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Effect of *Citrus reticulata* on Serum Protein Fractions of Mice After *Schistosoma mansoni* Infection

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Abstract: The effect of the ethanolic extract of *Citrus reticulata* roots or the oleo-resin extract from Myrrh of *Commiphora molmol* tree (Mirazid) were studied as new antischistosomal drugs. The total protein and the different serum proteins namely albumin, prealbumin, β -lipoproteins, macroglobulins, α -1-acid glycoprotein, α -1-antitrypsin, cholinesterase, ceruloplasmin, haemopexin, haptoglobin, transferrin and lipoprotein were measured in both infected and treated mice. Also, liver function parameters; AST, ALT, ALP and total bilirubin were studied in the different mice groups. Besides, the parasitological parameters, egg count and worm burden were estimated. The results showed a significant reduction in all protein fraction concentrations in *S. mansoni* infected mice and elevation in the liver function parameters AST, ALT, ALP and bilirubin. *Citrus reticulata* extract and mirazid showed an improvement in all the previous parameters which confirmed the medical importance and curative effects of *C. reticulata* and mirazid against *S. mansoni* infection. Also, the results showed a noticeable reduction in ova count and worm burden.

Key words: Schistosomiasis, *Citrus reticulata*, mirazid, serum proteins, liver function

INTRODUCTION

Schistosomiasis is considered a wide-spread problem that affects Egyptians at different ages (El-Sayed *et al.*, 1995). The chronic nature of the disease and its endemic property in Egypt affect both the patient and the society with regards to the cost of the treatment, especially in complicated cases (Mostafa *et al.*, 1998).

It is well known that the liver is one of the major target organs affected by schistosomiasis. Adult worms, that usually reside in portal and mesenteric venules of the host, lay large number of eggs that are trapped in hepatic and portal venules causing granulomatous inflammatory reactions followed by a characteristic pattern of hepatic fibrosis (Von Brand, 1979). The liver plays an important role in body metabolism and synthesizes the plasma proteins, fibrinogen, prothrombin and heparin and in addition, the exported serum proteins, albumin, prealbumin, transferrin, immunoglobulins IgG and IgM, hemopexin, haptoglobin, ceruloplasmin, α and β -lipoproteins, glycoprotein and α -antitrypsin (Miller *et al.*, 1964). Therefore, infection with schistosomes may result in hepatic disorders and metabolic disturbances in host livers.

A new trend for treatment of liver disorders as a result of *S. mansoni* infection is the use of natural extracts. In the present study *Citrus reticulata* roots and commiphora (Mirazid) plants are evaluated for their

antischistosomal activity. Citrus plants extract has been reported to have, antibacterial activity, (Tkachenko *et al.*, 1999), inhibit human cancer cell proliferation (Manthey and Guthrie, 2002), antioxidant activity (Tanizawa *et al.*, 1992; Hara *et al.*, 2004).

Commiphora extract (Mirazid) has been proved to be very effective in treatment of *S. haematobium* (El-Baz *et al.*, 2003). Also, Hassan *et al.* (2003) reported that Mirazid caused disruption of *S. mansoni* worms tegument and collapse of tubercles causing eradication in *S. mansoni* worm burden.

The present study was undertaken as a trial to understand and clarify the antibilharzial effect of *Citrus reticulata* extract roots in relation to *Commiphora molmol* tree extract (Mirazid). The measured parameters include serum protein fractions, prealbumin, albumin, α -lipoprotein, α -acid glycoprotein, haemopexin, 1-antitrypsin, ceruloplasmin, transferrin, cholinesterase, β -lipoprotein, haptoglobin and total protein content in addition to, liver function parameters, AST, ALT, ALP and total bilirubin, besides parasitological parameters egg count and worm burden.

MATERIALS AND METHODS

Chemicals: All chemicals were of analytical grade from Sigma Chemical Company.

- Mirazid (Purified *Commiphora molmol* extract from Pharco Pharmaceutical Company, Egypt).
- The dosages of the administered agents were: Mirazid: Two oral doses (600 mg kg⁻¹) purified commiphora extract for 3 consecutive days on empty stomach, at least one hour before eating (Haridy *et al.*, 2003).
- *Citrus reticulata*: Ethanolic extracts of citrus plants were prepared (Natural product Dep. National Research Centre). Oral doses of 10 µg/mL/mouse for 3 consecutive weeks were given daily eight weeks post infection (Nogata *et al.*, 2001; Manthey and Guthrie, 2002).

Animals: Forty eight male mice provided by lab-bred colony of similar age and weight (18-20 g) were selected for this study. They were obtained from Schistosome Biological Supply program (SBSP), Theodor Bilharz Institute, Cairo, Egypt. Animals were kept in a controlled environment and were allowed free access to diet and water during the study.

Plant material: *Citrus reticulata* Blanco cuv. Baladi Rutaceae, roots were collected from Moderey El Tahrir, Behera, Egypt in December, 2002. They were authenticated by Dr. Mohamed Abd El Ghaffar, Faculty of Agriculture, Al-Azhar University, Egypt. A voucher specimen is deposited at the Department of Chemistry of Natural Compounds, NRC, Dokki, Cairo, Egypt.

Extraction and isolation: Air-dried, powdered roots of *C. reticulata* (0.85 kg) were extracted with 80% EtOH. The ethanolic extract was evaporated and the aqueous residue extracted sequentially thrice with equal volumes of n-hexane, Et₂O, EtOAc and n-BuOH. The EtOAc extract was evaporated to dryness. The residue was monitored by TLC using precoated silica gel 60 F254 aluminium sheets (0.2 mm thickness, Merck) and it was found to contain flavonoids. The phenolic residue was subjected to bioassay testing.

Experimental design: Animals were divided into six groups, each of 8 animals. Group 1: served as normal healthy control. Group 2: Administered Mirazid (Purified *Commiphora molmol* extract) orally to mice to show its side effect. Group 3: administrated citrus extract orally to show its side effect. Group 4: *Schistosoma mansoni* infected mice with 100 cercariae by tail immersion method (Oliver and Stirewalt, 1952) and sacrificed after 2 months. Group 5: infected and treated with Mirazid. Group 6: infected mice treated with *Citrus reticulata* extract.

Liver perfusion: Worms were recovered by portomesentric technique of Smithers and Terry (1965). The degree of protection or the percent reduction in challenge was calculated from $P = C-V/C \times 100$, where P = % protection, C = mean number of parasites recovered from infected animals and V = mean number of parasites recovered from treated animals.

Ova count: The number of eggs per gram tissue was studied according to the procedure of Cheever and Anderson (1971).

$$\text{No. of Ova in 1g of liver} = \frac{\text{No. of Ova in liver digested in 5 mL KOH}}{\text{Weight of liver in grams recorded before digestion}}$$

Preparation of anti-mice sera in rabbits:

- Complete Freund's adjuvant and incomplete Freund's adjuvant were used to prepare polyspecific antisera directed against mice serum proteins.
- Rabbits weighing 1.5-2.0 kg were immunized against mice serum proteins to obtain antiserum, according to the method mentioned by Harlo and Lane (1988).

Two-dimensional (crossed) immunoelectrophoresis: It is the combination of electrophoretic separation of mice serum proteins (antigens) in agarose gel followed by electrophoresis in the second dimension in an antibody-containing gel according to the method of Clark and Freeman (1968) and Fouad *et al.* (1983).

A photocopy of the electropherogram was obtained. The areas under the individual protein peaks were measured using the planimeter. In each experiment the same patch of antibody was used for the control serum and sera obtained from other treatments. Since the area enclosed by each individual precipitate is proportional to the antigen/antibody concentration, hence the relative concentration of serum protein fractions was estimated by comparing given peak areas of the control serum and the sera obtained from different treatments. From the position of albumin and transferrin in the electrophoretic diagram the other proteins can be identified according to their mobility (Weeke, 1970). Albumin peak in the electropherogram is separated but can't be measured because of its high peak value so, it is estimated colorimetrically using the method of Spencer and Price (1977).

Biochemical assays: Estimation of serum total protein was carried according to the method of Bradford (1976). Bradford dye (Coomassie Brilliant blue G-250) dissolved

in ethanol-phosphoric acid mixture reacts with protein in the sample resulting an increase in absorbance due to the formation of protein-Bradford complex. The color developed is measured at 595 nm which is proportional to the protein concentration in the sample. Bovine serum albumin (BSA) is used as standard protein.

Serum albumin is estimated colorimetrically according to the method of Spencer and Price (1977). Bromocresol green (BCG) reacts with albumin in an acidic solution resulting in an increase in absorbance due to the formation of an albumin-BCG complex. The color developed has a maximum absorbance at 630 nm and is proportional to the albumin concentration in the sample.

Serum bilirubin was determined according to the method of Henry (1974) by reaction with diazotized sulfonilic acid, in the presence of caffeine, with the final production of an azo pigment that is measured at 478 nm.

Serum alanine and aspartate aminotransferases were determined according to the method of Bergmeyer *et al.* (1974) through measuring oxaloacetate and pyruvate respectively which are produced from the reaction in their derivative from 2,4 dinitrophenyl hydrazine that is measured at 505 nm. Pyruvate is usually used as standard

for both enzymes, since oxaloacetate is immediately converted to pyruvate.

Serum alkaline phosphatase is determined according to the method of Belfield and Goldberg (1971). The method described is based on measuring the liberated phenol at 510 nm in the presence of amino-4-antipyrine and potassium ferricyanide.

Statistical analysis: The statistical significance of the results was determined by analysis of variance (ANOVA) combined with post-hoc (SPSS computer program).

RESULTS

Table 1 shows the effect of *C. reticulata* and Mirazid on serum protein fractions in mice infected with *S. mansoni* as compared with normal control mice. The results revealed a significant reduction in all protein fraction concentrations in infected mice, while *C. reticulata* and Mirazid show an improvement in all fractions. Also the normal mice shows slight effect in most of protein fractions under the effect of the extracts. The results are shown in Fig. 1 and 2.

Table 1: Promoting effect of *C. reticulata* and mirazid extracts on serum protein fractions by two dimensional immunoelectrophoresis in infected mice with *S. mansoni*

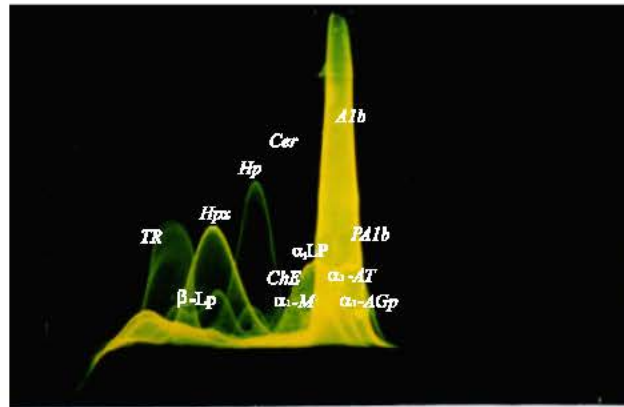
Parameters	Control (1)	Con. mirazid (2)	Con. extract (3)	Infected (4)	Inf mirazid (5)	Infe. extract (6)	ANOVA
Prealbumin (LSD)	11.48±0.21 (2 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	11.80±0.17 (1 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	11.67±0.16 (4 ^{***} , 5 ^{***} , 6 ^{***})	6.31±0.22 (1 ^{***} , 2 ^{***} , 3 ^{***} , 5 ^{***} , 6 ^{***})	11.01±0.12 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***})	9.70±0.23 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***})	<0.0001
Albumin	1.63±0.04 (2 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	1.72±0.02 (1 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	1.67±0.02 (4 ^{***} , 5 ^{***} , 6 ^{***})	0.97±0.04 (1 ^{***} , 2 ^{***} , 3 ^{***} , 5 ^{***} , 6 ^{***})	1.41±0.05 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 6 ^{***})	1.33±0.12 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***})	<0.0001
α-Lipoprotein	6.54±3.21 (2 ^{***} , 4 ^{***})	8.11±0.11 (1 ^{***} , 4 ^{***})	8.01±0.06 (4 ^{***})	4.56±0.24 (1 ^{***} , 2 ^{***} , 3 ^{***} , 5 ^{***} , 6 ^{***})	7.17±0.19 (4 ^{***})	6.82±0.21 (4 ^{***})	<0.0001
Macroglobin	13.96±0.08 (2 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	15.20±0.22 (1 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	14.26±0.73 (2 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	6.55±0.44 (1 ^{***} , 2 ^{***} , 3 ^{***} , 5 ^{***} , 6 ^{***})	12.25±0.64 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 6 ^{***})	10.27±0.37 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***})	<0.0001
Glycoprotein	13.31±0.17 (2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	15.58±0.72 (1 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	13.98±0.49 (1 ^{***} , 2 ^{***} , 3 ^{***} , 5 ^{***} , 6 ^{***})	6.31±0.33 (1 ^{***} , 2 ^{***} , 3 ^{***} , 5 ^{***} , 6 ^{***})	11.47±0.44 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 6 ^{***})	10.33±0.49 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***})	<0.0001
Antitrypsin	24.92±0.70 (2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	27.13±0.42 (1 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	26.08±0.19 (1 ^{***} , 2 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	10.89±0.31 (1 ^{***} , 2 ^{***} , 3 ^{***} , 5 ^{***} , 6 ^{***})	21.02±0.85 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 6 ^{***})	18.79±0.44 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***})	<0.0001
Cholinesterase	16.36±0.32 (2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	18.66±0.37 (2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	17.26±0.32 (1 ^{***} , 2 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	7.18±0.07 (1 ^{***} , 2 ^{***} , 3 ^{***} , 5 ^{***} , 6 ^{***})	12.71±0.50 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 6 ^{***})	10.28±0.50 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***})	<0.0001
Ceruloplasmin	8.90±0.35 (2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	11.19±0.22 (1 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	10.29±0.29 (1 ^{***} , 2 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	4.57±0.47 (1 ^{***} , 2 ^{***} , 3 ^{***} , 5 ^{***} , 6 ^{***})	7.68±0.18 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 6 ^{***})	6.81±0.36 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***})	<0.0001
Hemopexin	12.34±0.46 (2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	15.37±0.39 (1 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	14.25±0.29 (1 ^{***} , 2 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	6.35±0.55 (1 ^{***} , 2 ^{***} , 3 ^{***} , 5 ^{***} , 6 ^{***})	11.20±0.30 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 6 ^{***})	9.25±0.26 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***})	<0.0001
Haptoglobin	26.94±0.21 (2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	30.12±0.15 (1 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	28.84±0.36 (1 ^{***} , 2 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	10.81±0.18 (1 ^{***} , 2 ^{***} , 3 ^{***} , 5 ^{***} , 6 ^{***})	23.00±0.38 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 6 ^{***})	19.03±0.44 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***})	<0.0001
Transferrin	28.14±0.28 (2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	32.22±0.56 (1 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	29.86±0.44 (1 ^{***} , 2 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	12.78±0.45 (1 ^{***} , 2 ^{***} , 3 ^{***} , 5 ^{***} , 6 ^{***})	25.01±0.50 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 6 ^{***})	12.75±0.45 (1 ^{***} , 2 ^{***} , 3 ^{***} , 5 ^{***} , 6 ^{***})	<0.0001
β-lipoprotein	25.76±0.63 (2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	27.90±0.03 (1 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	27.05±0.20 (1 ^{***} , 2 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	9.38±0.35 (1 ^{***} , 2 ^{***} , 3 ^{***} , 5 ^{***} , 6 ^{***})	21.79±0.37 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 6 ^{***})	15.40±0.38 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***})	<0.0001
Total protein	115.45±3.36 (2 ^{***} , 3 ^{***} , 4 ^{***} , 6 ^{***})	141.07±2.03 (1 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	128.07±2.34 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***} , 6 ^{***})	67.90±7.43 (1 ^{***} , 2 ^{***} , 3 ^{***} , 5 ^{***} , 6 ^{***})	112.39±2.06 (2 ^{***} , 3 ^{***} , 4 ^{***} , 6 ^{***})	98.15±3.58 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***})	<0.0001

** means significant at < 0.01-0.001, *** means significant at < 0.001, Serum protein fractions are expressed in µg mL⁻¹ and albumin is expressed as mg⁻¹ dL. Total protein is expressed in mg mL⁻¹

Table 2: Effect of *C. reticulata* and mirazid extracts on liver function enzymes, ova Count and worm burden of infected mice with *S. mansoni*

Parameters	Control (1)	Con. mirazid (2)	Con. extract (3)	Infected (4)	Inf mirazid (5)	Infe. extract (6)	ANOVA
AST	48.34±0.64 (4 ^{***} , 5 ^{***} , 6 ^{***})	47.55±0.38 (4 ^{***} , 5 ^{***} , 6 ^{***})	48.61±0.38 (4 ^{***} , 5 ^{***} , 6 ^{***})	163.83±3.86 (1 ^{***} , 2 ^{***} , 3 ^{***} , 5 ^{***} , 6 ^{***})	57.27±2.31 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 6 ^{***})	75.31±3.49 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***})	<0.0001
ALT	26.22±0.28 (4 ^{***} , 5 ^{***} , 6 ^{***})	26.18±0.21 (4 ^{***} , 5 ^{***} , 6 ^{***})	25.88±0.18 (4 ^{***} , 5 ^{***} , 6 ^{***})	65.23±2.39 (1 ^{***} , 2 ^{***} , 3 ^{***} , 5 ^{***} , 6 ^{***})	33.53±1.97 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 6 ^{***})	43.92±2.02 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***})	<0.0001
ALP	181.45±1.11 (4 ^{***} , 6 ^{***})	181.74±1.25 (4 ^{***} , 6 ^{***})	180.73±0.51 (4 ^{***} , 6 ^{***})	398.15±2.24 (1 ^{***} , 2 ^{***} , 3 ^{***} , 5 ^{***} , 6 ^{***})	188.12±6.55 (4 ^{***} , 6 ^{***})	223.82±15.54 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***})	<0.0001
Bilirubin	51.83±2.14 (4 ^{***} , 6 ^{***})	49.67±1.87 (4 ^{***} , 5 ^{***} , 6 ^{***})	50.43±1.10 (4 ^{***} , 5 ^{***} , 6 ^{***})	200.56±5.01 (1 ^{***} , 2 ^{***} , 3 ^{***} , 5 ^{***} , 6 ^{***})	54.67±3.30 (2 ^{***} , 3 ^{***} , 4 ^{***} , 6 ^{***})	65.82±3.27 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***})	<0.0001
Egg count	- (4 ^{***} , 5 ^{***} , 6 ^{***})	- (4 ^{***} , 5 ^{***} , 6 ^{***})	- (4 ^{***} , 5 ^{***} , 6 ^{***})	(10) ³ 12.48±0.70 (1 ^{***} , 2 ^{***} , 3 ^{***} , 5 ^{***} , 6 ^{***})	(10) ³ 3.36±0.74 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 6 ^{***})	(10) ³ 5.06±1.55 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***})	<0.0001
Worm burden	- (4 ^{***} , 5 ^{***} , 6 ^{***})	- (4 ^{***} , 5 ^{***} , 6 ^{***})	- (4 ^{***} , 5 ^{***} , 6 ^{***})	17.09±1.19 (1 ^{***} , 2 ^{***} , 3 ^{***} , 5 ^{***} , 6 ^{***})	3.25±0.76 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 6 ^{***})	6.21±0.51 (1 ^{***} , 2 ^{***} , 3 ^{***} , 4 ^{***} , 5 ^{***})	<0.0001

** Means significant at < 0.01-0.001, *** Mean significant at < 0.001, Liver function enzymes are expressed in µ mL⁻¹, Total bilirubin is expressed in mg L⁻¹



<i>PAIb</i> = Prealbumin	<i>ChE</i> = Cholinesterase
<i>Alb</i> = Albumin	<i>Cer</i> = Ceruloplasmin
<i>alpha-Lp</i> = α_2 -Lipoprotein	<i>Hpx</i> = Haemopexin
<i>alpha-M</i> = α_1 -Macroglobulin	<i>Hp</i> = Haptoglobin
<i>alpha-AGp</i> = α_1 -Acid glycoprotein	<i>TR</i> = Transferrin
<i>alpha-AT</i> = α_1 -Antitrypsin	<i>beta-Lp</i> = β_2 -Transferrin

Fig. 1: Crossed two dimensional immunoelectrophoresis of control mice serum proteins

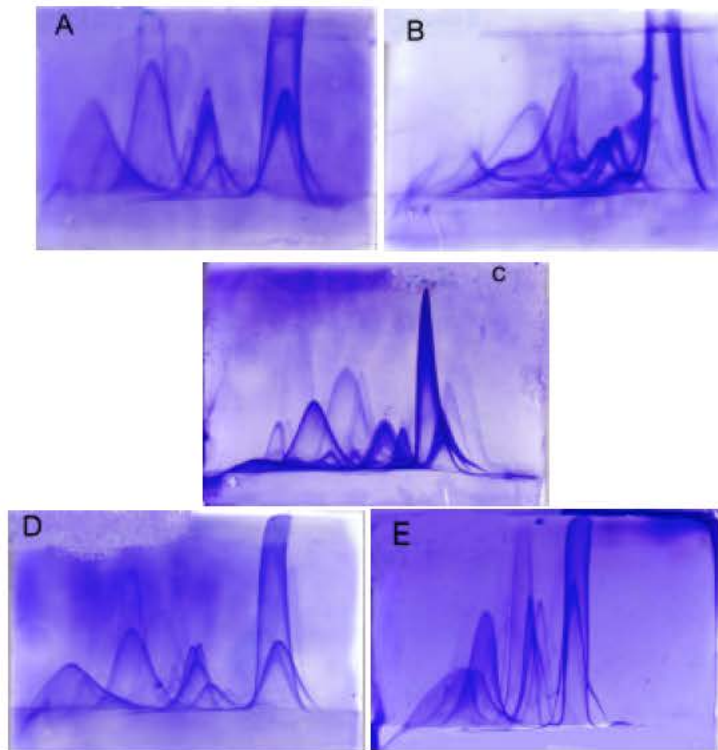


Fig. 2: Crossed two dimensional immunoelectrophoresis of infected and treated mice serum proteins

- A. Healthy mice treated with *Commiphora molmol* (mirazid)
- B. Healthy mice treated with *Citrus reticulata*
- C. Mice infected with *S. mansoni*
- D. Infected mice treated with *C. reticulata*
- E. Infected mice treated with *C. molmol* (Mirazid)

Table 2 shows an elevation in liver function parameters AST, ALT, ALP and bilirubin of infected mice as compared with normal group.

All parameters were ameliorated after treatment with *C. reticulata* and Mirazid extracts. The normal mice were not affected with the extracts. The table shows also the curative effects of both *C. reticulata* and Mirazid extracts through the reduction of ova count and worm burden.

DISCUSSION

In accordance with the present results, Mikhail and Mansour (1982) supported the present results mentioning that schistosomiasis and its complications tend to reduce the level of prealbumin, albumin and cholinesterase levels. The same results were also reported by Abdel-Rahim *et al.* (1990) who showed that the low serum albumin levels reflected an impaired protein synthesis of the liver destruction of parenchymal liver cells, reduction in hepatic protein secretion that is always associated with acceleration in catabolism of unsecreted plasma proteins and/or perturbations of the secretory process by schistosomiasis. The present results are also in accordance with Mahmoud *et al.* (2002) who found significant reduction in albumin level by *S. mansoni* infection and they found that the depression of albumin level was attributed to switching of albumin gene transcription to α -fetoprotein. In addition, Moota *et al.* (2001) reported that the responses of acute phase cholinesterase is greatly reduced in response to tissue injury. The present decreased serum α -lipoprotein (HDL) and β -lipoprotein (LDL) levels found support the previous findings of El-Marzouki and Amin (1997), who attributed the reduction in HDL and LDL to the presence of several metabolites released by *S. mansoni* which affect the host hepatic tissue resulting in decreased synthesis of these parameters and their release into the circulation. Lowering of HDL and LDL could also be supported by Dimenstein *et al.* (1992) who stated that the plasma concentrations of low and high-density lipoproteins were significantly reduced in patients with hepatosplenic *Schistosomiasis mansoni*. Low-density lipoproteins are important in human schistosomiasis because they bind to the surface of the parasite and inhibit the binding of anti-schistosomal antibodies. LDL may also serve as a source of lipids for the parasite membrane synthesis (Bennett and Caulfield, 1991). The reduction in LDL and HDL may be attributed to the inhibitory effects of different cytokines on; synthesis of the lipid and apoprotein portions, the lipid protein conjugation step, stability of lipoproteins and their release into the circulation (Feingold *et al.*, 1989a, b). Palmero *et al.*

(1999) suggested these inhibitory lipoprotein machineries by cytokines as different immunological signals in mice liver injury, could be a deleterious part of the acute phase response. Gillett and Carvalho (1985) referred the significant reduction in LDL and HDL to the abnormal apoprotein composition.

On the other hand, the significant reduction in α -1-macroglobulin in *S. mansoni* infected mice may be due to decrease in its production at the level which reached 50% by hepatocytes (Fouad *et al.*, 1983). Triolo (1979) reported that proteins of acute phase α -1-antitrypsin are deficient in serum of liver cirrhosis. Also, Poynard *et al.* (2002) found that the capacity for acute phase response of plasma level α -1-macroglobulin, haptoglobin and apolipoprotein (Fibrosis index) was significantly decreased in patients with chronic hepatitis and this may be due to dysfunction in protein synthetic machineries. In contrast to the present finding Mikhail and Mansour (1982) found elevated level of macroglobulin in schistosomiasis.

With regard to α -1-acid glycoprotein and α -1-antitrypsin. Onda (1977) suggested the significant reduction in their levels to the suppression of mitosis of the hepatocytes, while Milland *et al.* (1990) and Moota *et al.* (2001) reported that the responses of acute phase α -1-acid glycoprotein, α -1-antitrypsin are greatly reduced in response to tissue injury.

Concerning serum ceruloplasmin, Kwak and Jo (1976) previously explained the reduction in its level to the reduction in albumin synthesis. Milland *et al.* (1990) reported that acute phase response of ceruloplasmin is greatly reduced in inflammatory tissue. Lowering of transferrin could also be supported by Dinarello (2000) who stated its decrease in acute phase-response. In contrast to the present findings Princen *et al.* (1982) and Dickson *et al.* (1982) found that the production of ceruloplasmin increased strikingly by liver hepatocytes in response to tissue injury.

The present results indicated also significant reduction in serum haemopexin, haptoglobin and transferrin in *S. mansoni* infected mice.

As a matter of fact, haptoglobin, haemopexin and transferrin are the parameters concerned with carriage of free hemoglobin is particularly specified for heme carriage and recycling, while transferrin is for iron binding and transport (Ganong, 1999).

In this respect Mansour *et al.* (1985) stated that infection with *S. mansoni* is associated with high incidence of iron deficiency, that is reflected on haptoglobin, haemopexin and transferrin levels causing reduction in their concentrations.

Milland *et al.* (1990) found that haptoglobin and transferrin levels are greatly reduced in inflammatory tissue while Princen *et al.* (1982) and Dickson *et al.* (1982) claimed that transferrin produced by liver hepatocytes is decreased in response to tissue injury such as inflammation.

The obtained results are concerned with significant decrease in serum total protein content in *S. mansoni* infected mice. This could be attributed to cellular damage, caused by parasite toxins (Van Raaij *et al.*, 1994). This was similar to the results of El-Fakahani *et al.* (1993) and Farouk (2000) who reported low liver total protein in schistosomiasis. In addition, the main total protein content is albumin and the reduction in total protein is due to the reduced level of albumin that may in turn result from decreased anabolism or increased catabolism, hence malnutrition and/or malabsorption may contribute to decrease in biosynthesis of albumin. The significant decrease in total protein is mainly due to increase in messenger RNA degradation which is the possible cause for the hypoalbuminemia of murine schistosomiasis (Metwally *et al.*, 1990).

With respect to transaminase enzyme activities significant elevation was observed in both serum AST and ALT. As previously mentioned, the free radicals elaborated by *S. mansoni* toxicity lead to accumulation of calcium in the mitochondria that in turn leads to irreversible damage to the membrane and discharge of its enzymes content, since AST and ALT are sub-cellular enzymes localized in mitochondria and cytoplasm (Van Noorden and Frederiks, 1992).

Moreover, aminotransferase enzymes are considered as marker enzymes for cell toxicity and their elevated level in serum give an additional support for *S. mansoni* cytotoxicity (El-Shazly *et al.*, 2001). The diminution of AST was more manifested than that of ALT donating that, although the latter is more specific for liver cells, yet it is less sensitive than AST in detecting liver cell damage (Awadalla *et al.*, 1975).

Also, the activities of transaminases can serve as index of the metabolic aerobity degree or a relative role of aerobic and anaerobic pathways in the energetic metabolism of different animals, since AST can provide krebs cycle intermediates which in turn favour the succinate production, while ALT can be correlated to lactate production through transforming alanine to pyruvate. These observations lead to express a supposition about a regulatory role of AST in oxidative metabolism, while ALT participates in the regulation of glycolysis (Zayed, 1998). The observed reduction in AST and ALT in liver and their high relative concentration in serum are attributed to the hepatocellular damage

resulting from egg deposition where the transaminases level showed an intimate relationship to cell necrosis and/or increased cell membrane permeability to discharge of the enzyme to blood stream (El-Shazly *et al.*, 2001).

Serum alkaline phosphatase enzyme shows also a significant elevation in *S. mansoni* infected mice. Higher level of ALP in serum was observed by El-Aasar *et al.* (1989) and Abdel-Rahman *et al.* (1993) who attributed the elevation level to the irritation of liver cells by toxins or metabolic products of growing schistosomules of adult worms and eggs or due to increase loss of intracellular enzyme by diffusion through cell membrane which appear to act as a stimulus to the synthesis of more enzyme higher rate of formation would in turn, increase the rate of diffusion and hence increase serum enzyme activity. This observation was confirmed before by Kaplan (1972) who suggested that response of the liver to any forms of biliary tree obstruction is to synthesize more ALP.

Our data confirmed the medical importance and the curative effects of both *Mirazid* (*Commiphora*) and *Citrus reticulata* extracts in amelioration of serum protein fraction concentrations, total protein content, liver function enzyme activities and bilirubin. Previous reports indicated that *Mirazid* is an effective fasciolicidal drug, with no clinical side effect and is very effective and safe in treatment of *Schistosoma haematobium*. In addition, it causes disruption of *S. mansoni* worms and collapse of tubercles (Haridy *et al.*, 2003; Hegab and Hassan, 2003; Hassan *et al.*, 2003; El-Baz *et al.*, 2003; Massoud *et al.*, 2004).

The present results indicated also the curative effects of both *Commiphora molmol* and *C. reticulata* extracts through reduction of ova count and worm burden.

In various reports concerning *S. mansoni*, Sheweita *et al.* (1999; 1998) pointed out that toxic substances and free radicals elaborated from *S. mansoni* worms consume antioxidants and may affect the capacity of the liver to detoxify or neutralize the effect of the toxic endogenous and exogenous compounds.

The amelioration of protein fraction concentrations after citrus plant extracts treatment in infected mice may be resulted from *C. reticulata* which contain high concentrations of residues of phenols, flavonoid glycosides and polymethoxylated flavones. The flavones group occurs without glycosidic linkages and their antioxidant properties has been shown to protect protein against oxidative damage of free radicals (Akira *et al.*, 2000; Manthey *et al.*, 2001; Manthey and Guthrie, 2002; Ojewole, 2004; Hara *et al.*, 2004, respectively). El-Adawy *et al.* (1999) reported that citrus seeds and its flowers were rich in oil and protein and proved to be a good source for minerals K, Na, Fe, Ca, P and Mg. Based on these

findings, administration of citrus plants to healthy mice showed stimulatory effect of the protein synthetic machineries that stabilizes and protects the normal biological conditions of the body. Also, Whithman *et al.* (2005) showed that Citrus fruit-derived flavonoids have an inverse association with occurrence of coronary heart disease, via their ability to reduce plasma cholesterol concentrations. The total methanol extract of *Citrus juncos* had significant inhibitory effect of acetylcholinesterase *in vitro* (Heo *et al.*, 2004).

In conclusion, infection with *S. mansoni* caused significant depression in all protein fraction concentrations and elevation in liver function parameters in serum. Administration of both commiphora (*Mirazid*) and *C. reticulata* extracts to infected mice showed enhanced level of protein fraction concentrations and normalization in liver function parameters. These results are confirmed through the present finding in eradication of the number of ova and worm burden.

On the other hand commiphora and citrus plants extract administrated to healthy animals promotes total protein content and protein fraction concentrations and preserves normal liver function parameters.

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