Lubinsky & Bewley [1979]. The reaction for assaying catalase activity was initiated by adding 20 μL of 5% liver homogenate to 2.53 mL of the reaction mixture (0.1 mol/L sodium potassium phosphate buffer, pH 6.8 mixed with 120 mL H_2O_2). The disappearance of hydrogen peroxide was monitored by following the decrease in absorbance at 230 nm using molar extinction coefficient for hydrogen peroxide of 62.4 [Nelson & Kiesow, 1972].

Hepatic marker enzymes

Alanine and aspartate aminotransferases. ALT and ALT were measured by the Reitman & Frankel method [1957] using the bioMerieux kit. The colorimetric determination of

ALT and AST depends on determining amounts of oxaloacetate and pyruvate formed from the 2, 4-dinitrophenylhydrazine of oxaloacetate and pyruvate, the colour of which is read at 520 nm.

Lactate dehydrogenase. LDH was measured by monitoring the increasing intensity of NADH production as a result of oxidation of lactate to pyruvate. Reduction of NAD is coupled with reduction of tetrazolium salt (INT) with phenazine methosulphate as electron carrier; the result is the formation of formazan of INT. The developed colour is read at 503 nm [Babson & Babson, 1973].

Acid phosphatase. The enzyme was estimated by the method of Wattiaux & De Duve [1956] using Fiske-Subbarow reducing agent and sodium β-glycerophosphate as a substrate in the presence of Triton-X-100. The liberated inorganic phosphate was read at 660 nm.

5`Nucleotidase. The assay was carried out by the method of Bodansky & Schwartz [1963] depending on the hydrolysis of 5'AMP to adenine and inorganic phosphate in the presence of $MgCl_2$ as a catalyst. The developed colour of liberated inorganic phosphate was read colorimetrically at 660 nm using Fiske-Subbarow as the reducing agent.

Succinate dehydrogenase. The enzyme catalyses the oxidation of succinate to fumarate and reduction of FAD coupled with reduction of tetrazolium salt (INT). Tris buffer (0.2 mol/L) was added to 0.5 mol/L sodium succinate and 0.02 mol/L INT. The produced formazan was measured at 490 nm [Shelton & Rice, 1957].

Glucose-6-phosphatase. The enzyme is measured by the method of Swanson [1955] which depends on measuring the liberated inorganic phosphate from glucose-6-phosphate as a substrate using Fiske-Subbarow as a reducing agent, 0.2 mol/L tris buffer 0.001 mol/L sodium sulphate and 0.1 mol/L substrae reagent. The developed colour was read at 660 nm.

Alkaline phosphatase. The enzyme was estimated by the method of Belfield & Goldberg [1971] using disodium phenyl phosphate and 0.05 mol/L carbonate-bicarbonate buffer in a mixture of 1:2 (pH 10). The liberated phenol was measured at 510 nm in the presence of amino-4-antipyrene, sodium arsenate and potassium ferricyanide as the colour reagent.

Antischistosomal activity. Adult *S.mansoni* worms were recovered from the hepatic portal system and the liver by the perfusion technique and the number of ova/g liver tissue was calculated.

Estimation of protein. Protein was estimated by the method of Bradford [1976]. Bradford solution was added to 5% liver homogenate of the different mice groups and the blue colour developed was measured after 5 min at 595 nm in a spectrometer (Novaspec, LKB, Sweden) against blank containing water instead of the homogenate. The amount of protein was calculated from a standard curve using serial concentration of bovine serum albumin (1-10 μg).

Statistical analysis. Data were expressed as mean±S.D. One way analysis of variance (ANOVA) followed by LSD was used to show the statistical significance among the different groups. The Statistical Package for the Social Sciences (SPSS) version 8.0 was used in data analysis. The probability level of significance (p) was determined where $p<0.005$ was considered significant.

RESULTS

Table 1 and Figures 1 and 2 demonstrate the effect of both *A.altissima* and *Z.spina christi* extracts on the hepatic antioxidants of *S.mansoni* infected mice. The data obtained revealed that both extracts significantly ameliorated the elevated level of the lipid peroxides and the decreased levels of glutathione, vitamin C, vitamin E and catalase induced by infection. However, the chloroform extract of *A.altissima* caused a more pronounced improvement in the measured parameters.

Table 2 and Figures 3 and 4 show the effect of both extracts under study on the hepatic marker enzymes of *S.mansoni* infected mice. The results indicate that infection resulted in a significant increase in the activities of acid phosphatase and 5'nucleotidase and a significant decrease in the activities of glucose-6-phosphatase, lactate dehydrogenase and succinate dehydrogenase. Treatment with the two test extracts restored the activities of these enzymes to values near to normal.

TABLE 1. Effect of *Ailanthus altissima* and *Ziziphus spina christi* extracts on hepatic antioxidants in control and *Schistosoma mansoni* infected mice.

Parameters	Control	Control-chloroform	Control-ethanol	Infected	Infected-chloroform	Infected-ethanol	ANOVA
	G_1	G_2	G_3	G_4	G_5	G_6	
Lipid peroxide	0.77± 0.05 (4,5,6)	0.81 ± 0.04 (4,5,6)	0.80± 0.02 (4,5,6)	1.192 ± 0.03 (1,2,3,4,6)	0.87 ± 0.02 (1,2,3,4,6)	1.09 ± 0.03 (1,2,3,4,5)	< 0.0001
Glutathione	27.24 ± 0.61 (3,4,5,6)	27.08 ± 0.60 (3,4,5,6)	24.95 ± 0.72 (1,2,4,5,6)	15.98± 0.43 (1,2,3,5,6)	24.09 ± 0.52 (1,2,3,4,6)	20.22 ± 0.53 (1,2,3,4,5)	< 0.0001
Vitamin C	7.63 ± 0.31 (3,4,5,6)	7.37 ± 0.27 (4,5,6)	7.11 ± 0.28 (1,4,5,6)	5.32 ± 0.14 (1,2,3,5,6)	6.38 ± 0.15 (1,2,3,4,6)	5.93 ± 0.19 (1,2,3,4,5)	< 0.0001
Vitamin E	5.51 ± 0.06 (2,3,4,5,6)	5.10 ± 0.07 (1,4,6)	5.19± 0.12 (1,4,5,6)	3.41 ± 0.10 (1,2,3,5,6)	4.99 ± 0.14 (1,3,4,6)	4.3 ± 0.15 (1,2,3,4,5)	< 0.0001
Catalase	59.17± 1.55 (4,5,6)	57.74 ± 1.43 (4,5,6)	58.41 ± 2.65 (4,5,6)	35.55 ± 2.23 (1, 2,3,5,6)	48.66 ± 1.59 (1,2,3,4,6)	44.81 ± 1.87 (1,2,3,4,5)	< 0.0001

Data are means ± SD of six mice in each group. Values of Lipid peroxide are expressed as nm /mg protein, glutathione and vitamin (C &E) are expressed as μg /mg protein and Catalase are expressed as μmol /mg protein. P is level of significance, where $p<0.005$ is significant. Analysis of data is carried out by one way (ANOVA) (analysis of variance) accompanied by post hoc (spss computer programme).

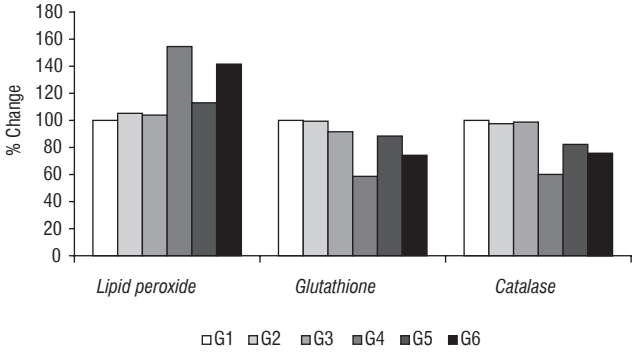

FIGURE 1. Percentage change in lipid peroxides, glutathione and catalase in different mice groups as compared to control.

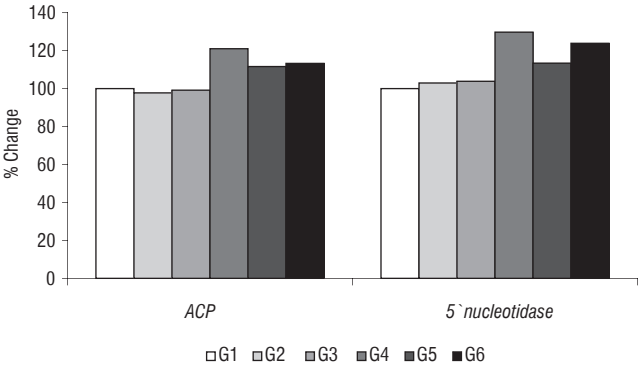

FIGURE 3. Percentage change in acid phosphatase (ACP) and 5`nucleotidase in mice livers of different groups as compared to control.

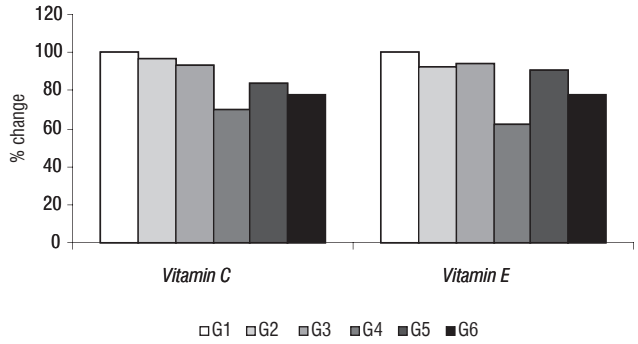

FIGURE 2. Percentage change in vitamin C and vitamin E in different mice groups as compared to control.

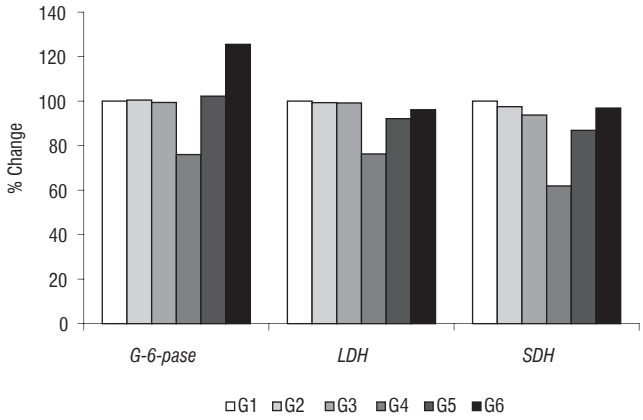

FIGURE 4. Percentage change of G-6-pase, LDH and SDH in mice livers of different groups as compared to control.

Table 3 and Figures 5 and 6 illustrate the efficiency of the two extracts under study as antischistosomal agents by the reduction in worm burden and egg counts and also by the improvement of the liver function enzyme levels, AST, ALT and alkaline phosphatase. The results obtained demonstrated the potency of these extracts with *A.altissima* chloroform extract revealing a more pronounced effect on all parameters while *Z.spina christi* ethanolic extract showing a more normalizing effect on alkaline phosphatase.

DISCUSSION

Previous studies have shown that the host's response to *Schistosoma mansoni* infection involves the production of reactive oxygen species [El-Sokkary *et al*., 2002]. The data in

TABLE 2. Effect of *Ailanthus altissima* and *Ziziphus spina christi* extracts on hepatic marker enzymes in control and *Schistosoma mansoni* infected mice.

Parameters	Control	Control-chloroform	Control-ethanol	Infected	Infected-chloroform	Infected-ethanol	ANOVA
	G_1	G_2	G_3	G_4	G_5	G_6	
Acid phosphatase	7.23± 0.37 (2,4,5,6)	6.85±0.20 (1,3,4,5,6)	7.17 ± 0.06 (2,4,5,6)	8.75± 0.29 (1,2,3,5,6)	8.07± 0.11 (1,2,3,4)	8.19± 0.07 (1,2,3,4)	< 0.0001
5`Nucleotidase	25.26 ± 0.72 (2,3,4,5,6)	25.89 ±0.46 (1,3,4,5,6)	24.29 ± 0.55 (1,2,,4,5,6)	32.77± 0.27 (1,2,,3,5,6)	28.65 ± 0.26 (1,2,,3,4,6)	31.29± 0.65 (1,2,3,4,5)	< 0.0001
Glucose-6-phosphatase	54.76 ±1.67 (4 ,6)	55.03±0.89 (4 ,6)	54.44 ±1.84 (4,6)	41.62 ± 0.45 (1,2,,3,5,6)	55.98 ± 0.98 (4,6)	68.73± 1.62 (1,2,,3,4,5)	< 0.0001
Lactate dehydrogenase	22.21 ± 0.56 (4,5,6)	22.06 ±0.44 (4,5,6)	22.03 ± 0.14 (4,5,6)	16.94 ± 0.17 (1,2,3,5,6)	20.46± 0.43 (1,2,,3,4,6)	21.35 ± 0.45 (1,2,3,4,5)	< 0.0001
Succinate dehydrogenase	0.16± 0.01 (3,4,5)	0.156± .002 (4,5)	0.148 ± 0.01 (1,4,5)	0.099± 0.01 (1,2,3,5,6)	0.139 ± 0.01 (1,2,3,4,6)	0.155± 0.004 (4,5)	< 0.0001

Data are means ± SD of six mice in each group. Values of the enzymes are expressed as μmole/min. /mg protein. p is level of significance, where $p<0.005$ is significant. Analysis of data is carried out by one way (ANOVA) (analysis of variance) accompanied by post hoc (spss computer programme).

TABLE 3. Effect of *Ailanthus altissima* and *Ziziphus spina christi* extracts on liver function enzymes (AST, ALT, ALP), egg count and worm burden in *Schistosoma mansoni* infected mice.

Parameters	Control	Control-chloroform	Control-ethanol	Infected	Infected-chloroform	Infected-ethanol	ANOVA
	G_1	G_2	G_3	G_4	G_5	G_6	
AST	11.15 ± 0.29 (3,4,5,6)	10.96± 0.59 (3,4,5,6)	12.23 ± 0.20 (1,2,4,5,6)	6.76 ± 0.49 (1,2,3)	7.43 ± 0.56 (1,2,3,6)	7.72 ± 0.69 (1,2,3,5)	< 0.0001
ALT	8.22 ± 0.41 (2,4,5,6)	7.02 ± 0.27 (1,3,4,5,6)	8.71 ± 0.24 (2,4,5,6)	5.50 ± 0.45 (1,2,3)	5.49 ± 0.46 (1,2,3,6)	6.19 ± 0.67 (1,2,3,5)	< 0.0001
ALP	1.92 ± 0.28 (4,5,6)	1.79 ± 0.21 (4,5,6)	1.76 ± 0.008 (4,5,6)	0.978 ± 0.006 (1,2,3,5)	1.412 ± 0.006 (1,2,3,4)	1.77 ± 0.002 (1,2,3)	< 0.0001
Egg count	–	–	–	$9.11x10^3$ ± 0.53 (5,6)	$5.16x10^3$ ± 0.73 (4,6)	$5.45x10^3$ ± 2.4 (4,5)	< 0.0001
Worm burden	–	–	–	20.3 ± 2.6 (5,6)	10.5 ± 1.9 (4,6)	14.17 ± 1.4 (4,5)	< 0.0001

Data are means ± SD of six mice in each group. Values of the enzymes are expressed as μmol/min /mg protein. P is level of significance, where $p<0.005$ is significant. Analysis of data is carried out by one way (ANOVA) (analysis of variance) accompanied by post hoc (spss computer programme). Egg count are expressed /gm tissue of liver.

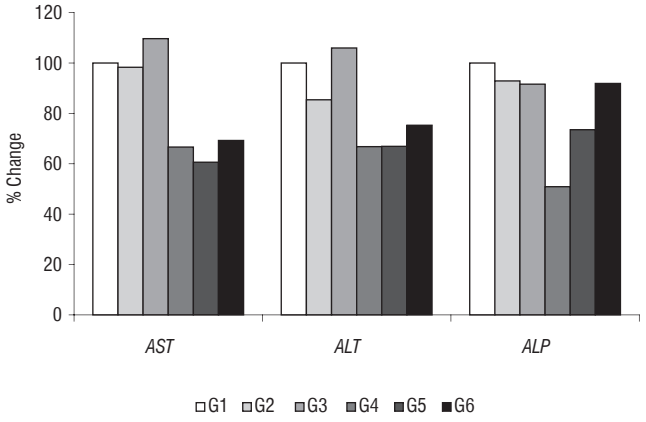

FIGURE 5. Percentage change in AST, ALT and ALP in different mice groups as compared to control.

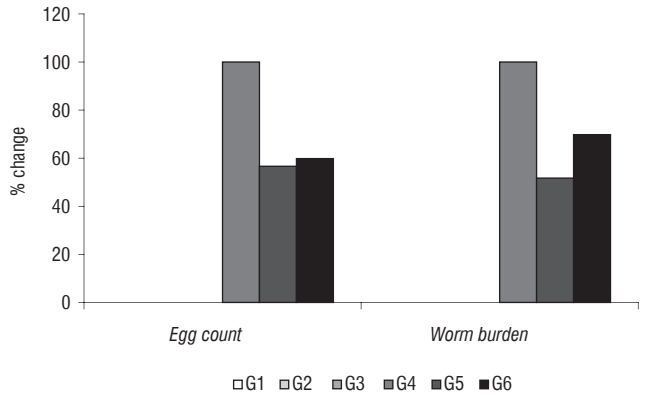

FIGURE 6. Percentage change in egg count and worm burden in different mice groups as compared to infected group.

the present work reveal that *S.mansoni* infection caused an elevation in lipid peroxides while glutathione, vitamin C, vitamin E and catalase activity were significantly reduced. These results suggest that infection with *S.mansoni* impairs the antioxidant system since the level of GSH depletion is used as an index of oxidative stress and a sign that hepatic cells are utilizing more antioxidant defences [Ip, 2000]. In a good agreement with the obtained data, Pascal *et al*. [2000] and Soliman *et al*. [2000] reported that oxidative stress due to schistosomiasis causes an elevation in lipid peroxides. On the other hand, Gharib *et al*. [1999] showed that liver GSH was drastically depleted in mice infected with *S.mansoni* due to increased cytotoxicity with H_2O_2 which is produced as a result of inhibition of glutathione reductase, that keeps glutathione in the reduced state.

With regards to vitamin C and coinciding with the present results, Frei *et al*. [1988] reported that peroxyl radicals are trapped by ascorbate and hence the level of the enzyme decreased during the free radical scavenging process. Also, the reduction in vitamin E after schistosomal infection occurs since the vitamin acts as a soluble antioxidant to protect biological membranes against oxidative stress which leads to disruption of cell function. In a related study, Sokol *et al*. [1998] reported that vitamin E protects hepatocytes against lipid peroxidation and toxic injury.

During oxidative stress, such as in the case of schistosomal infection, peroxide dismutation yields H_2O_2, which is detoxified by catalase and thus results in decline in its activity as previously indicated by Gharib *et al*. [1999]. Also Hahn *et al*. [2001] reported the participation of catalase in scavenging H_2O_2.

Administration of both the chloroform and ethanolic extracts to non-infected mice revealed a non toxic effect since the levels of the measured parameters were nearly similar to normal values. However, administration of these extracts to infected mice ameliorated the levels of these parameters since lipid peroxides level was reduced while vitamin C, vitamin E and catalase activity were elevated with respect to the infected groups, although the obtained data did not reach the normal values.

In accordance with this, Rizk [1998] reported the protective stress of the alcoholic extract of *Curcuma longa* against oxidative stress in *S. mansoni* infected mice and showed that this extract improved the elevated lipid peroxides and reduced glutathione, vitamin C, vitamin E and cat-

alase observed in the infected mice groups. Also, El-Sokkary *et al*. [2002] showed that melatonin reduces oxidative damage and increases survival of mice infected with *S.mansoni* and improved levels of lipid peroxides, glutathione vitamin E and superoxide dismutase. In a recent study, Yousif & El-Rigal [2004] studied the antioxidant activity of the aqueous and the flavone-containing fractions of the leaves of *C splendens G.* Don and reported that the aqueous fraction showed a more antioxidant powerful activity, probably due to a synergistic effect of all its constituents including the flavone-containing fraction, to diminish the adverse effects of free radicals on hepatic cells.

The data obtained in the present work for the hepatic marker enzymes revealed a marked increase in the activity of acid phosphatase and 5'nucleotidase in *S.mansoni* infected mice while the activities of aspartate and alanine aminotransferases, glucose-6-phosphatase, lactate dehydrogenase and succinate dehydrogenase were reduced.

With respect to transaminases, a significant reduction was observed in both AST and ALT activities following schistosomal infection while treatment with the two test extracts induced a moderate increase. As previously mentioned, the free radicals are elaborated by schistosomal infection and this may cause irreversible damage to the mitochondrial membrane which may lead to discharge of its enzyme content. In this regard, Ozares *et al*. [2003] stated that these enzymes were decreased relative to the lowered liver protein content either due to their release to the blood stream or to decreased synthesis. Since aminotransferases are marker enzymes for cell toxicity, this gives an additional support on the liver injury induced by infection. This hepatocellular damage results from egg deposition resulting in cell fibrosis and/or increased cell permeability leading to enzyme discharge to the blood stream [El-Shazly *et al*., 2001].

Concerning acid phosphatase and 5`nucleotidase, the elevation in their activity may be due to tissue catabolism resulting from increased worm and egg toxins of infection and due to the deranged metabolic functions as a result of liver injury. In a related study, Rizk [1998] indicated that all lysosomal enzymes are activated in conditions of increased tissue catabolism leading to enhancement of phagocytic phenomenon. The results also confirmed the earlier studies by Rodrigues [1988] who observed changes in the lysosomal membrane of *S.mansoni* infected mice which are provoked by the catabolites excreted by immature or adult worms present in the portal venous system.

The depletion in hepatic microsomal glucose-6-phosphatase was previously reported by Rizk [1998] who described a marked decrease in glycogen content due to disturbance in glycogen synthesis with progressive decrease in glucose-6-phosphatase activity in cases of heavy schistosomal infections. Also the decrease in the enzyme activity resulted from hepatic hypoglycemia occurring during *S.mansoni* infection, resulting from the inability of hepatic cells to dephosphorylate glucose-6-phosphate into glucose [Metwally & Fahim, 1994].

Lactate dehydrogenase is a glycolytic enzyme located in the cytoplasm. In the present work the depletion in enzyme activity in the direction of lactate oxidation may be correlated with glycogen depletion confirming inhibition of aerobic glycolysis and stimulation of anaerobic glycolysis induced by the developing parasite. This aerobic-anaerobic switch was previously reported by Tielens [1994, 1997] on the Crabtree effect of schistosomes through which lactate is accumulated and glycogen is depleted.

Concerning mitochondrial SDH, the activity of the enzyme was reduced followed by infection. This was supported by Van Hellemond & Tielens [1994] who reported that both SDH and Kreb's cycle enzyme activities are repressed when limiting amounts of oxygen are present, whreas Volpi *et al*. [1997] added that the increased NADH/NAD ratio causes shift to the left in the equilibrium of the oxidoreductive couple malate-oxaloacetate resulting in depression of citric acid cycle and may contribute to SDH lowered activity.

It should be pointed out that treatment with the test extracts significantly ameliorated the levels of the hepatic marker enzymes and decreased the derangements in the different subcellular fractions.

Previous research in the area of exploring new extracts from plant materials has proved a promising approach for the treatment of various diseases. Zaoui *et al*. [2002] studied the effect of *Nigella sativa* seed fixed oil on key hepatic enzymes in mice and supported the traditional use of this extract as a treatment of the dyslipedemia and hypoglycemia related abnormalities. A related study by Mahmoud *et al*. [2002] revealed that the same extract induced a hepatoprotective effect in liver damage caused by *S.mansoni* by ameliorating the liver function, the redox state and the liver granuloma.

In addition, El-Sokkary *et al*. [2002] measured alkaline phosphatase, total protein, albumin and other antioxidant parameters as evidences of liver damage in *S.mansoni* infected mice and concluded that melatonin administration prevented most of the changes that occurred. In a more recent study, Abo-Madyan *et al*. [2004] evaluated the efficacy of Mirazid (the resin extract from Myrrh of *Commiphora molmol* tree) on the treatment of both *S.mansoni* and *S.hematobium* and reported that the extract proved to be safe and very effective.

In good coincidence with the present data, Glombitza *et al*. [2002] reported that the butanol extract of *Z.spina-christi* leaves reduced the serum glucose level, liver phosphorylase and glucose-6-phosphatase activities in diabetic rats and also increased serum pyruvate level and glycogen content.

The present work was further extended to investigate the antischistosomal activity of the tested extracts by measuring the reduction in the worm and egg counts. It was found that both extracts exerted significant effects, the chlororoform extract showing a more potent activity. Similar studies have revealed the efficiency of different plant extracts on reducing the number of developed worms and the egg count of mature parasites. Thus, Massoud *et al*. [2004] and Haridy *et al*. [2003] indicated that mirazid is an effective fasciolicidal drug and also reported its use in treatment of *Schistosoma hematobium.* Also, Hassan *et al*. [2003] showed that the same drug caused disruption of *Schistosoma mansoni* worms.

CONCLUSIONS

It could be concluded that *Schistosoma* parasite generates free radicals in the infected host and these disorders are ameliorated by treatment with the two natural extracts under

study. Current estimates indicate that about 80% of people in developing countries still rely on traditional medicine based largely on various species of plants and animals for their primary healthcare. As a consequent, thirty per cent of the worldwide sales of drugs is based on natural products. Thus, the use of natural extracts as alternatives to the chemically synthesized formulations may prove a successful tool in drug technology for treatment of different diseases. Both the test extracts under study proved potent antioxidant and hepatoprotective activities and may be applied in folk medicine for improving liver disorders.

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