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Short Communication

Toxic nature of *Penicillium cyclopium*-contaminated feed

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Abstract

P. cyclopium-contaminated diet when fed to rats produced toxic symptoms like reduced growth rate and in some cases fatality. Characteristic histopathological lesions were observed in liver, kidney and intestine which led to a study of major cellular constituents and a few enzymes during toxicosis.

Key words : *P. cyclopium*, toxicity, pathology, cellular constituents and enzymes.

1. Introduction

Mold-contaminated foods are known to be toxic to animals. *Penicillium* and *Aspergillus* species are found to be more common fungal contaminants[1]. Brook and White[2] have tabulated 26 species of *Penicillium* as either proven or possible causes of toxicoses of domestic animals.

Holzapfel[3] reported *Penicillium cyclopium* as a common contaminant of stored grains. Certain isolates of *P. cyclopium* have been incriminated in mycotoxicoses in cattle[4]. Several strains of *P. cyclopium*, recovered from samples of domestic cereal products, produced acute toxicosis in ducklings. Reports indicate that this organism produces various secondary metabolites like cyclopiazonic acid[5], penicillic acid[6], frequentin, palitantin[7], cyclopenin and cyclopenol[8,9]. Of these various metabolites, only cyclopiazonic acid and penicillic acid have been reported to be toxic to living systems[6,10]. Regarding the other secondary metabolites there are no reports on their effect in living systems.

2. Materials and methods

2.1. *Maintenance of the strain*

P. cyclopium was isolated in our laboratory from a warehouse sample of rice and suitably confirmed. This fungus was maintained in Czapek–Dox agar slants and sub-cultured at every 14 days of interval.

2.2. *Preparation of contaminated bread*

Contaminated bread (manufactured by Modern Bakeries, India) which contained 0·03% calcium propionate as preservative was used for the culture. The bread pieces were taken in Haffkin flasks, the moisture content adjusted to about 40% and was sterilized in an autoclave at 15 lb/sq. inch pressure for 20 minutes. It was then inoculated with spores of *P. cyclopium* from a 14-day old culture. After incubation at 27–30° C for 14 days, the cultures were treated with 200 ml of chloroform for 48 hours to kill the organism and dried at 105° F for 5 days before grinding. The ground bread cultures were mixed with normal animal feed at a 25% level and used as the experimental diet.

Weanling albino rats of both sexes were fed with experimental diets and weighed at every 10-day intervals. Water was given *ad libitum*. After 40 days of treatment, the rats were sacrificed, liver, kidney and intestine were removed quickly. A portion of the tissue was processed in 10% formalin for histopathological examinations. A part of tissue was used for defatting with chloroform–methanol. The remaining tissue was used for the assay of various enzymes. The defatted material was used for the estimation of major cellular constituents.

3. Results and discussion

P. cyclopium-contaminated feed when fed to rats was found to be toxic as seen in less in weight of the experimental animals. Continued feeding produced 25% mortality in all the sets of experiments conducted. The morphology of cells of liver were found altered with pigment deposits ; red blood cells were in plenty in between lines indicating marked haemorrhage of the cells. The kidney tubules were found to be swollen but the glomeruli was seen to be intact. Tubules also showed extravasated red blood cells in the lumen. Large areas of intestine were normal but the epithelium in certain areas appeared degenerated.

Toxicity studies were conducted initially with culture filtrate concentrate and later with filtrate extract using ethylacetate and the solvent completely removed.

Chicks were found to be more affected than rats since mortality rate was high in them but the pathological changes were nearly the same.

Table I

Effect of feeding *P. cyclopium*-contaminated bread on liver, kidney and intestinal constituents of rats

The results are expressed in mg/gm of dry defatted tissue. The lipid components are expressed in mg/gm of wet tissue

Parameter	Liver		Kidney		Intestine	
	Control	Experimental	Control	Experimental	Control	Experimental
Protein	332·64±10·27	128·20±8·75	240·96±12·32	150·92±8·95	256·44±10·14	146·54±8·32
Total carbohydrate	65·44± 4·05	45·33±5·44	53·01± 5·78	28·86±4·01	66·35± 7·63	48·57±5·99
Glycogen	18·94± 2·02	7·94±1·34	9·36± 1·33	5·32±1·96	5·78± 1·47	4·00±1·03
DNA	15·23± 3·03	14·55±2·91	11·27± 2·76	9·20±2·11	27·26± 4·63	26·38±4·97
RNA	26·67± 5·02	23·11±4·77	37·47± 6·02	35·90±4·93	50·12± 6·98	48·03±7·46
Total lipid	52·63± 1·62	44·24±2·02	56·84± 1·52	52·20±1·93	50·13± 0·46	48·23± 0·52
Phospholipid	0·54± 0·03	0·53±0·05	1·05± 0·06	0·83±0·04	0·24± 0·06	0·34±0·08
Cholesterol	3·17± 0·10	3·54±0·12	6·74± 0·34	6·04±0·24	4·71± 0·04	4·41±0·05

Extraction by solvents and subsequent TLC studies showed that the strain of fungus used by us produced only cyclopenin and the other toxins such as cyclopiazonic acid and penicillic acid were absent. However, in our strain a few more unidentified secondary metabolites have been observed including a toxic yellow pigment.

Since histopathological studies indicate degeneration in the cell architecture it was of interest to study biochemical changes, the cellular constituents and certain key enzymes. The results are given in Tables I and II.

Significant decrease in the levels of total carbohydrates and glycogen are observed in experimental animals. A study of glycolytic enzymes (Fructose 1–6 diphosphatase[11], glucose-6 phosphatase[12], lactate dehydrogenase[13] and aldolase[13]) were made. From Table II it can be seen that the enzyme levels are low in test animals which explains the lower levels of glycogen present in the tissue during toxicosis.

Since the primary effect of any toxin in food is the absorption of the toxin by the intestinal membrane, membrane-bound enzyme alkaline phosphatase[14] was estimated. The enzyme activity is not altered in liver and kidney but gets decreased by 50% in intestine (Table II). The same observation has been noted when pure cyclopenin, a metabolite of *P. cyclopium* was administered with a more drastic lowering in the intestine only.

Table II

Effect of feeding *P. cyclopium*-contaminated bread on the activities of various enzymes in rats

Enzyme activities are expressed as micromoles of product formed under incubation conditions.

Enzymes	Activity in units/mg of protein					
	Liver		Kidney		Intestine	
	Control	Test	Control	Test	Control	Test
Alkaline phosphatase	7·55±0·07	7·68±0·15	10·65±0·21	11·11±0·33	9·23±0·2	5·35±0·23
FDP'ase	0·65±0·03	0·72±0·02	0·49±0·02	0·46±0·02	0·39±0·01	0·31±0·04
G6P'ase	1·05±0·02	1·22±0·05	1·21±0·05	1·04±0·06	1·07±0·23	0·52±0·11
LDH	7·59±0·21	8·14±0·26	8·02±0·23	9·36±0·32	9·61±0·27	8·90±0·22
Aldolase	4·85±0·14	3·85±0·17	3·46±0·10	3·02±0·11	4·98±0·22	4·14±0·27
GPT	3·74±0·07	3·47±0·05	2·73±0·11	1·49±0·10	2·80±0·13	1·04±0·14
GOT	3·99±0·05	2·49±0·05	3·46±0·05	2·87±0·07	2·89±0·04	2·33±0·03

FDP'ase : Fructose 1–6 diphosphatase; G6P'ase : Glucose 6 phosphatase; LDH : Lactate dehydrogenase; GPT : Glutamate-pyruvate transaminase; GOT : Glutamate-oxaloacetate transaminase.

The decreased glycolytic activity may cause reduced amounts of pyruvate formation, a precursor for lipogenesis leading to decreased levels in total lipid, phospholipids and cholesterol which are observed in our studies. However, the low levels of protein and transaminases[15] need to be looked into.

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