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EFFECT OF PENITREM A ON GLUCOSE TOLERANCE STUDIED IN RATS

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PENITREM A, a tremorgenic mycotoxin is a secondary metabolite of *Penicillium crustosum*[1], which is a natural contaminant of corn, cheese and cotton seeds[2-4]. Penitrem A ingestion is found to increase blood glucose concentration[5]. Hence it was of interest to study the effect of penitrem A on liver glycogen concentration and serum insulin levels.

P. crustosum was grown on Czapek-Dox liquid medium enriched with 2% corn steep liquor for 15 days at 25°C. The toxin was extracted from the mycelial mats[6] and purified[7]. Penitrem A was recrystallised from ethyl alcohol. Modern bread (containing 0.2% calcium propionate as the preservative) was sterilized with 30% moisture content at 15 psi for 10 min, was inoculated (after cooling) with 5 ml spore suspension of *P. crustosum* containing 1×10^6 spores/ml and kept at 25°C for 15 days. The infected bread was treated with diethyl ether to kill the spores and the ether was removed completely. The diet when tested was found to be free from any fungal spores, but contained the toxin and was mixed with the commercial rat feed in the ratio of 1:2 and fed to rats, as "fungal contaminated diet" containing 5 to 8 ppm of penitrem A. The composition of the "contaminated diet", was similar to that of the control diet.

Young albino rats of Wistar strain weighing about 40–50 g were divided into 4 groups. One group of rats was fed with contaminated bread. Another group was injected intraperitoneally with 0.1 ml of pure penitrem A, dissolved in sesame oil (1 mg/kg body weight). Proper controls were used in each case. After 24 weeks of experimentation, blood was collected from the tail of the rats and blood glucose concentration was estimated[8]. Since the fasting blood glucose concentrations were elevated, tolerance to glucose was tested[9]. The rats were starved overnight and the fasting blood samples were collected. Ten per cent glucose solution (3.5 g per kg body weight) was administered orally. Blood samples were collected thereafter over a period of 180 min. One set of blood samples was collected with sodium fluoride as anticoagulant for glucose estimation and another set was collected for the assay of serum insulin according to the modified method of Herbert *et al*[10]. The animals were later killed and the liver glycogen[11] and total lipids[12] were estimated.

The results on GTT are presented in figures 1 and 2. In normal rats, maximum blood glucose concentration reached after 30 min of glucose load and it comes back to the fasting level after 180 min. But in the experimental rats, blood glucose concentration progressively increased after glucose load and the fasting level is not reduced after 180 min, indicating poor response to glucose load. Though insulin concentration increases immediately after glucose load in the experimental animals (table 1), this increase is insufficient to utilize the ingested glucose, indicating

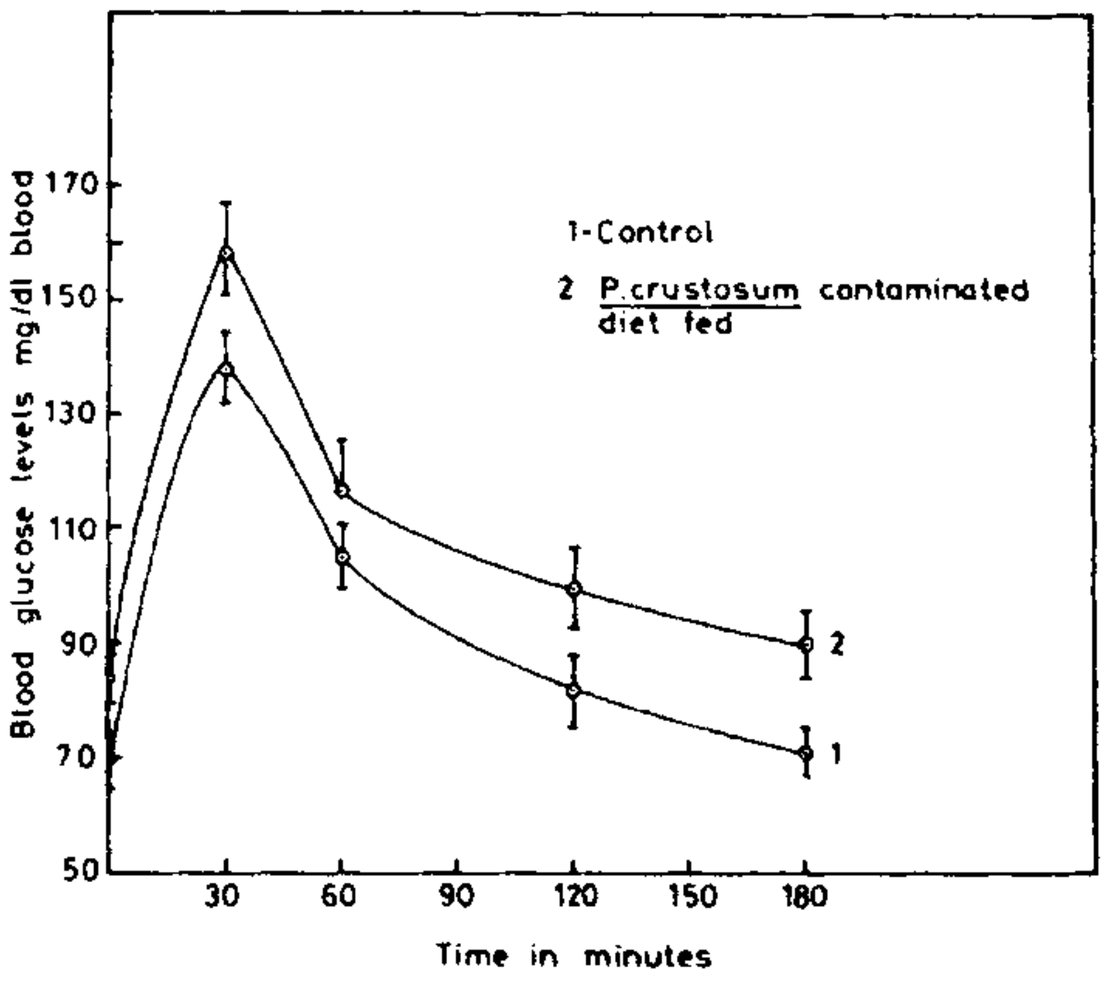

Figure 1. Glucose tolerance test conducted on control and *P. crustosum* contaminated diet fed rats.

Table 1. *Serum insulin levels of control and experimental rats during the performance of GTT. The values are given as μ IU per ml of serum.*

Time (min)	Control	*P. crustosum* contaminated diet fed	Control	Penitrem A injected
Fasting level	17.3 ± 1.2	13.7 ± 1.2*	17.5 ± 2.0	11.2 ± 1.0*
30	24.4 ± 3.8	17.8 ± 0.9**	24.0 ± 3.1	15.1 ± 1.43*
60	33.1 ± 5.3	22.5 ± 1.7**	34.3 ± 4.7	19.7 ± 3.6*
120	21.7 ± 2.6	14.8 ± 1.8*	22.8 ± 2.9	13.4 ± 1.12*
180	18.0 ± 1.3	11.9 ± 0.9*	17.4 ± 1.2	9.9 ± 2.80*

Statistical significance at $*p < 0.001$; $**p < 0.01$

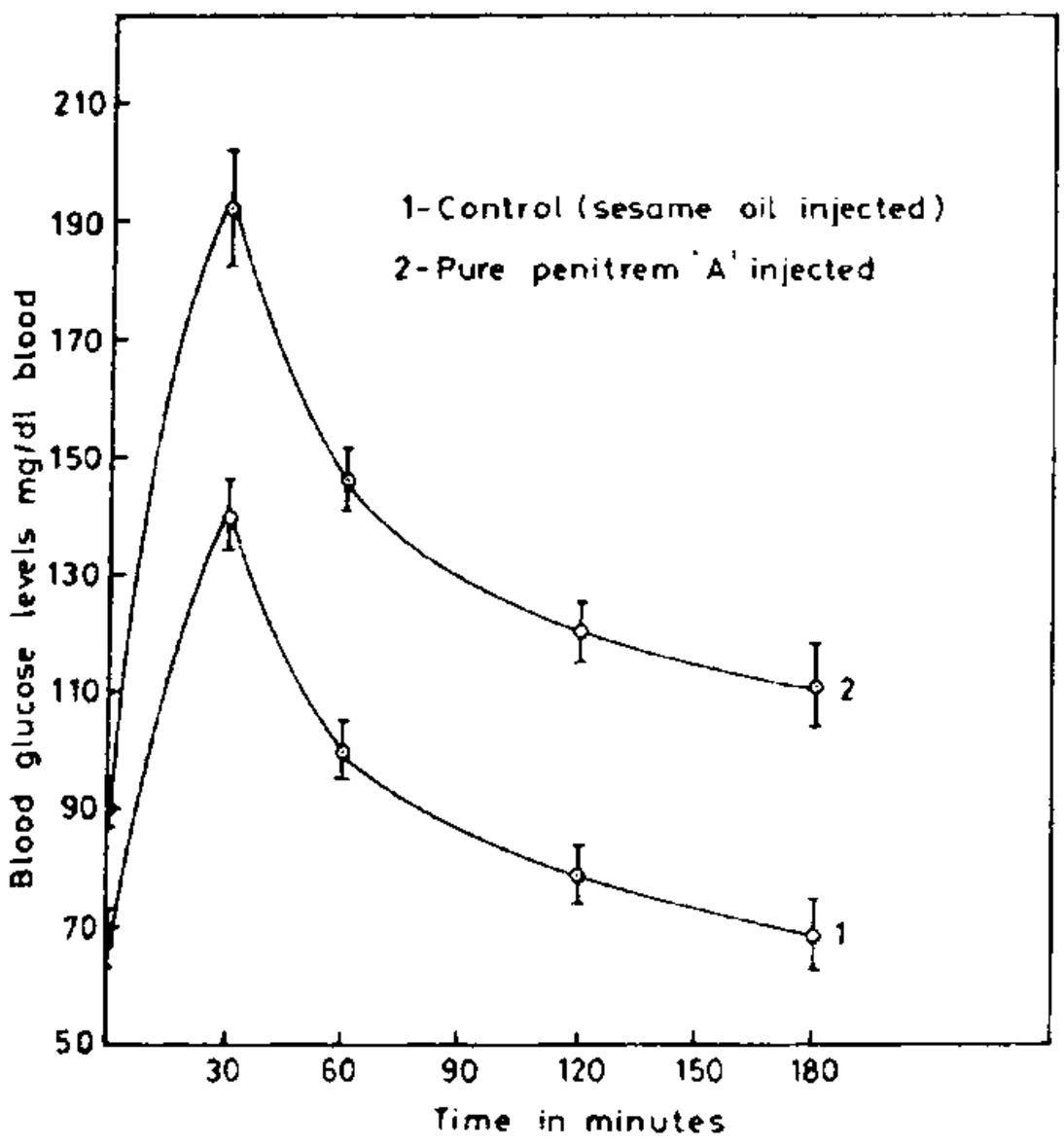

Figure 2. Glucose tolerance test conducted on control and penitrem A injected rats.

hyperglycemia accompanied by hypoinsulinemia. Such changes have been observed during other mycotoxicosis, patulin toxicosis[13], terreic acid toxicosis[14], and cyclopiazonic acid toxicosis[15]. A drastic reduction in liver glycogen and a significant increase in lipid concentration (table 2), hyperglycemia and hypoinsulinemia are the common observations during penitrem A toxicosis, indicating that the mycotoxin penitrem A, on long term ingestion in small amounts, may lead to disorders in glycose metabolism.

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Table 2. *Variation in the hepatic glycogen and total lipid levels during penitrem A toxicosis in rats.*

Group	Glycogen mg/g of wet tissue	Total lipid mg/g of wet tissue
Control	41.9 ± 3.5	54.2 ± 1.5
P. crustosum contaminated diet	32.5 ± 3.8**	61.9 ± 3.2**
Control	40.8 ± 3.2	55.8 ± 3.5
Penitrem A injected	28.5 ± 3.9*	67.4 ± 4.0*

Statistical significance at $*p < 0.001$; $**p < 0.01$

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PLANT REGENERATION FROM HYPOCOTYL PROTOPLASTS OF MOTHBEAN (*VIGNA ACONITIFOLIA*)

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EVER SINCE the successful demonstration of regeneration of isolated protoplasts in plants, much was speculated about its potential use for genetic manipulation of important crop plants namely legumes and cereals. It is essential to obtain reproducible differentiation of plants from protoplasts at will, before attempting any genetic manipulation. Unfortunately, unlike other plants, the regeneration of protoplasts from grain legumes and cereals have proved recalcitrant, though sporadic reports are available[1,2].

In our laboratory, we have initiated a programme to develop protoplast, cell and tissue culture technique in mothbean, a widely cultivated drought resistant grain legume so as to use the system for somatic cell hybridization and plant cell transformation. In previous studies on moth bean, regeneration of protoplasts has been reported from mesophyll[3] involving a complicated procedure of enzyme purification, and shoot-apex derived callus[4], by use of non-commercial enzymes. The aim of the present communication was to obtain reproducible plant differentiation from protoplasts of yet another source—hypocotyl-derived callus under simpler and well-defined conditions.

Seeds of mothbean (*Vigna aconitifolia* Jacq Marechal) cv No. 88 were surface-sterilized with 70% alcohol for 30 sec followed by treatment with mercuric chloride (0.1%) for 5 min. The seeds were rinsed five times with sterile distilled water and aseptically implanted on MS medium[5] for germination. Hypocotyl of 4–5 day old seedlings were cut into very fine pieces and cultured on MS medium supplemented with 2,4-D (2,4-dichlorophenoxyacetic acid at 2 mg/l) and coconut water (15%).

Protoplasts were isolated from 4–5 day old freshly raised callus. About 10 ml of filter-sterilized enzyme was used for a gram of callus tissue. Enzyme mixture comprised of 2.5% cellulase, 0.5% macerozyme (both from Kinki Yakult Biochemicals, Japan), 0.5% driselase (Kyowa Hakko Kogya, Japan) and 0.5% hemicellulase (Sigma Chemicals, USA) in 0.5 M. sorbital. Incubation was done overnight at 25 ± 1°C by keeping tissue stationary in enzyme mixture. At the end of the incubation, undigested material was separated by filtration through a 43 μ steel mesh. The resulting suspension was centrifuged at 300 g for 5 min and the pellet washed thrice by 0.5 M sorbitol. The final pellet was cleaned by floating on 25% sucrose solution[6] followed by centrifugation at 100 g for 5 min. In the process, the purified preparation formed supernatant and the pellet was discarded. Protoplasts were recovered by a Pasteur pipette and cultured in liquid media in glass petri dishes at a final concentration of 5×10^4 to 10^5 protoplasts/ml. Various media namely Kao and Michayluk[7], V-47[8], MS[5] and modified MS were used for culturing protoplasts. Hereafter the modified MS refers to a medium which contains mineral elements and iron of Murashige and Skoog[5], vitamins after Lin and Staba[9], along with 2,4-D (2 mg/l), kinetin (Kn 0.1 mg/l), caesin hydrolysate (100 mg/l), glutamine (800 mg/l), DL-tryptophan (Sigma Chemical Co. 25 mg/l), sucrose (3%) and sorbitol (9%). The petri dishes were sealed with parafilm and incubated in diffuse light at 25 ± 2°C. Protoplast-derived colonies were transferred to agar-jelled modified MS with 3% sucrose alone either by plating[10] or 'liquid over solid medium' method[11]. For organogenesis such calli were transferred to modified agar-jelled MS medium supplemented with cytokinins like BA (benzyladenine) Z(zeatin) Kn or 2,i-P (6-γ,γ-dimethylallylaminopurine) at 2 mg/l + 2,4-D (0.1 mg/l). After one month, protoclones were transferred to basal medium devoid of phytohormones.

With the above enzyme combination, protoplast yield varied between 3×10^6/g and 5×10^6/g of tissue used (figure 1). It was not found essential to add Ca^{++} in incubation period for their stabilization. However, Shekhawat and Galston[3] had to use purified Driselase and also add Ca^{++} in the incubation medium. Protoplast yield was found to be dependent upon age