

**ANTHELMINTIC EFFICACY OF *Flemingia vestita*: AN IN
VITRO STUDY ON CARBOHYDRATE METABOLISM IN
THE CESTODE, *Raillietina echinobothrida***

ABSTRACT

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ABSTRACT

The present work incorporates a study on the anthelmintic efficacy of *Flemingia vestita* Benth and Hooker (Family Fabaceae), a leguminous plant, the tuberous roots of which have a usage as vermifuge or vermicide in the indigenous traditional system of medicine in Meghalaya (Northeast India). In earlier studies, the root tuber peel extract of this plant and its major active component, genistein, were shown to cause flaccid paralysis and to be acting transtegumentally in trematode and cestode parasite. With a view to investing further the mode of action of these putatively anthelmintic phytochemicals, the crude-peel extract of *F. vestita*, genistein and the reference drug PZQ were tested in vitro against the cestode, *Raillietina echinobothrida*, in respect to the carbohydrate metabolism, the major energy yielding pathway in helminth parasites.

Alterations in:

- * glycogen level;
- * activities of glycogen phosphorylase (GPase) and glycogen synthase (GSase), both active and total;
- * physiological levels of some metabolites - glucose, lactate, pyruvate, malate and alanine;
- * activities of some regulatory glycolytic enzymes - hexokinase (HK), phosphofructokinase (PFK), pyruvate kinase (PK), phosphoenolpyruvate

carboxykinase (PEPCK), lactate dehydrogenase (LDH), malate dehydrogenase (MDH) and malic enzyme (ME);

- * activities of glucose 6-phosphate dehydrogenase (G6PDH), pyruvate carboxylase (PC) and fructose 1,6-bisphosphatase (FBPase) and
- * physiological levels of some trace elements and changes in Ca^{2+} concentration, in particular

in treated parasites in comparison to controls form the parameters of this study. Standard techniques were used for biochemical assays and histochemical demonstrations of the mentioned parameters.

1. *R. echinobothrida* were collected in 0.9% PBS (pH 7.2) from freshly slaughtered domestic fowl and incubated at 39 ± 1 °C with defined concentration of crude-peel extract (5 mg/ml), genistein (0.2 mg/ml) and PZQ (1 µg/ml) with simultaneous maintenance of controls till the onset of paralysis. Control parasites survived upto 72 h, whereas in the treated parasites paralysis set in about 5.9 h, 6.7 h and 2.9 h in case of crude root peel extract, genistein and PZQ, respectively. The treated parasites and controls were taken for various experiments.

2. After exposure to the plant crude-peel extract of *F. vestita* and genistein, the glycogen concentration was found to decrease by 15-44% in the parasites compared to their respective controls. The decrease in the physiological level of

glycogen was accompanied by an increase in the activity of GPase *a* by 29-39% and a decrease of activity of GSase *a* by 36-59% in treated parasites as compared to untreated controls, though without affecting the total activity of both the enzymes. PZQ also caused quantitative reduction in glycogen level and alterations in enzyme activities somewhat at par with the genistein treatment.

3. The glucose content in the treated worms decreased by 14-36%, whereas malate concentration increased by 49-134% as compared to controls. Both in controls and treated parasites, however, pyruvate content was not measurable, while alanine and lactate contents showed a decline by 7-31% in the parasites exposed to all test materials. Besides, the lactate content as effluxed into the incubation medium showed an increase of 9-44% in the case of treated parasites indicating an overall increase of lactate production.
4. While some enzymes of the glycolytic pathway showed an increase in their activities following exposure of the parasites to the various treatments, others showed a decline. 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 all treatments in comparison to the respective controls.
5. The cytosolic MDH got activated by 33-58% whereas mitochondrial MDH increased by 43-73% in the treated parasites. The MDH activity of in the tissue

homogenate was also found to be higher by 22-43% in treatments with crude-peel extract, genistein and PZQ. The ME activity in the tissue homogenate was found to be increased by 28-59% in the treated parasites; while the cytosolic ME activity showed an increase by 33-39%, there was no enhancement in mitochondrial ME activity.

6. The key regulatory enzyme of pentose phosphate pathway, G6PDH, was found to decrease by 23-31% in various treatments. FBPase, the enzyme of gluconeogenesis, did not show any changes during the treatment conditions while the activity of PC was significantly increased by 32-44% in treated parasites in comparison to the controls.
7. Using atomic absorption spectrophotometry, some trace elements, namely, lead (~ 25 µg/g dry tissue wt), iron (~ 1200 µg/g dry tissue wt), zinc (~ 400 µg/g dry tissue wt), magnesium (~1400 µg/g dry tissue wt), calcium (~ 400 µg/g dry tissue wt), chromium (~12 µg/g dry tissue wt) were detected in the cestode while manganese, cadmium and nickel were below level of detection.
8. The calcium concentration in the parasite tissue was found to decrease by 39-49% following with the test materials though the calcium concentration in the incubation media containing the test materials was found to increase by 94-118%, indicating thereby an efflux of Ca^{2+} from the parasite under the treatment itself.

9. The observations made in the present study are supported by six photomicrographs, eighteen tables and sixteen graphical column figures. Two hundred forty seven references are cited.
10. From the results obtained it can be hypothesized that due to the high energy demand of the parasites because of anthelmintic stress following exposure to the plant crude extract and genistein, the process of glycogenolysis and glycolysis got activated on the expense of other metabolic pathways of glucose utilization by regulating some key enzymes of these pathways. Changes in the homeostasis of Ca^{2+} could be one such regulating mechanism in the cestode parasites. The PEPCK/PK branch point provides an important clue for the switch over to the phosphoenolpyruvate-succinate pathway for the glucose metabolism and thus an avenue for anthelmintic attack. The phytochemicals of *F. vestita*, therefore, seem to influence the carbohydrate metabolism of the cestode, which may be a secondary target of action, the primary target being the tegumental interface of the parasite.

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