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ORIGINAL PAPER

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Anthelmintic efficacy of *Flemingia vestita* (Fabaceae): alteration in the activities of some glycolytic enzymes in the cestode, *Raillietina echinobothrida*

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Abstract The crude root-peel extract of *Flemingia vestita*, genistein and praziquantel were tested against some selected glycolytic enzymes—hexokinase (HK), phosphofructokinase (PFK), phosphoenolpyruvate carboxykinase (PEPCK), pyruvate kinase (PK), lactate dehydrogenase (LDH), malate dehydrogenase (MDH) and malic enzyme (ME)—of the fowl tape worm, *Raillietina echinobothrida*. Following exposure to the various treatments, the activities of HK, PFK, PEPCK and LDH increased by 33–39%, 41–125%, 44–49% and 55–67%, respectively, and that of PK decreased by 14–26% in the parasite at the time of paralysis. The MDH and ME activities of the tissue homogenate were also found to be higher by 22–43% and 28–59%, respectively, in the treatments. However, whereas the activity of both cytosolic and mitochondrial MDH increased by 33–58% and 43–73%, respectively, the cytosolic ME activity showed an increase of 33–39%, and there was no significant enhancement in the mitochondrial ME activity. Histochemically, the enhancement in the activities of HK, LDH and MDH was clearly discernible. The enhanced glycolytic activity seems to be a function of anthelmintic stress caused by the phytochemicals.

Introduction

Flemingia vestita(Fabaceae) is conventionally used as an anthelmintic in traditional medicine among the natives of Meghalaya (northeast India). The alcoholic crude root-peel extract of *F. vestita*, which contains genistein (0.25%) as the major isoflavone compound besides formononetin, pseudobaptigenin and diadzein (Rao and Reddy 1991), has been shown to act vermifugally/vermicidally against cestodes and trematodes but not against nematodes (Roy and Tandon 1996; Tandon et al. 1997). The plant-derived components were shown to induce flaccid paralysis and alterations in the tegumental architecture in the cestode of domestic fowl, *Raillietina echinobothrida* (Tandon et al. 1997) and also altered the activities of several enzymes that are associated with the tegument, namely acid and alkaline phosphatases, adenosine triphosphatase and 5′-nucleotidase (Pal and Tandon 1998a, 1998b). A pronounced decline in the activity of the non-specific esterases that are associated with the nervous system, acetylcholine esterase in particular, was observed in the cestode (Pal and Tandon 1998c). Alteration in the activity of nitric oxide synthase was also observed in *Fasciolopsis buski* following treatment with the same plant materials and genistein (Kar et al. 2002). In addition, in vitro treatment of *R. echinobothrida* with the crude root-peel extract and genistein also caused alterations in the levels of the free amino acid pool and tissue ammonia (Tandon et al. 1998). In an earlier study, we also showed that glycogen was decreased significantly due to increased glycogenolysis and decreased glycogenesis during treatments of *R. echinobothrida* with these plant materials (Tandon et al. 2003).

In the quest to find the mode of action of the putative anthelmintic active principles of *F. vestita*, and genistein in particular, the parameters physical motility, paralysis, survival time, alterations in the surface ultrastructure, enzymes associated with the tegument and co-ordination system and glycogen metabolism in *R. echinobothrida* were chosen in earlier studies. As a sequel to these studies, it seems important to ascertain the effect of these phytochemicals on the energy-yielding pathways in the parasite. The activities of some glycolytic enzymes in the cestode were determined in the present study.

Glycolysis is the major energy-yielding pathway in these parasites since the Krebs' cycle and hexose monophosphate pathways are less functional. In most

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cestodes, glucose and other simple carbohydrates are metabolized following type 2 glucose fermentation, which is characterized by a CO_2-fixation step (by phosphoenolpyruvate carboxykinase) and malate dismutation (Bryant and Flockhart 1986). Glycolytic enzymes, such as hexokinase (HK), phosphofructokinase (PFK), aldolase, glyceraldehyde 3-phosphate dehydrogenase (G3PDH), pyruvate kinase (PK), phosphoenolpyruvate carboxykinase (PEPCK), lactate dehydrogenase (LDH), and malic enzyme (ME) are reported to be present in cestodes, and the operation of the Embden-Meyerhof pathway has been confirmed (Arme and Pappas 1983; Smyth and McManus 1989). HK (ATP: D-hexose 6-phosphotransferase; EC 2.7.1.1) was partially purified from *Hymenolepis diminuta* and *Bothriocephalus scorpii* (Vykhrestyuk and Klochkova 1984). PFK (ATP: D-fructose 6-phosphate 1-phosphotransferase; EC 2.7.1.11), the pacemaker enzyme of the glycolytic pathway, has been investigated in adult *Moniezia expansa* and in the plerocercoids of *Schistocephalus solidus* (Beis and Theophilidis 1982). The properties of PK (ATP: pyruvate 2-0-phosphotransferase; EC 2.7.1.40) and LDH (L-lactate: NAD^+ oxidoreductase; EC 1.1.1.27) have been investigated in several cestodes, although only for LDH in detail in *H. diminuta* (Burke et al. 1972). The presence of malate dehydrogenase (MDH; L-malate: NAD^+ oxidoreductase; EC 1.1.1.37) and ME (L-malate: $NADP^+$ oxidoreductase; EC 1.1.1.40) has been investigated in *H. diminuta* and also in a range of cestodes (Fioravanti and Saz 1980). PEPCK (orthophosphate: oxaloacetate carboxykinase; EC 4.1.1.32), a rate-limiting enzyme at the branch point of phosphoenolpyruvate, has been reported from a number of cestodes such as *M. expansa*, *H. diminuta* and *Spirometra erinacei* (Fukumoto 1985). The main role of PEPCK in cestodes appears to be the opposite to that in vertebrates; in the former it is involved in the degradation of the glucose molecule, whilst in the latter its main role is in gluconeogenesis. Because of the differing primary functions of PEPCK in cestodes and their hosts, this enzyme might be inhibited selectively and thus provide an avenue for anthelmintic attack (Reynolds 1980).

Materials and methods

Parasites and treatment in vitro

Live *R. echinobothrida* were obtained from the intestines of freshly killed domestic fowl into 0.9% phosphate buffered saline (PBS, pH 7.2). The freshly obtained live parasites (≈0.2 g fresh weight) were treated in vitro in 10 ml of PBS (pH 7.2) at 39 ± 1°C with either 5 mg/ml crude peel extract, 0.2 mg/ml genistein or 1 μg/ml praziquantel (PZQ) dissolved in 1% dimethylsulfoxide (DMSO), with the simultaneous maintenance of controls for each treatment in PBS containing 1% DMSO, within 1 h of collection. As reported in a previous study by Tandon et al. (1997), these concentrations caused paralysis in the cestode in 5.9 ± 0.05 h, 6.7 ± 0.04 h, and 2.9 ± 0.05 h, respectively, while the controls survived in vitro for 72 ± 0.05 h. For each set of treatments, the parasites were taken from a single host. As soon as paralysis set in post-incubation (~6 h in case of crude peel extract and genistein and ~3 h for PZQ), the treated parasites and their respective controls were processed for the enzyme assays.

Drugs and chemicals

The alcoholic crude peel extract of *F. vestita* and genistein were obtained as reported earlier (Tandon et al. 1997). Synthetic genistein (G 6649) was obtained from Sigma Chemicals (St. Louis, Mo., USA). PZQ (from Droncit), a broad-spectrum cestocide, served as the reference drug. All enzymes and co-enzymes were obtained either from Sigma Chemicals or from Roche (Germany). Other chemicals used were of analytical grade and obtained from local sources such as Sisco Research Laboratory, Merck, etc. Deionized double glass-distilled water was used for all preparations.

Enzyme assays

Tissue processing

A 10% homogenate (w/v) of the parasite tissue was prepared in a homogenizing buffer containing 50 mM Tris-HCl (pH 7.4), 0.3 M sucrose, 1 mM EDTA, 2 mM $MgCl_2$ and 3 mM 2-mercaptoethanol. The homogenate was treated with 0.5% Triton X-100 in 1:1 ratio for 30 min, followed by sonication for 30 s (Soniprep 150) for proper breakage of the mitochondria. The homogenate was then centrifuged at 10,000 *g* for 15 min (Beckman J2-HS) and the resultant supernatant was used for all enzyme assays. All of these steps were carried out at 4°C.

Sub-cellular fraction

Mitochondrial and cytosolic fractions were prepared by differential centrifugation of a 20% homogenate of the parasite tissue in a fractionating buffer containing 50 mM Tris-HCl (pH 7.4), 0.3 M sucrose, 1 mM EDTA, 2 mM $MgCl_2$ and 3 mM 2-mercaptoethanol. LDH was used as the cytosolic marker and glutamate dehydrogenase [GDH; L-glutamate: $NAD(P)^+$ oxidoreductase; EC 1.4.1.3] as the mitochondrial marker to access the complete separation process of the different sub-cellular fractions.

Assays

The enzymes were assayed spectrophotometrically (Beckman DU 640, fitted with a Peltier temperature

controlled system) at 340 nm, keeping 1 ml as the final volume of the reaction mixture.

1. HK (Bergmeyer 1974).Tris-HCl buffer (pH 7.4) 50 μmol, D-glucose 5 μmol, $NADP^+$ 0.2 μmol, ATP 0.9 μmol, $MgCl_2$ 5 μmol, G6PDH 10 units and tissue extract 50 μl.
2. PFK (modified method of Buckwitz et al. 1988). Tris-HCl (pH 7.2) 80 μmol, fructose 6-phosphate 5 μmol, NADH 0.2 μmol, ATP 0.8 μmol, $MgCl_2$ 0.9 μmol, KCl 90 μmol, K_2HPO_4 1 μmol, aldolase 8.0 μg, triose phosphate isomerase 3.3 μg, glycerophosphate dehydrogenase 3.3 μg and tissue extract 100μl.
3. PEPCK (Mommsen et al. 1985). Tris-HCl (pH 7.4) 50 μmol, phosphoenolpyruvate 4.5 μmol, NADH 0.15 μmol, GDP 0.6 μmol, $NaHCO_3$ 20 μmol, $MnCl_2$ 1 μmol, MDH 5 units and tissue extract 20 μl.
4. PK (Bücher and Pfleiderer 1955). Imidazole buffer (pH 7.6) 50 μmol, phosphoenolpyruvate 5 μmol, NADH 0.2 μmol, ADP 1.5 μmol, KCl 120 μmol, $MgSO_4$ 30 μmol, LDH 10 units and tissue extract 50 μl.
5. LDH (Vorhaben and Campbell 1972). Sodium phosphate buffer (pH 7.4) 30 μmol, pyruvate 5 μmol, NADH 0.2 μmol and tissue extract 50 μl.
6. MDH (Kun and Volfin 1966). Sodium phosphate buffer (pH 7.4) 100 μmol, oxaloacetate 6 μmol, NADH 0.2 μmol and tissue extract 50 μl.
7. ME (Bergmeyer 1974). Tris-HCl (pH 7.4) 50 μmol, pyruvate 5 μmol, NADPH 0.2 μmol, $NaHCO_3$ 5 μmol and tissue extract 50 μl.
8. GDH (Olson and Anfinsen 1952 with certain modifications by Das et al. 1991). Potassium phosphate buffer (pH 8.5) 100 μmol, ammonium chloride 50 μmol, α-ketoglutarate 25 μmol, NADH 0.2 μmol, EDTA 0.2 μmol and tissue extract 50 μl.

The enzyme activity was calculated by taking 6.22×10^6 as the molar extinction coefficient value for NADH or NADPH.

One unit of enzyme activity is defined as the amount of the enzyme (mg) which catalysed 1 μmol of $NADP^+$ reduction (in the case of HK) or NADH oxidation (in the case of PFK, PEPCK, PK, LDH and MDH) or NADPH oxidation (in the case of ME) per min at 38°C.

Protein estimation and specific activity

Protein was estimated following the method of Lowry et al. (1951), using bovine serum albumin as the standard. Specific activity of the enzymes was expressed as the units of enzyme activity per mg protein.

Histochemistry

HK, LDH and MDH were also demonstrated histochemically following the standard methods described by Pearse (1972). Cryostat sections of the fresh frozen parasitic material 15 μm thick were incubated in the respective incubation medium. HK: 30 mg D-glucose, 2.5 mg $NADP^+$, 5.5 mg ATP, 20 mg $MgCl_2$, 2.5 mg nitro BT, 2 ml 40 mM imidazole buffer (pH 7.5), 3.8 ml 6% gelatin and 5 μl G6PDH per 10 ml of incubation mixture. LDH: incubation medium containing equal quantities of solution A and solution B and 1.5 mM NAD^+ (added just before incubation). Solution A contained 200 mM L-lactate, 9.8 mM nitro BT, 10 mM NaCN, 0.66 mM PMS, 33 g polyvinyl alcohol in 100 ml of 0.1 M Tris buffer (pH 7.2). Solution B contained 40 g polyvinyl alcohol in 100 ml of 50 mM Tris buffer (pH 7.2). MDH: Each 10 ml of incubation medium contained 2 mg NAD^+, 0.1 ml of stock substrate solution and 0.9 ml of stock incubating medium. In 10 ml of stock substrate solution was 1 M L-malate, which was neutralized by 0.9 ml of 40% NaOH. The stock incubating medium (9 ml) contained 2.5 ml of nitro BT (4 mg/ml), 2.5 ml 0.2 M Tris-HCl buffer (pH 7.4), 1 ml of 5 mM $MgCl_2$ and 3 ml distilled water.

Statistical analysis

Data collected from three to five replicates were statistically analysed and are presented as means ± SEM. Comparisons of the paired mean values between the experimental data and respective controls were made using Student's *t*-test (Croxton et al. 1982) with $P < 0.05$ regarded as significant.

Results

Table 1 shows the effect of the crude peel extract of *F. vestita*, genistein and PZQ on the tissue activities of the glycolytic enzymes in *R. echinobothrida*. At the physiological level, the activity of PEPCK in the parasite was found to be highest, averaging about 18.85 units/g wet weight, followed by LDH (4.53 units/g wet wt), HK (1.68 units/g wet wt) and PFK (1.52 units/g wet wt). The activity of PEPCK in the parasite increased significantly by 49%, 45% and 44%, respectively, in treatments with crude peel extract, genistein and PZQ. Whereas a significant decrease in the activity in PK (of about 26%) was observed when the parasite was treated with crude peel extract, only a little decrease in activity (of about 15%, not significant) was observed in treatments with genistein and PZQ. The tissue activity of HK in the parasite increased significantly under all three treatments. The percentage increase of HK activity was 33%, 39% and 36% during treatment with crude peel extract, genistein and PZQ, respectively, compared to the controls. The activity of PFK increased by 49% and 41% in genistein and crude peel extract treatments, respectively, and by 125% with PZQ, in comparison to the respective controls. The activity of LDH increased by 67% in the genistein treatment, and by 55% and 58% in treatments

Table 1 Effects of different test materials on tissue activity (units/g wet weight) and specific activity (units/mg protein) of hexokinase (HK), phosphofructokinase (PFK), phosphoenolpyruvate carboxykinase (PEPCK), pyruvate kinase (PK) and lactate dehydrogenase (LDH) of *Raillietina echinobothrida* in vitro. Values are expressed as mean ± SEM ($n=4$). Percentage increases (+) or decreases (−) of tissue activity compared to respective controls is given in parentheses. One unit of enzyme activity is defined as that amount of enzyme which catalysed 1 μmol of $NADP^+$ reduction (in the case of HK) or NADH oxidation (in the case of PFK, PEPCK, PK and LDH) per min at 38°C. Specific activity of the enzymes is expressed as the units of enzyme activity per mg protein. NS indicates not significant

Treatment (mg/ml)	Enzyme activity (tissue/specific)				
	HK	PFK	PEPCK	PK	LDH
1.a. Control (in 0.9% PBS)	1.96 ± 0.02/0.041 ± 0.002	1.45 ± 0.17/0.03 ± 0.001	15.87 ± 0.11/0.33 ± 0.02	1.07 ± 0.16/0.022 ± 0.001	4.51 ± 0.06/0.094 ± 0.001
1.b. Crude peel extract (5.0)*P*	2.61 ± 0.12/0.055 ± 0.001 (+33) <0.05	2.04 ± 0.1/0.043 ± 0.001 (+41) <0.05	23.68 ± 1.18/0.5 ± 0.03 (+49) <0.01	0.79 ± 0.13/0.017 ± 0.001 (−26) <0.05	7 ± 1.05/0.15 ± 0.002 (+55) <0.01
2.a. Control	1.79 ± 0.13/0.037 ± 0.003	1.77 ± 0.06/0.037 ± 0.004	16.74 ± 0.37/0.35 ± 0.03	1.07 ± 0.16/0.022 ± 0.002	4.51 ± 0.06/0.094 ± 0.002
2.b. Genistein (0.2) *P*	2.48 ± 0.23/0.052 ± 0.002 (+39) <0.05	2.64 ± 0.15/0.055 ± 0.001 (+49) <0.01	24.33 ± 0.64/0.51 ± 0.04 (+45) <0.05	0.92 ± 0.15/0.019 ± 0.001 (−14) NS	7.55 ± 0.66/0.158 ± 0.003 (+67) <0.01
3.a. Control	1.28 ± 0.12/0.029 ± 0.001	1.34 ± 0.04/0.03 ± 0.004	23.93 ± 0.97/0.38 ± 0.02	1.09 ± 0.12/0.025 ± 0.001	4.57 ± 0.04/0.103 ± 0.004
3.b. PZQ (0.001) *P*	1.74 ± 0.03/0.039 ± 0.003 (+36) <0.05	3.01 ± 0.3/0.068 ± 0.003 (+125) <0.001	34.31 ± 0.37/0.52 ± 0.03 (+44) <0.05	0.93 ± 0.12/0.021 ± 0.002 (−15) NS	7.21 ± 0.25/0.163 ± 0.002 (+58) <0.01

Table 2 Effects of different test materials on tissue activity (units/g wet weight) and specific activity (units/mg protein) of malate dehydrogenase (MDH) and malic enzyme (ME) in *R. echinobothrida* in vitro. Values are expressed as mean ± SEM ($n=4$). Percentage of tissue activity in the mitochondrial and cytosolic fraction compared to the activity in the homogenate is given within parentheses. Percentage increase of tissue activity compared to respective controls is given within square brackets. One unit of enzyme activity is defined as that amount of enzyme which catalyzed 1 μmol of NADH oxidation (in the case of MDH) or NADPH oxidation (in the case of ME) per min at 38°C. Specific activity of the enzymes was expressed as the units of enzyme activity per mg protein. *NS* indicates not significant

Treatment (mg/ml)	Enzyme activity (tissue/specific)					
	MDH			ME		
	Homogenate	Mitochondrial fraction	Cytosolic fraction	Homogenate	Mitochondrial fraction	Cytosolic fraction
1.a. Control (in 0.9% PBS)	73.23 ± 4.32/1.6 ± 0.03	26.89 ± 0.75/0.59 ± 0.05 (37)	37.77 ± 1.25/0.83 ± 0.025 (52)	0.18 ± 0.005/0.005	0.08 ± 0.002/0.002 (44)	0.11 ± 0.002/0.003 (61)
1.b. Crude peel extract (5.0)*P*	89.61 ± 2.68/1.96 ± 0.02 [22] <0.05	39.4 ± 1.52/0.86 ± 0.02 (44) [47] <0.05	51.32 ± 0.98/1.12 ± 0.09 (57) [36] <0.05	0.23 ± 0.009/0.006 [28] <0.05	0.09 ± 0.001/0.0025 (39) [13] NS	0.15 ± 0.005/0.004 (65) [+36] <0.05
2.a. Control	65.28 ± 2.41/1.43 ± 0.04	22.01 ± 1.09/0.48 ± 0.009 (34)	38.8 ± 1.02/0.85 ± 0.02 (59)	0.29 ± 0.007/0.008	0.07 ± 0.003/0.002 (24)	0.18 ± 0.003/0.005 (62)
2.b. Genistein (0.2) *P*	81.69 ± 0.99/1.79 ± 0.03 [25] <0.05	31.51 ± 0.98/0.69 ± 0.008 (39) [43] <0.05	51.7 ± 0.56/1.13 ± 0.05 (63) [33] <0.05	0.37 ± 0.006/0.01 [28] <0.05	0.08 ± 0.004/0.002 (22) [14] NS	0.25 ± 0.001/0.007 (68) [+ 39] <0.05
3.a. Control	46.39 ± 4.12/1.18 ± 0.02	18.95 ± 0.65/0.48 ± 0.06 (41)	24.93 ± 0.79/0.64 ± 0.009 (54)	0.22 ± 0.005/0.006	0.1 ± 0.008/0.003 (36)	0.15 ± 0.002/0.004 (54)
3.b. PZQ (0.001)*P*	66.56 ± 2.55/1.69 ± 0.05 [43] <0.05	32.71 ± 2.05/0.83 ± 0.05 (49) [73] <0.01	39.44 ± 2.03/1.01 ± 0.03 (59) [58] <0.01	0.35 ± 0.008/0.01 [59] <0.05	0.11 ± 0.006/0.003 (31) [10] NS.	0.2 ± 0.003/0.005 (57) [33] <0.05

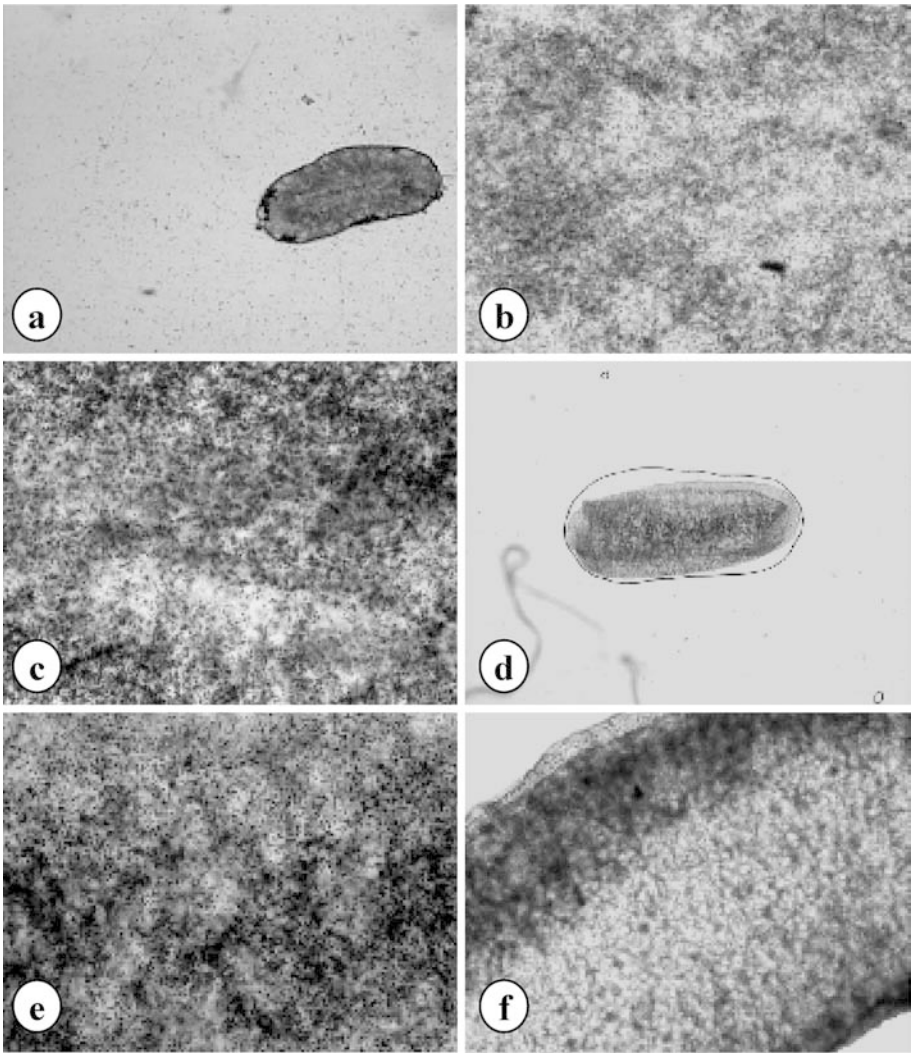

Fig. 1a–f *Raillietina echinobothrida*: Hexokinase (HK) activity in control and treated parasites. Photomicrographs show cross sections through a mature proglottid. **a** Control whole section ×110; **b** magnified view of a portion of the same ×1,100; **c** crude peel extract ×1,100; **d** genistein whole section ×110; **e** magnified view of a portion of the same. ×1,100; **f** praziquantel ×1,100. Increased HK activity is noticeable in all treatments in comparison to control

with crude peel extract and PZQ, respectively. The specific activity of HK, PFK, PK, PEPCK and LDH in the parasite under different treatments showed an almost similar pattern of change to that of the tissue activity (Table 1).

The MDH activity in *R. echinobothrida* occurred both in the mitochondria and cytosol, with 40% of the activity in the former and 50% in the latter (Table 2). Both the mitochondrial and cytosolic MDH were affected in the parasite by the different treatments. The mitochondrial MDH activity was stimulated significantly, with an increase by 47%, 43% and 73%, respectively, during treatment with crude peel extract, genistein and PZQ, while the percentage increase in the cytosolic MDH activity was 36%, 33% and 58%, respectively, under similar conditions. Like MDH, ME was also found in the mitochondria and cytosol of this parasite, the activity distribution being about 35% and 60%, respectively (Table 2). Only the cytosolic ME was found to be affected significantly under different treatment conditions in the parasite; the percentage increase in the activity of the cytosolic ME was 36%, 39% and 33%, respectively, during treatment with crude peel extract, genistein and PZQ. The specific activity of MDH and ME, both cytosolic and mitochondrial, showed similar changes to the tissue activity (Table 2).

Histochemically, alterations in the activity of HK, LDH and MDH activity in the parasite tissue were also shown; the activities of the enzymes increased in all of the treatments in comparison to the control (Figs. 1, 2, 3).

Discussion

Glycogen, which is stored in the parenchymal tissue, serves as the most important energy reserve in cestodes (Smyth and McManus 1989). In an earlier study, it was found that at the time of paralysis, the glycogen concentration in *R. echinobothrida* decreased by 19–44% under the influence of *F. vestita* derived components. This was accompanied by the stimulation of glycogenolytic enzymes, particularly the active form of glycogen phosphorylase (Tandon et al. 2003).

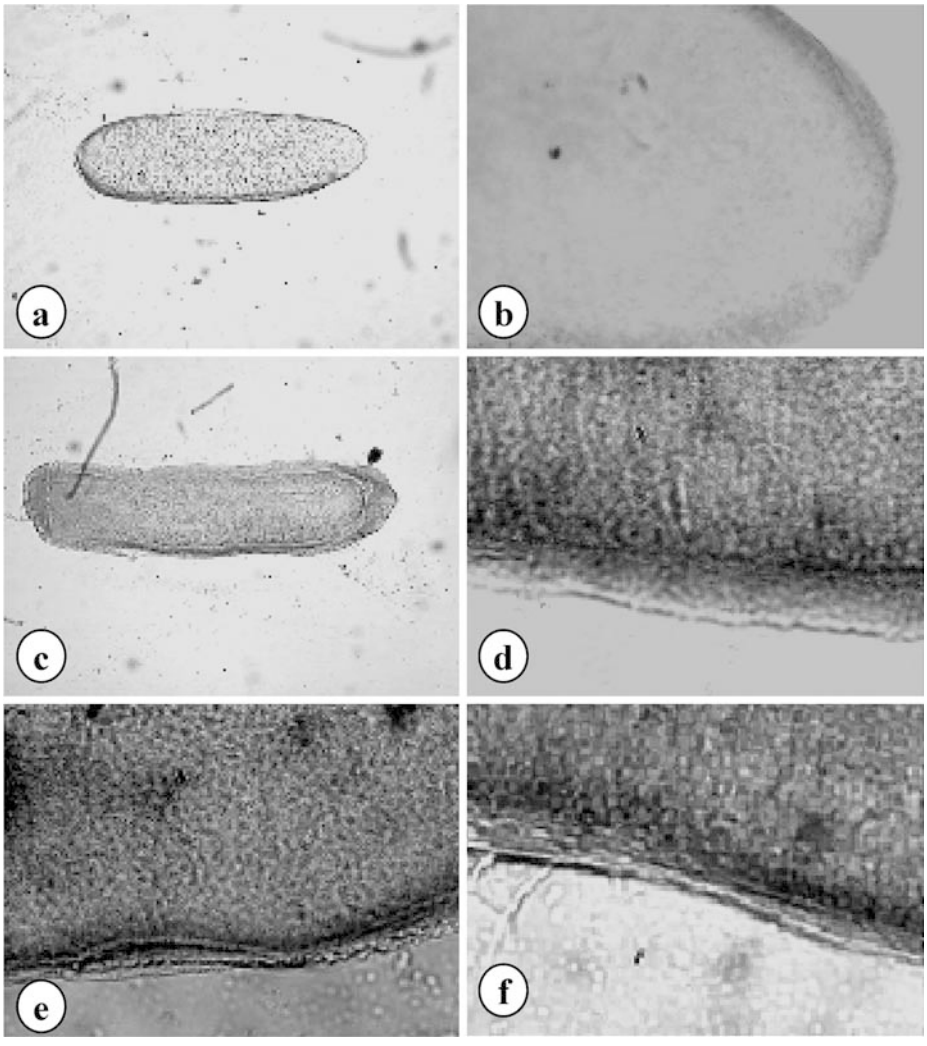

Fig. 2a–f *R. echinobothrida*: Lactate dehydrogenase (LDH) activity in control and treated parasites. Photomicrographs show cross sections through a mature proglottid. **a** Control whole section ×110; **b** magnified view of a portion of the same ×1,100; **c** crude peel extract whole section ×110; **d** magnified view of a portion of the same.×1,100; **e** genistein ×1,100; **f** praziquantel ×1,100. Increased stain intensity, indicative of increased LDH activity, is noticeable in all treatments in comparison to control

Glycolysis, the main energy-generating pathway, with lactate as an end product in nematode parasites (Omar et al. 1996), seems to be a promising target for new drugs against trypanosomatid parasites, because this pathway plays an essential role in ATP supply (Lakhdar-Ghazal et al. 2002). Some carboline antifilarials, e.g. 90/55 (7-oxo-1-phenyl-8, 14-dihydropyrido (3,4-b) imidazo (1,2-c) quinazolo (4,5-g) and 87/639 (6-nitro-1-phenyl-9H-pyrido (3,4-b) indole, significantly lowered the HK activity but increased the activities of GPase, PFK and PK. However, mebendazole had no effect on HK in *Avitellina lahorea* but it influenced the activities of some glycolytic enzymes such as phosphorylase, phosphoglucomutase and G6Pase, and also caused glycogen depletion and the inhibition of glucose uptake in vitro (Ahmad and Nizami 1987). In *Hymenolepis microstoma*, the specific activity of HK, PFK and PK was not stimulated by 5-hydroxytryptamine, but CO_2 fixation by PEPCK was inhibited (Rahman and Mettrick 1982). The HK activity in the present study increased significantly by between 33% and 39% in treatments with crude peel extract, genistein and PZQ. This suggests that more glucose was being utilized by the parasite during exposure to the various treatments so as to yield more energy.

Phosphorylation of PFK is believed to play an important role in the regulation of carbohydrate metabolism, particularly the glycolytic pathway, in nematodes (Srinivasan et al. 1988). In the present study, the PFK activity increased by 125% in the PZQ-treated parasites, whereas a 49% and 41% increase was observed in the genistein- and crude peel extract-treated parasites, respectively, indicating that the rate of glycolysis was also stimulated in the parasites under the treatment conditions. Although the possible mechanism(s) of stimulation of PFK in *R. echinobothrida* under all the treatments is difficult to explain, a change in the phosphorylation status of this enzyme seems to be a plausible reason.

PEPCK's main role in cestodes is in the degradation of glucose rather than its synthesis through the gluconeogenic pathway (Smyth and McManus 1989). However, in *Schistosoma mansoni*, experiments with inhibitors of PEPCK gave no indication that this enzyme is involved in the degradation of glucose, and it was confirmed that lactate is formed from

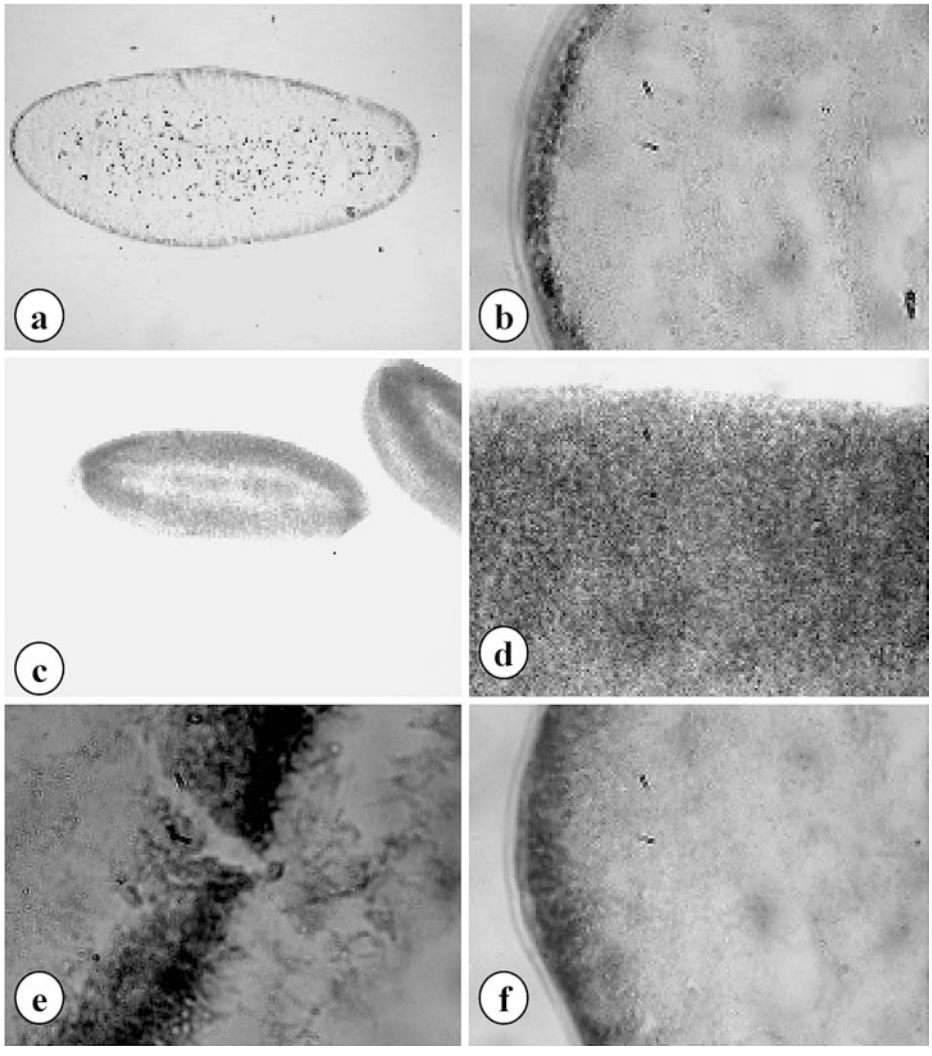

Fig. 3a–f *R. echinobothrida*: Malate dehydrogenase (MDH) activity in control and treated parasites. Photomicrographs show cross sections through a mature proglottid. **a** Control whole section ×110; **b** magnified view of a portion of the same ×1,100; **c** crude peel extract whole section ×1,100; **d** magnified view of a portion of the same ×1,100; **e** genistein ×1,100; **f** praziquantel ×1,100. Increased stain intensity in all treatments (**c–f**) indicates enhanced MDH activity in comparison to control

phosphoenolpyruvate via the actions of PK and LDH (Tielens et al. 1991). In the present study, PEPCK activity was found to be high in *R. echinobothrida*, averaging about 18.85 units/g wet wt in control parasites. The activity of PEPCK was stimulated by 44–49% in the parasites treated with the various test materials. The low concentration of pyruvate (below the level of biochemical detection, unpublished data) in the parasite can also be explained in light of the higher activity of PEPCK over PK in controls as well as in all treatments.

PK, another potential regulatory enzyme of glycolysis, converts phosphoenolpyruvate to pyruvate. The PK, glyceraldehyde-phosphate dehydrogenase and phosphoglycerate kinase activities were inhibited by artemether both in male and female *Schistosoma japonicum* (Xiao et al. 1998). Sumarmin and centperazine caused a marked inhibition in most of the enzymes of phosphoenolpyruvate-succinate pathway in *Setaria cervi*, although diethylcarbamazine and levamisole were found to be more or less ineffective at a lower concentration against all enzymes of the glycolytic pathway (Hussain et al. 1990). In the present study, the PK activity decreased by about 14–15% in *R. echinobothrida* in treatments with genistein and PZQ, and by 26% in the case of crude peel extract. The lower activity of PK and the inhibitory action of the test materials on the enzyme also corroborate the presence of a very low concentration of pyruvate in the parasite tissue (unpublished observations). Thus, the branch point of PEPCK/PK perhaps forms the basis of the anthelmintic attack by the plant-derived components.

Many anthelmintics are believed to work via an alteration in the activity of parasite LDH. For example, benzimidazoles act primarily via activation of LDH, catalysing the conversion of pyruvate to lactate (Veerakumari and Munuswamy 2000). Artmether, a derivative of *Artemesia* and well known for its antimalarial properties, was shown to exert a potent inhibitory action on the LDH and G6PDH activities in *Schistosoma japonicum* (Xiao et al. 1999). Filarin (a drug of herbal origin and used in Siddha medicine) and diethylcarbamazine inhibited pyruvate reduction rather than lactate oxidation in *Setaria digitata* by altering the activity of LDH (Banu et al. 1989). With treatment by isatin, the LDH activity decreased in the metacestodes of *Echinococcus multilocularis*, in which glucose and glycogen

stores also declined significantly (Delabre-Defayolle et al. 1989). In *R. echinobothrida*, the activity of LDH increased by 67% with genistein treatment but by 55% and 58% in treatments with crude peel extract and PZQ, respectively. The higher activity of LDH under stress conditions can be explained by the possible formation of pyruvate following the increased activity of cytosolic ME. The increased net production of lactate in the parasite tissue under various similar treatments also corroborates this (unpublished data).

The presence of MDH, which converts oxaloacetate to malate, has also been demonstrated in several cestodes. Drugs like oxyclozanide, hexachlorophene, nitroxynil, rafoxamide and diamphenethide inhibited the MDH activity in trematodes (Probert et al. 1981). Following in vitro treatment with cambendazole and tiabendazole, moderate inhibition of the MDH activity was reported in fowl nematodes, although haloxon had little effect on the worms (Sharma et al. 1986). Diethylcarbamazine citrate did not appreciably change the activity of either mitochondrial MDH or mitochondrial ME, while filarin effectively inhibited mitochondrial MDH in *S. digitata*. The leaf extracts of *Ocimum sanctum*, *Lawsonia inermis* and *Calotropis gigantea* and leaf and flower extracts of *Azadirachta indica* were, however, found to inhibit both mitochondrial MDH and mitochondrial ME (Banu et al. 1992). The MDH activity was suppressed by mebendazole, albendazole and PZQ, but no apparent effects on succinate dehydrogenase or fumarate reductase activities in *Echinoccocus granulosus* hydatid cysts were found (Xiao et al. 1993). In the present study, the MDH activity in *R. echinobothrida* increased in all treatments, which can be attributed to the increase in the concentration of malate in the parasite tissue. The ME activity in the tissue homogenate was also found to increase by 28% in treatments with crude peel extract and genistein, and by 59% in treatment with PZQ in comparison with the respective controls. While there was no significant increase in the activity of ME in the mitochondrial fraction, enzyme activity increased by 33–39% in the cytosolic fraction with these treatments. However, most of the synthetic anthelmintics tested against *H. diminuta* did not show any inhibitory effect on the ME activity (Wani and Srivastava 1994).

The low activities of LDH and PK, and the high activities of MDH and PEPCK, suggest that anaerobic carbohydrate catabolism follows the fumarate-reductase pathway (Vykhrestyuk et al. 1984). In the present study, the PEPCK activity was found to be increased and the PK activity decreased under various treatment conditions, suggesting that glucose breakdown followed the PEPCK-malate pathway. Thus, from the results obtained in various in vitro experiments, it may be postulated that the phytochemicals of *F. vestita*, which seem to have the tegumental interface of the parasite as the primary target, also influence carbohydrate metabolism towards PEPCK-malate formation in *R. echinobothrida*. It may be further hypothesized that to meet the high energy demands of the parasite during anthelmintic stress following exposure to the plant crude peel extract and genistein, the glycolytic pathway is activated by regulating some key enzymes. The phytochemicals of *F. vestita*, and genistein in particular, may act upon PEPCK, the latter being one of several secondary drug targets in cestode parasites. The enhanced glycolytic activity, as evidenced in the treated parasite, seems to be a function of anthelmintic stress caused by the test phytochemicals.

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