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Research note

Anthelmintic efficacy of *Flemingia vestita* (Fabaceae): effect of genistein on glycogen metabolism in the cestode, *Raillietina echinobothrida*

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Abstract

The edible root-tuber peel of *Flemingia vestita* and its major active component, genistein, have been earlier shown to have a vermifugal/vermicidal effect on cestodes in vitro by causing a flaccid paralysis and alterations in the tegumental architecture and activity of several enzymes associated with the tegumental interface of the parasite. Pursuing further investigation on the mode of action of this putative anthelmintic, the crude peel extract and pure genistein were further tested in respect of glycogen metabolism in the fowl tapeworm, *Raillietina echinobothrida*. On exposure to the plant root peel crude extract (5 mg/ml) and genistein (0.2 mg/ml), the glycogen concentration was found to decrease by 15–44%, accompanied by an increase of activity of the active form of glycogen phosphorylase (GPase *a*) by 29–39% and decrease of activity of the active form of glycogen synthase (GSase *a*) by 36–59% in treated parasites as compared to untreated controls, but without affecting the total activity ($a+b$) of both the enzymes. Praziquantel (1 μg/ml), the reference drug, also caused quantitative reduction in glycogen level and alterations in enzyme activities somewhat at par with the genistein treatment. These results suggest that this plant-derived component may influence the glycogen metabolism of the parasite by directing it towards utilization of glycogen.

Keywords: *Raillietina echinobothrida*; Glycogen phosphorylase; Glycogen synthase; *Flemingia vestita*; Anthelmintic; Genistein; Praziquantel; Phytochemical

The edible root-tuber peel of *Flemingia vestita* (Fabaceae), an indigenous leguminous plant of Meghalaya, is conventionally used in local traditional medicine as an anthelmintic against intestinal worms. The active principles of the root-tuber peel extract were isolated by Rao and Reddy [1].

The crude extract of the root-tuber peel of *F. vestita* and its major active isoflavone component, genistein, have been shown to have a vermifugal/vermicidal effect on *Raillietina echinobothrida*, cestode of domestic fowl, in vitro by causing a flaccid paralysis accompanied by alterations in the tegumental architecture [2,3]. Alterations were also observed in the activity of several enzymes associated with the tegumental interface and nervous coordination of this cestode [4,5].

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With a view to understanding the mode of action of these plant-derived components, it seems desirable to further investigate their effect on the energy-yielding pathway in the parasite. With limited ability to metabolize lipids and amino acids, cestodes and trematodes mainly ferment glucose and other simple carbohydrate molecules to meet their energy requirements [6]. The carbohydrate occurs mainly as glycogen in both larval and adult cestodes, which serves typically as the most important energy reserve. Several chemotherapeutic agents have been shown to influence glycogen metabolism in helminths [7].

The two key regulatory enzymes in glycogen metabolism are glycogen phosphorylase (1,4-α-D-Glucan: orthophosphate α-D-glucosyltransferase, EC 2.4.1.1, GPase) and glycogen synthase (UDP glucose: glycogen 4-α-glucosyltransferase, EC 2.4.1.11, GSase). The degradation and synthesis of glycogen are regulated depending upon the energy need under various physiological conditions mainly by regulating the activity of these two enzymes that are normally present in two forms—active (*a*) and inactive (*b*), which are interconverted by phosphorylation by protein kinase, and dephosphorylation by protein phosphatase [8]. The presence of GPase activity and glycogenesis in cestodes have been reported in vitro [9]. The glycogen content and the activity of these two enzymes form the parameters for the present study.

Live specimens of *R. echinobothrida* were collected from the intestine of freshly slaughtered domestic fowl at local abattoirs in 0.9% phosphate buffered saline (PBS, pH 7.2). The alcoholic crude root-tuber peel extract of *F. vestita* was obtained as reported previously [2]. Genistein was obtained from Sigma Chemicals (Code no. 6649). Praziquantel (PZQ, Droncit), a broad-spectrum cestocide, served as the reference drug. All enzymes, co-enzymes and substrates were either obtained from Sigma Chemicals (St. Louis, USA) or Sisco Research Lab (Mumbai, India). All other reagents used were of high quality and obtained from indigenous sources.

The worms ($\approx$0.2 g fresh wt.) were incubated in 5 ml of the medium at 38 ± 1 °C with 5 mg/ml crude peel extract, 0.2 mg/ml genistein and 0.001 mg/ml PZQ dissolved in dimethylsulfoxide (DMSO, final concentration 1% in PBS), with simultaneous maintenance of controls for each treatment kept in PBS containing 1% DMSO. The concentrations of the test materials were determined on the basis of a previous study, wherein they were shown to cause paralysis in 5.9 ± 0.05, 6.7 ± 0.04 and 2.9 ± 0.05 h, respectively, while the controls survived in vitro for 72 ± 0.05 h [2]. For each set of treatment cestodes were taken from a single host.

Alkali-soluble glycogen was estimated using anthrone reagent following the method of Seifter et al. [10]. For enzyme extract and assay 10% homogenate (w/v) of the treated worms and controls was prepared as per the procedure of Russel and Storey [11]. The homogenate was centrifuged at $10\,000\times g$ at 0 ± 2 °C for 10 min and the supernatant was used for enzyme activity determinations. GPase was assayed spectrophotometrically (Beckman DU 640) following the method of Moon et al. [12]; the reaction mixture also contained 10 mM caffeine for measurement of GPase *a* alone. GSase was also assayed spectrophotometrically following the method of Passoneau and Rottenberg [13]; the reaction mixture did not contain 5 mM glucose 6-phosphate while measuring the GSase *a* activity alone. Percent GPase *a* or GSase *a* represents the ratio of the active form to the total $(a+b)$ enzyme$\times100$.

Following treatment with root peel extract and genistein, paralysis ensued after approximately 6 h of incubation, whereas in PZQ-containing medium paralysis set in after 3 h of exposure. Table 1 shows the glycogen content in the control and treated parasites. The physiological level of glycogen in the parasite was found to be quite high and averaged to approximately 66 mg/g wet wt., which decreased significantly 12 h onwards post incubation in controlled condition. The glycogen level in the parasite decreased significantly compared to the respective controls at different hours of treatment. The contents of glycogen in cestodes can range up to 50% or more of dry wt. [7]. The amount of glycogen stored at a given time, however, is affected by a number of environmental factors and varies with the feeding cycle and physiological status of the host, the number of parasites present (crowding effect) and the stage

Table 1
Effects of root-tuber peel extract of *F. vestita*, genistein and PZQ on glycogen content (mg/g wet wt.) in *R. echinobothrida* in vitro

Treatment (mg/ml)	Concentration of glycogen* post incubation				
	0 h**	3 h	6 h	12 h	24 h
1a Control (in 0.9% PBS)	66.53±4.17	–	56.97±4.41	36.04±2.64[1]	20.9±2.71[2]
1b Crude peel extract (5.0)	–	–	47.93±2.32[a] (−16)	20.24±2.73[b] (−44) [−46]	14.54±2.05[a] (−30) [−69]
2a Control	65.74±4.44	–	57.28±4.12	40.14±1.62[1]	25.89±3.36[2]
2b Genistein (0.2)	–	–	48.83±1.94[a] (−15)	26.19±2.18[b] (−35) [−39]	18.74±1.36[a] (−28) [−61]
3a Control	67.56±5.00	63.78±3.59	45.42±2.90[1]	–	–
3b PZQ (0.001)	–	51.16±3.91[a] (−20)	32.53±0.44[a] (−28)	–	–

Values are expressed as mean±S.E.M. ($n=3$). [1,2]P value significant at <0.05 and <0.01, respectively, in starved controls compared with the 0 h control. [a,b]P value significant at <0.05 and <0.01, respectively, in treatments compared with their respective controls.

* Percentage decrease (−) in glycogen content in treated worms from respective controls is given in parentheses and that to 0 h, in square brackets.

** Glycogen content in freshly recovered parasite.

of development of the worms [14]. In the present study, a significant decrease in the glycogen level in the control parasite to approximately 40–70% within 24 h of starvation suggests that, as true for platyhelminths, carbohydrate is the prime source of energy in this cestode, too [6]. Also a significant decrease (by 15–44%) was recorded in treated parasites in paralytic state. A number of anthelmintic agents have been shown to affect the glycogen concentration and metabolism in helminths [6,15], though several others do not cause any such change. While no drug-induced effect in respect to glycogen content was demonstrable in *Schistosoma mansoni* from mice dosed with Ro 15-5458 [16], schistosomes recovered from artemether-treated hosts showed an increased activity of glycogen catabolism and decreased glucose uptake [17].

As shown in Table 2, treatment of the parasite with crude peel extract, genistein and PZQ caused significant change only in the activity of the active forms of GPase and GSase, with no significant effect on the total activity of either enzyme. GPase *a*, which constituted approximately 72–74% of the total GPase activity in the control parasite, increased by 29, 39 and 30% in the parasite treated with crude peel extract and genistein for 6 h and PZQ for 3 h, respectively. However, GSase *a* activity, which constituted approximately 56–68% of the total GSase activity, decreased by 59, 36 and 38%, respectively, in similar treatments. The decrease in glycogen content accompanied by a significant decrease in GSase *a* activity and an increase in GPase *a* activity in the parasite under different treatment conditions suggest that the energy demand by the cestode was perhaps enhanced under test conditions leading to stimulation of glycogenolysis and inhibition of glycogenesis. Chemotherapeutics like levamisole showed a decrease in GPase activity and an increase in activity of GSase in nematodes [7,18,19]. However, GPase activity was found to be 25–30% higher in *H. diminuta* from amoscanate-treated hosts [20]. An increase in the activity of GPase *a* in *R. echinobothrida* during the treatments suggests that the phytochemicals, as also PZQ, influence activation of this enzyme.

It is interesting to note that the treatment in vitro of *R. echinobothrida* with the crude peel extract of *F. vestita*, and genistein affected only the active forms of GPase and GSase, without causing any change of their total activities. It appears that the changes of the phosphorylation status could be one of the possible mechanisms of regulation of these two enzymes, thereby converting the inactive to active form and vice versa

Table 2
Effects of root-tuber peel extract of *F. vestita*, genistein and PZQ on the activity (units/g wet wt) of GPase and GSase of *R. echinobothrida* in vitro

Treatment (mg/ml)	Enzyme activity[a] of			
	GPase		GSase	
	Total ($a+b$)	Active (a)	Total ($a+b$)	Active (a)
1a Control (in 0.9% PBS)	1.92±0.12	1.43±0.02 (74)	0.73±0.04	0.41±0.06 (56)
1b Crude peel extract (5.0)	2.09±0.25 [±9]	1.84±0.25 (88) [±29][b]	0.65±0.03 [−10]	0.17±0.04 (27) [−59][c]
2a Control	2.28±0.43	1.66±0.31 (73)	1.68±0.17	1.15±0.08 (68)
2b Genistein (0.2)	2.49±0.27 [±9]	2.30±0.17 (92) [±39][b]	1.54±0.13 [−8]	0.74±0.14 (48) [−36][b]
3a Control	2.42±0.53	1.74±0.49 (72)	1.29±0.02	0.86±0.08 (67)
3b PZQ (0.001)	2.45±0.28 [±1]	2.26±0.33 (92) [±30][b]	1.16±0.09 [−10]	0.53±0.07 (46) [−38][b]

Values are expressed as mean±S.E.M. ($n=3$). One unit of enzyme activity is defined as 1 μmol of $NADP^+$ reduced in case of GPase or NADH oxidized in case of GSase per minute at 38 °C.

[a] Percentage of GPase a and GSase a activities out of the total ($a+b$) is given in parentheses. Percentage increase (+) or decrease (−) of GPase a and GSase a activities in the treated worms compared to their respective controls is given in square brackets.

[b] $P<0.05$.

[c] $P<0.01$.

under the various treatments. Chemotherapeutics like PZQ are known to cause vacuolization and disruption of the surface tegument and also muscular paralysis in several parasite species [17,21]. Treatment of *R. echinobothrida* in vitro with *F. vestita* crude peel extract and its active principle also causes alterations in tegumental organization of the parasite [3]. Considering that altered influx of Ca^{2+} may account for these changes in the treated cestode, alteration in the activity of the enzymes of glycogen metabolism may also be related to Ca^{2+} influx, which needs to be further investigated.

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