TOXICITY OF PHYTOALEXIN TO BACTERIAL PATHOGENS OF MAN

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PHYTOALEXINS have been defined as antibiotics which are produced during the interaction of two metabolic systems, host and parasite, and which inhibit the growth of microorganisms pathogenic to plants[1]. Because of their antifungal properties, phytoalexins are implicated in plant disease resistance[2]. Although these accumulate in plants reacting hypersensitively to bacterial infections[3-5], their toxic effect on bacteria has remained uncertain[6-7], except for two recent reports[8-9] which report on the selective toxicity of phytoalexins, *viz.*, kievitone, phaseollin, wyerone, etc., to gram-positive bacteria. Hence it was felt that phytoalexins could be used in treating plant diseases caused by species of *Corynebacterium* and *Streptomyces* and could have some medical value[8]. Whether the plant produced antibiotic would be toxic to gram-positive bacteria, pathogenic to man, was investigated and our results with kievitone, a potent isoflavonoid phytoalexin produced by French bean, *Phaseolus vulgaris* L., seem to support this.

Kievitone was extracted from rotting cowpea seeds or bean seeds infected with *Rhizoctonia solani*[10-11]. The gram-positive human pathogens used as test organisms were: *Corynebacterium diphtheriae* var. *mitis*, *Staphylococcus aureus* and *Streptococcus haemolyticus*. These organisms were supplied by the Director, King Institute, Madras-25. A plant pathogen of the gram-positive group, *Corynebacterium flaccumfaciens* (a gift from Dr. K. Rudolph, George August University, W. Germany) was also included in one set of experiments.

The standard antibiotic disc assay, liquid culture assay and a modified paper-disc assay were employed to measure the antibacterial activity of kievitone. In the first method, sterile paper discs (Whatman A. A. discs, 6 mm dia.) were loaded with the prescribed amount of phytoalexin in absolute ethanol (maximum volume of ethanol, 25 μl/disc). In each plate, discs treated with ethanol (25 μl) alone served as controls. When sufficient time to allow the ethanol to evaporate had lapsed, the discs were arranged in Petridishes on a layer of soft agar (nutrient agar with 0·75% agar) seeded with the test bacterium (0·1 ml of 16 h grown nutrient broth culture)[8]. Diameters of inhibition zones were calculated (Tables I and II). In the liquid culture assay, kievitone was dissolved in minimum quantity of ethanol and added to the medium to give a final concentration of 25 μg/ml. The same amount of ethanol was added to control flask. *C. diphtheriae* var. *mitis* and *S. haemolyticus* were grown to logarithmic phase and 0·1 ml of each (*ca.* 10^8 cells/ml) was added to the medium. Cultures were incubated at 30° C and growth was measured after 0, 6, 12, 24 and 48 h as Klett units using a photoelectric colorimeter

TABLE I

Toxicity of kievitone to gram-positive bacterial pathogens of man

Organism	Area of inhibition (mm²)* Kievitone μg/disc 50	μg/drop 0	5	12·5
1. *Corynebacterium diphtheriae* var. *mitis*	131	0	137	301
2. *Streptococcus haemolyticus*	126	0	110	220
3. *Staphylococcus aureus*	112	0	67	205

*Area of inhibition = area of total inhibition minus the area of disc (applies to AA disc assays only); each value is the mean of duplicate values rounded off to the nearest whole number.

TABLE II

Toxicity of low concentrations of kievitone to gram-positive bacterial pathogens of man

Organism	Area of inhibition (mm²)* Kievitone (μg/disc) 0	2	4	6	8	10	20
1. *Corynebacterium diphtheriae* var. *mitis*	0	0	16	22	32	58	76
2. *C. flaccumfaciens*	0	0	22	35	43	94	137

*Area of inhibition = total area of inhibition minus area of disc; each value is a mean of duplicate values rounded off to the nearest whole number.

(Klett-Summerson, USA; model 800-3). The experiment was repeated using identical conditions. In the third method, kievitone was placed as ethanol droplets (5 μl droplets) on a soft agar surface[7]. Inhibition zones which developed around droplets containing kievitone was measured after 20 h and areas calculated (Table I)

Kievitone at 50 μg/disc inhibited the growth of all the human pathogens tested (Table I). Inhibition zones were also induced by kievitone in *C. diphtheriae* var. *mitis* and *C. flaccumfaciens* at as low a concentration as 4 μg/disc, although the plant pathogen exhibited larger areas of inhibition (Table II). In liquid culture assay, at 25 μg/ml, the kievitone-induced growth inhibition was both clear and distinct within 6 h after inoculation (Fig. 1). After 48 h of incubation, kievitone-treated cultures of *C. diphtheriae* var. *mitis* and *S. haemolyticus* had 22 and 23 Klett units of growth whereas the non-treated controls had 182 and 98 Klett units, respectively.

When droplets carrying kievitone were placed directly on the agar surface, 5 and 12·5 μg of kievitone likewise induced much larger zones of inhibition in the test organisms (Table I). For example, the area of inhibition induced by 5 μg of kievitone in this method was 137 mm^2 in *C. diphtheriae* var. *mitis* which was larger than the area of inhibition induced by 20 μg of kievitone in the same organism when the compound was added to the AA disc (Tables I and II).

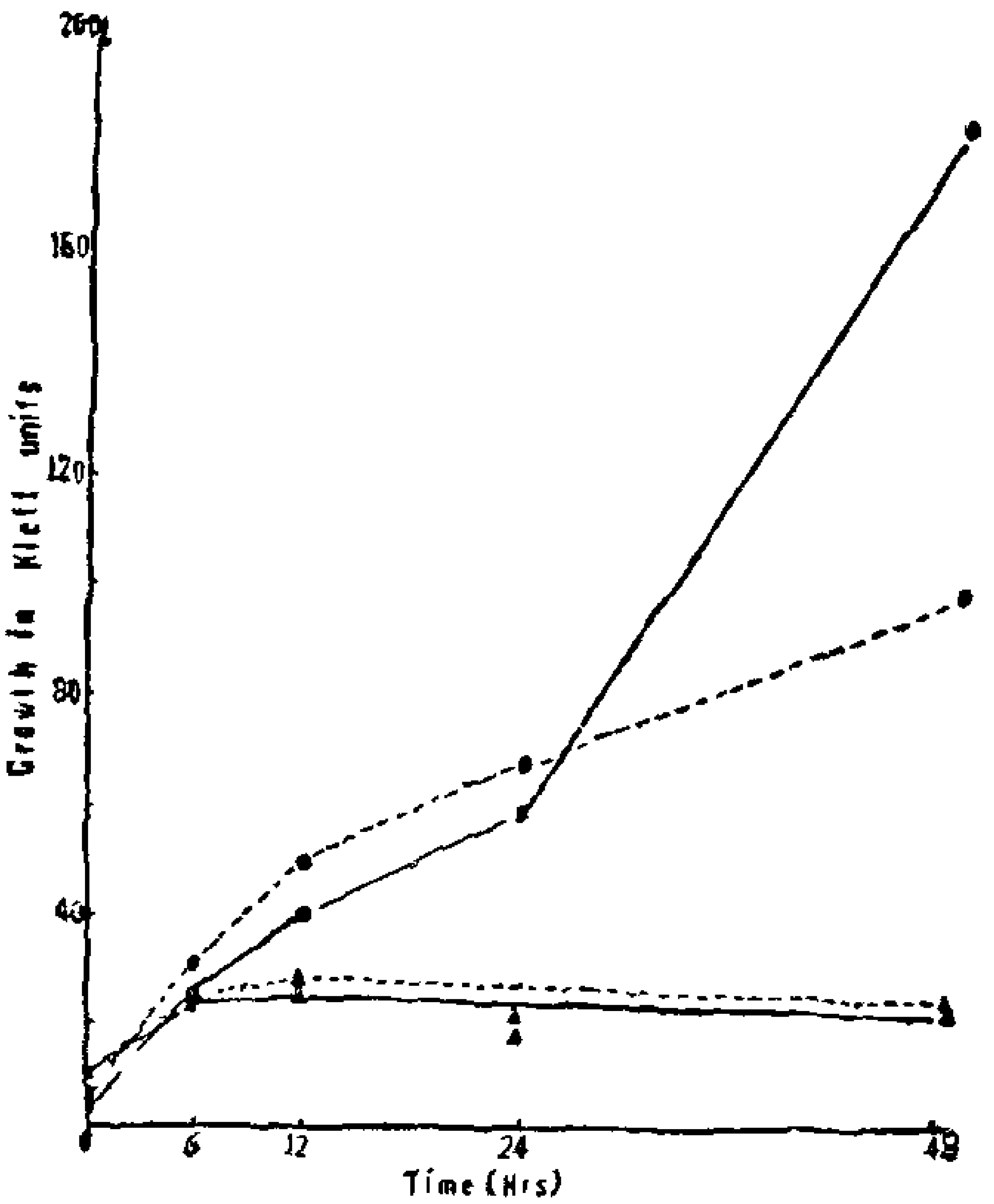

FIG. 1. Liquid culture assay for kievitone toxicity (——)—*Corynebacterium diphtheriae* var. *mitis*. (– – –)—*Streptococcus haemolyticus*. Lines with ▲ represent growth in kievitone-treated flasks and lines with ● represent non-treated controls.

The demonstration of toxicity of kievitone to bacterial pathogens of man heralds another era in chemotherapy. That toxicity is expressed at very low concentrations, and these compounds are produced by plants at fairly large concentrations offer much scope in chemotherapy and this may cause medical scientists to have a fresh look at the value of phytoalexins. The recent observation on the selective toxicity of phytoalexins to gram-positive bacteria[8-9] appears to apply to both plant and human pathogens equally, and thus suggests that the underlying mechanism of toxicity to bacteria of diverse pathogenic potentials is about the same at least in the *in vitro* situation.

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