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In vivo intestinal absorption of ^{14}C-labelled amino acids, alanine and methionine during *P. cyclopium* toxicosis in rats

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Abstract

Penicillium cyclopium is a common food contaminant and one such isolate from rice has been used in the present investigation. Feeding of *P. cyclopium*-contaminated diet to rats is found to affect the intestinal cellular integrity as shown by histopathological study, followed by analysis of major intestinal macromolecular constituents. The *in vivo* intestinal absorption studies using ^{14}C alanine and methionine indicate a significant reduction in the absorption of these amino acids during toxicosis and this is corroborated by the changes observed in the membrane-bound enzyme activities which are involved in the transport.

Key words: *P. cyclopium*, toxicity, pathology, *in vivo* intestinal absorption, cellular constituents and membrane-bound enzymes.

1. Introduction

Mycotoxins are known to affect the structural and functional aspects of different organ systems. Various studies conducted on mycotoxins indicate that some of the mycotoxins like aflatoxin[1] and rubratoxin[2] are hepatotoxins, while ochratoxin[3] is a nephrotoxin and penitrem A[4] is a neuro-toxin. Patulin[5], a potent mycotoxin, affects the intestinal cellular integrity which is reflected by the lowered transport of metabolites through intestinal membrane into portal circulation. Rubratoxin, a hepato-toxin, is also found to cause intestinal tissue damage and lowers the intestinal absorption of nutrients[6].

The toxic nature of *P. cyclopium* has been well documented by Wilson[7]. This organism produces penicillic[8] and cyclopiazonic acids[9], cyclopenin and cyclopenol[10] as its major secondary metabolites. A strain of *P. cyclopium* isolated by us produced in large amounts an yellow compound along with cyclopenin[11]. The purified yellow compound at a concentration of 800 mcg per rat was administered (ip) on alternate days[11]. Mortality was observed at the end of the sixth dose of administration of the compound. Histopathological examination of the liver, kidney and intestine showed evidence of cell damage in all cases with inflammatory cellular infiltration in liver and kidney.

The present investigation deals with the toxic nature of this strain of *P. cyclopium* on the intestinal tissue of rats which are fed with diet mixed with *P. cyclopium*-contaminated diet.

2. Materials and methods

One of the common contaminants of rice is *P. cyclopium*. 500 g of rice having a high moisture content was artificially contaminated with a 2 ml spore suspension (10^7 spores/ml) of *P. cyclopium* and incubated for 15 days at 28° C. The microbial contamination is freed from the rice by shaking it with chloroform. The rice was dried as such to remove the chloroform completely. The chloroform treatement did not remove any of the toxic metabolites. Apart from the presence of killed fungal spores, the rice looked normal and only a portion of this is added as a powder to the normal pelleted rat diet purchased from Hindustan Lever Ltd., Bombay, in the ratio of 1:2 and fed to animals as '*P. cyclopium*-contaminated diet'. This diet has the necessary food composition as normal diet fed to rats.

Thirty weanling albino rats were divided into two groups. One served as control receiving normal pelleted rat diet purchased from Hindustan Lever Ltd., Bombay, while the other, the experimental was fed with contaminated diet. 10 g of the contaminated diet was fed per day per rat which contains nearly 30 mcg of the yellow compound whereas the other secondary metabolites were detectable as trace amounts. The yellow compound was estimated by the method of Ramani[12]. Food consumption of both control and experimental rats was identical but the growth rate was lowered.

After sixty days of experimental period, one set of animals was sacrificed and the intestinal tissue was removed. A part of it was used for histopathological examinations and the rest for estimating the cellular constituents like protein[13], alkali extractable carbohydrate and glycogen[14], and lipid[15] and also for determining the activities of membrane-bound enzymes such as different ATPases[16] like total and $Na^+ K^+$-dependent ATPase and alkaline phosphatase[17] and 5′ nucleotidase[18].

In vivo intestinal absorption of ^{14}C – methionine and alanine was carried out by perfusion technique in one set of rats. The method followed is that of Younoszai and Schedl[19] with slight modification. Rats starved for 24 hours were anaesthetized by intraperitoneal injection of sodium phenobarbitone (50 mg/kg body weight). The animals were perfused while lying on their back. The abdominal cavity was opened with a longitudinal incision, the common bile duct was ligated and an inlet cannula was inserted at the pylorus and an outlet cannula at the terminal ileum. The entire small intestine was perfused at a constant rate of one ml/mt with the perfusion solution, after flushing the intestine with 0.9% saline. The perfusion solution contained 135 mM sodium chloride, 5 mM potassium chloride, 5 mM unlabelled amino acids and 20 mc Ci of the corresponding ^{14}C – labelled amino acid in 150 mM phosphate buffer of pH 7.4. The effluent samples (or the perfusates) were collected at 0,10,20,30,40,50 and 60 mts. From the effluent solution, 0.1 ml was taken for the measurement of radioactivity in the liquid scintillation counter with 10 ml of scintillation fluid. The scintillation fluid was a mixture of dioxane and ethylene glycol (50: 1 v/v) containing PPO (4g/litre) POPOP (200 mg/litre) and naphthalene (60g/litre). At the end of the experiment, the length of the intestines of control as well as experimental rats were recorded and the

results expressed as per 100 cm length. The results are expressed in terms of uptake (radioactive counts per minute (CPM)/100 cm intestinal length) which are arrived at from the difference between the counts of the perfusion solution and that of the samples at various time intervals per 100 cm length of intestine.

3. Results and discussion

The growth rate of the normal and *P.cyclopium*-contaminated diet fed rats (fig. 1) indicates that there is a slight retardation in the growth rate of experimental animals. Reduced growth may be due to reduced absorption of nutrients from the intestine since the histopathological investigation of the intestinal tissue shows desquamation of intestinal cells (fig. 2) which denotes an altered cellular integrity due to toxicosis.

The intestinal macromolecular cellular constituents presented in Table I show a significant reduction in the levels of glycogen, protein and lipid during toxicosis indicating intestinal damage. This is further confirmed by the lowered levels of membrane-bound enzymes involved in transport across membranes like Na^+ K^+-dependent ATPase and alkaline phosphatase and increased activity of 5′ nucleotidase (Table II). That the intestinal absorption is retarded has been further confirmed by the uptake studies, the results of which are given in figs. 3 and 4. In the case of experimental animals, there is a definite lowered absorption of both amino acids, alanine and methionine during toxicosis. The lowered level of Na^+ K^+-dependent ATPase seen in the present study may thus indirectly affect the uptake of amino acid.

From figs. 3 and 4, it is evident that the absorption of alanine is delayed when compared with that of methionine as shown by the delayed peak period of alanine absorption. According to the classification of amino acid transport systems elucidated by Their *et al*[20] and Penrose *et al*[21], alanine and methionine (neutral amino acids) share a common transport protein for their transport across membranes. Although the absorption of both amino acids

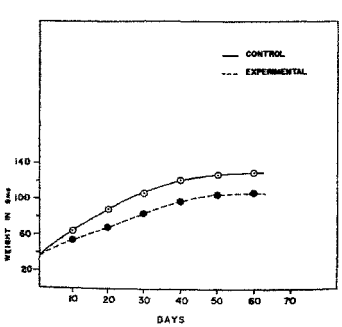

FIG. 1. Growth curve of control rats and *P.cyclopium*-contaminated diet-fed rats.

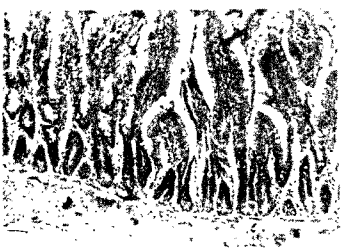

FIG. 2a. Section of intestine from control rat. Haematoxylin-Eosin

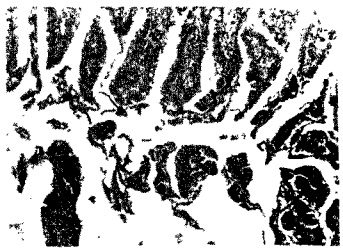

FIG. 2b. Section of intestine from rat fed with *P. cyclopium*-contaminated diet. Haematoxylin-Eosin (Desquamation of intestinal cells)

Table I

Effect of *P. cyclopium* toxicity on macromolecular cellular constituents of intestine of control and experimental animals

The mean values ± S.D. are expressed as mg/g defatted dried tissue

Parameters	Control	Experimental
Protein	337.0 ± 11.5	298.01 ± 7.5
Alkali extractable carbohydrate	13.89 ± 1.24	12.2 ± 1.06
Glycogen	11.63 ± 0.52	10.52 ± 0.65
Lipid (mg/g wet tissue)	42.29 ± 0.78	35.39 ± 0.67

Table II

Effect of *P. cyclopium* toxicity on the activity of some membrane-bound enzymes in the intestine of control and experimental animals

Parameters	Control	Test
ATPase (mc moles of Pi liberated/mg protein)		
Total ATPase	4.21 ± 0.15	3.1 ± 0.1
Na^+ K^+-dependent ATPase	1.85 ± 0.3	1.1 ± 0.2
Alkaline phosphatase (mc moles of phenol liberated/mg protein)	3.40 ± 0.1	2.24 ± 0.16
5′ nucleotidase (mc moles of Pi liberated/mg protein)	1.52 ± 0.3	1.64 ± 0.14

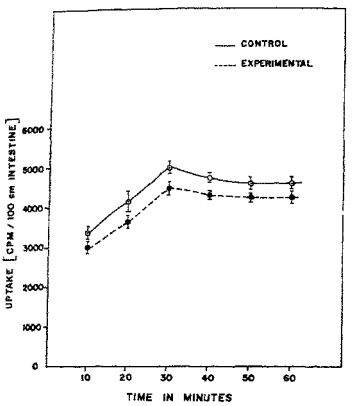

FIG. 3. *In vivo* absorption of ^{14}C methionine by the intestine of control and *P.cyclopium*-infected-diet-fed rats expressed as counts per minute per 100 cm intestine at various time intervals.

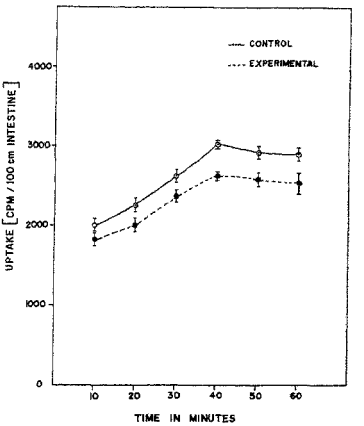

FIG. 4. *In vivo* absorption of ^{14}C alanine by the intestine of control and *P.cyclopium*-infected-fed rats expressed as counts per minute per 100 cm intestine at various time intervals.

was found to be lowered, the uptake of alanine was found to be delayed suggesting not only a change in carrier protein due to *P.cyclopium* toxicosis but also impaired substrate affinity of the carrier protein towards alanine. Our experiment is *in vivo*. However, a similar observation was encountered by Ramani[12] during *in vitro* uptake studies of histidine and tryptophan during *P.cyclopium* toxicosis where the uptake of histidine was delayed when compared with that of tryptophan.

Crude toxic extract of the culture filtrate of *P.cyclopium*, when given orally in a vehicle produced similar toxic effects on cellular constituents, on the activities of membrane-bound enzymes and on intestinal absorption of rats like that of contaminated-diet-fed rats[22].

It may be pointed out that patulin[23,24], rubratoxin[25] and citreovirdin[26] were also shown to inhibit ATPase activities of various tissues. Patulin interference in membrane permeability and alkaline phosphatase activity has been reported by Ciegler *et al*[27].

It looks from our studies as well as of others that the primary site of action of mycotoxins will be on the intestinal membrane integrity.

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