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Effects of phytochemicals of *Flemingia vestita* (Fabaceae) on glucose 6-phosphate dehydrogenase and enzymes of gluconeogenesis in a cestode (*Raillietina echinobothrida*)

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Abstract

The crude root-peel extract of *Flemingia vestita*, containing genistein as the major isoflavone, has a vermifugal/vermicidal effect. It acts by causing flaccid paralysis accompanied by alterations in the activities of several tegumental enzymes and other metabolic activities in the fowl tapeworm, *Raillietina echinobothrida*. To elucidate the mode of action of the putative phytochemicals on energy metabolism, crude root-peel extract, pure genistein and praziquantel were tested on glucose 6-phosphate dehydrogenase (G6PDH) and enzymes of gluconeogenesis—pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK) and fructose 1,6-bisphosphatase (FBPase)—in *R. echinobothrida*. The activities of G6PDH, PEPCK and FBPase were largely restricted to the cytosolic fraction, while PC was confined to the mitochondrial fraction. Following treatments, the G6PDH activity was decreased by 23–31%, whereas the activities of PC and PEPCK were increased by 32–44% and 44–49%, respectively. There was no significant effect by any of the treatments on FBPase activity. We hypothesize that the phytochemicals from *F. vestita*, genistein in particular, influence the key enzymes of these pathways, which is perhaps a function of high energy demand of the parasite under anthelmintic stress.

Keywords: *Flemingia vestita*; Anthelmintic; *Raillietina echinobothrida*; Hexose monophosphate pathway; Gluconeogenesis; Cestode; Genistein; Parasite

1. Introduction

The fleshy edible tuberous root of *Flemingia vestita* (Fabaceae) is usually used against intestinal parasites in local traditional medicine among the native people of Meghalaya, India. The ethanolic crude root-peel extract of *F. vestita*—which contains genistein as the major isoflavone compound, besides formononetin, pseudobaptigenin and daidzein (Rao and Reddy, 1991)—exerts vermifugal/vermicidal effects against intestinal trematodes and cestodes (Roy and Tandon, 1996; Tandon et al., 1997). In earlier studies, these phytochemicals caused flaccid paralysis in *Raillietina echinobothrida*, with accompanying alterations in the tegumental architecture and activities of several tegumental enzymes, namely, acid—and alkaline phosphatases, adenosine triphosphatase and 5′-nucleotidase (Tandon et al., 1997; Pal and Tandon, 1998a,b). There was a decline in the activity of the enzymes associated with the coordination system, nonspecific esterases, acetylcholine esterease in particular, in the cestode during the treatments with these phytochemicals (Pal and Tandon, 1998c); the activity of nitric oxide synthase in *Fasciolopsis buski* was also influenced following treatment with the same plant materials and pure genistein (Kar et al., 2002). In vitro treatment of *R. echinobothrida* and *F. buski* with crude root-peel extract and genistein also caused alteration in the free amino acid pool and tissue ammonia (Tandon et al., 1998; Kar et al., 2004). These phytochemicals also influence energy-yielding mechanisms of the parasite, as revealed by our studies on

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carbohydrate metabolism in *R. echinobothrida*; the treatments caused a significant decline in glycogen levels (Tandon et al., 2003), and glycolysis was enhanced by regulating key enzymes of the pathway (Das et al., 2004).

However, scant information is available regarding the hexose monophosphate pathway (HMP) in cestodes. This pathway converts hexoses to pentoses and yields NADPH as a carrier of chemical energy in the form of reducing power (Nelson and Cox, 2000). A complete sequence of enzymes for HMP has been demonstrated only in larval *Echinococcus granulosus* (Agosin and Aravena, 1960; Agosin and Repetto, 1961). However, the presence of the first two enzymes of this pathway, namely, glucose 6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PDH) has been reported in several species including both pseudophyllidean and cyclophyllidean cestodes (Körting and Barrett, 1977; McManus and Smyth, 1982; McManus and Sterry, 1982; Roberts, 1983).

Regarding the gluconeogenic pathway, the presence of pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK), fructose 1,6-bisphosphatase (FBPase) and glucose 6-phosphatase (G6Pase) has been reported in some cestodes (Behm and Bryant, 1975; Moon et al., 1977; Fukumoto, 1985; Unnikrishnan and Raj, 1995).

In the present study, one key regulatory enzyme of the HMP and enzymes of gluconeogenesis were studied as a preliminary step to elucidate the presence of these pathways and the influence, if any, of the phytochemicals of *F. vestita* on them in the cestode, *R. echinobothrida*.

2. Materials and methods

2.1. Drugs and chemicals

The ethanolic crude root-peel extract of *F. vestita* and pure genistein were obtained as reported (Tandon et al., 1997). Synthetic genistein was obtained from Sigma (St. Louis, USA). Praziquantel (PZQ; Droncit, Bayer, India) was used as the reference drug. All enzymes and coenzymes were obtained either from Sigma or Roche (Germany). Other chemicals (analytical grade) were obtained from local sources. Deionized double glass-distilled water was used for all preparations.

2.2. Parasites and in vitro treatment

Live parasites, collected in 0.9% phosphate-buffered saline (PBS, pH 7.2) from the intestines of freshly slaughtered domestic fowl (*Gallus domesticus*), were exposed to different treatments immediately after collection. The parasites (approximately 0.2 g fresh mass) were incubated in 10 mL of PBS at 38±1 °C with defined concentrations of different test materials, i.e., 5 mg/mL crude root-peel extract, 0.2 mg/mL genistein or 1 μg/mL PZQ dissolved in 1% dimethylsulfoxide (DMSO), with respective controls containing 1% DMSO in PBS. As reported earlier (Tandon et al., 1997), the fore-mentioned concentrations of test materials caused paralysis in the cestode in about 6, 6 and 3 h in case of crude root-peel extract, genistein and PZQ, respectively, while death occurred at about 46, 50 and 10 h, post incubation; controls survived in vitro for about 72 h. Each set of treatments contained the parasites from a single host. Treated parasites and their respective controls were retrieved from the incubation media at the time when paralysis set in and were processed for different biochemical studies.

2.3. Enzyme assays

2.3.1. Tissue processing; subcellular fractionation

Fresh parasites were minced and homogenized in homonizing buffer (1:2.25 ratio, g/v) containing 50 mM Tris–HCl (pH 7.4) buffer, 0.3 M sucrose, 1 mM EDTA, 2 mM $MgCl_2$ and 3 mM 2-mercaptoethanol with a motor-driven Potter–Elvehjem glass homogenizer with a loosely fitted Teflon pestle. The subcellular fractions such as mitochondrial and cytosolic (soluble) were separated out by differential centrifugation of the homogenate. The homogenate was first centrifuged at 600×*g* for 10 min to pellet out the cell debris and nuclei. The loose pellet was resuspended in equal volume of the homogenizing buffer, homogenized a second time as described above to facilitate breakage of unbroken cells and centrifuged at 600×*g* for 10 min. The supernatant was decanted, combined with the first supernatant and centrifuged at 18,000×*g* for 20 min to give a well-defined and firm pallet (mitochondrial fraction); the supernatant from this centrifugation step was the cytosolic fraction. The mitochondrial fraction was resuspended in the same buffer as described above. The mitochondrial and cytosolic fractions as well as a portion of the crude homogenate (kept separately before centrifugation) were treated with 0.5% Triton X-100 (prepared in the same homogenizing buffer) in 1:1 ratio for 30 min. The crude homogenate and the mitochondrial fraction were sonicated (Soniprep 150) for 30 s for proper breakage of mitochondria. Glutamate dehydrogenase (GDH) and lactate dehydrogenase (LDH) were used as mitochondrial and cytosolic markers, respectively. All the steps were carried out at 4 °C.

2.3.2. Assays

Enzymes were assayed spectrophotometrically at 340 nm with a Beckmann UV-Visible spectrophotometer (Model DU 640) fitted with a Peltier temperature-controlled system. For all assays, the total volume of the reaction mixture was adjusted to 1 mL.

G6PDH (EC 1.1.1.49) activity was measured following DeMoss (1955); the reaction mixture contained 50 μmol Tris–HCl buffer (pH 7.4), 10 μmol $MgCl_2$, 0.2 μmol $NADP^+$, 5 μmol D-glucose 6-phosphate and 50 μL of the

enzyme source. PC (EC 6.4.1.1) was measured following Moon and Mommsen (1987); the reaction mixture contained 100 μmol Tris–HCl buffer (pH 7.8), 0.1 μmol acetyl CoA, 15 μmol $NaHCO_3$, 10 μmol pyruvate, 0.15 μmol NADH, 5 μmol $MgCl_2$, 10 units of MDH and 20 μL of the enzyme source. PEPCK (EC 4.1.1.32) was assayed following Mommsen et al. (1985); the reaction mixture contained 50 μmol Tris–HCl (pH 7.4), 4.5 μmol phosphoenolpyruvate, 0.15 μmol NADH, 0.6 μmol GDP, 20 μmol $NaHCO_3$, 1 μmol $MnCl_2$, 5 units of MDH and 20 μL of the enzyme source. FBPase (EC 3.1.3.11) was measured following Mommsen et al. (1985); the reaction mixture contained 50 μmol Tris–HCl buffer (pH 7.4), 0.15 μmol F1,6-bisphosphate, 0.2 μmol $NADP^+$, 15 μmol $MgCl_2$, 10 units of G6PDH, 400–600 units of phosphoglucose isomerase and 50 μL of the enzyme source. LDH (EC 1.1.1.27) was assayed following Vorhaben and Campbell (1972); the reaction mixture contained 30 μmol Na-phosphate buffer (pH 7.4), 5 μmol pyruvate, 0.2 μmol NADH and 50 μL of the enzyme source. GDH (EC 1.4.1.3) was assayed following Olson and Anfinsen (1952) with certain modifications by Das et al. (1991); the reaction mixture contained 100 μmol K-phosphate buffer (pH 8.5), 50 μmol ammonium chloride, 25 μmol α-ketoglutarate, 0.2 μmol NADH, 0.2 μmol EDTA and 50 μL of the enzyme source.

The linear increase (FBPase and G6PDH) or decrease (PEPCK, PC, LDH and GDH) in optical density was taken for expressing the enzyme activity, using 6.22×10^6 as the molar extinction coefficient for NADPH or NADH. One unit of enzyme activity is the amount of enzyme catalyzing 1 μmol of $NADP^+$ reduction or NADH oxidation per min at 38 °C.

2.4. Protein estimation and specific activity

Protein content in the samples was estimated following Lowry et al. (1951), and specific activity of the enzymes was expressed as the units of enzyme activity per mg protein.

2.5. Statistical analysis

Statistical data from five replicates were presented as mean±S.E.M., and $p<0.05$ was regarded as statistically significant. Comparisons of the paired mean values between

Table 1
Effects of different test materials on tissue activity (units/g wet wt) and specific activity (units/mg protein) of glucose 6-phosphate dehydrogenase (G6PDH) and pyruvate carboxylase (PC) in *R. echinobothrida* in vitro

Treatment (mg/ml)	Enzyme activity (tissue/specific)					
	G6PDH			PC		
	Homogenate	Mitochondrial fraction	Cytosolic fraction	Homogenate	Mitochondrial fraction	Cytosolic fraction
1.a. Control (in 0.9% PBS)	1.27±0.02/ 0.028±0.002	0.24±0.001/ 0.007±0.001 (19)	1.08±0.13/ 0.024±0.004 (85)	6.39±0.38/ 0.14±0.03	5.79±0.28/ 0.13±0.01 (91)	0.52±0.04/ 0.012±0.003 (8)
1.b. Crude peel extract (5.0)	0.88±0.006/ 0.019±0.006 [−31]	0.16±0.002/ 0.006±0.002 (18)	0.69±0.002/ 0.015±0.003 (78) [−36]	9.21±0.2/ 0.2±0.02 [+44]	8.65±0.12/ 0.19±0.03 (94) [+49]	0.81±0.02/ 0.018±0.001 (9)
p	<0.05		<0.05	<0.05	<0.01	
2.a. Control	1.55±0.1/ 0.034±0.001	0.29±0.001/ 0.01±0.002 (19)	1.33±0.23/ 0.027±0.003 (86)	6.68±0.29/ 0.15±0.02	5.93±0.21/ 0.13±0.01 (89)	0.59±0.02/ 0.013±0.001 (9)
2.b. Genistein (0.2)	1.12±0.15/ 0.024±0.001 [−28]	0.2±0.06/ 0.005±0.001 (18)	0.94±0.19/ 0.021±0.002 (84) [−29]	8.82±0.38/ 0.19±0.03 [+32]	8.01±0.15/ 0.178±0.003 (91) [+35]	0.91±0.03/ 0.02±0.003 (10)
p	<0.05		<0.05	<0.05	<0.05	
3.a. Control	3.2±0.29/ 0.04±0.003	0.36±0.02/ 0.005±0.001 (11)	2.78±0.32/ 0.037±0.003 (87)	8.46±1.32/ 0.18±0.01	7.89±0.78/ 0.168±0.03 (93)	0.65±0.02/ 0.014±0.001 (8)
3.b. PZQ (0.001)	2.48±0.45/ 0.031±0.004 [−23]	0.26±0.04/ 0.004±0.001 (10)	2.06±0.39/ 0.026±0.002 (83) [−26]	11.23±1.03/ 0.25±0.03 [+33]	10.57±0.63/ 0.225±0.01 (94) [+34]	0.96±0.03/ 0.02±0.002 (9)
p	<0.05		<0.05	<0.05	<0.05	
GDH activity (mitochondrial marker)	89±4.12/1.48±0.65	84.26±2.59/ 1.43±0.58 (95)	3.52±0.45/ 0.06±0.001 (4)	89±4.12/ 1.48±0.65	84.26±2.59/ 1.43±0.58 (95)	3.52±0.45/ 0.06±0.001 (4)
LDH activity (cytosolic marker)	41±2.14/0.69±0.01	2.46±0.85/ 0.042±0.001 (6)	39.77±3.68/ 0.67±0.02 (97)	41±2.14/ 0.69±0.01	2.46±0.85/ 0.042±0.001 (6)	39.77±3.68/ 0.67±0.02 (97)

Percentage of tissue activity in the mitochondrial and cytosolic fractions compared to the activity in the homogenate is given within parentheses. Percentage decrease or increase of tissue activity compared to respective controls is given within square brackets only if $p<0.05$.
One unit of enzyme activity is the amount of enzyme catalyzing 1 μmol of $NADP^+$ reduction (in case of G6PDH) or NADH oxidation (in case of PC, LDH and GDH) per minute at 38 °C.
Specific activity of the enzymes is expressed as the units of enzyme activity per mg protein.
Values are expressed as mean±S.E.M. (n=5).

the treatments and respective controls were calculated using student's *t*-test (Croxton et al., 1982).

3. Results

The subcellular distribution of G6PDH and PC in the cestode, *R. echinobothrida* and the effect of the test materials on the activity of these two enzymes are presented in Table 1. G6PDH activity was predominantly (78–87%) localized in cytosolic fraction. G6PDH activity in the whole tissue homogenate was significantly decreased by 31%, 28% and 23%, respectively, in treatments with crude root-peel extract, genistein and PZQ; this was accompanied by the decrease of enzyme activity in the cytosolic fraction by 36%, 29% and 26%, respectively. PC activity, mainly demonstrated in the mitochondrial fraction (about 89–94%), was significantly increased by 44%, 32% and 33% in the whole homogenate in treatments with crude root-peel extract, genistein and PZQ, respectively. Under similar treatments, the mitochondrial PC activity was increased by 49%, 35% and 34%, respectively. PEPCK activity was predominantly detected in the cytosolic fraction (77–91%), whereas that measured in the mitochondrial fraction was negligible. In treatments with crude root-peel extract, genistein and PZQ, PEPCK activity in the whole tissue homogenate increased significantly by 44–49% and that in the cytosolic fraction by 56%, 63% and 46%, respectively (Table 2). Activity of FBPase was also primarily detected in the cytosolic fraction (72–81%); however, there was no significant effect of any treatment on total tissue activity or cytosolic FBPase (Table 2).

Specific activities of the enzymes showed similar changes (decrease or increase) as the respective tissue activities of homogenate/fractions (Tables 1 and 2).

4. Discussion

HMP is an important carbohydrate metabolic pathway providing NADPH for fatty acid synthesis and pentoses for nucleic acid synthesis (Smyth and McManus, 1989). The functional significance of this pathway in cestodes is debatable. The presence of significant activity of G6PDH in *Angiostrongylus cantonensis* and 6-PDH in *Onchocerca fasciata* indicates that HMP might be operative in helminths

Table 2
Effects of different test materials on tissue activity (units/g wet wt) and specific activity (units/mg protein) of phosphoenolpyruvate carboxykinase (PEPCK) and fructose 1,6-bisphosphatase (FBPase) in *R. echinobothrida* in vitro

Treatment (mg/ml)	Enzyme activity (tissue/specific)					
	PEPCK			FBPase		
	Homogenate	Mitochondrial fraction	Cytosolic fraction	Homogenate	Mitochondrial fraction	Cytosolic fraction
1.a. Control (in 0.9% PBS)	15.87±0.11/ 0.33±0.02	1.68±0.39/ 0.04±0.002 (10)	13.42±0.9/ 0.28±0.035 (85)	1.1±0.02/ 0.024±0.002	0.26±0.03/ 0.006±0.002 (24)	0.89±0.023/ 0.02±0.002 (81)
1.b. Crude peel extract (5.0)	23.68±1.18/ 0.5±0.03 [+49]	2.57±0.52/ 0.05±0.003 (11)	20.9±0.47/ 0.44±0.02 (88) [+56]	0.98±0.15/ 0.022±0.001	0.21±0.01/ 0.005±0.002 (21)	0.76±0.12/ 0.017±0.002 (78)
p	<0.01		<0.01	N.S.	N.S.	N.S.
2.a. Control	16.74±0.37/ 0.35±0.03	2.96±0.48/ 0.06±0.001 (18)	12.89±0.56/ 0.27±0.032 (77)	1.1±0.02/ 0.024±0.002	0.26±0.03/ 0.006±0.002 (24)	0.89±0.023/ 0.02±0.002 (81)
2.b. Genistein (0.2)	24.33±0.64/ 0.51±0.04 [+45]	4.5±0.83/ 0.085±0.004 (19)	21.02±0.92/ 0.44±0.019 (86) [+63]	1.05±0.08/ 0.023±0.001 N.S.	0.22±0.05/ 0.005±0.003 (21)	0.83±0.04/ 0.018±0.003 (79)
p	<0.05		<0.01		N.S.	N.S.
3.a. Control	23.93±0.97/ 0.38±0.02	2.45±0.29/ 0.039±0.002 (10)	21.31±0.79/ 0.34±0.002 (89)	1.68±0.09/ 0.037±0.001	0.32±0.03/ 0.007±0.002 (19)	1.24±0.07/ 0.028±0.002 (74)
3.b. PZQ (0.001)	34.31±0.37/ 0.52±0.03 [+44]	3.82±0.72/ 0.06±0.004 (11)	31.1±0.23/ 0.49±0.012 (91) [+46]	1.45±0.06/ 0.032±0.003 N.S.	0.25±0.04/ 0.006±0.001 (17)	1.06±0.03/ 0.024±0.001 (72)
p	<0.05		<0.01		N.S.	N.S.
GDH activity (mitochondrial marker)	89±4.12/ 1.48±0.65	84.26±2.59/ 1.43±0.58 (95)	3.52±0.45/ 0.06±0.001 (4)	89±4.12/ 1.48±0.65	84.26±2.59/ 1.43±0.58 (95)	3.52±0.45/ 0.06±0.001 (4)
LDH activity (cytosolic marker)	41±2.14/ 0.69±0.01	2.46±0.85/ 0.042±0.001 (6)	39.77±3.68/ 0.67±0.02 (97)	41±2.14/ 0.69±0.01	2.46±0.85/ 0.042±0.001 (6)	39.77±3.68/ 0.67±0.02 (97)

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 only if $p<0.05$.
N.S.—not significant.
One unit of enzyme activity is the amount of enzyme catalyzing 1 μmol of NADH oxidation (in case of PEPCK, LDH and GDH) or $NADP^+$ reduction (in case of FBPase) per minute at 38 °C.
Specific activity of the enzymes is expressed as the units of enzyme activity per mg protein.
Values are expressed as mean±S.E.M. (n=5).

(Shih and Chen, 1982; Omar et al., 1996). Histochemically, G6PDH, PFK and G3PDH were demonstrated in the hypodermal tissue, somatic musculature and reproductive organs of the adult female *O. fasciata*, indicating that both the glycolytic pathway and HMP are active in various tissues of the worm (Omar and Raoof, 1994a). Sensitivity of these enzymes to anthelmintics has been tested in vitro. The drug thiophenate was reported to cause an increase in the activities of G6PDH and GDH, and a decrease in NADPH-diaphorase, although after febendazole treatment, the activities of these enzymes, except GDH, remained unaltered in *Haemonchus contortus*, a trichostrongylid nematode (Kaur and Sood, 1992). Artemether, a well-known antimalarial drug derived from the plant genus *Artemesia*, exerts a potent inhibitory action on G6PDH and LDH activities in *Schistosoma japonicum* (Xiao et al., 1999). G6PDH is the first and regulatory pacemaker enzyme of HMP. As expected, G6PDH activity in *R. echinobothrida* tissue was predominantly localized in the cytosolic fraction, with a trifling activity occurring in the mitochondrial fraction. G6PDH activity in the treated homogenate was significantly decreased by 23–31%; in the cytosolic fraction, this decrease was 26–36%. These results suggest that, under stress conditions, NADPH synthesis may get inhibited due to lowered activity of this key regulatory enzyme of HMP in the parasite.

PC, FBPase and G6Pase have been reported in several helminth parasites (Pampori et al., 1985; Tielens et al., 1991; Omar and Raoof, 1994b). The activities of PC, FBPase and G6Pase along with PEPCK were demonstrated in homogenates of adult *S. mansoni*; all four gluconeogenic enzymes were present in the worm, although experiments with ^{14}C-labelled substrates failed to demonstrate the actual occurrence of gluconeogenesis in the parasite (Tielens et al., 1991). Treatment of isolated brush border membrane of the cestode, *Cotugnia digonopora* with mebendazole, niclosamide and PZQ in vitro did not alter the activity of these enzymes, although treatment of intact worms drastically affected the integrity of the membrane (Pampori et al., 1985). In the present study, PC activity in the tissue homogenate and mitochondrial fraction of the parasite showed a significant increase ($p<0.05$) in all treatments. However, the involvement of the enzyme in the gluconeogenesis is difficult to explain, as it participates in anaplerotic reactions in the mitochondria. FBPase activity along with that of G6Pase, LDH and ATPase were decreased during the treatments with hetrazan, levamisole and tetramisole in adult *Setaria cervi* worms (Khatoon et al., 1983). However, in the present study, although the presence of FBPase was demonstrated, with its activity primarily limited to the cytosolic fraction of the parasite tissue homogenate, there was no significant change in the activity of the enzyme in either fraction. PEPCK, another important gluconeogenic enzyme, was detected mainly in the cytosolic fraction (about 77–91%) in the cestode; however, in the host, the enzyme is localized in the mitochondrial fraction (Nelson and Cox, 2000). In view of the differing primary functions of PEPCK in cestodes and their hosts, this enzyme might be selectively inhibited and thus provide an avenue for anthelmintic attack (Reynolds, 1980). Involvement of PEPCK in glycolysis has been shown in the protoscoleces of the horse and sheep strains of *E. granulosus* and the closely related *E. multilocularis* (McManus and Smyth, 1982). Also, in adult *Fasciola hepatica* and *Dipetalonema viteae*, PEPCK takes part in degradation of glucose (Tielens et al., 1987; Christie et al., 1987) rather than in gluconeogenesis. In contrast, in *S. mansoni*, experiments with inhibitors of PEPCK were not conclusive that this enzyme was involved in glycolysis (Tielens et al., 1991). In the present study, PEPCK activity in the parasite tissue homogenate and cytosolic fraction was increased significantly ($p<0.01$) in all treatments. The presence of these gluconeogenic enzymes in *R. echinobothrida* and significant increase of their activities, except for FBPase, under treatment suggest that glucose production by gluconeogenic pathway might be affected by the test phytochemicals.

Thus, from the results obtained, it may be postulated that the phytochemicals of *F. vestita*, particularly genistein, influence both HMP and gluconeogenesis in the cestode, which seems to be a response to high energy demand of the parasite under anthelmintic stress.

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References

Agosin, M., Aravena, L., 1960. Studies on the metabolism of *Echinococcus granulosus*: IV. Enzymes of the pentose phosphate pathway. Exp. Parasitol. 10, 23–38.

Agosin, M., Repetto, Y., 1961. Studies on the metabolism of *Echinococcus granulosus*: VI. Pathways of glucose ^{14}C metabolism in *Echinococcus granulosus* scolices. Biologica 23, 33–38.

Behm, C.A., Bryant, C., 1975. Studies of regulatory metabolism in *Moniezia expansa*: general conditions. Int. J. Parasitol. 5, 209–217.

Christie, D.A., Powell, J.W., Stables, J.N., Watt, R.A., 1987. A nuclear magnetic resonance study of the role of phosphoenolpyruvate carboxykinase (PEPCK) in the glucose metabolism of *Dipetalonema viteae*. Mol. Biochem. Parasitol. 24, 125–130.

Croxton, F.E., Cowden, D.J., Klein, S., 1982. Applied General Statistics. Prentice Hall, New Delhi.

Das, J.R., Saha, N., Ratha, B.K., 1991. Tissue distribution and sub-cellular localization of glutamate dehydrogenase in a freshwater air breathing teleost, *Heteropneustes fossilis*. Biochem. Syst. Ecol. 19, 207–212.

Das, B., Tandon, V., Saha, N., 2004. Anthelmintic efficacy of *Flemingia vestita* (Fabaceae): alteration in the activities of some glycolytic enzymes in the cestode, *Raillietina echinobothrida*. Parasitol. Res. 93, 253–261.

DeMoss, R.D., 1955. Glucose 6-phosphate and 6-phosphogluconic dehydrogenases from *Leuconostoc mesenteroides*. Methods Enzymol. 5, 328.

Fukumoto, S., 1985. Pyruvate kinase isoenzymes and phosphoenolpyruvate carboxykinase during development of *Spirometra erinacei*. Yonago Acta Med. 28, 89–105.

Kar, P.K., Tandon, V., Saha, N., 2002. Anthelmintic efficacy of *Flemingia vestita*: genistein-induced effect on the activity of nitric oxide synthase and nitric oxide in the trematode parasite, *Fasciolopsis buski*. Parasitol. Int. 51, 249–257.

Kar, P.K., Tandon, V., Saha, N., 2004. Anthelmintic efficacy of genistein, the active principle of *Flemingia vestita* (Fabaceae): alterations in the free amino acid pool and ammonia levels in the fluke, *Fasciolopsis buski*. Parasitol. Int. 53, 287–291.

Kaur, M., Sood, M.L., 1992. Histoenzymic effects of thiophenate and fenbendazole on the absorptive surfaces of *Haemonchus contortus*. Acta Vet. Hung. 40, 63–70.

Khatoon, H., Baqui, A., Wajihullah, Ansari, J.A., 1983. Effect of anthelmintics on the enzyme activities of *Setaria cervi* (Nematoda: Filarioidea). Angew. Parasitol. 24, 72–75.

Körting, W., Barrett, J., 1977. Carbohydrate metabolism in the plerocercoids of *Schistocephalus solidus* (Cestoda: Pseudophyllidea). Int. J. Parasitol. 7, 411–417.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with Folin-phenol reagent. J. Biol. Chem. 193, 265–275.

McManus, D.P., Smyth, J.D., 1982. Intermediary carbohydrate metabolism in protoscoleces of *Echinococcus granulosus* (horse and sheep strains) and *E. multilocularis*. Parasitology 84, 351–366.

McManus, D.P., Sterry, P.R., 1982. *Ligula intestinalis*: intermediary carbohydrate metabolism in plerocercoids and adults. Z. Parasitenkde. 67, 73–85.

Mommsen, T.P., Walsh, P.J., Moon, T.W., 1985. Gluconeogenesis in hepatocytes and kidney of atlantic salmon. Mol. Physiol. 8, 89–100.

Moon, T.W., Mommsen, T.P., 1987. Enzymes of intermediary metabolism in tissues of little skate, *Raja erinacea*. J. Exp. Zool. 244, 9–15.

Moon, T.W., Mustafa, T., Hulbert, W.C., Podesta, R.B., Mettrick, D.F., 1977. The phosphoenolpyruvate branchpoint in adult *Hymenolepis diminuta* (Cestoda): a study of pyruvate kinase and phosphoenolpyruvate carboxykinase. J. Exp. Zool. 200, 325–326.

Nelson, D.L., Cox, M.M., 2000. Lehninger's Principles of Biochemistry, 3rd edn. Macmillan, Worth Publications, Hampshire, New York.

Olson, J.E., Anfinsen, C.B., 1952. The crystallization and characterization of L-glutamic acid dehydrogenase. J. Biol. Chem. 197, 67–79.

Omar, M.S., Raoof, A.M., 1994a. Histochemical localization of key glycolytic and related enzymes in adult *Onchocerca fasciata*. J. Helminthol. 68, 337–341.

Omar, M.S., Raoof, A.M., 1994b. Histochemical distribution of hydrolytic enzymes in adult *Onchocerca fasciata* (Filarioidea: Onchocercidae). Parasitol. Res. 80, 216–222.

Omar, M.S., Raoof, A.M., Al-Amari, O.M., 1996. *Onchocerca fasciata*: enzyme histochemistry and tissue distribution of various dehydrogenases in the adult female worm. Parasitol. Res. 82, 32–37.

Pal, P., Tandon, V., 1998a. Anthelmintic efficacy of *Flemingia vestita* (Fabaceae): genistein-induced alterations in the ultrastructure of the tegument in the cestode, *Raillietina echinobothrida*. J. Parasit. Dis. 22, 104–109.

Pal, P., Tandon, V., 1998b. Anthelmintic efficacy of *Flemingia vestita* (Fabaceae): genistein-induced alterations in the esterase activity in the cestode, *Raillietina echinobothrida*. J. Biosci. 23, 25–31.

Pal, P., Tandon, V., 1998c. Anthelmintic efficacy of *Flemingia vestita* (Fabaceae): genistein-induced alterations in the activity of tegumental enzymes in the cestode, *Raillietina echinobothrida*. Parasitol. Int. 47, 233–243.

Pampori, N.A., Singh, G., Srivastava, V.M., 1985. Enzymes of isolated brush border membrane of *Cotugnia digonopora*, and their insensitivity to anthelmintics in vitro. Vet. Parasitol. 18, 13–19.

Rao, H.S.P., Reddy, K.S., 1991. Isoflavones from *Flemingia vestita*. Fitoterapia 62, 458.

Reynolds, C.H., 1980. Phosphoenolpyruvate carboxykinase from the rat and from the tapeworm, *Hymenolepis diminuta*. Comp. Biochem. Physiol. 65B, 481–487.

Roberts, L.S., 1983. Carbohydrate metabolism. In: Arme, C., Pappas, P.W. (Eds.), Biology of the Eucestoda. Academic Press, New York, pp. 343–390.

Roy, B., Tandon, V., 1996. Effect of root-tuber extract of *Flemingia vestita* a leguminous plant, on *Artyfechinostomum sufrartyfex* and *Fasciolopsis buski*: an electron microscopy study. Parasitol. Res. 82, 248–252.

Shih, H.H., Chen, S.N., 1982. Glycolytic enzymes in juvenile and adult *Angiostrongylus cantonensis*. Southeast Asian J. Trop. Med. Public Health 13, 114–119.

Smyth, J.D., McManus, D.P., 1989. The Physiology and Biochemistry of Cestodes. Cambridge University Press, Cambridge.

Tandon, V., Pal, P., Roy, B., Rao, H.S.P., Reddy, K.S., 1997. In vitro anthelmintic activity of root tuber extract of *Flemingia vestita*, an indigenous plant in Shillong, India. Parasitol. Res. 83, 492–498.

Tandon, V., Pal, P., Saha, N., 1998. Anthelmintic efficacy of *Flemingia vestita* (Fabaceae): genistein-induced alterations in the free amino acid pool of the cestode, *Raillietina echinobothrida*. J. Parasit. Dis. 22, 110–115.

Tandon, V., Das, B., Saha, N., 2003. Anthelmintic efficacy of *Flemingia vestita* (Fabaceae): effect of genistein on glycogen metabolism in the cestode, *Raillietina echinobothrida*. Parasitol. Int. 52, 179–183.

Tielens, A.G., Van Den Heuvel, J.M., Van Den Bergh, S.G., 1987. Differences in intermediary energy metabolism between juvenile and adult *Fasciola hepatica*. Mol. Biochem. Parasitol. 24, 273–281.

Tielens, A.G., Van Der Meer, P., Van Den Heuvel, J.M., Van Den Bergh, S.G., 1991. The enigmatic presence of all gluconeogenic enzymes in *Schistosoma mansoni* adults. Parasitology 102, 267–276.

Unnikrishnan, L.S., Raj, R.K., 1995. Transhydrogenase activities and malate dismutation linked to fumarate reductase system in the filarial parasite *Setaria digitata*. Int. J. Parasitol. 25, 779–785.

Vorhaben, J.E., Campbell, J.W., 1972. Glutamine synthetase: a mitochondrial enzyme in uricotelic species. J. Biol. Chem. 247, 2763–2767.

Xiao, S.H., You, J.Q., Guo, H.F., Mei, J.Y., Jiao, P.Y., Yao, M.Y., Zhuang, Z.N., Feng, Z., 1999. Effect of artemether on phosphorylase, lactate dehydrogenase, adenosine triphosphatase, and glucosephosphate dehydrogenase of *Schistosoma japonicum* harbored in mice. Zhongguo Yaoli Xuebao 20, 750–754.