

**AN IN-VITRO STUDY ON THE ANTHELMINTIC EFFICACY
OF THE ROOT TUBER PEEL EXTRACT OF *FLEMINGIA VESTITA*
AGAINST THE CESTODE, *RAILLIETINA ECHINOBOTHRIDA***

ABSTRACT



BY

PAPRI PAL

DEPARTMENT OF ZOOLOGY

SUBMITTED

IN

**FULFILMENT OF THE REQUIREMENT OF THE DEGREE OF
DOCTOR OF PHILOSOPHY IN ZOOLOGY**

OF

NORTH-EASTERN HILL UNIVERSITY

SHILLONG - 793022

ABSTRACT

The present work incorporates a study on the anthelmintic efficacy of *Flemingia vestita* Benth and Hooker, an indigenous leguminous plant consumed by the natives in North East India. The in-vitro activity of the root-tuber peel extract of this plant and its active chemical principles was tested against the cestode, *Raillietina echinobothrida*. Changes in physical motility, histomorphological alterations, activity of non-specific and specific esterases that are associated with the nervous system, activity of some tegumental enzymes and changes in the levels of free amino acid pool and tissue ammonia are the parameters for this study.

(I) Live parasites from domestic fowl were collected in 0.9% physiological buffered saline (PBS) and maintained at $37 \pm 1^{\circ}\text{C}$. In-vitro treatment of the parasite with the crude extract (50 mg/) in PBS revealed complete immobilization of the cestode in about 20 min. Exposure of *R. echinobothrida* to genistein (0.5 mg/ml), an active principle isolated from the root-tuber peel, caused spontaneous loss of movement (paralysis) in 4.5 h, which was slower than the time required for praziquantel, the reference cestodicide.

(II) Stereoscan and transmission electron microscopic observations revealed several tegumental alterations and deformity in the treated worms. Alterations in the contour of microtriches and disorganization of the tegumental region were conspicuously evident; the microtriches showed deformity and clumping in the parasite exposed to the crude peel extract. The tegument, inner subtegumental region and muscle layers were the sites predominantly affected by the genistein treatment (0.5 mg/ml); the severe distortion and disorganization occurred in the region of microtriches, and the inner subtegumental region showed pronounced vacuolization in comparison to controls.

(III) With the localization of non-specific esterases and cholinesterases, the organization of cholinergic components of the nervous system in toto could be visualized in the cestode; a pattern in conformity with the general plan of nervous system in cyclophyllidean cestodes was revealed. The cholinesterase in the parasite is acetylcholinesterase. Both non-specific esterases and cholinesterase were found in close association with the central and peripheral nervous components, besides being present in the tegument and

muscular parts of the terminal male genitalia. The whole tissue homogenate of the parasite also showed a high acetylcholinesterase activity. After exposure to the crude peel extract (50 mg/ml) and genistein (0.5 mg/ml), a pronounced decline in the visible stain intensity in the cholinergic components of the nervous system and tegument was noticeable, indicating extremely reduced activity of nonspecific esterases and cholinesterase in these sites. The total acetylcholinesterase activity was also reduced to 49.07% and 56.77%, following treatment with the peel extract and genistein, respectively. The reference drug, praziquantel (0.01 mg/ml) also caused a reduction in the enzyme activity, somewhat at par with the genistein treatment.

Alteration in the acetylcholinesterase activity points towards acetylcholine, an inhibitory neurotransmitter in cestodes, as a potential target of action.

(IV) Acid phosphatase, alkaline phosphatase, adenosine triphosphatase and 5'-nucleotidase are predominantly distributed in the tegument, subtegument and somatic musculature. After exposure to the crude extract and

genistein, a pronounced decline in the visible stain intensity was observed indicating very less or no activity in these sites. Quantitatively the activity of AcPase, AlkPase, ATPase and 5'-Nu was found to be suppressed by 97%, 95%, 88% and 57%, respectively, following genistein treatment. The reference drug, praziquantel also caused a reduction in the enzyme activity, somewhat similar with the genistein treatment.

(V) Using high performance liquid chromatography phosphoserine, taurine, phosphoamine, threonine, serine, glutamate, proline, glycine, alanine, citrulline, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, β -alanine, α -aminobutyric acid, γ -aminobutyric acid, tryptophan, histidine, ornithine, arginine and ammonia were detected in the tissue homogenate of the parasite both qualitatively and quantitatively. After exposure to the crude extract (50 mg/ml) and genistein (0.5 mg/ml), alterations were noticeable in the free amino acid pool. Following genistein treatment, quantitatively the contents of phosphoserine, taurine, β -alanine, α -aminobutyric acid, tryptophan, histidine and valine were significantly lower.

and glutamate, methionine, isoleucine, γ -aminobutyric acid and ammonia were slightly higher than those in the control; phosphoamine, citrulline and ornithine were not detectable. 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.

(VI) The active principle (genistein) of *F. vestita* root peel suggestively acts transtegumentally on the parasite. In view of the known effects of genistein on cellular physiology, anthelmintic role of this chemical of plant origin needs to be further ascertained.

UNIVERSITY LIBRARY
Acc. No. 103.263
Acc. by
Date 3-3-2007
Class by
Sub Heading by
Enter by
Transcribed by