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ESSENTIAL ROLE OF *Citrus reticulata* AND MIRAZID IN TREATMENT OF *Schistosoma mansoni* INFECTED MICE: BIOCHEMICAL AND PARASITOLOGICAL STUDIES

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Key words: *Schistosoma mansoni,* adenine nucleotides, adenylate energy charge, glycogen, AMP-deaminase, adenosine deaminase

This study was designed to evaluate the changes in adenylate levels, adenylate energy charge (AEC) and glycolytic enzymes in control and infected mice and treatment with *Citrus reticulata* or the oleo-resin extract from Myrrh of *Commiphora molmol* tree (Mirazid), as a new antischistosomal drug. Alternations in the hepatic content of adenine nucleotides (ATP, ADP and AMP), Pi, phosphate potential, AEC, glucose, glycogen, AMP-deaminase, adenosine deaminase and total protein contents were investigated in a trial to point out the effect of infection with *Schistosoma mansoni* parasite as a hypoxia inducer in mice liver tissues. Moreover, the role of *Citrus reticulata* and *Commiphora* (Mirazid), natural plant extracts in improving the energy status in *S. mansoni* infected mice was studied, since *Citrus reticulata* was previously reported to possess anti-leukemia, antimicrobial and antibacterial activities, whereas *Commiphora* extract (Mirazid) – antifasciolicidal ones.

INTRODUCTION

The control of *Schistosomiasis* infection relies on the use of praziquantel (PZQ) chemotherapy. However, PZQ treatment cannot prevent re-infection and progressive development of the pathology [Johansen, 1998; Dupre *et al*., 1999]. PZQ therapy has been reported to have side effects; hemorrhage in the lung tissue of the host and cytotoxicity [Malheiros *et al*., 2000].

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 vennules causing granulomatous inflammatory reactions followed by a characteristic pattern of hepatic fibrosis [Von Brand, 1979]. Since the liver plays the crucial maintaining role in energy potentials in the body, therefore infection with schistosomes may result in hepatic disorders and metabolic disturbances in host liver.

The adenylate energy charge (AEC) has been proposed as an indicator of both sublethal stress and physiological well-being in aquatic animals [Atkinson & Walton, 1967]. The AEC calculated as ATP +½ [ADP]/ [ATP] + [ADP] + [AMP] is a measure of the amount of energy available from the adenylate pool. The phosphate potential given by [ATP]/ [ADP] [Pi] regulates the flux through the electron transport chain (ETC) and hence its can reflect the rate of oxygen consumption [Van Waarde *et al.,* 1990].

A new trend for treatment of liver disorders as a result of *S. mansoni* infection is the use of natural plant extracts of *Citrus* and *Commiphora* (Mirazid). *Citrus* has been reported to have anti-leukemic properties [Maknai *et al*., 1999], to inhibit human cancer cell proliferation [Tian *et al*., 2001], as well as to display antioxidant activity [Tanizawa *et al*., 1992], antibiotic properties [Tkachenko *et al*., 1999], antimicrobial and antibacterial activities [Fathih *et al*., 2000; Guddadarangavvanahally *et al*., 2000]. Commiphora extract (Mirazid) has been proved to be safe antifasciolicidal drug without any side effects [Massoud *et al*., 2004; Hegab & Hassan, 2003; Hassan *et al*., 2003]. Moreover, it is very effective in treatment of *Schistosoma haematobium* [El-Baz *et al*., 2003]. Also, Hassan *et al*. [2003] reported that Mirazid caused disruption of *S. mansomi* worms' tegument and collapse of tubercles causing eradication in worm burden.

The present study was undertaken as a trial to understand and clarify the antibilharzial effect of *Citrus* in relation to *Commiphora* extract (Mirazid). The measured parameters include: adenine nucleotides (AMP, ADP and ATP), adenylate energy charge (AEC), phosphate potential, glycogen, glucose, AMP deaminase, adenosine deaminase and protein content.

MATERIAL AND METHODS

Chemicals. All chemicals were of analytical grade from Sigma Chemical Company. Mirazid (the oleo-resin extract from Myrrh of *Commiphora molmol* tree, family: Burseraceae) is a product of Pharco Pharmaceutical Company, Egypt. The dosages of the administered agents were: Mirazid: two equal 600 mg oral doses of purified *Commiphora* extract for 3 consecutive days [Haridy *et al*., 2003] on empty stomach, at least one hour before eating; Citrus plants: ethanolic extracts

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of citrus plants were prepared (Natural Product Dep. National Research Centre), oral doses of 10 μg/mL were given for 3 consecutive weeks eight weeks post infestation [Manthey & Guthrie, 2002; Nogata *et al*., 2001].

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), Theodore Bilharz Research 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 Modereyet El-Tahrir, Behera, Egypt in December 2002. It was authenticated by Dr. Mohamed Abd El Ghaffar, Faculty of Agriculture, Al-Azhar University, Egypt. A voucher specimen is deposited at the Dept. of Natural Compounds, NRC, Dokki, and Cairo, Egypt.

Extraction and isolation *Citrus reticulata*. Air-dried, powdered roots of *Citrus reticulata* (0.45 kg) were extracted with 80% ETOH. The ethanolic extract was evaporated and the aqueous residue extracted sequentially thrice with equal volumes of *n*-hexane, Et_2O, EtOAC and *n*-BuOH. The EtOAC extract was evaporated to dryness. The residue monitored by TLC using precoated silica gel 60 F 254 aluminum sheets (0.2 mm tickness, Merck), was found to contain flavonoids. The phenolic residue was subjected to biochemical determinations.

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

Liver perfusion. Worms were recovered by portomesentric technique of Smithers & Terry [1965]. The degree of protection or the percent reduction in challenge was calculated from P=C-V/C x 100, where: P=% protection, C=mean number of parasites recovered from infested animals and V=mean number of parasites recovered from treated animals.

Ova count. The number of eggs per gram of tissue was studied according to the procedure of Cheever & Anderson [1971]. No. of ova in 1 gm of liver = No. of ova in liver digestion 5 mL KOH/ Weight of liver in grams recorded before digestion.

Preparation of liver tissue homogenates. *Energetic parameters* (ATP, ADP, AMP and Pi): 0.25 g of liver tissues were homogenized using 7% TCA for extraction of adenosine nucleotides, according to the method of Wijsman [1976]. *Glucose and protein*: Liver tissue was homogenized in normal physiological saline solution (0.9N NaCl) at a ratio 1:9 w/v. The homogenate was centrifuged for 5 min at 3000 × g at 4°C and the supernatant was used. *Glycogen:* One gram of liver tissue was boiled in 5 mL of 30% KOH for estimation of glycogen. *AMP deaminase and adenosine deaminase:* A liver piece was homogenized in 20 mmol/L cold potassium phosphate buffer, pH 7.1 containg 100 mmol/L KCl and 0.1% 2 mercaptoethanol, centrifuged and filtered for 10 min at 3000 × g at 4°C and the supernatant was used .

PARAMETER ASSAYS

Determination of adenosine nucleotides. *Determination of ATP*: The method used is based on the procedure of Lamprecht & Trauschold [1974]. *Determination of ADP and AMP*: ADP and AMP were both assayed in a single assay system according to the method of Jaworek *et al.* [1974].

Determination of inorganic phosphate. Inorganic phosphate was determined in the same extract in which, ATP, ADP and AMP were assayed by the method of Fiske & Subbarow [1925].

TABLE 1. Effect of mirazid and *Citrus reticulata* on glucose, glycogen AMP-deaminase, Adenosine-deaminase and protein in liver of control and *S. mansoni*-infected mice.

Parameters	Control (G1)	Control mirazid (G2)	Control extract (G3)	Infected (G4)	Infected- mirazid (G5)	Infected- extract (G6)	ANOVA
Glucose	37.9 ± 3.1 $(3,5,6)^{***}(2,5)^{**}$	42.7±1.8 $(3,4,5,6)^{***}(1)^{**}$	31.4±1.8 $(1,2,4)^{***}$	18.9±2.4 $(1,2,3,5,6)^{***}$	33.3±1.5 $(2.4)^{***}(1,6)^{**}$	29.4±1.0 $(1,2,4)^{***}(5)^{**}$	<0.0001
Glycogen	3.3±0.3 $(2,3,4,6)^{***}(15)^{**}$	4.14±0.19 $(1,4,5,6)^{***}$	4.14±0.18 $(1,4,5,6)^{***}$	1.9±0.11 $(1,2,3,5,6)^{***}$	2.8±0.21 $(2,3,4)^{***}(1)^{**}$	2.7±0.26 $(1,2,3,4)^{***}$	<0.0001
Protein	9.69±0.43 $(3,4,5,6)^{***}$	9.37±0.83 $(3,4,5,6)^{***}$	10.98±0.13 $(1,2,4,5,6)^{***}$	4.95±0.24 $(1,2,3,5,6)^{***}$	7.68±0.29 $(1,2,3,4)^{***}$	8.18±0.27 $(1,2,3,4)^{***}$	<0.0001
AMP-deaminase activity	24.3±3.3 $(4)^{***}$	31.1±3.07 $(3,4)^{***}(5,6)^{**}$	14.3±0.50 $(2,4)^{**}$	101.7±14.3 $(1,2,3,5,6)^{***}$	20.2±1.48 $(4)^{***}(2)^{**}$	16.5±0.52 $(4)^{***}(2)^{**}$	<0.0001
Adenosine-deaminase activity	11.7±1.2 $(2,3,4,5,6)^{***}$	23.6±1.3 $(1,4,6)^{***}(3)^{**}$	18.7±1.7 $(1,4)^{***}(2,5)^{**}$	47.1±3.4 $(1,2,3,5,6)^{***}$	23.7±2.5 $(1,4,6)^{***}(3)^{**}$	17.8±0.92 $(1,2,4,5)^{***}$	<0.0001

Data are mean ± SD of eight mice in each group. Glucose, glycogen and protein are expressed as mg/gm tissue. AMP-deaminase and adenosine deaminase activity are expressed as μM moles ammonia (min/mg protein). ** means significant at <0.01 – 0.001 and *** means significant at < 0.001. Analysis of data is carried out by one way (ANOVA) (analysis of variance) accompanied by post hoc (spss computer programme).

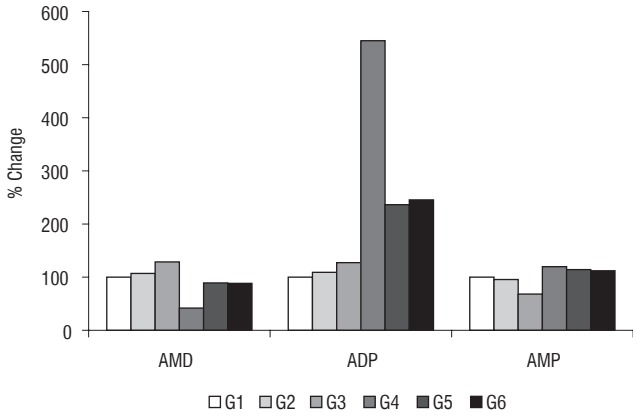

FIGURE 1. The percentage change of hepatic ATP, ADP and AMP parameters in mice of different groups as compared to control.

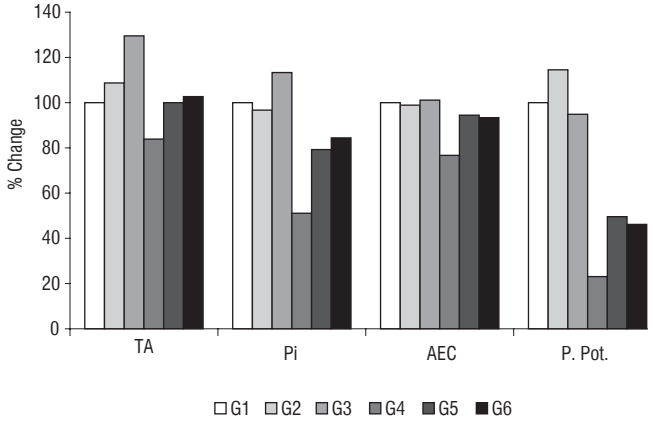

FIGURE 2. The percentage change of hepatic TA, Pi, AEC and P. Pot. parameters in mice of different groups as compared to control.

Determination of glycogen. Glycogen was assayed according to the method of Nicholas *et al*. [1956].

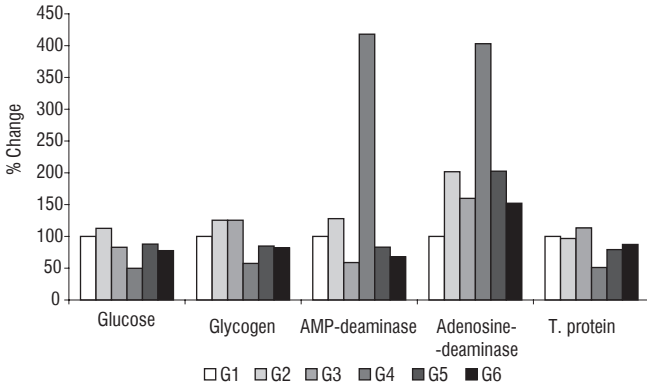

FIGURE 3. The percentage change of some hepatic glycolytic parameters in mice of different groups as compared to control.

Determination of glucose. Glucose was assayed according to the method of Trinder [1969].

Determination of AMP and adenosine deaminases. The assay was carried out by measuring the ammonium ion liberated using phenol hypochlorite reagent according to Fijisawa &Yoshino [1987]. Enzyme activities were evaluated using end point assay method. Protein was estimated according to the method of Bradford [1976].

Statistical analysis. Data in the reported study are presented as mean±SD, the statistical significance of the results was determined using the analysis of variance (ANOVA) combined with post-hoc (SPSS computer program).

RESULTS

Table 1 as well as Figures 1 and 2 indicate that *S. mansoni*-infected mice recorded a significant reduction in ATP,

TABLE 2. Levels of adenylates (ATP, ADP, AMP), adenylate energy charge (AEC) and phosphate potential in liver mice treated with mirazid and *Citrus reticulata*.

Parameters	Control (G1)	Control mirazid (G2)	Control extract (G3)	Infected (G4)	Infected mirazid (G5)	Infected extract (G6)	ANOVA
ATP	1.29±0.039 (3,4,5,6)***(2)**	1.38±0.094 (3,4,5,6)***(1)**	1.66±0.037 (1,2,4,5,6)***	0.54±0.36 (1,2,3,5,,6)***	1.15±0.039 (1,2,3,4,6)***	1.14±0.034 (1,2,3,4,5)***	<0.0001
ADP	0.11±0.0085 (4,5,6)***	0.12±0.0084 (4,5,6)***	0.14±0.010 (4,5,6)***	0.60±0.0234 (1,2,3,5,6)***	0.26±0.039 (1,2,3,4)***	0.27±0.034 (1,2,3,4)***	<0.0001
AMP	0.091±0.0086 (4)***	0.087±0.0081 (4)***(3)**	0.12±0.0084 (4)***(2)**	0.109±0.0071 (1,2,3,5,6)***	0.04±0.012 (4)***	0.102±0.019 (4)***	<0.0001
TA	1.49±0.0038 (3,4)***(2)**	1.62±0.086 (3,4)***(1,5)**	1.93±0.034 (1,2,5,6)***	1.25±0.052 (1,2,5,6)***	1.49±0.061 (3,4)***(2)**	1.53±0.071 (3,4)***	<0.0001
Pi	9.69±0.43 (3,4,5,6)***	9.37±0.83 (3,4,5,6)***	10.98±0.13 (1,2,4,5,6)***	4.95±0.24 (1,2,3,5,6)***	7.68±0.29 (1,2,3,4)***	8.18±0.27 (1,2,3,4)***	<0.0001
AEC	0.90±0.0081 (4,5,6)***	0.89±0.032 (4,5,6)***	0.91±0.0063 (4,5,6)***	0.69±0.015 (1,2,3,5,6)***	0.85±0.013 (1,2,3,4)***	0.84±0.011 (1,2,3,4)***	<0.0001
Phosph-p	1.17±0.064 (4,5,6)***(2)**	1.34±0.11 (3,4,5,6)***(1)**	1.11±0.046 (2,4,5,6)***	0.27±0.05 (1,2,3,5,6)***	0.58±0.095 (1,2,3,4)***	0.54±0.073 (1,2,3,4)***	<0.0001

Data are mean ± SD of eight mice in each group. ATP, ADP, AMP and Pi are expressed as moles /gm tissue. AEC and Phosph-P concentration are without dimensions. ** means significant at <0.01 – 0.001 and *** means significant at < 0.001. Analysis of data is carried out by one way (ANOVA) (analysis of variance) accompanied by post hoc (spss computer programme).

TABLE 3. Effect of mirazid and *Citrus reticulata* on egg count and worm burden *S. mansain* infected mice.

Parameters	Control (G1)	Control mirazid (G2)	Control extract (G3)	Infected (G4)	Infected mirazid (G5)	Infected extract (G6)	ANOVA
Egg count	-	-	-	$12.48\pm0.70\times(10)^3$ (1,2,3,5,6)***	$3.36\pm0.74x(10)^3$ (1,2,3,4,6)***	$5.06\pm1.55x(10)^3$ (1,2,3,4,5)***	< 0.0001
Worm burden	-	-	-	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

Data are means ± SD of eight mice in each group. P is level of significance, where P<0.001 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.

TA, Pi, phosphate potential level and AEC with different percentage -58.14, -16.11, -48.9, -76.92 and -23.33%, respectively. After given mirazid, these parameters changed by -10.85, 20.74, -50.43, and -5.55%. Whereas, when treated with, *Citrus reticulata* they changed by -11.63, 2.68, -15.58, -53.8 and -6.67%, respectively, as compared with the control.

Table 2 and Figure 3 show that in the livers of infected animals glycolytic enzymes were disturbed whereas glucose, glycogen and total protein contents were reduced by -50.13, -42.42, and -48.92% respectively, while AMP-deaminase and adenosine deaminase, significantly elevated by 318.5% and 302.6% with respect to the control group.

Table 3 and Figure 4 illustrate that Mirazid induced reduction in egg count and worm burden recorded a significant reduction by -73% and -81%, whereas *Citrus reticulata* recorded -59.5% and -63.7% in egg count and worm burden, respectively as compared to the infected group.

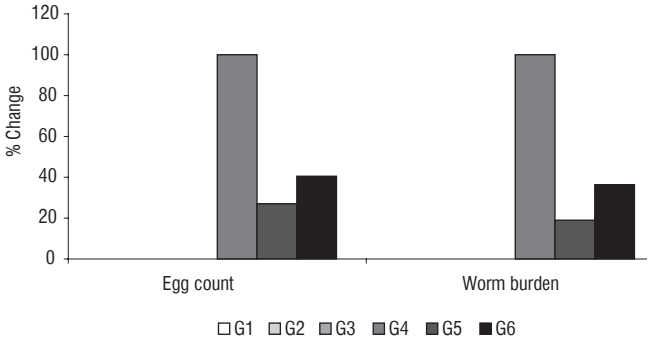

FIGURE 4. The percentage change of egg count and worm burden on hepatic mice at different groups as compared to infected group as control.

DISCUSSION

The dynamic interaction between molluscs and their respective parasites leads either to a state of co-existence or to incompatibility. In the first case the parasite thrives and produces subsequent stages of its life cycle. On the other hand, in incompatible snails, the parasite is either destroyed or eliminated by the snail defense response or fails to develop because the host is physiologically unsuitable [El-Ansary *et al.*, 2001].

In the current study, infection caused a significant depletion in ATP, TA, Pi and phosphate potential level accompanied by an increase in ADP and AMP levels. As early as one month after infection prior to maturation of *S. mansoni* and egg deposition, marked periportal inflammation and tissue necrosis are known to occur. Such tissue reactions have been claimed to be related to toxic products, secreted by maturating schistosomules [Tielens *et al*., 1994].

The effect of infection on ATP levels was directly proportional to the duration post infection. Reduction in the ATP level accompanied by an increase in ADP and AMP concentrations could easily be correlated to the aerobic-anaerobic transition (decrease in rate of O_2 consumption and induction of anaerobic metabolism) induced by the developing parasites [Tielens *et al*., 1994]. El-Ansary *et al*. [2001] reported that a general feature for anaerobiosis is the decrease in ATP and increase in ADP and AMP levels. Moreover, the decline in the ATP level during infection stages is consistent with the previous results of Daugherty [1955] who observed a decrease in the activity of Krebs' cycle enzymes, succinc dehydrogenase and the mitochondrial enzyme cytochrome oxidase following infection of mice with schisotsomiasis. El-Ansary *et al*. [2001] added additional support to the present finding by stating that the significant depletion in ATP in infection with *S. mansoni* may be attributed to a marked depletion in the level of phosphoarginine (PA) which has an important role in buffering changes in ATP by serving as a metabolic pool of inorganic phosphates for transphosphoration of ADP to ATP. Mantawy *et al*. [2004] reported that AEC was decreased following the decrease in cellular ATP level. The decreased ATP level is due to its high utilization as a result of the induced stress by *S. mansoni*. AEC has been assessed as a potentially useful indicator of physiologically stressful conditions [El-Ansary *et al.*, 2001]. In infected group AEC was severely decreased in the present results. Chapman & Atkinson [1977] reported that AEC values below 0.55 are apparently incompatible with maintenance of the minimal level of homeostasis required for viability. Thus, the decreases in AEC of infected mice liver in this study (0.69) are still within the non-stressed range and hence permit recovery. Regulation of the AEC with the non-stressed range can be easily attributed to the remarkable increase in the activities of the glycolytic enzymes, pyruvate kinase and phosphofructokinase previously reported by Ahmed & Gad [1995].

The significant reduction in the inorganic phosphate concentration, in spite of the reduced level of ATP could be explained on the basis that, when the liver is subjected to metabolic stress, this leads to trapping of a large amount of phosphate that results from the presence of abnormally high levels of phosphoryl acceptor [Chapman & Atkinson, 1977].

Phosphate potential is an alternative useful indicator for the energy status of the tissue as well as a regulator of the electron transport system in the mitochondria [Tielens, 1997]. The decline in phosphate potential in the present study may be attributed to the increase of ATP hydrolysis to ADP and Pi with lower turnover rate of ATP indicating

a strong inhibition of oxidative phosphorylation [El-Ansary *et al*., 2001]. These observations are consistent with findings of Ahmed & Gad [1995] who reported a remarkable reduction in the activity of they key enzyme of Krebs' cycle, citrate synthase by the 10th week of inhibition.

The present results revealed a significant increase also in AMP and adenosine deaminases. Higher deaminases activity will increase the glycolytic flux to compensate the decrease in the ATP concentration. This usually occurs through the production of IMP and ammonia as a strong activator of phosphofractokinase, a rate limiting enzyme of glycolysis [Yoshino & Murakami, 1982]. The stimulation of both deaminases in infection has been supported by Tanabe *et al*. [1989].

El-Ansary *et al*. [2000] stated that the significant increase in gluconeogenic enzymes fructose 1-6,diphosphatase is due to depletion of glucose in the infected group and the ratio of glycogen to glucose. Their levels in liver are known to be regulated by the balance between glycogen synthesis and degradation capacities. The increased influx of glucose enhanced glycogen stored leads to stimulation of the enzymes. In addition, glycogen and glucose together with glucose-6-phosphate can favour the Crabtree effect needed by the developing schistosoma parasite. This can be explained on the basis that more active glucose-6-phosphate will lead to minimization of the concentration of G-6-phosphate, an inhibitor for hexokinase [Horemans *et al*., 1992] a rate limiting enzyme of glycolysis. High glycolytic flux will simultaneously occur in the developing parasite and its moluscan hosts, leading to increased lactate production and inhibition of respiration, by glucose as a product of glucose-6-phosphatase [Nabih *et al.*, 1998].

El-Ansary *et al*. [2003] suggested that in order to accurately identify and quantify the contribution of gluconeogensis to glucose synthesis it is important to consider factors potentially mediating the effects of infection on glucose synthesis such as a relative level and utilization of glycogen and the level of anaerobic metabolism. In the present study, the reduction in glycogen content is consistent with glucose, as building units of glycogen and this may reflect the high utilization of ATP due to the stress induced by infection [El--Ansary *et al*., 2001]. In addition, Tielens [1997] previously mentioned lactate accumulation and glycogen depletion confirming inhibition of aerobic respiration and stimulation of anaerobic glycolysis through hexokinase – a rate limiting enzymes of glycolysis.

In the present study, hepatic total protein was reduced in bilharzail infection. This could be attributed to cellular damage caused by parasite toxins [Van Raaij *et al*., 1994]. This was similar to results of El-Fakahani *et al.* [1993] and Rizk *et al*. [2000] who reported low liver total protein in schistosomaisis.

The main fraction of total protein content is albumins and the reduction in total protein may be due to the reduction in albumin fraction level that in turn may result from decreased anabolism or increased catabolism; hence, malnutrition and/or malabsorption may contribute to decreased biosynthesis of albumin [El-Fakahani *et al*., 1993]. The significant decrease in total protein is mainly due to an increase in messenger RNA degradation which is the possible cause for the hypoalbuminemia of murine schistosomiasis [Metwally *et al*., 1990].

On studying the effect of both Natural Purified Commiphora and citrus plants extract on control mice, citrus plants caused a significant increase in ATP, TA, Pi, glucose, glycogen, adenosine deaminase and protein. While purified Commiphora extract showed a significant increase in phosphate potential in addition to the previous parameters. This enhancing ability of both extracts may be related to antioxidantive activities [Tanizawa *et al*., 1992].

On the other hand, Citrus plants given to infected mice were noticed to cause significant enhancement in energetic parameters ATP, ADP, AMP, TA, Pi, AEC and phosphate potential in addition to glucose, glycogen both deaminases and protein, with percentage improvement recorded 46.51, 300, 7.69, 18.79, 33.33, 16.66, 23.16, 27.70, 24.24, 350.62, 250.43, 33.33%, respectively.

In various reports concerning *S. mansoni*, Sheweita *et al*. [1997, 1998] pointed out that levels of reduced glutathione and glutathione reductase increased, while the activity of glutathione S-transferase decreased in human and mice infected with *S. mansoni*. In this respect S. *mansoni* infection alters and consumes the hepatic levels of glutathione, superoxide dismutase, catalase and glutathione metabolizing enzymes (antioxidant system) and these alterations may affect the capacity of the liver to detoxify or neutralize the effect of toxic endogenous and exogenous compounds. Thus the significant improvement of *Citrus* plants on all the previous mentioned parameters in infected mice may be attributed high concentrations of several classes of phenols, including numerous hydroxycinnamates, flavonoid glycosides and polymethoxylated flavones, in *Citrus* fruits. The latter group of compounds occurs without glycosidic linkages and has been shown to inhibit the proliferation of a number of cancers and to protect protein against oxidative damage [Manthey & Guthrie, 2002]. The anti-inflammatory activities of citrus flavonoids arise from the antioxidant properties of these compounds [Manthey *et al*., 2001].

Citrus flavonoids revealed antibiotic, free radical scavenger, anti-leukemia and antibacterial activities [Akira *et al*., 2000]. In addition, the citrus seeds and its flours were rich in oil and protein and proved to be a good source for such minerals as K, Ca, P, Na, Fe and Mg [El-Adawy *et al*., 1999]. The essential oil of citrus plants was shown to possess also antimicrobial activities [Fathih *et al*., 2000]. The potential activity of this plant extract in inducing glycogen and glucose levels could be easily correlated to the previous reports of El-Ansary & Farouk [2001] who reported that a *C. longa* extract was effective in restoring normal adenylate energy charge (AEC), through the activation of the oxidative phosphorylation pathway. Stimulation of oxidative phosphorylation as the main ATP-generating pathway could explain the glycogen repletion observed in the present study due to *C. longa* treatment of schistosome-infected mice.

With respect to the Commiphora extract (Mirazid) given to infected mice, a significant improvement was recorded at levels of 47.29, 309, 5.49, 16.11, 28.17, 17.78, 26.49, 37.99, 27.27, 335.39, 200 and 28.17% for ATP, ADP, AMP, TA, Pi, AEC, phosphate potential glucose, glycogen, AMP deaminase, adenosine deaminase and total protein, respectively.

Eradication of the number of egg count and worm burden is shown in infected mice treated with Mirazid more than citrus plants extracts, which gives an additional support for the curative effects of both extracts.

Massoud *et al*. [2004], Haridy *et al*. [2003] and Hegab & Hassan [2003] reported that Mirazid is an effective fasciolicidal drug, with no clinical side effect. On the other hand, Hassan *et al*. [2003] stated that Mirazid has the ability to contract *S. mansoni* worms muscle and affect the worm surface ultra-structure, causing disruption of the tegument and collapse of tubercles. El Baz *et al.* [2003] proved that Mirazid was very effective and safe in treatment of *Schistosoma haematobium.*

CONCLUSION

Infection with *S. mansoni* caused inhibition of energetic parameters ATP, AEC, phosphate potential total adenylate, Pi, glucose, glycogen and total protein and elevation of AMP and adenosine deaminases. Administration of both Commiphora and Citrus plants extracts showed improvement in most parameters under investigations. These results are confirmed through the present finding in eradication of the number of ova and worm count. On the other hand, administration of both extracts to normal healthy mice enhanced total adenylate glucose, glycogen and adenosine deaminase levels.

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