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Effect of *Curcuma longa* or parziquantel on *Schistosoma mansoni* infected mice liver — Histological and histochemical study

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Effect of drug praziquantel (PZQ) and *C. longa* extract on *S. mansoni* infected mice is reported. The level of glycogen, alkaline and acid phosphatases (ALP and ACP respectively), and body weight, liver weight and liver weight/body weight ratio were studied in mice infected with *S.mansoni*. ALP level was increased after infection. *C. longa* treated mice showed marked reduction in ALP level more than after PZQ-treatment. *C. longa* enhanced the concentration of glycogen after being reduced by infection, while PZQ-treatment revealed more reduction. *C. longa* caused enhancement in body weight while PZQ treatment had no effect. The formation of granuloma around schistosome eggs in the liver produced inflammation. *C.longa* extract and PZQ were effective in reducing granuloma size in infected mice.

Keywords: Acid phosphatase, Alkaline phosphatase, *Curcuma longa*, Glycogen, Granuloma, Paraziquantel, S*chistosoma mansoni*

Schistosomiasis is one of the most common parasitic diseases, which mostly affect the liver and intestine, causing granuloma formation and hepatic fiibrosis. Schistosomiasis also causes certain necrotic changes in the liver tissues[1]. Liver cells undergo some hisopathological changes which frequently lead to elevations in the serum enzymes, aspartate amino transferase (AST) and alkaline phosphatase (ALP)[2].

Serum ALP is useful in the diagnosis of various types of liver disease. Granulomas contain neutral and acid mucopolysaccharides, the ova contain lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), acid phosphatase and esterase. The worms contain increased alkaline phosphatase[3]. Recent evidence suggests that resistance to praziquantel (PZQ) may be developing in countries like Egypt where the drug has been in use for more than 10 years[4].

Treatment of schistosomiasis relies only on the use of praziquantel (PZQ) chemotherapy. However, PZQ treatment can't prevent reinfection and progressive development of the pathology[5]. The necrotic tissue was invaded by leucocytes and macrophages but neovascularization of the necrotic areas was observed only in mice that had been infected with 50 cercariae after egg granuloma formation as the hyperinfected animals[6]. In addition to these lesions, *in vivo* microscopy revealed dilatation and sacculation of sinusoids. These lesions were associated with varying degrees of reduction of blood flow due to schistosomules[7]. *S. mansoni* infection in animals resulted in a marked decrease in liver glycogen[8]. When infected animals were treated with PZQ (500 mg/kg body weight), there was a marked increase in liver glycogen content[9]. *Curcuma comosa* used in traditional medicine as an anti-inflammatory agent and to treat postpartum urine bleeding (480 mg /kg rats), is highly effective in elevating the glycogen content[10]. In hepatosplenic *Schistosomiasis mansoni* high serum levels of ALP were observed. This observation reflects the degree of inflammatory reaction (granulomata) in the liver towards egg or worm antigens or both[11].

El-Sharabasy *et.al*[12] concluded that effect of PZQ on ALP activity is minimal while Fallon *et.al*[13] revealed that ALP activity increased progressively with increasing doses of PZQ in infected animals. *Curcuma longa* exhibited the strongest antithrombotic activity in mice[14]. Antioxidative and hypolipidaemic action of curcumin is responsible for its protective role against ethanol induced brain injury and decreased ALP activity, which was elevated by

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ethanol[15]. The detected effects of schistosome infection were a reduction of body weight in 8 weeks infected mice. Differences in behavioral abnormalities between 8 and 15 weeks infected mice may be associated with modifications in the levels of nerve growth factor and cytokines induced by granulomas[16]. The liver weight increased significantly in infected as compared to control mice starting from the sixth week post infection. These changes may be attributed to several metabolites released by *S. mansoni* which affect the host hepatic tissue[17]. PZQ increased each liver and body weights, improved liver function parameters in mice infected with *S. mansoni* treated with PZQ (500 mg/kg for 2 successive days)[18].

Curcuma amada and curcumin reduced liver total lipids and free fatty acids on the standard diet and decreased liver weight and serum total lipids on the high sucrose diet[19]. The major pathologic changes in infection by S. *mansoni* are not caused by the adult worm itself but by eggs which do not reach the intestinal lumen and become trapped in other body tissues[20]. A primary site for such inflammatory reactions is the liver, as it filters the blood and thus receives the many eggs washed back through the portal channels[21]. At these sites, areas of local inflammation are produced, culminating in the formation of granulomas around the eggs[22]. Higher worm burden was associated with increased levels of hepatic granuloma, chronic cholecystopathy and oedema petechiae, fibrosis and pseudopolys of rectosigmoid mucosa[23]. The histopathological examination of liver sections revealed moderate to small sized hepatocellular granulomas when PZQ chemotherapy is administered[24]. PZQ reduced the number, diameter and cellularity granulomata[25]. The liver of infected, untreated rabbits had superficial necrotic foci and large numbers of worms in the mesenteric veins, while PZQ treated rabbits had smaller necrotic foci in the liver and few worms in the mesenteric veins, and nodules were formed around dead worms[26]. A histopathological study confirmed that PZQ did not exhibit hepatotoxicity. There was no evidence of hepatic pathology when PZQ was given for 4 weeks but PZQ administered for 8 weeks post-infection resulted in the arrested formation of new granulomata fibrosis, existing granulomata and the disintegration of schistosome ova[27]. The late administration of PZQ at 12 or 16 weeks post infection resulted in only mild to moderate improvement in the histopathology. It is concluded that the earlier PZQ is administered in the course of infection, the less serious hepatic pathology develope; PZQ is only effective against mature eggs[28]. Curcumin and capsaicin significantly lowered the secretion of lysosmal enzyme collagenase, elastase and hyaluronidase from macrophages in male wistar rats[29]. Cardioprotective effects of *C.longa* correlates with the improved ventricular function. Histopathological examination further confirmed the protective effects of *C.longa* on the heart[30]. *C.longa* has anti-inflamatory, antioxidant and anti-cancer activities[31].

Materials and Methods

Experimental animals—The animals used were healthy male albino mice of CD strain weighting 20–25g, obtained from the Schistosome Biological Supply Programmes (SBSP), Theoder Bilharz Institute. They were fed 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 Pharmaceutical Industries Company (E.I.P.I. Co). *Curcuma longa*, crude material (obtained from Chemistry and Pharmacognosy department, National Research Centre) was reduced to a moderately coarse powder; 100g of powder was immerged with 500 ml. of 70% ethyl alcohol for 72 hr. with occasional shaking. The extract was concentrated to dryness[32].

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 *Biomphalaria alexandrina* snails were placed in a beaker containing 200 ml dechlorinated water. In order to shed cercariae, snails were exposed to sunlight at 0800- 0900 hrs. Each mouse was subjected to subcutaneous injection with 50 cercariae[33].

Animal treatments—Animals were divided equally into three batches first, second and third. These batches were of one, two and three month's age respectively. Each batch was subdivided into 4 groups. For the 1st and 2nd batches, these 4 groups included group I, control, group II, infected group, group III, 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,

served as control, II infected group, III served as *C. longa*-treated control while IV, was used as *C. longa*-treated infected group.

Statistical analysis—The statistical significance of the results was determined by Ronald *et al*[34].

Histopathological and histochemical studies—All liver samples were studied histopathologically to evaluate structural alterations of the hepatic parenchymal cells and to clarify the presence of multiple schistosome eggs and granulomata in the liver by Haematoxylin and Eosin stained sections. Also, the present study included the histochemical observations for glycogen, acid and alkaline phosphatases activities, in the liver.

Cryostat sections—Cryostat sections were prepared from frozen tissue kept at –80°C.Tissue sections were cut with 5 µm thickness at a slow but constant speed. The sections were stained with Hematoxylin and Eosin[21].

Glycogen[35,] alkalinephosphatase[36] and acid phosphatase[37] were estimated.

Results

Glycogen was elevated (34.9%) in mice after one month of post infection and the increase was more (47.6%) after treatment with PZQ as compared with control. A marked reduction was observed after 2 months of post infection. When control group were given PZQ, a marked decrease in glycogen was observed (with percentage change of 51.8 and 42.2% after 1st and 2nd month respectively, as compared to the control untreated; Table 1).

Treatment of infected mice with *C. longa* produced significant reduction of glycogen (31.68%) as compared to control (Table 2). When compared to infected group significant elevation in glycogen (28 %) was recorded. The control group, which were given *C. longa* showed elevation of glycogen concentration of 33.9% as compared to group I.

A significant decrease in alkaline phosphatase activity one month of post infection was observed (Tables 1 and 2). A slight reduction of acid phosphatase activity reaching 7.6% with respect to control was also recorded. At 2nd month of post infection, ALP activity was elevated to 6.59 µ moles in infected mice which after PZQ treatment reached 1.90 µ moles. 3months post infection ALP showed 27% elevation compared to control. This value was reduced only to 3% in *C.longa* treated group, while a slight reduction in case of ACP at different durations of infection was observed.

Table 1—Effect of Praziquantel drug (PZQ) treatment on liver glycogen and alkaline and acid phosphatases activities of *S. mansoni* in infected mice

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

Parameters	Durations (months)	Control	Experimental groups		
			Infected	Control-PZQ	Infected-PZQ
Glycogen*	1	5.15±0.83	6.95 ± 1.2^{a}	2.48 ± 0.259^{a}	$7.60\pm0.44^{a,ns}$
	2	7.97±0.54	3.46 ± 0.54^{a}	4.61 ± 0.23^{a}	$3.15\pm0.17^{a,ns}$
Alkaline	1	1.94±0.41	1.34 ± 0.18^{a}	2.48 ± 0.40^{b}	$1.39\pm0.25^{a,ns}$
phosphatase**	2	1.41±0.16	6.59 ± 1.47^{a}	1.90 ± 0.25^{a}	$4.64\pm0.39^{a,al}$
Acid phosphatase**	1	0.131±0.02	0.121 ± 0.01^{ns}	0.065 ± 0.01^{a}	$0.093\pm0.01^{a,al}$
	2	0.132±0.01	0.130 ± 0.018^{ns}	0.102 ± 0.05^{a}	$0.191\pm0.05^{a,al}$

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

Table 2—Effect of *C. longa* extract treatment for 3 months on liver glycogen and alkaline and acid phosphatases activities of *S. mansoni* in infected mice.

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

Parameters	Control	Experimental Groups		
		Infected	Control-*C. longa*	Infected- *C. longa*
Glycogen*	4.45±0.27	2.37 ± 0.29^{a}	5.96 ± 0.64^{a}	$3.04\pm0.27^{a,al}$
Alkaline Phosphatase**	1.33±0.14	1.69 ± 0.13^{a}	0.29 ± 0.02^{a}	$1.37\pm0.16^{ns,al}$
Acid Phosphatase**	0.084±0.01	0.080 ± 0.01^{ns}	0.092 ± 0.005^{c}	$0.096\pm0.007^{b,al}$

*mg/g tissue, ** µ moles phosphate liberated/min/mg protein
Other values are same as in Table 1

Increase in liver weight and decrease in body weight in experimental mice are shown in Table 3 and 4.

Histopathological and histochemical observations—Liver sections from the control group stained with haematoxylin and eosin showed normal histological features. Effect of *S. mansoni* on hepatic cells 1st month of post infection showed early formation of granulomatous lesions and migration of infiltrative cells including lymphocytes, monocytes and eosinophils. On the other hand, the effect of PZQ treatment on two months post infection showed well defined granuloma. During the 3rd month of infection, fibrosis appeared as, peirportal thickened sheath. *C.longa* extract on hepatocyte of mice 3rd month of post infection, stimulated fibrotic reaction, accompanied by early dissolution of the cellular granulomatous reactions around dissolute ova. Fig. 1. The examination of cryostat sections of the liver of control mice revealed that parenchymal cells acquired deep stain ability indicating rich glycogen stores. Hepatic sections from experimental group PZQ-treated 1st month post infection showed focal degenerative parenchymal change with increase of glycogen concentration as compared to control. By the 2nd month post infection histopathological lesions associated with infection were characterized by large granulomatous lesions around intact ova; glycogen was significantly reduced. PZQ-treatment caused a complete disintegrating ovum. *C.longa* showed early dissolution of the granulomatous lesions and a complete disintegration of schistosome ova beside schistosomal pigments (Fig. 2).

ALP enzyme is present in cytoplasm of hepatic cell which acquire deep stain ability. Highly significant elevation in ALP activity, which accumulated around bile canaliculi in the liver as compared to uninfected group. PZQ and *C. longa* showed a marked decrease in ALP activity as compared to infected group (Fig. 3). Enzyme ACP accumulated around granulomatous lesions in infected mice. PZQ-treated animals examined after the 2nd month post infection showed an increased ACP activity as compared to control group. *C.longa* treatment proved to be highly effective against *S.mansoni* in mice showing complete disintegrating ova and reduction in granulomatous size and consequently histochemical stain ability of ACP was almost normal. (Fig. 4).

Discussion

The mice infected with *Schistosoma mansoni* cercariae, showed a significant changes in all parameters. Praziquentel (PZQ) has become the drug of choice in most endemic areas because of its efficacy, ease of administration, tolerable side-effects

Table 3—Effect of Praziquantel drug (PZQ) treatment on liver weight, body weight and liver/body weight ratio of *Schistosoma mansoni* infected mice

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

Parameters	Durations (months)	Control	Experimental Groups		
			Infected	Control-PZQ	Infected-PZQ
Liver weight (g)	1	1.69±0.16	$1.65±0.07^{ns}$	$1.67±0.16^{ns}$	$1.67±0.30^{ns, ns}$
	2	1.80±0.22	2.17±0.30	$1.89±0.20^{ns}$	$1.76±0.13^{ns, al}$
Body weight (g)	1	29.9±1.90	$30.2±0.93^{ns}$	$31.2±2.03^{ns}$	$30.1±3.13^{ns,ns}$
	2	35.6±2.4	$30.0±4.14^{ns}$	$34.7±2.35^{ns}$	$29.0±1.27^{a,ns}$
Liver/body weight	1	0.057±0.003	$0.052±0.008^{b}$	$0.054±0.005^{ns}$	$0.055±0.006^{ns,ns}$
	2	0.051±0.006	$0.073±0.011^{a}$	$0.054±0.004^{ns}$	$0.061±0.006^{ns, bl}$

P values : $^{a}< 0.001$; $^{b}< 0.010$; $^{c}< 0.050$ ^{ns} non significant
$^{al}< 0.001$; $^{bl}< 0.010$; $^{cl}< 0.050$ – as compared to infected group

Table 4—Effect of *Curcuma longa* extracts *(C. longa)* treatment on liver weight, body weight and liver/body weight ratio of *Schistosoma mansoni infected* mice:

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

Parameters	Control	Experimental groups		
		Infected	Control (*C. longa*)	Infected (*C. longa*)
Liver weight (g)	1.88±0.19	$2.21±0.42^{c}$	$1.94±0.44^{ns}$	$2.6±0.61^{b,ns}$
Body weight (g)	37.5±1.18	$34.0±2.82^{a}$	$36.7±1.39^{ns}$	$35.4±4.7^{ns,ns}$
Liver/body weight	0.050±0.005	$0.064±0.007^{a}$	$0.053±0.01^{ns}$	$0.073±0.01^{ns, cl}$

Other details are same as in Table 1

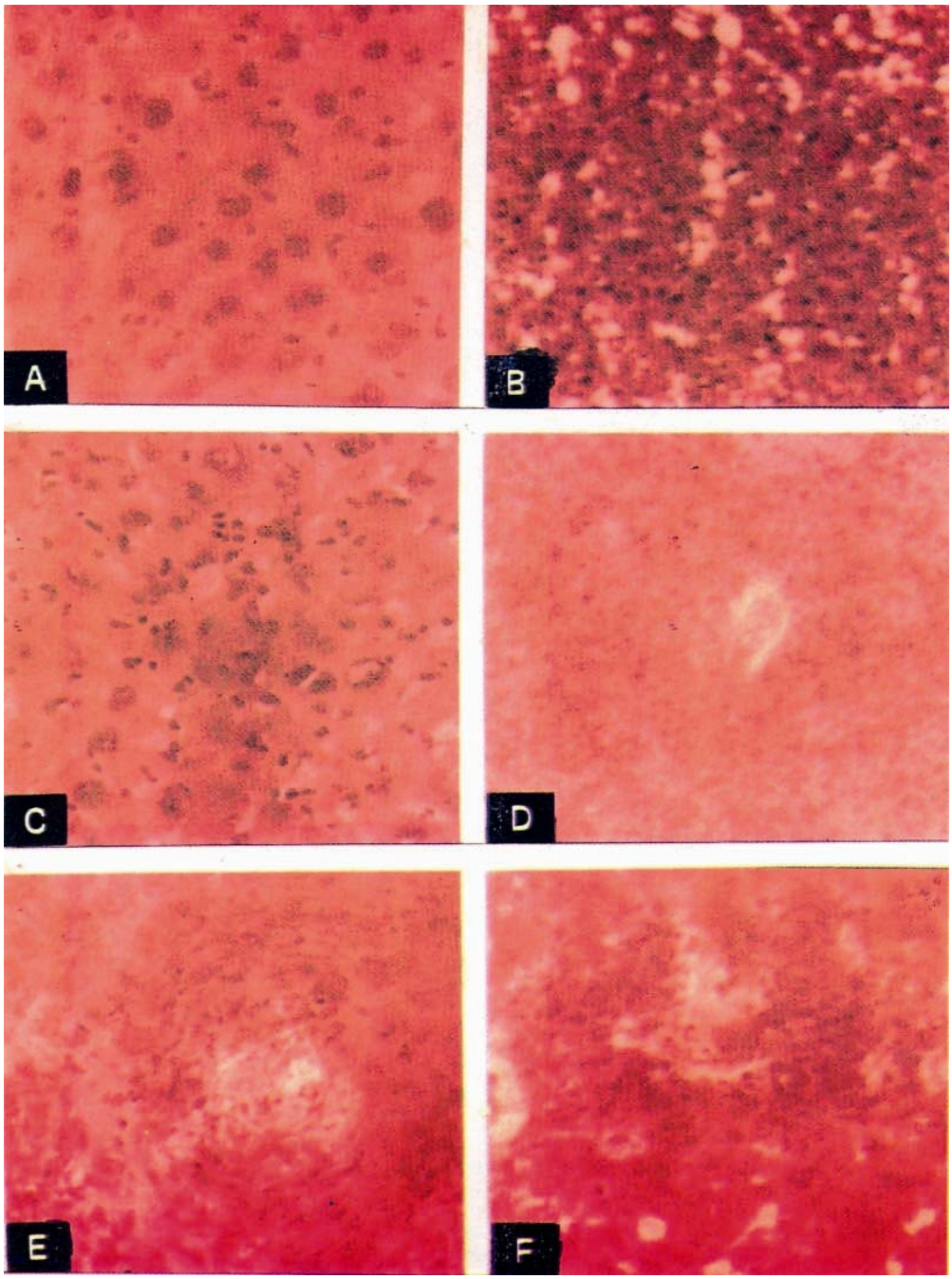

Fig. 1—Sections through liver of mice. (A) Control group showing normal hepatic architecture (200 ×). (B) 1st month post infection treated (PZQ) showing fatty infiltration. (100 ×). (C) one month infected animal showing early formation of granulomatous lesion and migration of infiltrative cells including lymphocytes, monocytes and easinophils. (200 ×).(D) two months post infection showing schistosomal granuloma surrounding ovum (100 ×) .(E) granulomatous frequency in schistosoma 3rd month post infection around early disintegrating ova (200 ×). (F) 3rd month post infection treated *C. longa* showing early dissolution of the granulomatous lesions (200 ×) [H & E]

and cost. As a consequence of this positive trend, two potential dangers have emerged. The possibility that other existing drugs may be discontinued and the diminished interest of major pharmaceutical companies in the quest for novel active compounds[38]. Yang *et al*.[39] recorded that the contents of glycogen

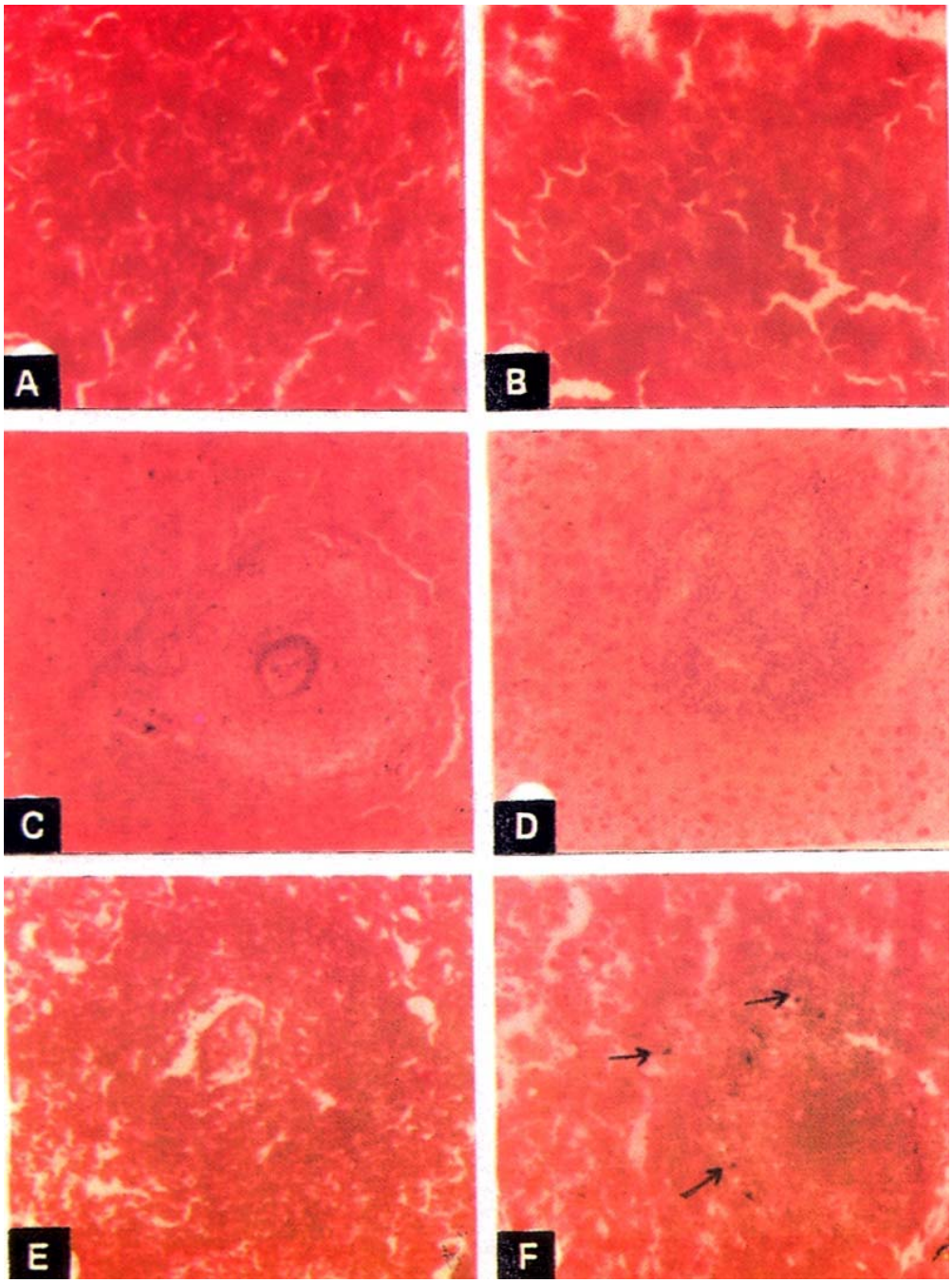

Fig. 2—Sections of mice liver. (A) Uninfected animal showing normal hepatic glycogen stores (200 ×). (B) one month infected animal, a marked increase in glycogen as compared to control (100 ×). (C) two months infected mouse , granulomatous lesion around intact ovum and a marked decrease in glycogen content beside schistosomal pigment (100 ×). (D) two months infected mouse PZQ - treated showing disintegrating ovum and a marked reduction in glycogen (100 ×). (E) 3rd month post infection, showing intact ovum with lateral spine. Glycogen is faintly stained in the parenchymal cell surrounding granulomatous lesions (100 ×). (F) three months infected animal treated with *C. longa* ,the disintegration of schistosome ovum and a marked increase of glycogen when compared to untreated animal beside schistosomal pigments (→) (100 ×)

had notably decreased or even disappeared, after administration of PZQ at a single oral dose of 300mg/kg to mice infected with *Schistosoma japonicum*. These results are not in good agreement

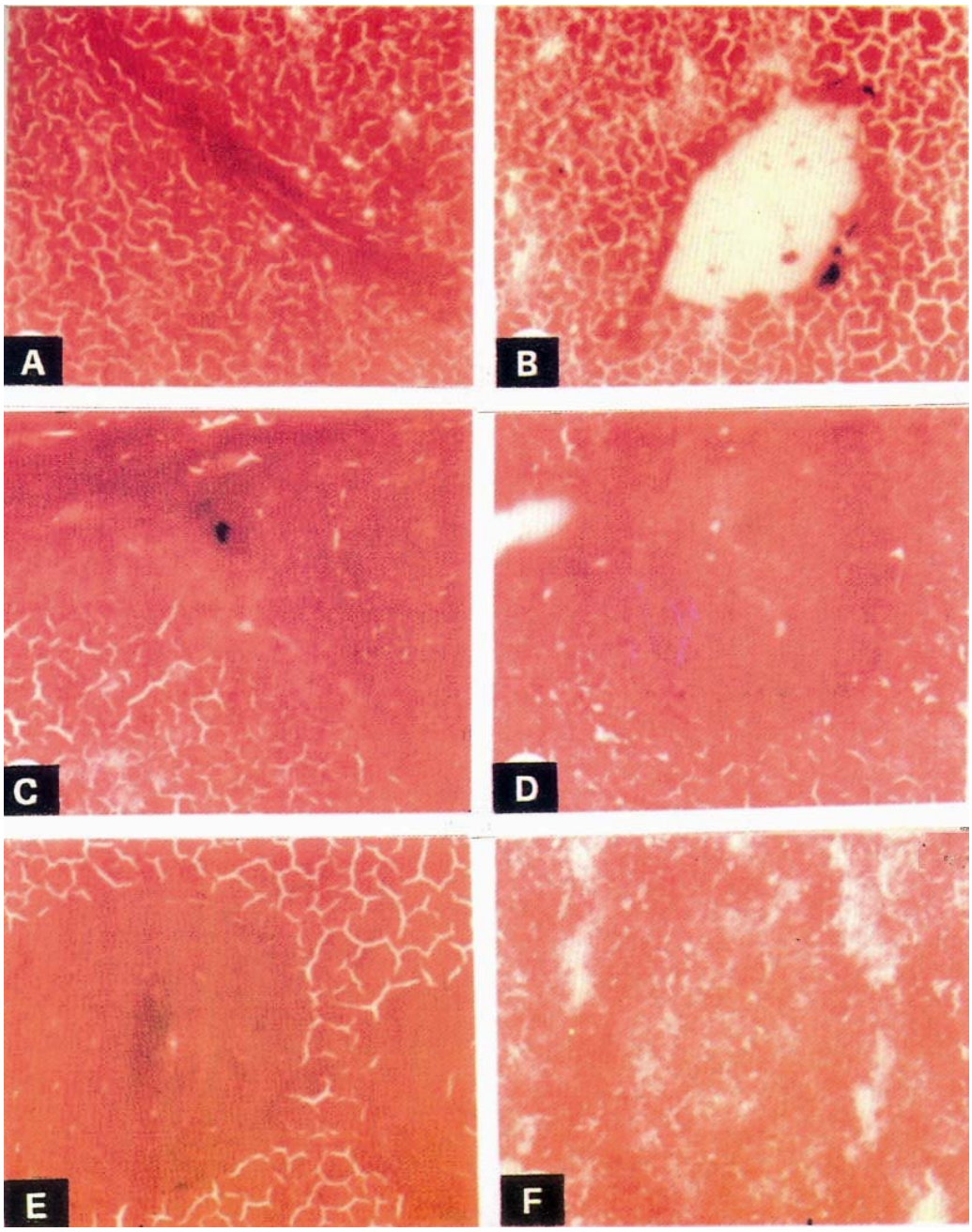

Fig. 3—Cryostat section through a liver stained for alkaline phosphatase activity (ALP): (A) one month infected animal showing focal degenerative (100 ×). (B) one month post infection, mouse treated with PZQ showing slight increase in ALP (100 ×). (C) Two months infected mouse, highly significant elevation in ALP activity which accumulated around bile canaliculated in the liver as compared to uninfected group (100 ×). (D) two months infected animal treated PZQ showing a marked decrease in ALP activity as compared to infected group (100 ×). (E) three months post infection showing multiple granulomatous lesions around intact ova, also showing a marked increased of ALP activity as compared to uninfected group. (100 ×). (F) three months infected animal treated with *C. longa* showing a disintegrating ovum and a marked decrease in ALP activity as compared to untreated groups (100 ×).

with those of EL-Hawy *et al.*[40] and Ahmed and Gad[41] who recorded that PZQ administration resulted in reaccumulation of glycogen pathways as early as the 4th week of infection. Data of the present study could be acertained through the previous reports of Cunha and Noel[42] who showed that PZQ has no direct effect

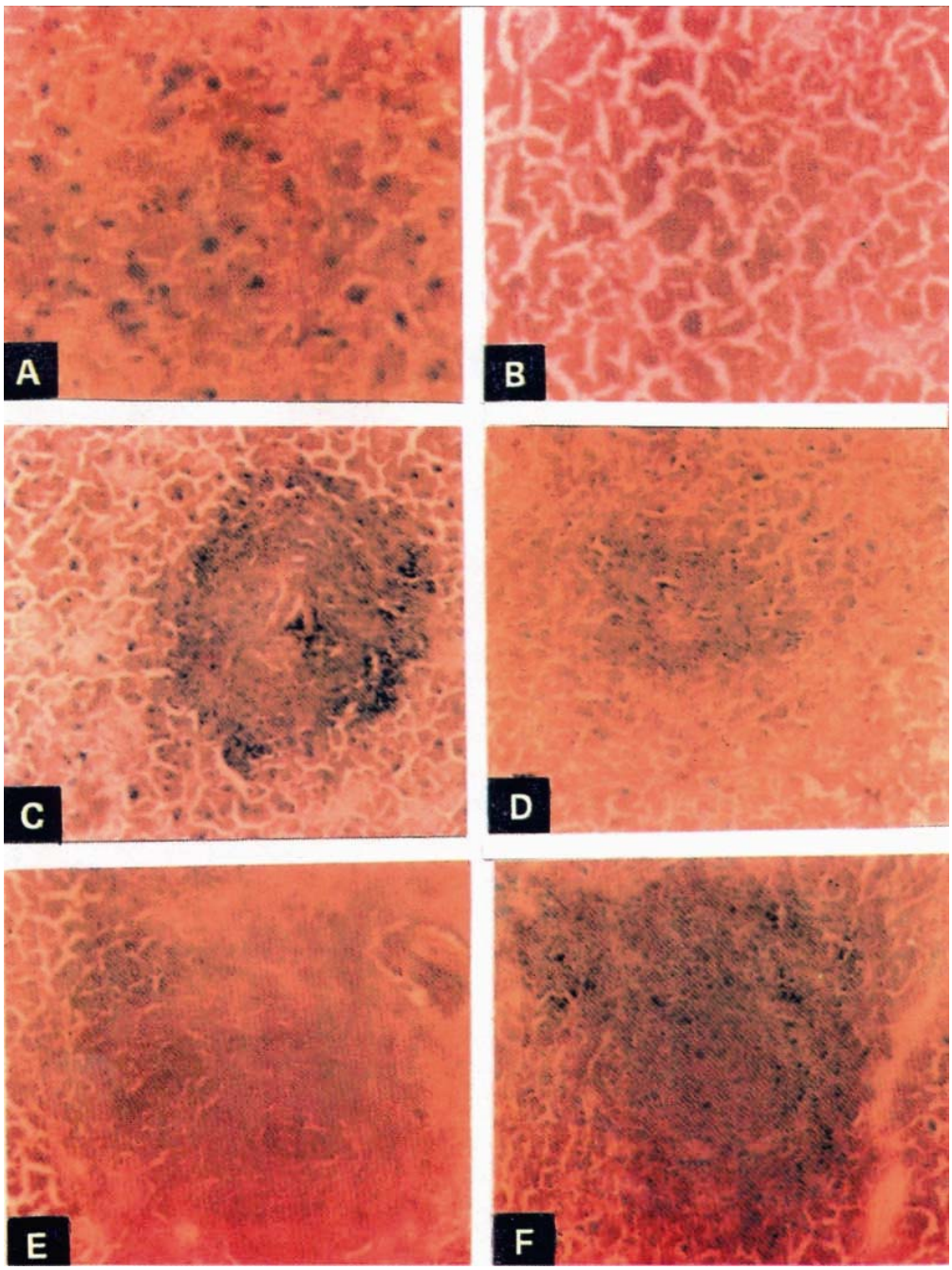

Fig. 4—Cryostat section through liver stained for acid phosphatase activity (ACP): (A) uninfected liver of mouse showing normal hepatic architecture for ACP activity (100 ×). (B) one month infected mouse treated with PZQ showing mild decrease in the activity of ACP activity (100 ×). (C) two months infected animal showing granulomatous lesions around intact ovum a non significant decrease was observed in the ACP activity (100 ×). (D) two months infected mouse treated with PZQ showing increased enzyme activity as compared to control (100 ×). (E) three months post infection showing multiple granulomatous lesions around intact and disintegrating ova with a slight reduction in activity of the enzyme (100 ×).(F) 3rd month post infection treated with *C. longa* showing complete disintegrating ovum with reducing granuloma size and increasing of enzyme activity (100 ×).

on Na-K-ATPase and Ca-Mg-ATPase activities, the two enzymes of critical importance for glucose transport and glycogen synthesis. Schistosome infection significantly stimulated glycogen after one month, and caused its depletion after two months, PZQ-treatment showed reduction in glycogen level in

infected animals (Table 1). This is not in agreement with the result of Ahmed and Gad[41] who recorded depletion of glycogen one month post infection. Regarding the effect of PZQ, it can easily be noticed that drug induce more depletion. El-Sharkawy *et al*.[43] attributed the increased activity to the effect that occurs on the membrane of endoplasmic reticulum or to the elevation of cytosolic calcium that can trigger the conversion of the enzyme phosphorylase b (inactive form) to phosphorylase a (active form) which degrades glycogen into glucose. Lower adenylate energy charge (AEC) is usually accompanied by activated glycogen phosphorylase and glycolytic enzymes and inhibited glycogen synthase and gluconeogenic enzymes[44].

There is still intensive search for effective anti-schistosomal drugs with minimal side effects[45]. Natural health products have become increasingly important in the lives in the past few years[46,47]. The huge global economic potential for the production and processing of medicinal plants has led to important initiatives in research, development and regulatory procedures[48]. In the present study *C. longa* was tested as antibilharzial drug. The results show that *C.longa* extract was efficient in the repletion of the depleted glycogen reserves and induced a significant elevation of glucose concentration in control and infected *C. longa*-treated animals[49]. The potential activity of this plant extract in inducing glycogen and glucose levels could be easily correlated to the previous reports of El-Ansary and Farouk[50]. They reported that *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. Higher glycogen reserves in *C. longa*- treated control animals could ascertain the mode of action of this extract.

Regarding the enzymatic activities of alkaline phosphatase (ALP) and acid phosphatase (ACP) bilharzial infection was found to variably affect these enzymes. While ACP was non-significantly inhibited, Li *et al*.[51] showed a non-significant change in ACP activity in control and schistosomes infected animals[52]. ALP was markedly inhibited one month post infection with *S. mansoni* while it was highly induced two and three months post infection. This could be attributed to the fact that at this stage of infection (8th week), the disease begins with the onset of egg production, and subsequently evolves into a chronic phase with the development of late squeal[53]. ALP elevation could be correlated to the acute chronic state transition, in response to elevation of egg-secreted toxins. The remarkable increase of ALP reported in the present study two months-post infection could be confirmed through the previous reports of Gazayerli[54] who showed an increase in ALP activity in schistosome-infected mice. They attributed the increase of ALP activity to irritation of the liver cells by toxins or metabolic products of growing schistosomules, of adult worms and eggs. Increased loss of intracellular enzyme diffused through cell membrane would appear to act as a stimulus to the synthesis of more enzyme proteins[55]. Moreover, elevation of ALP could be of parasitic origin since this enzyme is a marker for tegumental membranes of *S. mansoni*[56]. They detected ALP activity in cercariae, schistosomula, adult schistosomes and their eggs and concluded that this enzyme is not exposed at the schistosome's surface, and is probably buried in the tegumental membrane network. Hamdy and Saleh[57] in histochemical studies showed that there was a progressive increase in the level of ALP in the liver within 7 days of hamsters being injected with 200 *S. mansoni* cercariae, which persisted for 120 days (the period of study). In control animals, ALP of the liver stained only in the endothelial cells of the blood vessels and blood sinusoids but after infection, in addition to these sites ALP was also detected around the ova and some cells around periportal tissues. In addition, a significant elevation of total, hepatic and bone ALP isoenzymes were seen in hepatosplenic schistosomiasis[58]. The significant elevation of ALP reported in the present study was also ascertained histochemically. Infected mice showed higher ALP activity which was localized around the bile ducts. Mansy *et al*[52] showed proliferation of bile ductules and canaliculi may explain the increased activity of ALP. Bile ductule proliferation, related to hepatic fibrosis, was noticed at the 8th week post infection of mice with *S. mansoni* (80 cercariae) and was marked in late stage of schistosomal infection. A correlation between elevated ALP activity and high morbidity in patients infected with *S. mansoni*[59].

The non-significant decrease in ACP reported in the present study is in good agreement with many previous studies, which showed same number of lysosomes and more or less similar ACP activity as a

lysosomal marker in control and schistosomes infected animals[51]. Also, Hara *et al*.[60] showed an insignificant decrease in enzyme activity of *S. mansoni* infected mice liver. They attributed this decrease to molecular and biological changes in hepatic and granulomatous cells as a result of infection. Contradictory, El-Gowhary *et al*.[61] found that the enzyme activity was increased in *S. mansoni* infected mice liver. They attributed this increase to the proliferation and deletion of rough and smooth endoplasmic reticulum as well as evident increase of ribosomes accompanied by proliferation and dilation of microsomal membrane where ACP enzyme is usually located and considered as microsomal marker enzyme. 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[62]. These results suggest that distinct cell membrane interaction pathways lead to drug-specific mechanisms of cytotoxicity. The change in the activity of ACP 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 ALP and ACP in control-treated mice. In case of infected animals, *C. longa* extract could have significantly reactivated ACP, which showed its potent effect in restoring normal hepatocyte permeability. On the other hand, elevated ALP activity in schistosome-infected mice was lowered by *C. longa* extract. Reduced ALP activity in *C. longa*-treated controls reported in the present study was previously reported by Joe and Lokesh[29] when this extract was given to rats in amounts normally taken by man or in amounts 1.25-125 times higher. Reduction of ALP of infected mice is supported by the previous records of Rajakrishnan *et al*.[63] who demonstrated the efficacy of *C. longa* in lowering increased ALP levels induced by ethanol. Moreover, Jayadeep *et al*.[15] revealed that the antioxidative and hypolipidaemic action of *C. longa* is responsible for its protective role against ethanol induced brain injury. Park *et al.*[64] concluded that curcumin improved both acute and subacte liver injury induced by carbon tetrachloride in rats by lowering ALP levels induced by CCl_4.

The present study showed that due to schistosome infection, both the mean body weight (BW), liver weight (LW) and the relative liver weight /body weight (LW/BW) were greatly changed from normal controls. These results are in accordance with those of Soliman *et al*.[65] who noticed the failure of infected animals to gain weight with initiation of schistosomal egg deposition. PZQ treatment has no improvement effect on BW, LW and LW/BW values of control and infected animals. A more or less similar effect on body weight was recorded in case of *C. longa* treatment. This is in accordance with the results of Bhavanishankar and Murthy[66] who reported that body weight were not significantly different from rats fed *C. longa. C. longa* induced a significant increase in the liver weight and this in turn led to a significantly higher LW/BW ratio. The increase in liver weight could be attributed to the glycogen repletion previously reported in the present study for infected *C. longa* treated mice compared to control infected ones.

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[67]. The formation of granuloma around schistosome eggs in the liver and the intestine is the major cause of pathology in schistosome infections. Granuloma and the subsequent fibrosis in the liver appear to be primarily responsible for mortality and morbidity by this highly endemic parasitic disease. Also, El-Sharkawy *et al*.[43] attributed the increase activity to the effect that occurs on the membrane of endoplasmic reticulum or to the elevation of cytosolic calcium that can trigger the conversion of the enzyme phosphorylase b (inactive form) to phosphorylase a (active form) which degrades glycogen into glucose. Lower AEC usually accompanied by activated glycogen phosphorylase and glycolytic enzymes and inhibited glycogen synthase and gluconeogenic enzymes[44].

The histological observations revealed that Schistosomiasis affects the liver causing granuloma formation and hepatic fibrosis. Schistosomiasis also causes certain necrotic changes in the liver tissues 2nd and 3rd month post infection. These results agree with those obtained by El-Assar *et al*[1] who recorded more or less same effect. PZQ caused reduced number, diameter and cellularity of granuloma, and these results are in agreement with Kresina *et al.*[69] and Badawy *et al.*[18] who recorded that PZQ therapy

caused excess pigmentation in macrophages and kupffer cells, binucleation and large sized hepatocytic nuclei were evident. The histopathological feature of the liver in response to *Schistosoma mansoni* infection and PZQ treated 1st month post infection was in agreement with Yang *et al*[39].

Li *et al*[70] showed a significant improvement in ultra parenchyma images after treatment of PZQ also showed significant improvement of periportal fibrosis. The present results confirm these findings. In the present study PZQ (500 mg/kg body wt. for 2 consecutive days) 2nd month post infection reduced the hepatic granuloma in histopthological sections of liver which revealed a small fibrocellular granuloma with few inflammatory cells and excess fibrous collagen tissue deposition was supported by Kamel *et al*.[71] and Nessin and Demerdash[72].

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%)[73]. Curcumin, obtained from powdered rhizomes of plant *C. longa linn*, is commonly used as coloring agent in food, drugs and cosmetics[74]. *C. longa* extract is thus effective in the treatment of schistosomiasis and should be explored further to identify the potential active principle(s).

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