Efficacy of *Citrus reticulata* and Mirazid in treatment of *Schistosoma mansoni*

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This work has been carried out to investigate the effect of Schistosoma mansoni *infection on mice livers after treatment with the ethanolic extract of* Citrus reticulata *root or the oleo-resin extract from Myrrh of* Commiphora molmol *tree (Mirazid), as a new antishistosomal drug. Marker enzymes for different cell organelles were measured; succinate dehydrogenase (SDH); lactate dehydrogenase (LDH) and its isoenzymes; glucose-6-phosphatase (G-6-Pase); acid phosphatase (AP) and 5'- nucleotidase. Liver function enzymes; aspartate aminotransferase (AST); alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were also estimated. Parasitological studies through ova count and worm burden will also be taken into consideration. The results showed a marked reduction in SDH, LDH, AST, and ALT enzyme activities and a significant increase in G-6-Pase, AP, 5'- nucleotidase, and ALP after* S. mansoni *infection. A noticeable alteration in LDH subunits were also noticed. Treatment with* C. reticulata *or Mirazid improved all the previous enzyme activities with a noticeable reduction in ova count and worm burden.*

Key words: *Citrus reticulata* - Mirazid - schistosomiasis - enzymes, worm burden - ova count

Schistosomiasis, a chronic and debilitating parasitic disease, affects approximately 200 million people in the developing world and imposes a substantial public health and economic impact, despite the continuous control efforts (Wang et al. 2004).

Current control of the disease by chemotherapeutic agents is impractical because of the common occurrence of re-infection after treatment due to the relative resistance of the larval stages of *Schistosoma mansoni* to schistosomicide drugs (Silva et al. 2003). Praziquantel, the currently used drug for chemotherapeutic control, was reported to induce hemorrhage in the lung tissue of the host (Flisser & McLaren 1989) as well as abdominal pain and diarrhea (Kabatereine et al. 2003). The new trends nowadays is the use of natural plant extracts as new safe and effective drugs.

In this study, *Citrus reticulata* root extract and Myrrh from *Commiphora molmol* tree (Mirazid) are evaluated for their antischistosomal activity. Extract of *C. reticulata* roots has been reported as anticancer (Manthey & Guthrie 2002), antibacterial (Tkachenko et al. 1999) as well as antioxidants activity (Hara et al. 2004). In addition, Withman et al. (2005) recorded the ability of citrus fruit-derived flavonoids to reduced plasma cholesterol concentration. Moreover, commiphora extract has been reported as a new safe and effective drug against *S. mansoni* and *S. heamatobium* (Abo-Madyan et al. 2004).

The present study is a trial to clarify the antischistosomal effect of *C. reticulata* root extract compared to commiphora extract (Mirazid). Enzyme markers for different cell organelles were measured in liver of *S. mansoni* infected mice; succinate dehydrogenase (SDH) for mitochondria; lactate dehydrogenase (LDH), and its isoenzymes for cytoplasm; glucose-6-phosphatase (G-6-Pase) for microsomes; acid phosphatase (AP) for lysosomes and 5'- nucleotidase for plasma membrane. Liver function enzymes; aspartate aminotransferase (AST); alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were also measured. Parasitological studies through ova count and worm burden will take into consideration.

MATERIALS AND METHODS

Chemicals - All chemicals used in the present study were of high analytical grade, products of Sigma (US), Merck (Germany), BDH (England).

Mirazid (the oleo-resin extract from Myrrh of *C. molmol* tree, family: Burseraceae) is a product of Pharco Pharmaceutical Company, Egypt.

Animals - The animals used were intact male Swiss albino mice of CDI strain of similar age (8 weeks) and weight (18-20 g). They were obtained from Theodor Bilharz Research Institute, Cairo, Egypt. Animals were kept in a controlled environment and were maintained on water and stock commercial pellet diet ad libitum.

Plant material - C. reticulata (Family: Rutaceae) roots were collected from Modereyet El Tahrir, Behera, Egypt. It was authenticated by Dr Mohamed Abdel Ghaffar, Faculty of Agriculture, Al- Azhar University, Egypt. A voucher specimen is deposited at Chemistry of Natural Compounds Dept., National Research Center, Dokki, Cairo, Egypt.

Extraction and isolation - Air dried powered roots of *C. reticulata* (0.85 kg) were extracted with 80% ethyl alcohol. The ethanolic extract was evaporated and the aqueous residue extracted sequentially thrice with equal volumes of n-hexane, ether, ethyl acetate, and n-butanol. The ethyl acetate extract was evaporated to dryness. The residue monitored by TLC using precoated silica gel 60 F254 aluminium sheets (0.2 mm thickness, Merk), was found to

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contain flavonoids. The phenolic residue was subjected to biochemical determinations.

Doses and route of administration - Oral dose of 10 µg/ml/mice of the phenolic extract of *C. reticulata* root was given daily for three consecutive weeks which the more effective dose as described by (Nogata et al. 2001).

Oral dose of Mirazid (600 mg/kg body weight) was given for three consecutive days on empty stomach, at least 1 h before meal regarding to the concentration of Mirazid illustrated in the brochure of the drug (Haridy et al. 2003).

Experimental design - The animals were divided into six groups each of eight mice. Group 1: normal healthy control. Group 2: received *C. reticulata* extract daily for three weeks, left one week and sacrificed. Group 3: received Mirazid daily for three days, left for 27 days and sacrificed. Group 4: *S. mansoni* infected mice with 100 cercariae of Egyptian strain with tail immersion technique (Oliver & Stirewalt 1952) and sacrificed after two months. Group 5: received *C. reticulata* extract daily for three weeks post two months of *S. mansoni* infection. Mice left for one week after treatment and sacrificed. Group 6: received Mirazid daily for three days after two months of *S. mansoni* infection. Animals left for 27 days after treatment and sacrificed.

Preparation of tissue homogenates - Liver tissue was homogenized in 0.9N NaCl by a ratio 1:10 w/v for estimation of all enzymes under investigation, while it was homogenized in 0.01M tris-glycine buffer by a ratio 1:10 w/v for estimation of LDH isoenzymes, where 100 µg protein was applied to each gel.

Parameter assays - Enzyme activities were evaluated using end point assay method. SHD: the reduction of FAD is coupled with a reduction of tetrazolium salt as INT, the produced formazan of INT is measured colorimetrically at 490 nm (Shelton & Rice 1957). LDH: the reduction of NAD coupled with the reduction of tetrazolium salt with PMS serving as an intermediate election carrier, the resulted formazan of INT was measured colorimetrically at 503 nm (Babson & Babson 1973). LDH isoenzymes (Dietz & Lubrano 1967). The three enzymes, G-6-Pase, AP, and 5'-nucleotidase were measured colorimetrically at 660 nm through measuring the inorganic phosphorus release (Swanson 1955, Wattiaux & De Duve 1956, Bodansky & Schwartz 1963, respectively). AST and ALT were estimated through measuring oxaloacetate and pyruvate produced respectively (Reitman & Frankel 1957). ALP were measured as a liberated phenol in the presence of amino-4-antipyrine and sod-arsenate as a blocking agent and potassium ferricyanide as a color reagent. The developed color measured at 510 nm (Kind & King 1954). Total protein was estimated by the method of Bradford (1976) using Coomassie Blue Dye in the present of bovine serum albumin. The developed color was measured at 595 nm.

Worm counting - Worms were recovered by liver perfusion as described by Smithers and Terry (1965). The percent of reduction in worm number after challenge was calculated by the method of Tendler et al. (1986) as follows :

$$P = C - V/C \times 100$$

where P = % of protection, C = mean number of parasite recovered from infected animals and V = mean number of parasite recovered from treated animals.

Ova count - The number of ova/g tissue was counted by the method of Cheever and Anderson (1971), where

$$\text{Number of ova in 1 g of liver} = \frac{\text{Number of ova in 5 ml KOH}}{\text{Weight of liver in grams recorded before digestion in KOH}}$$

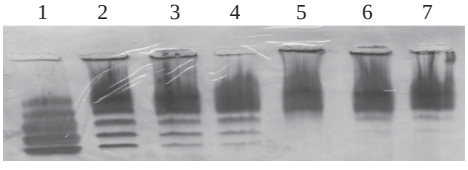
Statistical analysis - Data in the present study are presented as mean ± S.D. Statistical significance values were determined by one way analysis of variance (ANOVA) accompanied by post-hoc (SPSS Computer Program).

RESULTS

Table I illustrates significant reduction of SDH and LDH, while significant increase in G-6- Pase, AP, and 5'-nucleotidase enzyme activities of *S. mansoni* infected mice. Infected treated mice recorded amelioration levels after treatment with *C. reticulata* and Mirazid amounting 16.27 and 23.25% for SDH, 8.40 and 15.29% for LDH, 56.15, and 60.91% for G-6- Pase, 12.82 and 17.94% for AP, 10.91 and 14.54% for 5'-nucleotidase, respectively. Normal healthy control mice received *C. reticulata* and Mirazid showed insignificant changes.

Table II and the Figure show significant variation in LDH subunits in *S. mansoni* infected mice. Treatment of infected mice with *C. reticulata* and Mirazid recorded improvement in LDH_4 and LDH_5, the subunits responsible for liver inflammation, by 74.10 and 75.02% for LDH_4 and 14.66 and 14.84% for LDH_5, respectively. Insignificant changes were recorded in LDH_4 and LDH_5 after treatment of healthy normal mice with both plant extracts.

Table III demonstrates significant reduction in AST and ALT, while a significant increase in ALP was recorded after bilharzial infection. *S. mansoni* infected mice treated with *C. reticulata* and Mirazid show enhancement levels amounting 25.93 and 29.56% for AST, 27.28, 38.98% for ALT, and 20.16, 29.83% for ALP, respectively. Healthy control mice administered with both extracts recorded insignificant change.



Electophoretic separation pattern of lactata dehydrogenase isoenzymes in mice liver. 1: hearth (standard); 2: control liver; 3: control treated with *Citrus reticulata* extract; 4: control treated with Mirazid; 5: infected; 6: infected treated with *C. reticulata* extract; 7: infected with Mirazid.

TABLE I

Effect of *Citrus reticulata* and Mirazid on different marker enzymes in mice livers

Enzymes	Control	Control+ *C. reticulata*	Control + Mirazid	Infected	Infected + *C. reticulata*	Infected+ Mirazid	Improvement percent		ANOVA
	(1)	(2)	(3)	(4)	(5)	(6)	*C. reticulata*	Mirazid	
Succinate dehydrogenase	0.43 ± 0.03 (4, 5, 6)	0.39 ± 0.02 (4, 5)	0.40 ± 0.03 (4, 5, 6)	0.28 ± 0.03 (1, 2, 3, 5, 6)	0.35 ± 0.01 (1, 2, 3, 4, 6)	0.38 ± 0.02 (1, 3, 4, 5)	16. 27	23.25	0.0001
Lactate dehydrogenase	67.20 ± 2.61 (4, 5, 6)	65.26 ± 2.17 (4, 5, 6)	66.70 ± 2.20 (4, 5, 6)	52.60 ± 1.86 (1, 2, 3, 5, 6)	58.25 ± 1.44 (1, 2, 3, 4, 6)	62.70 ± 1.33 (1, 2, 3, 4, 5)	8.40	15.29	0.0001
Glucose-6-Pase	16.40 ± 0.47 (4, 5, 6)	17.21 ± 0.81 (4, 5, 6)	17.00 ± 0.52 (4, 5, 6)	28.71 ± 0.87 (1, 2, 3, 5, 6)	19.50 ± 0.63 (1, 2, 3, 4, 6)	18.72 ± 0.42 (1, 2, 3, 4, 5)	56.15	60.91	0.0001
Acid phosphatase	0.39 ± 0.04 (4, 5, 6)	0.41 ± 0.04 (4, 5, 6)	0.40 ± 0.03 (4, 5, 6)	0.51 ± 0.02 (1, 2, 3.5, 6)	0.46 ± 0.04 (1, 2, 3, 4, 6)	0.44 ± 0.03 (1, 2, 3, 4, 5)	12.82	17.94	0.0001
5'- nucleotidase	0.55 ± 0.04 (4, 5, 6)	0.58 ± 0.05 (4, 5)	0.57 ± 0.03 (4, 5)	0.66 ± 0.02 (1, 2, 3)	0.06 ± 0.03 (1, 2, 3, 4, 6)	0.58 ± 0.02 (1, 4, 5)	10..91	14.54	0.0001

Data are means ± S.D. of eight mice in each group; all values are expressed as µmol/ min/mg protein; statistics is carried out using one way ANOVA test and the significant difference between groups is analyzed by Post-Hoc (SPSS Computer Program).

TABLE II

Effect of *Citrus reticulata* and Mirazid on lactate dehydrogenase (LDH) isoenzymes in mice livers

Enzymes	Control	Control+ *C. reticulata*	Control + Mirazid	Infected	Infected + *C. reticulata*	Infected+ Mirazid	Improvement percent		ANOVA
	(1)	(2)	(3)	(4)	(5)	(6)	*C. reticulata*	Mirazid	
LDH_1 (H_4M_O)	1.22 ± 0.23 (4)	1.15 ± 0.26 (4,)	1.18 ± 0.23 (4,)	1.85 ± 0.14 (1, 2, 3, 5, 6)	1.35 ± 0.36 (4)	1.23 ± 0.11 (4)	40.98	50.81	0.0001
LDH_2 (H_3M_1)	1.61 ± 0.17 (4)	1.78 ± 0.40 (4)	1.60 ± 0.22 (4)	3.47 ± 0.42 (1, 2, 3, 5, 6)	1.65 ± 1.50 (4)	1.63 ± 0.25 (4)	113.04	114.28	0.0001
LDH_3 (H_2M_2)	3.07 ± 0.42 (4, 5, 6)	3.13 ± 0.52 (4, 5, 6)	3.3 1 ± 0.24 (4, 5, 6)	2.76 ± 0.52 (1, 2, 3)	2.00 ± 0.41 (1, 2, 3)	2.10 ± 0.37 (1, 2, 3)	24.75	21.98	0.0001
LDH_4 (H_1M_3)	13.02 ± 1.51 (4, 5, 6)	12.05 ± 1.42 (4, 5, 6)	12.39 ± 1.14 (4, 5, 6)	23.18 ± 1.52 (1, 2, 3, 5, 6)	14.25 ± 1.79 (1, 2, 3, 4)	14.14 ± 0.98 (1, 2, 3, 4)	74.10	75.02	0.0001
LDH_5 (H_0M_4)	81.08 ± 1.52 (4)	81.89 ± 1.24 (4, 5, 6)	81.52 ± 1.28 (4, 5, 6)	68.74 ± 3.84 (1, 2, 3, 5, 6)	80.75 ± 1.05 (2, 3, 4)	80.90 ± 1.02 (2, 3, 4)	14.66	14.84	0.0001

Data are means ± S.D. of eight mice in each group; all values are expressed as percentages; statistics is carried out using one way ANOVA test and the significant difference between groups is analyzed by Post-Hoc (SPSS Computer Program).

TABLE III

Effect of *Citrus reticulata* and Mirazid on liver function enzymes in mice

Enzymes	Control	Control+ *C. reticulata*	Control + Mirazid	Infected	Infected + *C. reticulata*	Infected+ Mirazid	Improvement percent		ANOVA
	(1)	(2)	(3)	(4)	(5)	(6)	*C. reticulata*	Mirazid	
Aspartate aminotransferase	40.21 ± 2.66 (4, 5, 6)	38.50 ± 2.62 (4, 5)	39.40 ± 2.14 (4, 5)	26.11 ± 1.98 (1, 2, 3, 5, 6)	36.50 ± 2.33 (1, 2, 3, 4, 6)	38.00 ± 2.68 (1, 4, 5)	25.93	29.56	0.0001
Alanine aminotransferase	26.50 ± 1.16 (2, 4, 5, 6)	24.70 ± 1.12 (1, 3, 4, 5)	25.96 ± 1.24 (2, 4, 5, 6)	14.27 ± 0.98 (1, 2, 3, 5, 6)	21.50 ± 1.13 (1, 2, 3, 4, 6)	24.60 ± 1.22 (1, 3, 4, 5)	27.28	38.98	0.0001
Alkaline phosphatase	4.76 ± 0.22 (4, 5, 6)	4.90 ± 0.23 (4, 5, 6)	4.85 ± 0.22 (4, 5, 6)	7.18 ± 0.21 (5, 6)	6.22 ± 0.24 (6)	5.76 ± 0.23 (4, 5)	20.16	29.83	0.0001

Data are means ± S.D. of eight mice in each group; all values are expressed as µmol/ min/mg protein; statistics is carried out using one way ANOVA test and the significant difference between groups is analyzed by Post-Hoc (SPSS Computer Program).

Table IV shows worm burden and ova count in *S. mansoni* infected mice treated with *C. reticulata* and Mirazid. Significant reduction in worm and ova count amounting 63.66 and 59.45% for *C. reticulata* and 81.10 and 73.07% for Mirazid, respectively.

DISCUSSION

The liver plays an important role in the vital activities of the body where its hepatocytes show differences in the localization and concentration of some enzyme systems (Gumucio & Chiamale 1988). Many of these enzymes served as marker enzymes for different cell organelles and any defect of them will be reflected to the enzyme activity itself (Van Noorden & Frederiks 1992). Hence, studying changes in these enzymatic activities could be helpful in evaluating the possible side effects of different treatments on different cell organelles after *S. mansoni* infection and the improvement occurring in such enzymes after treatment.

Concerning SDH enzyme activity, the present results revealed a significant decrease in its activity two months post infection. This is in agreement with Ismail et al. (1991). Daugherty (1955) attributed this failure to *Schistosoma* toxins which accumulated with the mitochondrial fraction and reflected on enzyme activity. SDH is the marker enzyme of the mitochondria (Van Noorden & Frederiks 1992), so any changes in the enzyme activity should be accompanied with changes in the mitochondria (Jones & Bogitsh 1979, El-Gowhary 1993). This was confirmed by the same authors, who noticed that mitochondria appeared swollen with fewer cristae and pale matrix with the subsequent leakage of significant amounts of their enzyme content. An additional evidence of increased mitochondrial membrane permeability was shown by the increased release of mitochondrial enzyme ornithine carbamoyl transferase and isocitrate dehydrogenase into sera of human patients and experimental animals after infection with schistosomes (Rizk et al. 2000).

The mitochondrial changes could originate from the relative anoxia and irritation caused by toxic or metabolic products of adult worm and/or eggs, hence inhibition of mitochondrial oxidation (Ahmed & Gad 1995). When limiting amounts of oxygen are present, both SDH and Krebs cycle enzyme activities are repressed (Van Hellemond & Tielens 1994).

As concerning LDH enzyme activity the obtained results showed a significant decrease in enzyme activity after infection.

In agreement with the present results Awadalla et al. (1975) and Metwally et al. (1990) mentioned that the level of LDH was decreased in the liver tissue and increased in the serum of mice infected with *S. mansoni*. They attributed the decrease of enzyme activity to tissue damage caused by larvae in the infection period, led to the release of enzyme from the necrotic tissue to blood stream or due to the increased cell anoxia and irritation by toxic or metabolic products of the worm.

LDH is one of the glycolytic enzymes and is associated mainly with the cytoplasm (De Duve et al. 1962). Lawson and Wilson (1980) added that LDH is not a regulatory factor for glycolysis but its activity is normally re-

TABLE IV
Effect of *Citrus reticulata* and Mirazid on worm burden and ova count

Parameters	Infected	Infected + *C. reticulata*	Infected + Mirazid	Reduction percent		ANOVA
	(1)	(2)	(3)	*C. reticulata*	Mirazid	
Worm burden	17.09 ± 1.19 (2,3)	6.21 ± 0.51 (1,3)	3.23 ± 0.76 (1,2)	63.66	81.10	0.0001
Ova count $\times 10^3$	12.48 ± 0.70 (2,3)	5.06 ± 1.55 (1,3)	3.36 ± 0.74 (1,2)	59.45	73.07	0.0001

Worm burden is expressed as means ± S.D. of eight mice in each group; ova count is expressed as (means ± S. D.) $\times 10^3$ of eight mice in each group; statistics is carried out using one way ANOVA test and the significant difference between groups is analyzed by Post-Hoc (SPSS Computer Program).

lated to the maximum rate of glycolysis. LDH inhibition revealed the aerobic-anaerobic switch induced by the developing parasite (Tielens et al.1994). Moreover, lower activity in LDH is in the direction of lactate oxidation which could be easily correlated to the crabtree effect of schistosomes (Tielens 1997) through which lactate is accumulated and glycogen depleted confirming inhibition of aerobic respiration and stimulation of anaerobic glycolysis through hexokinase, a rate limiting enzymes of glycolysis. The decreased enzyme activity due to the increase in anaerobic glycolysis may be attributed also to provision of energy, inhibition of Kreb's cycle, decrease NAD/NADH ratio due to inhibition of mitochondrial oxidation which favors the conversion of pyruvate to lactate, increased metabolic activity of infected liver tissues due to associated inflammation lesions, decreased hepatic blood flow, decreased utilization efficiency of other energy sources such as fat and proteins or from worms themselves which depend on anaerobic glycolysis as the main source of energy production (Ahmed & Gad 1995).

Results of bilharzial infection on the activity of LDH-isoenzymes showed that LDH_4 is found to be significantly higher when compared to control, while LDH_5 isoenzyme which plays the main role in the anaerobic glycolysis was significantly reduced. This is in agreement with Salah et al. (1976) who reported an increase in serum LDH_5-isoenzyme in bilharzial infected patients. The reduction in the percentage contribution of LDH_5 in liver may explain the false higher contribution of LDH_1 which is concerned with aerobic respiration and this is not in contrast to the measured LDH enzyme activity in lactate oxidation direction previously discussed. This confirmed the opinion of Varley et al. (1980) who stated that LDH_5 is increased in case of liver disease; even the total LDH activity is still within normal limits.

Maly and Toranelli (1993) reported that the distribution pattern of LDH activity was functionally connected with other enzyme activities involved in gluconeogenesis as pyruvate derived from lactate and alanine. In both, man and rat, more than 98% of LDH activity is represented by LDH_5. This enzyme reached its maximum exactly in periportal zone of liver acinus which receives the highest oxygen supply. According to "anaerobic/aerobic" theory H-monomere is supposed to be principally involved in the oxidation of lactate and should therefore be predominant in tissues with a high oxygen supply. In contrast to this, the M-4 isoform and other isoforms rich in M subunits are geared to the reduction of pyruvate and should be predominant in tissues that are subjected to more anaerobic conditions. This led to consider the possible distribution pattern of LDH isoenzyme forms which are characterized by high values of H-subunits. They added that LDH isoenzyme shows species dependent difference in the distribution pattern where the cattle liver show LDH_1, LDH_2 and LDH_3 activity rich in H-subunits, while rat liver contain LDH_5 only which rich in M-subunit. They explain this phenomena as the isoenzymes rich in H-subunit could represent a safety mechanism in that they protect the liver of some species from an excessive buildup of lactate from skeletal muscle under heavy working conditions.

Concerning G-Pase enzyme activity, the present results revealed significant increase after infection with *S. mansoni*. Shaheen et al. (1989) and El-Gowhary et al. (1993) found that the enzyme activity was increased in *S. mansoni* infected mice liver. They attributed this increase to the proliferation and dilation of rough and smooth endoplasmic reticulum as evident by increase of ribosomes accompanied by proliferation and dilation of microsomal membranes where the enzyme is usually located and considered as microsomal marker enzyme (Van Noorden & Frederiks 1992). Also Trela et al. (1985) attributed the increase in enzyme activity to the effect that occurs on the membrane of endoplasmic reticulum or to the elevation of cytosolic calcium that can trigger the conversion of the enzyme phosphorylase b (inactive form) to phosphorylase a (active form) which degrades glycogen into glucose (Exton 1982).

Regarding AP enzyme activity, the present results showed a significant increase in enzyme activity after infection. This elevation in AP activity may be due to increased tissue catabolism resulting from increased worm and egg toxins by infection, since all the lysosomal enzymes are activated in conditions characterized by increased tissue catabolism led to enhancement of phagocytic phenomenons (Salah et al. 1976) and or due to aberration of the lysosomes, where AP is the lysosomal marker enzyme (Frederiks & Marx 1988). This is in accordance with Jones and Bogitsh (1979) who found histochemically an increase in the number of mature lysosomes of the liver cells in mice after *S. mansoni* infection. These

results confirmed those of Rodrigues (1988) who observed an important change on the lipidic constitution of the lysosomal membrane of *S. mansoni* infected mice. These changes are provoked by the catabolites excreted by immature or adult worms of *S. mansoni* present in the portal venous system, but El-Sharkawy et al. (1993) attributed the increase in the enzyme activity to deranged metabolic function as a result of liver cell injury.

As concerning the 5'-nucleotidase enzyme activity, the present results recorded a significant increase in its post infection. This increase in the 5'-nucleotidase enzyme activity may be due to enhancement of the active transport process through the plasma membrane where the enzyme appeared in accurate localization at bile canalicular and sinusoidal plasma membrane of liver parenchymal cells (Frederiks & Marx 1988) and/or due to accelerated nucleic acid metabolism where the catabolism of purine and pyrimidine nucleotides starts with the action of 5'-nucleotidase (Hardonk 1968). Also, the enzyme activity may be increased due to cellular injury (Michael et al. 1979). These results were confirmed by El-Aasar et al. (1978) who demonstrated histochemically that the sinusoids of infected mice livers showed an increased 5'-nucleotidase enzyme activity.

It is concerned to study transaminases enzyme activities which showed a significant decrease after infection. El-Aasar et al. (1989) attributed the decrease of transaminase enzyme activities in mice livers to the decrease in hepatic cell population due to liver fibrosis or due to the release of the enzyme from the damaged livers into the circulation as a result of increased cell membrane permeability. The observed diminution of AST was more manifested than that of ALT denoting that, although the later is more specific for liver cells, yet it is less sensitive than AST in detecting liver cell damage (Awadalla et al. 1975). Moreover, the presence of considerably more AST in human hepatic tissue indicated that the released ALT is too diluted in the extracellular compartment to cause significant increase in the ALT activity in *S. mansoni* patients. Therefore, variations in the release, destruction or excretion of the two enzymes or an unknown metabolism aberration are probably important contributory mechanisms (Salah et al. 1976).

In the present study, ALP enzyme activity in infected mice showed a significant increase. Awadalla et al. (1975) and El-Aasar et al. (1989) observed an elevation in ALP activity in murine liver after *S. mansoni* infection. They attributed the increase in enzyme activity to the irritation of the liver cells by toxins or metabolic products of growing schistosomules, adult worms and eggs or due to increased loss of intracellular enzyme by diffusion through cell membranes which appears to act as a stimulus to the synthesis of more enzyme protein. Higher rates of formation would, in turn, increase the rate of diffusion and hence increase serum activity (Wilkinson 1962). Abdel-Rahman et al. (1993) mentioned a significant rise in liver ALP isoenzyme in patients having hepatosplenic schistosomiasis. Mansour et al. (1982) added that the elevation of ALP enzyme activity in *S. mansoni* infected human is of intestinal origin especially since *S. mansoni* is a disease which primarily affected the intestine, while this elevation is not of hepatic origin as it is observed in both patients of *S. mansoni* and hepatosplenomegaly disease.

Hunter et al. (1973) showed by histochemical studies an increase in ALP activity in experimental infection with schistosomiasis and in the late stage of human lesions by liver biopsy. Mansy et al.(1990) attributed the increase in enzyme activity to the proliferation of bile ductules and bile canaliculi as a result of schistosomiasis by ultrastructural examination of the liver specimens. This result confirmed the observation of Kaplan (1972) who suggested that the response of the liver to any form of bilary tree obstruction is to synthesize more ALP.

The present results recorded improvement levels of all enzymes under investigation after treatment of *S. mansoni* infected mice with *C. reticulata* and Mirazid. This amelioration was confirmed by a significant reduction of worm burden and ova count after treatment with both extracts. Our data is confirmed by the previous reports indicated that Mirazid is an effective drug for treatment of *Fasciola hepatica, S. mansoni,* and *S. haematobium* through worm disruption, collapse of tubercles, and reduction of worm burden as well as reduction in ova count in urine and stool (Haridy et al. 2003, Abo-Madyan et al. 2004, Massoud et al. 2004). In addition, *C. reticulata* contain high concentration of flavonoid, glycosides, and polymethoxylated flavones which recorded an antioxidant properties, acts as free radical scavenger and has anti-inflammatory activity (Hara et al. 2004 & Ojewole 2004). Toxic substances and free radicals elaborated by worms consume antioxidants and may affect the capacity of the liver to detoxify or naturalize the effect of the toxic endogenous and exogenous compounds (Sheweita et al. 1998). This gives an additional support that the improvement pattern in liver enzyme after treatment with *C. reticulata* resulted from its antioxidant capacity.

In conclusion, *C. reticulata* and Mirazid have no side effects on normal healthy mice and succeeded to reduce the hazardous effect of *S. mansoni* through improvement of liver enzymes, reduction of worm burden, and ova count. Our recommendation is to increase the treatment time of both extracts for complete eradication of worm and ova to preserve normal liver function parameters.

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