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Antischistosomal and liver protective effects of *Curcuma longa* extract in *Schistosoma mansoni* infected mice

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With a view to clarify the induction of the "Crabtree consequence" in liver cells of *S. mansoni* infected mice, the curative effect of oil extract of *C. longa* was tested and compared to praziquantel (PZQ) the effective drug against all schistosome species occurring in man. Protein, glucose, glucose-6-phopsphatase, AMP-deaminase, adensoine deaminase, urea concentration, pyravate kinase (PK), phosphoenol pyruvate carboxykinase (PEPCK) and PK/PEPCK ratio were estimated. In addition, worm burden and ova count in mice infected with *S.mansoni* were elucidated. The result showed that *C. longa* normalized the concentration of protein, glucose, AMP-deaminase and adenosine deaminase, which were changed by infection. Moreover, it lowered pyruvate kinase level, while PZQ-treatment induced more elevation of this enzyme. PZQ was more effective in lowering worm burden while *C. longa* extract was more potent in reducing egg count.

Keywords: Adensoine deaminase, AMP-deaminase *Curcuma longa*, Glucose, Glucose-6-phopsphatase, Praziquantel, Protein, *Schistosoma mansoni*, Schistosomiasis

Schistosomiasis is one of the most common parasitic diseases which mostly affects the liver and intestine, causing granuloma formation and hepatic fibrosis.Schistosomisis also causes certain necrotic changes in liver tissues[1, 2].

The chemotherapy of schistosomiasis has been reviewed[3]. praziquantel (PZQ) compound is highly active against *Schistosoma haematobium, S. japonicum* and *S. mansoni* in the hamster with no apparent significant differences against the different geographical strains of the parasites[4].

A new trend for treatment of liver disorders as a result of *S. mansoni* infection is the use of natural plant extracts. Curcumin, a yellow colouring agent from turmeric (*Curcuma longa* linn, Zingiberaceae) has been shown to inhibit tumour formation in diverse animal models[5]

Curcuma x*anthorrbiza* improved the diabetic symptoms such as growth retardation and elevation of glucose in the serum[6]. Curcumin (diferuloyl methane) a natural product obtained from the rhizomes of *Curcuma longa*, enhanced wound repair in diabetic impaired healing and could be developed as a pharmacological agent in such clinical settings[7]. Cardioprotective effects of *C.longa* correlate with the improved ventricular function. Histopathological examination further confirmed the protective effects of *C.longa* on the heart[8]. *C. longa* has anti-inflammatory, antioxidant and anti-cancer activities[9].

Total liver protein content shows non-significant differences in schistosoma infected mice as compared to control[10]. A significant decrease in concentration of serum total proteins and albumin has also been observed in patients with active schistosomiasis (either *S. mansoni* alone or mixed with *S. haematobium*)[11].

Glucose-6-phosphatase(G-6-Pase) activity shows a significant increase in hepatic cells of *S. mansoni* infected animals[12]. After PZQ administration, a marked decrease of hepatic G-6-Pase was observed[13]. It has been recognized that the chief source of the ammonia produced by working muscle is the reaction catalyzed by AMP deaminase[14]. There is a high correlation between AMP-deaminase activity and phosphofructokinase activity. This lends credence to the idea that one function of AMP-deaminase may be regulated flux through the glycolytic pathway[15]. Since ammonia is formed by many parasites, it seems probable that competition for substrates by phosphoenol pyruvate carboxykinase (PEPCK) and pyravate kinase (PK) could account for the apparent

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shift in the proportions of succinate and lactate formed when oxygen is present in the maintenance medium[16]. However, there is a group of parasites which have been described as homolactate fermenters; *Schistosoma mansoni,* which possesses PEPCK, belongs to this group[17].

The liver is the main organ responsible for the biosynthesis, uptake and degradation of proteins and enzymes. Liver infection, may therefore, be reflected to some extent on the levels and/or the activities of these circulating biochemical compounds in serum. Hepatic schistosomiasis is one of the most prevalent forms of human hepatic fibrosis in the world[18] and a reproducible experimental model of disease can be obtained in infected mice[19].

The present study has been undertaken to evaluate antischistosomal and liver protective effects of *Curcuma longa* extract in *S. mansoni* infected mice. The parameters selected were protein, glucose, glucose-6-phopsphatase (G-6-Pase), AMP-deaminase, adensoine deaminase, urea concentration, pyravate kinase , phosphoenol pyruvate carboxykinase and PK/PEPCK ratio. As worm burden and ova count are closely related to the glycolytic flux which could reflected the Crabtree effect usually induced by the schistosome parasite in the definite host,they were also estimated.

Materials and Methods

Experimental animals—Healthy male albino mice of CD strain weighting 20–25 g obtained from the Schistosome Biological Supply Programmes (SBSP), Theoder Bilharz Institute, were used. They were feed stock commercial pellets (El– Kahira Company for Oil and Soap) and water was supplied *ad libitum*.

Drugs—Praziquantel drug (suspension), a product of Egyptian International Pharmacetical Industries Company (E.I.P.I. Co) was purchased locally. *Curcuma longa* crude material (obtained from Chemistry and Pharmacognosy Department, National Research Centre) was reduced to a moderately coarse powder. The powder (100 g) was immerged with 500 ml of 70% ethyl alcohol for 72 hr, with occasional shaking. The extract was concentrated to dryness[20].

Chemicals—All the reagents used were of analytical grade obtained from Sigma (USA), Merck (Germany), BDH (England), Reidel (Germany) and Fluka (Switzerland) chemical companies.

Infection—For the infection of mice, 10–20 *Biomphalria alexandrina* snails were placed in a beaker containing 200 ml dechlorinated water. In order to shed cercariae the snails were exposed to sunlight at 0800–0900 hrs. Each mouse was subjected to subcutaneous injection with 50 cercariae[21].

Animal treatments—Animals were divided equally into three batches of one, two and three month's age animals respectively. Each batch was subdivided into 4 groups. For the 1st and 2nd batches, these 4 groups included group I as control, group II as infected group, group III as control treated with praziquantel (PZQ), while group IV was given (PZQ) post infection. Animals of groups III and IV served as control and infected were given PZQ and sacrificed after 7 days of treatment. Mice of the third batch of 3 months age were subdivided into the following four groups: I, control, II infected group, III served as *C. longa*–treated control while IV, was used as *C. longa*–treated infected group.

The experimental design and groups' distribution can be summarized as follows:

Batches*	Treatment	Duration
Batch 1		
Group I (control)	----	
Group II (infected)	----	One month
Group III (control treated)	Praziquantel drug 500 mg/kg body weight on two successive days	
Group IV (infected treated)		
Batch 2		
Group I	----	Two months
Group II	----	
Group III	Praziquantel drug 500 mg/kg body weight on two successive days	
Group IV		
Batch 3		
Group I	----	
Group II	----	Three months
Group III	*Curcuma longa* extract300 mg /kg body weight after one month post infection twice a week for two months.	
Group IV		

Each group contains six animals.

Praziquantel suspension (500 mg/kg body weight) was given orally, on two successive days[22]. *Curcuma longa* extract was given orally to control and infected mice, one month post infection period of two months, twice per week extending up to 300 mg/kg body

weight[20].

At the end of each period, a group of six mice were weighed and sacrificed and the liver was taken for the biochemical analyses of protein, glucose, glucose-6-phosphatase, AMP–deaminase, adenosine deaminase, urea, pyruvate kinase (PK), phosphoenol pyruvate carboxykinase (PEPCK), liver and body weights. In addition, worm burden and ova count in infected mice were recorded.

Preparation of tissue homogenates for biochemical analysis

Liver tissue (1g) was homogenized in 9 ml bi-distilled water to yield 10% homogenate, the homogenate was used for determination of protein, glucose, glucose–6-phosphatase, and urea concentration.

Protein was estimated by the method of Bradford[23], as modified by Gogstad and Krutnes[24]. Glucose[25], inorganic phosphate[26], glucose -6- phosphatase (EC 3.1.3.9) activity[27] and urea[28] were determined.

The hepatic tissue was homogenized in 10 volume of 20 mM cold potassium phosphate buffer (*p*H 7.1) containing 100 mM KCl and 0.1%, 2-mercaptoethanol. The mixture was centrifuged for 10 min. The filtrate was used for the measurment of adenylate degrading enzymes[29].

AMP-deaminase (EC 3.5.4.6) activity was determined through the measurement of ammonium ion liberated using phenol hypochlorite reagent[30].

Adenosine deaminase activity (EC 3.5.4.4) was determined by enzymatic colorimetric method of Fijisawa and Yoshino[29].

One gram tissue of the liver was homogenized in 5 ml Tris-HCl buffer *p*H 7.6 according to Umezurike and Anya [31]**,** the homogenate was centrifuged at 4000 rpm for 15 min, and the supernatant was used for the measurment of pyruvate kinase and phosphoenol pyruvate carboxykinase.

Pyruvate kinase (EC 2.7.1.40) activity was measured according to the method of Bucher and Pfleiderer[32].

Phosphoenol pyruvate carboxykinase (EC 4.1.1.49) was assayed spectrophotometrically by the procedure of Suarez *et al.*[33]. OAA formation from PEP and $NaHCO_3$ were determined by measuring the oxidation of NADH in presence of malate dehydrogenase at 340 nm.

Recovery of adult worms of infected mice (liver perfusion)—After infection of mice with 50 cercariae, adult *Schistosoma mansoni* worms were recovered from hepatic portal system and liver by the perfusion technique described by Smithers and Terry[34].The infected mouse was killed by intraperitoneal injection of 0.15 ml of thiopental (0.5 µg). The adult worms were recovered from the hepatic system and the liver by perfusion with citrate saline (0.85% sodium chloride, 1.5% sodium citrate). When the liver, kidney and gut become pale, the perfusion process was stopped. The perfusate was collected in a container attached to the perfusion plate. The coils of the intestine were lifted from the tray and washed down in order to dislodge any worms adhering to them.

Worm counting—The degree of protection or the percent reduction in challenge was calculated as follows[35]: P = C-V/C × 100

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

Ova count—The number of ova in each tissue was counted in the slides, and the average was calculated [36, 37].

Statistical analysis—The statistical significance of the results was determined by the method of Ronald *et al*[38].

Results and Discussion

Previous studies have shown that the interaction between schistosomal parasites and the mammalian host is extremely complex. Many parasitologists have focussed their studies on the epidemiology of schistosomiasis or the physiology of the parasites neglecting to some extent the metabolic changes developed in the host in consequence to infection or drug treatment. Despite the possession of a mouth and functional gut, glucose is taken up by the schistosome parasites across their outer body surface or tegument. All cells appear to move glucose molecules across specific glucose transporter proteins (GTPs)[39]. Because mammalian stage of schistosomes live in a high glucose medium, it seems likely that uptake of host glucose accelerated the synthesis of these transporters[40].

In the present study, as early as one month post infection of mice with *Schistosoma mansoni* cercariae and prior to maturation and egg deposition, significant elevation of protein and glucose was observed in the tissue homogenates of schistosome-infected animals The results showed significant elevation of protein

and glucose concentrations after one month post infection when compared with control group of mice, while at 2nd month post infection, a marked reduction in protein, glucose levels and glucose-6-phosphatase activity with respect to control mice was observed (Table1). Treatment of infected mice with PZQ caused a pronounced reduction of the elevated level of protein and reduction of glucose reaching to the level of control mice. While on the other hand, glucose was elevated after treatment with PZQ as compared with control. Increase in protein concentration, and marked decrease in glucose at the first month, when compared to the untreated control, was observed (Table1).

This may prove that at this stage of infection, the parasite has no disturbing effect on host metabolism. The elevation of the measured parameters could be attributed to the presence of the parasite itself. Total liver protein content showed non significant differences in schistosome infected as compared to control. A significant reduction of protein, glucose and G-6-Pase after 3 months of infection and these reductions was observed when treated with *C. longa* extract (Table 2).

These findings may be due to either the decrease in hepatic cell population due to the liver fibrosis or the release of the enzyme from the damaged livers into the circulation[10]**.** El-Hawry *et al.*[41] recorded that the decrease in albumin fraction may be due to decreased anabolism or increased catabolism, Malnutrition and/or malabsorption may contribute to the decreased biosynthesis of albumin.

Praziquentel (PZQ) has become the drug of choice in most endemic areas because of its efficacy, ease of administration, tolerable sideeffects and cost. As a consequence of this positive trend, two potential dangers have emerged. The possibility that other existing drugs may be discontinued. Further, there is diminished interest of major pharmaceutical companies in the quest for novel active compounds[42]. In the present study, PZQ was not effective in restoring normal glucose level. Control, one and two months-infected animals were found to have lower glucose concentration. Moreover, PZQ- treatment reduced the protein content of hepatic cells either in control or infected animals. This could be explained on the basis that this drug may interfere with amino acid and protein metabolism of treated animals.

There is still intensive search required for effective anti-schistosomal drugs with minimal side effects. Natural health products have become increasingly

Table 1—Effect of praziquantel drug (PZQ) treatment on liver protein, glucose and glucose-6-phosphatase enzyme of *S. mansoni* infected mice

[Values are mean ± SD from 6 mice in each group]

Parameters	Durations	Control	Experimental groups		
			Infected	Control-PZQ	Infected-PZQ
Protein*	One month	11.6±0.0046	13.2±0.0039^{a}	12.4±0.0107^{ns}	11.6±0.0042^{al}
	Two months	12.0±0.0061	9.4±0.0056^{a}	11.4±0.0037^{ns}	7.0±0.0034^{a,al}
Glucose*	One month	72.5±9.3	88.7±9.69^{a}	45.00±2.91^{a}	81.3±3.75$^{b, cl}$
	Two months	87.25±3.89	41.7±1.95^{a}	77.2±12.05^{b}	37.3±3.64^{a,al}
Glucose-6-phosphatase**	One month	4.62±0.68	3.51±0.21^{a}	3.22±0.17^{a}	3.07±0.151^{al}
	Two months	2.96±0.27	1.94±0.31^{a}	3.68±0.22^{a}	2.49±0.48^{b,bl}

* mg/g tissue;** μ mol glucose released/min/mg protein.
P values*:* ^{a}<0.001; ^{b}<0.01;^{c}<0.05; ^{*ns} non-significant as compared with control
^{a1}<0.001; ^{b1},<0.01; ^{c1} <0.05 as compared to infected group

Table 2—Effect of *C. longa* extract treatment (3 months) on liver protein, glucose and glucose-6 phosphatase enzyme of *S. mansoni* infected mice

[values are mean ± SD from 6 mice in each group]

Parameters	*Control*	Experimental groups		
		Infected	Control- *C.longa*	Infected-*C.longa*
Protein*	13.0±0.0054	11.4±0.03 ^{a}	14.2±0.0056 ^{b}	11.6±0.0039 $^{a, ns}$
Glucose*	68.8±1.0	33.9±1.15 ^{a}	69.69±11.78 ^{ns}	39.5±4.41 $^{a, al}$
Glucose-6-phosphatase**	2.39±0.064	1.79±0.21 ^{a}	2.96±0.25 ^{a}	2.07±0.24 $^{a, bl}$

Other details are same as in Table 1

important during the past few years[43,44] The huge global economic potential for the production and processing of medicinal plants has led to important initiatives in research, development and regulatory procedures. In the present study, *Curcuma longa* extract having medicinal properties[45,46], was used against *Schistosoma mansoni*. The results showed proved that *C.longa* extract induced a significant elevation of glucose concentration in control and infected *C. longa*-treated animals. A significant elevation in AMP-deaminase in infected mice and those infected and given PZQ was observed. Also, adenosine deaminase activity and total urea concentration revealed increase with different percentage at different durations (Table 3). The activity of G 6-Pase, was found to be variably affected by bilharzial infection [47.] In the present study, G-6-Pase was found to be greatly inhibited in schistosome-infected mice (1, 2 and 3 months post infection). The pronounced decrease in G-6-Pase activity reported in the present study due to infection seems to be consistent with the lower adenylate energy charge (AEC) previously reported in schistosome-infected animals at the same experimental durations[48]. Lower AEC usually accompanied by activated glycogen phosphorylase and glycolytic enzymes and inhibited glycogen synthase and gluconeogenic enzymes[49]. G-6-Pase as a gluconeogenic enzyme was inhibited with infection. This led to the availability of glucose-6-phosphate as a substrate for phosphohexoisomerase the glycolytic enzymes. Reduced G-6-Pase activity recorded in the present study is in full agreement with El-Merzabani *et al*[50] and Daniele *et al*.[51] who reported a significant reduction in G-6-Pase activity between normal and schistosome infected livers, respectively.

The decrease of G-6-Pase activity indicates the damaging effect of schistosomiasis on liver parenchymal cells. The hepatic hypoglycemia reported in the present investigation in infected animals may be a normal consequent to the decrease of G-6-Pase activity level. This could be supported through the work of El-Haieg *et al.*[10] who recorded the inability of hepatic cells to dephosphorylate glucose-6-phosphate into glucose, the marked decrease in glycogen, total liver protein content. Moreover it is good with Hara *et al.*[52] who found a significant decrease of G-6-Pase enzyme activity in the liver of mice infected by subcutaneous injection with *S. mansoni* cercariae. They attributed the decrease in enzyme activity to molecular and biological changes occurring in hepatic and granulomatous cells.

PZQ as uncharged compound was found to induce some morphological alterations in human erythrocytes. Haemolysis of erythrocytes and release of membrane lipids (phospholipids and cholesterol) were shown to be concentration-dependent[53]**.** These results suggest that distinct cell membrane interaction pathways lead to drug-specific mechanisms of cytotoxicity. The change in the activity of G-6-Pase observed in control and infected animals-treated with PZQ could be correlated to its membrane interaction and increase of hepatocytes permeability. *C. longa* on the other hand was effective in activating G-6-Pase in control-treated mice. In case of infected animals, *C. longa* extract significantly reactivated G-6-Pase which showed its potent effect in restoring normal hepatocyte permeability[54, 55]. Moreover, Jayadeep *et al.*[56] revealed that the antioxidative and hypolipidaemic action of *C. longa* is responsible for its protective role against ethanol induced brain injury. In recent years, clinical interest has been directed to the study of other liver enzymes which may offer an improved specificity over the conventional liver function enzymes for the

Table 3—Effect of Praziquantel drug (PZQ) treatment on AMP-deaminase, adenosine deaminase and urea of *S. mansoni* infected mice

[Values are mean ± SD from 6 mice in each group]

Parameters	Durations	Control	Experimental groups		
			Infected	Control-PZQ	Infected-PZQ
AMP-deaminase*	One month	4.98±0.46	6.01±0.81 [b]	3.75±0.42 [a]	5.75±0.06 [a, ns]
	Two months	3.68±0.68	4.84±0.46 [a]	5.0±0.66 [a]	5.20±0.71 [a, ns]
Adenosine deaminase*	One month	3.79±0.28	4.77±0.66 [a]	3.01±0.42 [a]	4.96±0.83 [b , ns]
	Two months	3.06±0.36	4.02±0.46 [a]	3.95±0.42 [a]	4.30±0.48 [a , ns]
Urea*	One month	136.6±6.1	137.1±2.6 [ns]	170.7±13.6 [b]	162.6±6.19 [a , al]
	Two months	178.1±5.9	165.0±6.1 [c]	209.9±3.21 [a]	322.0±15.0 [a, al]

*μ moles ammonia/min/mg protein.
Other details are same as in Table 1

confirmation of the degree and activity of liver disease and the success of its treatment. In the present study, AMP deaminase and adenosine deaminase as adenylate degrading enzymes were studied. These two enzymes were selected since they have a regulatory role on the glycolytic pathway as the most important metabolic pathway in schistosome-infected definitive or intermediate hosts[57–59]. AMP deaminase and adenosine deaminase were biochemically detected in the liver tissues of normal-healthy animals. In the present investigation both enzymes were significantly stimulated in schistosome- infected mice at the three different durations studied (Table 3). Activation of these two enzymes is consistent with the stimulation of the glycolytic flux previously reported in schistosome infected animals[60]. AMP deaminase activity is closely correlated with the activity of phosphofructokinase and pyruvate kinase as key enzymes of glycolysis in mammalian cells. The ammonia formed by deamination of AMP may accelerate the rate of glycolysis through these two enzymes[61,62]**.** The presence of an activated AMP-deaminase in schistosome-infected liver cells confirms the dependence of energy metabolism of infected animals on the glycolytic flux.

Moreover, because the liver plays a central role in detoxication of free ammonia absorbed from gastrointestinal tract or derived from amino acid breakdowns in a large number of higher organisms, hepatic damage often results in elevation of blood ammonia levels[63]**.** Actually, hyperammonemia and abnormal ammonia tolerance have been demonstrated in mice experimentally infected with *S. mansoni* and patients with hepatosplenic schistosomiasis[64]. Such alterations in the amino acid catabolism and in the detoxication of free ammonia are considered to be related with the activity of urea cycle.

In the present study, urea levels was related to enzymes AMP deaminase and adenosine deaminase which produce ammonia as a metabolic product, since urea is the detoxified product of ammonia. The recorded lower urea concentration in schistosome-infected mice could be easily correlated to the significant decrease in both total and specific activities of carbamayl phosphate synthetase and ornithine carbamyl transferase[63] of female mice infected with cercariae of *S. mansoni*, Moreover, this decrease in the urea concentration confirmed the work of Rizk *et al.*[65] who reported inhibition of carbamayl phosphate synthetase, argininosuccinate lyase and arginase as urea cycle enzymes greatly affected with schistosome infection. Inhibition of the ammonia detoxifying activity in hepatocytes of infected mice could be explained on the basis that infections significantly inhibit glutathione-S-transferase, glutathione reductase, reduced glutathione together with cytochrome P450 and NADH-cytochrome c reductase as a group of drug metabolizing enzymes[66]. The alteration in the activities of these drug-metabolizing enzymes as a result of infection with different levels of *S. mansoni* may thus change the liver's capacity to detoxify endogenous compounds as ammonia through its conversion to urea.

The effectiveness of PZQ as an antibilharzial drug was noticed in the present investigation. PZQ reduced the enzymatic activities of AMP-deaminase and adenosine deaminase to a certain extent. Inhibition of these two enzymes could be related to its inhibitory effect of glycolysis and stimulation of Krebs cycle previously reported by Ahmed and Gad[67]. PZQ also was effective in elevating urea concentration in control and infected mice livers. Elevation of urea concentration with PZQ treatment, two and three months post infection confirmed that liver cells restored its NH_3 detoxifying ability. *C. longa* extract was more effective in normalizing the enzymatic activities of AMP deaminase and adenosine deaminase when compared to PZQ. This could be easily explained on the basis that *C. longa* has potential role in the stimulation of oxidative phosphorylation and stabilization of adenylate energy charge (AEC) as a biochemical parameter controlling the flux of metabolites through the glycolytic pathway[48, 68]. *C. longa* was also effective as PZQ in initiating the impaired NH_3 detoxifying mechanism in schistosome-infected animals. Hasmeda and Polya[69] reported that curcumin, a major bioactive secondary metabolite isolated from rhizomes of *C. longa* inhibits cyclic-AMP dependent protein kinase. Lower protein kinase activity could be helpful through the cascade mechanism to keep glycogen phosphorylase and glycogen synthase in the dephosphorylated form. This in turn may suppress glycogen breakdown and stimulates glycogen synthesis which could explain the glycogen repletion in *C. longa* treated-infected animals[49].It was suggested that an important control point in the respiration of parasitic helminthes exist at the level of phosphoenol pyruvate (PEP)[70]. The nature

of the end product formed is dependent on the competition for PEP by the two enzymes pyruvate kinase (PK) and phosphoenol pyruvate carboxykinase (PEPCK). Stimulation of PK in schistosome-infected animals ascertained the stimulation of the glycolytic flux[67]. Stimulation of PK was reflected on the substrate availability of PEP for PEPCK and resulted in its remarkable inhibition two and three months post infection[71]. This in turn led to significantly higher PK/PEPCK ratios in schistosome-infected mice. PK/PEPCK ratio is described as potential regulatory site. It may give an indication about a major pathway *in vivo*[72]. Animals that rely on glycolysis generally have a ratio in the region 2-10, whilst those that rely on Krebs cycle have a much lower ratio (0.05-0.1) in the region[73]. Data of the present investigation confirmed the stimulation of the glycolytic flux with bilharzial infection. Values of 2.76, 8.79 and 4.5 PK/PEPCK ratios were recorded for one, two and three months infected mice respectively compared to values of 2.15, 3.75 and 1.95 for the corresponding age-matching controls. PZQ was effective in lowering PK as a rate limiting enzyme of glycolysis. PK and PEPCK activities significantly reduced in control-treated (group III). On the other hand PK/PEPCK ratio was significantly elevated in the two groups III and IV as compared to group I (Table 4) able to switch on/off the whole metabolic pathway. This finding is in good agreement with Ahmed and Gad[67] who reported a more or less same effect of PZQ on phosphofructokinase (PFK) another glycolytic regulatory enzyme.

In the present study, PZQ caused a significant inhibition of the gluconeogenic enzyme PEPCK in control and infected animals. Inhibition of PEPCK was reflected on the PK/PEPCK ratio resulted in ratios of 19.11 and 11.39 for control and infected PZQ-treated animals. This may be helpful to clarify the impairment of the gluconeogenic activity in PZQ-treatment. Inhibition of PEPCK could be easily correlated to the inhibition of G-6-Pase, discussed earlier. *C. longa* was highly effective in normalizing PK activity of infected mice (3 months post infection) and at the same time it has less inhibitory effect on PEPCK showing a value of 40.7% decrease compared to a value of -62.3% in PZQ-treated infected animals. The slight effect of *C. longa* on this respect could be easily noticed in the remarkable lowering of PK/PEPCK ratio of 3.26 which showed a slightly impaired gluconeogenic activity in infected mice treated with this extract.

PK and PEPCK activities were decreased in both control and infected groups treated with *C.longa*. *C.longa* can be easily noticed through the reduction of PK and PEPCK activities when compared to the control group. AMP-deaminase, adenosine deaminase activities and urea concentration were elevated in infected group treated with *C.longa*[80] (Table 5). In infection by *S. mansoni,* the major pathologic changes are not caused by the adult worm itself but by eggs which do not reach the intestinal lumen, but instead, become trapped in other body tissues. At these sites, areas of local inflammation are produced, cumulating in the formation of granulomas around eggs[74]. The formation of granuloma around schistosome eggs in the liver and the intestine is the major cause of pathology in schistosome infections[75]. It is the result of a classic Th2 cytokine-mediated immune response that was shown to involve tumor necrosis factor-α (TNF-α)[76, 77].

In the present investigation, PZQ was effective in reducing worm burden, ova count and granuloma size in infected -treated mice that PZQ treatment post infection induced reduction in worm burden when

Table 4—Effect of praziquantel drug (PZQ) treatment on pyruvate kinase (PK), phosphoenol pyruvate carboxykinase (PEPCK) activities and PK/PEPCK ratio of *S. mansoni* infected mice

[Values are mean ± SD from 6 mice in each group]

Parameters	Durations	Control	Experimental groups		
			Infected	Control-PZQ	Infected-PZQ
PK*	One month	3.43±0.169	5.76±0.48 [a]	1.54±0.07 [a]	1.30±0.05 [a, al]
	Two months	3.45±0.296	4.83±0.33 [a]	2.57±0.27 [a]	6.61±0.50 [a ,al]
PEPCK*	One month	1.61±0.066	2.09±0.14 [a]	1.45±0.05 [a]	1.22±0.04 [a ,al]
	Two months	0.93±0.070	0.55±0.03 [a]	0.23±0.03 [a]	0.35±0.03 [a, al]
PK/PEPCK	One month	2.14±0.172	2.76±0.18 [a]	1.06±0.06 [a]	1.06±0.039 [a, al]
	Two months	3.75±0.497	8.79±0.95 [a]	11.35±1.42 [a]	19.11±0.49 [a, al]

* μ moles $NADH.H^+$ formed/min/mg
Other details are same as in Table1

Table 5—Effect of *C longa extract* treatment (3 months) on AMP-deaminase, adenosine deaminase, urea, pyruvate kinase (PK), phosphoenol pyruvate carboxykinase (PEPCK) activities and PK/PEPCK ratio of *S. mansoni infected* mice

[Values are mean ± SD from 6 mice in each group]

Parameters	Control	Experimental groups		
		Infected	Control -*C. longa*	Infected-*(C .longa)*
AMP deaminase*	3.41±0.36	3.89±0.33 [b]	3.29±0.46 [ns]	3.83±0.39 [b, ns]
Adenosine-deaminase*	2.26±0.33	3.11±0.40 [a]	2.70±0.15 [a]	3.76±0.35 [a, al]
Urea*	109.9±9.1	81.1±2.18 [a]	84.1±3.2 [b]	120.9±4.4 [ns, al]
PK**	5.50±0.18	8.42±0.39 [a]	4.53±0.45 [a]	5.05±0.47 [b, al]
PEPCK**	2.65±0.47	1.87±0.07 [a]	0.57±0.066 [a]	1.57±0.16 [a, al]
PK/PEPCK	1.95±0.15	4.5±0.36 [a]	7.89±0.61 [a]	3.26±0.63 [a, al]

* μ moles ammonia/min./mg protein; ** μ moles $NADH.H^+$ formed/min./mg protein
Other details are same as in Table 1.

Table 6—Worm burden and ova count in liver of mice infected with S. mansoni in groups treated with praziquantel or C. longa

[Values are mean ± SD from 6 mice in each group]

Parameters	Infected and treated groups					
	Infected 1 month	Infected 1 month PZQ- treated	Infected 2 months	Infected 2 month PZQ treated	Infected 3 months	Infected 3 months *C.longa* treated
Worm burden	6.25±2.24	5.25±1.49	16.75±3.30[bl]	0.75±0.75[al]	15.75 ±1.37[al]	7.0 ±1.35[al]
Ova count	—	—	$0.456\times10^{4}\pm0.127$	$0.229\times10^{4}\pm0.0162$	$0.64\times10^{4}\pm0.027$	$0.109\times10^{4}\pm0.025$ [al]

*OVA count is expressed/g tissue of liver.
P values: [al] < 0.001; [bl] < 0.010; [cl] < 0.050 as compared to infected one month group

compared to infected groups. *C.longa* treatment showed a significant reduction in worm burden and ova count compared to the three months infection duration (Table 6). These results coincide with Gerges *et al*.[78,79]and Farah *et al.*[80] who reported that worm burden and the number of eggs were significantly reduced in PZQ-treated animals when compared to untreated groups. Moreover, Botros *et al.*[81] discussed that PZQ given orally (500 mg/kg) over 2 consecutive days at 7 weeks to mice caused complete eradication of worms, disappearance of immature egg stages, and decrease in the number of mature eggs and an increase in the number of dead eggs. The result of the present study showing that after one month post infection in mice treated with PZQ, there was few worms still living, is in agreement with Yang *et al.*[82] who revealed that male worms which were still living but encapsulated by connective tissue, were observed along the liver margin of some rabbits. Histopathological examination of liver sections revealed moderate to small-sized hypocellular granulomas[83, 84].

Although *C. longa* extract was less effective in reducing the worm burden (-55.5%) in schistosome-infected-treated animals when compared to PZQ (-95.5%), it was about 2 fold higher in reducing ova count (-83.0%) in treated animals compared to PZQ treatment (-49.8%). This could be more promising in controlling the pathology of this disease which is mostly due to the toxins released by the eggs and could be attributed to the antifecundity effect of curcumin. Curcumin, obtained from powdered rhizomes of *Curcuma longa* linn, which is commonly used as coloring agent in food, drugs and cosmetics[85]**.** In conclusion, it can be proposed that *C. longa*, a remarkable non-toxic plant with many medicinal properties should be explored for possible intervention in schistosomiasis as a disease which involves impairment of metabolism of infected subjects. These inspire more hope for further study on *C. longa*.

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