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Anthelmintic efficacy of *Flemingia vestita* (Fabaceae): Genistein-induced Alterations in the Free Amino Acid Pool of the Cestode, *Raillienttina echinobothridia*

V. TANDON*, P. PAL AND N. SAHA
Department of Zoology, North Eastern Hill University, Shillong 793 002, Meghalaya, India

The crude root-tuber peel extract of *Fleminigia vestita* and its active principle, genistein, have shown paralyzing effect on trematode and cestode parasites. Ultrastructurally, alterations in the tegumental architecture were evident in the *in vitro* treated cestode, *Raillietina echinobothrida*. With a view to investigating the mode of action of the plant - derived component, its effect on the changes of free amino acid pool of the parasite was studied, both qualitatively and quantitatively, using high-performance liquid chromatography (HPLC). Phosphoserine (Ph. ser), taurine (Tau), phosphoethanolamine (Ph.NH_2), threonine (Thr), serine (Ser), glutamate (Glu), proline (Pro), glycine (Gly), alanine (Ala), citrullin (Cit), valine (Val), methioine (Met), isoleucine (Ile), leucine (Leu), tyrosine (Tyr), phenylalanine (Phe), ß-alanine (ß-Ala), a-aminoisobutyric acid (a-AiBA), t-aminobutyric acid (GABA), tryptophan (Trp), histidine (His), ornithine (Orn), arginine (Arg), and ammonia were detectable in the tissue homogenate of the parasite. After exposure to the crude extract (50 mg/ml) and genistein (0.5 mg/ml), alterations were noticeable in the free amino pool. Following genistein treatment, quantitatively the contents of Ph.ser, Tau, ß-Ala, a-AiBA, Trp, His and Val were significantly lower and Glu, Met Ile, GABA and ammonia were slightly higher than those in the control; Ph.NH_2, Cit and Orn were not detectable in the treated parasite, The amino acids excreted by the control worm and as detected in the effluent were Ph.ser, Glu, Val, Met, Ile, Leu, Tyr, Phe, ß-Ala, a-AiBA, GABA, His, Orn, Arg and ammonia; in the effluent of the genistein- treated parasite Gly, Ala, Met and Orn were below the level of detection. The reference drug, praziquantel (0.01 mg/ml) also caused a quantitative reduction in the free amino acid contents of the parasite, somewhat at par with the genistein treatment. The results suggest that the free amino acid pool of the cestode may be a possible target of action of genistein.

Key words: Anthelmintic, *Flemingia vestita*, genistein, free amino acids, cestode, *Raillietina echinobothrida*.

Flemingia vestita Benth and Hooker (Family: Fabaceae) is an indigenous medicinal plant of Meghalaya, North-East India, the tuberous roots of which are considered to have anthelmintic properties and hence eaten unpealed by the natives as a traditional cure against worm infections. The crude extract of the root-tuber peel of *F. vestita* has been shown to induce paralysis and pronounced tegumental damage and disruption in Fasciolopsis buski and *Artyfechinostomum sufrartyfex* after *in vitro* treatment (Roy and Tandon, 1996). Besides the crude peel extract, the major active component of the root peel that has been identified as an isoflavone - genistein, induces similar effects in the cestode, *Raillietina echinobothrida*; using scanning and transmission electron microscopy genistein - induced tegumental damage in this cestode was demonstrated (Tandon *et al.*, 1997). After exposure to the crude peel extract and genistein, alterations were also observed in the acetylcholinesterase activity in the tegument and nervous components and tegumental enzymes, namely, acid phosphatase, alkaline phosphotase, adenosine triphosphatase and 5'-nucleotidase (Pal and Tandon 1998a, b). Further investigation is required to know the mode of action of this putative anthelmintic of plant origin.

As major constituent of biological materials and with a range of properties and functions, amino acids have recently emerged as potential targets for anthelmintic development. Differences between the pathways of amino acid metabolism in helminths and their mammalian hosts are being exploited in newer drug designs. Newer information is now accumulating with regard to amino acid metabolism in helminths (Barrett, 1991), more so for cestodes. There are reports that in *Hymenolepis diminuta* the major free mino acid (FAA) is alanine (Daugherty, 1952; Foster and Daugherty, 1959; Campbell,1963; Chappel and Read, 1973; Wack *et al.*, 1983; Webb, 1986). There are published reports on the FAA pool of several cestode species (Dobrowski, 1980; Pathak et al., 1980; Soutter *et al.*, 1980; Gaur and Agarwal, 1981; Bhalya *et al.*, 1983, 1984, 1985; Niyogi and Agarwal, 1983; Nanda *et al.*, 1987) and evidences suggest that FAAs may be involved in osmotic regulation (Lussier *et al.*, 1978; Wack *et al.*, 1983). Many amino acids and their metabolic products are known to serve as precursors for various metabolic reactions involving the synthesis of chemical

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*Corresponding author

energy and many important biomolecules. Of the hitherto studied helminths, most have been found to excrete significant amounts of nitrogenous wastes in the form of amino acids in addition to ammonia. IN cestodes, too, FAAs constitute a large proportion of their nitrogenous wastes (Barrett, 1991). Ammonia, which results as the first end product of protein and amino acid catabolic, is known to cause various neurological disorders in addition to other toxic affects (Lussier *et al.*, 1978; Nanda *et al.*, 1987). Excretion of amino acids as such, instead of converting to ammonia, could be one of the physiological adaptations in helminths including cestodes to avoid any neurological disorder under normal physiological conditions (Cooper and Plum, 1987; Campbell, 1991).

In view of the functional significance of FAAs in protein metabolism of helminths, we report herein the changes in the levels of different FAAs and tissue ammonia in *R. echinobothrida* following exposure in vitro to the crude root-tuber peel extract of *F. vestita* and its active component-genistein, both qualitatively and quantitatively.

MATERIALS AND METHODS

Drugs: The root-tuber peel extract and active component (genistein) were obtained from *F. vestita*, following the method of Rao and Reddy (1991) and as per the detailed procedure previously described by Tandon et al. (1997).

Experimental parasites and treatment: The adult cestodes, *R. echinobothrida* (Megnin, 1888) were collected from the intestine of domestic fowl in 0.9% phosphate buffered saline (PBS, pH 7.3) from freshly slaughtered hosts at local abattoirs in Shillong. They were incubated in 5 ml of the medium at 37 ± 1°C for treatment with 50 mg/ml crude extract, 0.5 mg/ml genistein and 0.01 mg/ml praziquantel, all made in PBS with 1% dimethylsulphoxide (DMSO) (three replicates for each incubation medium). After exposure to the treatment the paralyzed worms were processed for analysis of FAAs and ammonia by high-performance liquid chromatography (HPLC). The FAAs and ammonia excreted out were also analyzed in lkthe incubation medium.

Tissue processing for amino acid analysis by HPLC: A 10% (w/v) tissue homogenate was prepared in distilled water water with a Potter-Elvenjem motor driven glass homogenizer with a teflon pestle at 2 ± 1°C. Protein was precipitated out by adding 5% perchloric acid (prepared in 0.4 N lithium citrate) in the ratio of 1:1, followed by centrifugation at 10,000 x g for 20 min at 2 ± 1°C to pellet out the precipitated protein. The clear supernatant was then filtered through Whatman microfilter (0.45 um pore size) and the pH of the filtrate was adjusted to 2.2 by adding known volume of 0.2 N lithium hydroxide. All the processed samples were preserved in deep freeze at-20°C until used for amino acid analysis. All the analyses were completed within 2-3 days after processing the sample.

Processing of incubation medium for amino acid analysis by HPLC: For analysis of amino acids excreted by the parasite in the incubation medium, the medium was collected after 1 h of incubation and assumed to have contained the excreted material. The medium was briefly centrifuged at 10,000 x g and 3 ml of supernatant was concentrated to a volume of 0.5 ml by overnight lyophilization in a lyophilizer. The pH of the lyophilized sample was also adjusted to 2.2 with a known volume of 0.2 N lithium hydroxide and passed through Whatman microfilter (0.45 um pore size) before being injected to HPLC for amino acid analysis.

FAA analysis by HPLC: FAAs and also ammonia, both in the tissue as well as in the incubation medium, were analyzed with a Schimadzu HPLC (Model LC 4A) with a post column fluorimetric detection system by using O-phthaldehyde reagent as a fluorescent dye following the method of Fugiwara et al. (1987) with certain modifications. A cation-exchange column (Shim - Pack ISC - 07 Li) was used for separation of FAAs. The mobile phases were (A) lithium citrate (0.16 N, pH 2.5) containing 7% methyl cellulose, (B) lithium citrate (0.32 N, pH 10.0) containing 12.4% boric acid, and (C) lithium hydroxide (0.2N). A gradient of buffer A to B for 190 min was used for analysis of FAAs, other amino compounds and ammonia. A standard physiological amino acid mixture (Sigma) was also run under identical conditions for both qualitative and quantitative analysis of FAAs.

Ammonia estimation: Ammonia was also estimated both in the worm and incubation medium by enzymatic method following Kun and Kearney (1974). For estimation in the worm, a 20% homogenate was prepared in Tris-HCI buffer (100 mM, pH 8.0). The homogenate was treated with 5% PCA in 1:0.5 ratio to precipitate out the protein. followed by the centrifugation at 10,000 x g for 10 min. The supernatant was neutralized with known volume of 2 N NaOH before estimation of ammonia.

One ml of incubation medium, collected after 1 h of incubation, was treated with 10 ul of 2 M PCA, and centrifuged to precipitate out the protein (if any) and other debris. The supernatant was neutralized with 10 ul of 2 N NaOH before estimation of ammonia.

The reaction mixture for estimation of ammonia contained 66 mM Tris-HCI buffer (pH 8.0), 2.5 mM a-ketoglutarate. 0.2 mM EDTA, 1mM ADP, 0.4 mM NADH, 10 units of glutamate dehydrogenase (GDH) and the sample in a final volume of 1 ml. The reaction mixture was incubated at 37°C for 30 min (sufficient to convert all the ammonia) and the decrease in optical density, which was equivalent to the concentration of ammonia in the reaction mixture was recorded at 340 nm in a uv-visible spectrophotometer (Beckman, DU 640).

Chemicals: Genistein, physiological free amino acid mixture and GDH were obtained from Sigma, St. Louis, U.S.A., and praziquantel was from Bayer, Germany. Other chemicals used were of analytical grades and obtained from indigenous sources. For all preparations deionized double glass-distilled

Table-I

Levels of different free amino acids and ammonia (nmol/g wet wt*) in *R. echinobothrida*

Amino acid	Control (0.9% PBS in 1% DMSO)	Crude extract (50mg/ml)	Genistein (0.5mg/ml)	Praziquantel (0.01 mg/ml)
1. Ph.ser	254 ± 45	130 ± 14(-49)	165 ± 12(-35)	185 ± 14(-27)
2. Tau	2334 ± 220	1088 ± 68(-53)	1733 ± 125(-26)	1442 ± 115(-38)
3. Ph.NH$_2$	251 ± 25	66 ± 11(-76)	BLD	BLD
4. Thr	206 ± 31	138 ± 12(-33)	195 ± 15(-5)	116 ± 7(-44)
5.Ser	922 ± 108	385 ± 28(-58)	859 ± 58(-7)	344 ± 28(-68)
6.Glu	667 ± 44	739 ± 47(+10)	856 ± 46(+29)	953 ± 59(+43)
7. Pro	1756 ± 142	1930 ± 156(+10)	1988 ± 155(+34)	2641 ± 190(-19)
8. Gly	3802 ± 311	1001 ± 101(-74)	2517 ± 202(-34)	2641± 190(-19)
9. Ala	6001 ± 421	3560 ± 285(-41)	5342 ± 335(-11)	1168 ± 105(-81)
10. Cit	11 ± 1	BLD	BLD	5 ± 1(-54)
11. Val	4584 ± 337	455 ± 33(-90)	467±44(-90)	1424 ± 122(-68)
12. Met	116 ± 12	223 ± 25(+92)	143±11(+23)	136 ± 12(+17)
13. Ile	348 ± 24	554 ± 36(+59)	358 ± 22(+3)	1535± 125(+54)
14.Leu	756 ± 41	364 ± 38(-52)	141 ± 9(-81)	153 ± 14(-80)
15. Tyr	1486 ± 88	734 ± 55(-51)	671 ± 51(-54)	1047 ± 85(-30)
16. Phe	555 ± 29	284 ± 18(-49)	449 ± 36(-19)	173 ± 15(-69)
17. ß-Ala	619 ± 32	257 ± 21(-58)	548 ± 57(-11)	266 ± 20(-57)
18. a-AiBA	769 ± 37	484 ± 33(-37)	556 ± 61(-28)	901 ± 66(-17)
19. GABA	167 ± 18	110 ± 12(-34)	105 ± 12(-37)	60 ± 8(-64)
20. Trp	138 ± 8	202 ± 14(+46)	135 ± 18(-2)	426 ± 32(-208)
21. His	289 ± 25	106 ± 8(-63)	187 ± 15(-35)	554 ± 48(-92)
22. Orn	1070 ± 94	1217 ± 121(+14)	BLD	1085 ± 24(-11)
23. Arg	435 ± 38	183 ± 10(-58)	154 ± 10(-64)	161 ± 12(-63)
Total FAA	27536	14210(-48)	17569 (-37)	17000(-38)
24. Ammonia	467 ± 65	2208 ± 188(+373)	1064 ± 85(+128)	709 ± 58(+52)

*Values are expressed as mean ± SEM (n=3) ;

BLD-Below level of detection % increase (+) or decrease (-) ofFAA levels is given in parentheses.

water was used.

RESULTS

Levels of FAAs and ammonia in the parasite maintained in controlled medium and their excretion pattern: As given in Table 1, the levels of different FAAs in the control cestode included phosphoserine (Ph. ser), taurine (Tau), phosphoethanolamine (Ph. NH$_2$), threonine (Thr), serine (Ser), glutamic acid (Glu), proline (Pro), glycine (Gly), alanine (Ala), citrulline (Cit), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), tyrosine (Tyr), phenylalanine (Phe), ß-alanine (ß-Ala), a-aminoisobutyric acid (a-AiBA), t-aminohutyric acid (GABA), tryptophan (Trp), hisidine (His), ornithine (Orn) and arginine (Arg). Out of the amino acids detected, Ala was found to be at the maximum level, followed by Val, Tau, Pro, Tyr, Ser, a-AiBA, Leu, Glu, and Phe, respectively in a decreasing order. Other amino acids, as mentioned above, were at comparatively lower levels. A low level of ammonia was also detected in the worm.

Amino acids which were excreted by *R. echinobothrida* in the incubation medium include Ph.ser, Glu, Val, Met, Ile, Leu, Tyr, Phe, ß-Ala, a-AiBA, GABA, His, Orn and Ary (Table 2). Significant level of ammonia was also excreted by the parasite which could be detected both by HPLC and enzymatic methods (Table 2,3).

Effect of various treatments on the FAA and ammonia levels in the parasite and their excretion pattern: As shown in Table 1, the level of different FAAs and ammonia in the parasite was significantly affected by the treatment with crude extract of *F. vestita*, genistein and praziquantel. When the parasite was treated with the crude extract, the levels of most of the amino acids were markedly decreased except for Glu, Pro, Met, Ile, Trp, and Orn, which either did not change much or increased in certain cases (Met, Ile and Trp). Almost similar decrease of FAA levels was also noticed when the parasite was treated with either genistein or praziquantel except for Trp, the level of which markedly decreased in the case of praziquantel. The

Table-II

Amino acids excreted excreted by *R. echinobothrida*

Amino acid	Control	Crude extract	Genistein	Praziquantel
Ph.ser	+	-	-	-
Tau	-	+	-	+
$Ph.NH_2$	-	-	-	-
Thr	-	+	-	+
Ser	-	+	-	+
Glu	+	+	+	+
Pro	-	-	-	-
Gly	-	+	-	+
Ala	-	+	-	+
Cit	-	-	-	-
Val	+	+	+	+
Met	+	+	-	+
Ile	+	+	+	+
Leu	+	+	+	+
Tyr	+	+	+	+
Phe	+	+	+	+
ß-Ala	+	+	+	+
a-AiBA	+	+	+	+
GABA	+	+	+	+
Trp	-	-	-	-
His	+	+	+	-
Orn	+	-	-	-
Arg	+	+	+	+
NH_3	+	+	+	+

-below level of detection

Table-III

Rate of excretion of ammonia (µmol/g wet wt/h)
R. echinobothrida during *in vitro* treatment

Treatment (mg/ml)	Ammonia in incubation medium
Control	77.30 ± 3.21
Crude extract (50.0)	79.20 ± 2.92
Genistein (0.50)	76.70 ± 1.19
Praziquantel (0.01)	97.33 ± 2.3

levels of Glr, Pro, Met and Ile, were not affected much by either of these treatments. Interestingly, however, the ammonia level in the parasite was increased by 373%, 128% and 52% following treatment with the crude extract, genistein and praziquantel, respectively. The ammonia excreted by R. echinobothrida in the incubation medium is depicted in Table 3.

DISCUSSION

The FAA composition of *R. echinobothrida* detected in the present study includes 23 amino acids. Of these, 15 (Thr, Ser, Glu, Pro, Gly, Ala, Val, Met, Ile, Leu, Tyr, Phe, Trp, His and Arg) represent common or standard amino acids. Besides, Tau, Cit, a-AiAB, GABA and Orn are non-protein amino acids which could appear as metabolic intermediates of neurotransmitters, as reported in other cestodes (Eriksson *et al.*, 1995).

Like in other cestodes (Barrett, 1991), Ala, Pro and Gly, the three non-essential amino acids, were also found to be predominantly present in this parasite. In addition, Tau and Val also constituted a major part of its FAA pool. Relatively high levels of Tau reported in this bird cestode might play a significant role for osmotic balance, since birds predominantly synthesize and excrete uric acid instead of urea as major nitrogenous waste. The size of the total FAA pool in this cestode was also high (about 2.7 mmols/100 g fresh wt), as is the case in most cestodes. A high level of FAA pool may be physiologically significant in several ways - (i) it can provide a means to detoxify ammonia, (ii) may play an important role in osmotic balance, and/or (iii) may serve as an alternate source of energy supply. Il'yasov (1978) earlier reported the occurrence of 17 amino acids in *R. echinobothrida*, of which one. i.e., lysine was not detected in the present study. In quantitative analysis, Ala, Val and Orn emerged as the major amino acids. There are reports that in *H. diminuta*, that major amino acid is Ala (Campbell, 1963, Chappel and Read, 1973; Wack *et al.*, 1983; Webb, 1986).

The levels of most of the amino acids detected in the control parasite decreased markedly (about > 30 to 90%), with the total decrease of about 50% when the parasite was treated with the crude extract of *F. vestita*. A reduction in the FAA pool was also noticed in the parasite treated with genistein and praziquantel, with a total decrease of about 40% in both the cases. This decrease in FAA pool in the treated parasite could be due to an increase in the catabolic rate of different amino acids after exposure to treatment, since this decrease in the amino acid levels was accompanied by a marked increase in the level of ammonia which would be formed as a first end product in amino acid catabolism. Higher accumulation of ammonia in the parasite treated with the crude extract and genistein might be one of the causes of paralysis noticed in the present study, as ammonia known to have a neurotoxic effect in animals (Cooper and Plum, 1987). It may be so that the carbohydrate stored in the parasite is not sufficient to supply enough energy needed to survive in these stressful conditions, hence the amino acids are tapped as an alternate source of energy through gluconeogenic pathway. However, to conclude this definitely carbohydrate metabolism in this parasite under similar conditions will have to be studied. In the crude root peel extract of *F. vestita* active compounds other genistein, i.e. formononetin pseudobaptigenin and diadzein (Rao and Reddy, 1991), though present in much less amount than genistein, may also have influenced the amino acid metabolism, hence more decrease in the amino acid pool in the crude extract-treated parasite than the genistein-treated one. Another reason for the decrease in the FAA pool could also be an increase in the rate of excretion of amino acids (Fairbairn *et al.*, 1961).

Of the helminths that have so far been studied, most excrete significant amounts of nitrogen in the form of amino acids, peptides or protein (Barrett, 1991). Amino nitrogen constitutes about 28% of the total nitrogenous end products of *H. diminuta* (Webster and Wilson, 1970). The amino acids excreted by *R. echinobothrida* were Ph.ser, Glu, Val, Met, Ile, Leu, tyr, Phe, ß-Ala, a-AiBA, GABA, His, Orn and Arg and showed a similar trend as that in H. diminuta. The excretion of amino acids can provide a means of detoxifying ammonia. There was no significant difference in the level of excreted ammonia in the control and genistein-treated parasite (Table 3). Some amino acids such as Ala and Pro may represent true metabolic end products; the two amino acids have been found to be the major component in th protonephridial tissue fluid of cestode and trematode parasites (Webster and Wilson, 1970; Lutz and Siddiqi, 1971; Zavras and Roberts, 1984). Though not detected in the incubation medium of the control, Thr and Ser were detectable in the effluent of the parasite treated with the crude extract and praziquantel; the effluent of the treated parasite also did not contain Orn, which was present in the control. In many invertebrates, and possibly in helminths as well, amino acids are involved in the regulation of intracellular osmotic pressure; disruption of the osmotic balance when helminths are removed from their host and incubated *in vitro* could lead to amino acid leakage (Barrett, 1991).

The differences between the pathways of amino acid metabolism in helminths and their mammalian hosts, particularly in the metabolism of the sulfur amino acids and Arg and Pro may be exploited in newer drug design. Proline biosynthesis inhibitors have shown promise as potential fasciolicides (Sheers *et al.*,1982). In the presence of hexachloroethane in the amphistomid fluke, Fischoederius cobboldi, valine amino transferase levels increased and leucine- and isoleucine aminotransferases were inhibited, whereas rafoxamide and albendazole caused high leucine- and isoleucine aminotransferase activity (Vanaja and Rao, 1992). Alterations in the FAA content of the cestode on exposure to genistein indicate an antagonistic potential of this phytochemical towards the cestode parasite.

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REFERENCES

Barrett, J. 1991. Amino acid metabolism in helminths. In Advances in Parasitology 30. eds. Baker, J.S. and Muller, M., London, New York: Academic Press.

Bhalya, A., Seth, A., Capoor, V.N. and Malhotra, S.K. 1984. Chaemotaxonomic studies on davaineid tapeworms based on amino acid analysis. Journal of Helminthology *58*: 325-326.

Bhalya, A., Seth, A., Malhotra, S.K. and Capoor, V.N. 1983. Amino acids of *Amoebotaenia cuneata* (Cestoda: Dilepidoidea Wardle, McLeod & Radinovski, 1974). Journal of Helminthology *57*: 9-10.

Bhalya, A., Seth, A., Malhotra, S.K. and Capoor, V.N. 1985. Amino acids of *Hymenolepis palmarum* (Johri, 1956) and chaemotaxonomic studies on hymenolepidid cestodes. Journal of Helminthology 59: 39-42.

Campbell, J.W. 1963. Amino acids and nucleotides of the cestode, *Hymenolepis diminuta*. Comparative Biochemistry and Physiology *8*: 181-185.

Campbell, J.W. 1991. Environmental nitrogen metabolism. In Environmental and Metabolic Animal Physiology, ed. Processer, C.L.pp 277-324, New York: Wiley-Liss Inc.

Chappell, L.H. and Read, C.P. 1973. Studies on the free pool of amino acids of the cestode, *Hymenolepis diminuta*. Parasitology *67*: 289-305.

Cooper, A.J.L. and Plum, F. 1987. Biochemistry and physiology of brain ammonia. Physiological. Review *67*: 440-519.

Daugherty, J.W. 1952. Studies on the protein metabolism of certain helminth parasites. Journal of Parasitology *38*: 32.

Dobrowski, K.R. 1980. Amino acid composition of *Ligula intestinalis* (L.) (Cestoda) plerocercoids and of the host parasitized by these cestodes. Acta Parasitologica Polonica *27*: 45-48.

Eriksson, K.S., Maule, A.G., Halton, D.W., Panula, P.A.J. and Shaw, C. 1995. GABA in the nervous system of parasitic flatworms. Parasitology *110*: 339-346.

Fairbairn, D., Werthheim, G., Harper, R.P. and Schiller, E.L. 1961. Biochemistry of normal and irradiated strains of *Hymenolepis diminuta*. Experimental Parasitology *11*: 248-263.

Foster, W.B. and Daugherty, J.W. 1959. Estimation and distrbution of *Raillietina cesticillus* in the fowl and comparative studies on amino acid metabolism of *Raillietina cesticillus* and *Hymenolepis diminuta*. Experimental Parasitology 8: 413-426.

Fugiwara, M., Ishida, Y., Nimura, N., Toyama, A. and Kinoshita, T. 1987. Postcolumn fluorometric detection system for liquid chromtographic analysis of amino and imino acids using o-phthaldehyde/N-acety-L-cysteine reagent. Analytical Biochemistry *116*: 72-78.

Gaur, A.S. and Agarwal, S.M. 1981. Studies on amino acids and sugars in larval *Hydatigera taeniformis* (Batsch, 1758). Proceedings of Indian Academy of Parasitology *2*: 4-6.

Il'yasov, I.N. 1978. Amino acid composition of Raillietina tetragona (Molin, 1858) and *Raillietina echinobothrida* (Megnin, 1881). Trudy Nauchno-Issledovatel: Skog Veterinarnogo Instituta Tadzhikskoi SSR *8*: 75-79.

Kun, E. and Kearney, E.B. 1974. Ammonia. In Methods of Enzymatic analysis, ed. Bergmeyer, H.U. New York: Academic Press.

Lussier, P.E., Podesta, R.B. and Mettrick, D.F. 1978. *Hymenolepis diminuta*: Amino acid transport and osmoregulation. Journal of Parasitology *64*: 1140-1141.

Lutz, P.L. and Siddiqi, A.H. 1971. Nonprotein nitrogenous composition of the protonephridial fluid of the trematode *Fasciola gigantica*. Comparative Biochemistry and Physiology 40A: 453-457.

Nanda, S., Bhalya, A., Gairola, D., Malhotra, S.K. and Capoor, V.N. 1987. Comparative analysis of amino acids of three species of *Gangesia* (Cestoda: Proteocephalata). Journal of Helminthology 61: 233-239.

Niyogi, A. and Agarwal, S.M. 1983. Free and protein amino acids in *Lytocestus indicus*, *Introvertus raipurensis* and *Lucknowia indica* parasitizing *Clarias batrachus* (Linn) (Cestoda: Caryophyllidea). Japanese Journal of Parasitology *32*: 341-345.

Pal, P. and Tandon, V. 1998a. Anthelmintic efficacy of *Flemingia vestita* (Fabaceae): Genistein-induced alterations in the esterase activity in the cestode, *Raillietina echinobothrida*.Journal of Biosciences *23*: 25-31.

Pal, P. and Tandon, V. 1998b. Anthelmintic efficacy of *Flemingia vestita* (Leguminoceae): Genistein-induced alterations in the activity of tegumental enzymes in the cestode, *Raillietina echinobothrida*. Parasitology International *47*: 233-243.

Pathak, K.M., Gaur, S.N.S.and Verma, H.C. 1980. Quantitative estimation of amino acids in cysticercus of *Taenia hydatigena*. Veterinary Parasitology *7*: 375-378.

Rao, H.S.P. and Reddy K.S. 1991. Isoflavones from *Flemingia vestita*. Fitoterapia *63*: 458.

Roy, B. and Tandon, V. 1996. Effect of root-tuber extract of *Flemingia vestita*, a leguminous plant, on *Artyfechinostomum sufratyfex* and *Fasciolopsis buski*: A scanning electron microscopy study. Parasitology Research *82*: 248-252.

Sheers, M., Campbell, J., Beames, D.J., Edwards, S.R., Meare, R.J. and Montague, P.E. 1982. Fasciolicidal potential of proline analogues and proline biosynthesis inhibitors. International Journal for Parasitology *12*: 47-52.

Soutter, A.M., Walker, M. and Arme, C. 1980. Amino acids in the plerocercoid of *Ligula intestinalis* (Cestoda: Pseudophyllidea) and its fish host *Rutilus rutilus*. Zeitschrift fur Parasitenkunde 63: 151-158.

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

Vanaja, D. and Rao, B.R. 1992. Effects of flukicides on the transmission of *Fischoederius cobbolde*. Comparative Physiology and Ecology 17: 16-20.

Wack, M., Komumiecki, R. and Roberts, L.S. 1983. Amino acid metabolism in the tapeworm, *Hymenolepis diminuta*. Comparative Biochemistry and Physiology *74B*: 399-402.

Webb, R.A. 1986. The uptake and metabolism of L-glutamate by tissue slices of the cestode *Hymenolepis diminuta*. Comparative Biochemistry and Physiology *85C*: 151-162.

Webster, L.A. and Wilson, R.A. 1970. The chemical composition of protonephridial canal fluid from the cestode *Hymenolepis diminuta*. Comparative Biochemistry and Physiology *35A*: 201-209.

Zavras, E.T. and Roberts, L.S. 1984. Developmental physiology of cestodes: characterization of putative crowding factors in *Hymenolepis diminuta*. Journal of parasitology *70*: 937-944.