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Toxicity of Allyl Isothiocyanate and Cinnamic Aldehyde Assessed Using Cultured Human KB Cells and Yeast, *Saccharomyces cervisiae*

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The main components of mustard and cinnamon oils are allyl isothiocyante (AIT) and cinnamic aldehyde (CA), substances used as food additives. The acute toxicity of these substances has been noted in rats (Jenner *et al.* 1964) and it is desirable to obtain information on the toxic effects of these compounds in *in vitro* systems.

We now report the toxicity of AIT and CA on human KB cells and *Saccharomyces cervisiae* cultivated in culture systems.

MATERIALS AND METHODS

Allyl isothiocyanate (AIT) and cinnamic aldehyde (CA), obtained from Wako Pure Chemical. Ind., Osaka, Japan were dissolved in ethyl alcohol (final concentration was under 0.5%).

Saccharomyces cervisiae (IFO 2260) was obtained from the Institute for Fermation, Osaka (IFO), Japan and was used throughout. This strain was cultivated in yeast extract-malt extract (YM) medium (3 g yeast extract, 3 g malt extract, 5 g peptone and 10 g glucose in 1 liter of distilled water) at 25°C. The inhibition of growth of the yeast was estimated in YM liquid culture media. After 48-h of incubation, the cells were suspended 1 X 10^5 cells per 1 mL of YM medium containing serial dilutions of compounds and 5 mL volumes of this YM medium were added to the test tubes (18 X 150 mm).
Cultures were incubated at 25°C on a shaking incubator. After 72-h of additional incubation, the cell density was determined by culture absorbance at 610 nm. The inhibition of cell growth was determined by comparing the cell density in substance-treated cultures with the cell density in cultures that had been treated only with ethyl alcohol (control).
The resultant inhibition, as related to the substance concentration, was then plotted on long-probit paper.
The dose-response curve obtained when the substance caused a fifty per cent inhibition of cell growth (ID50) was deterined.

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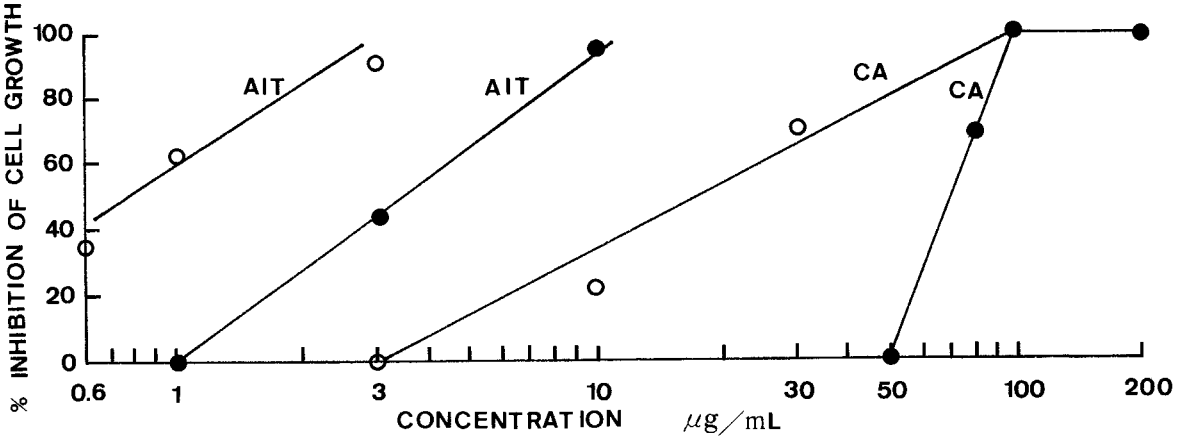

Figure 1. Dose-response curves obtained after 72-h exposure of KB cells (O) and *Saccharomyces cervisiae* (●) to various concentrations of AIT and CA. AIT : allyl isothiocyanate. CA : cinnamic aldehyde.

Table 1. ID50 of allyl isothiocyanate and cinnamic aldehyde.

substances	72-h ID50 (μg/mL)[1]	
	KB cells	*Saccharomyces cervisiae*
allyl isothiocyanate	0.78	3.5
cinnamic aldehyde	19.50	70.0

1 72-h ID50 : 50% inhibitory dose to growth of KB cells and *Saccharomyces cervisiae* after 72-h of incubation.

Each point on the resulting curves represents the average of five replicates.

The KB human cells were used throughout this work (Mochida *et al.* 1985). Methods for toxicity testing were as described (Mochida *et al.* 1985).

The 72-h ID50 values (50% inhibitory dose to growth of *Saccharomyces cervisiae* and KB cells) were used as an index of the toxicity of these substances.

RESULTS AND DISCUSSION

Dose-response curves (Figure 1) and 72-h ID50 values (Table 1) for human KB cells and *Saccharomyces cervisiae* are shown. AIT is apparently more toxic than CA to human KB cells. Jenner *et al.* (1964) reported that the LD50 value of AIT and CA in rats was 339 mg/kg and 2220 mg/kg, respectively. Thus the toxicity rankings, determined from the *in vitro* and *in vivo* assays show a good parallel.

We found that CA 100 μg/mL completely inhibited the growth of *Saccharomyces cervisiae* (Figure 1). The concentration of CA above 200 μg/mL completely inhibited the growth of *Aspergillus parasiticus* (Bullerman *et al.* 1977). The *Saccharomyces cervisiae* seems to be more sensitive than *Aspergillus parasiticus* to CA.

Our previous study (Mochida *et al.* 1985) showed that the ID50 values of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) of food additives obtained using the same KB cell culture system were 12.5 μg/mL and 43.0 μg/mL, respectively. Our present results show that AIT (ID50 values : 0.78 μg/mL) is more toxic than BHA and BHT to KB cells. This is a aggreement with results of acute oral toxicity (LD50) in rats (Jenner *et al.* 1964; Karplyuk 1959).

In 72-h ID50 values (Table 1), the KB cell culture system had a 4.5 times higher value than the *Saccharomyces cervisiae* assay to AIT substance, and KB cells a 3.6 times higher value than the *Saccharomyces cervisiae* to CA substance.

We found that the cell culture system using KB cells is more sensitive to both substances than was the *Saccharomyces cervisiae* test.
We propose that the dose of AIT used be lower than the of CA to ensure safety for human consumption.

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