Inhibitory activity of *A. marmelos* aqueous leaf extract when exposed under UV was superior over the other treatments. Aqueous extract without either autoclaving or exposing under germicidal UV lamp showed equally good inhibition with those of chloroform, ethanolic and acetone extracts. However, none other than UV exposed aqueous extract treatment was free from bacterial contamination. Obviously bacterial contaminants were sterilized due to UV irradiation treatment. This is important in the present context so as to attribute a single factor (plant extract) as growth inhibitor.

Although, growth of *D. oryzae* and *R. solani* was not effectively inhibited as *P. oryzae*, there was no contamination in UV-exposed aqueous extracts. A similar technique can also be used to study inhibitory efficacy of other plants.

The data on efficacy of UV irradiated *A. marmelos* aqueous extract are given in table 1, where higher inhibition of *P. oryzae* growth free from bacterial contaminants was observed (22.9^{-}) than all the other except in benzene extracted treatment (14.31^{+}). Later treatment could not be considered ideal owing to its inability in preventing bacterial contamination. Development of bacterial colony (+, ++, +++) as recorded in various treatments shown in the table was thought to interfere in the growth of the test pathogen. Hence, in such cases it would be paradoxical to ascertain inhibitory activity due to plant extract, bacterial contaminants or synergistic effect of plant extract + bacterial contaminants. Since UV-irradiated plant extract treatment alone inhibited radial growth of *P. oryzae* free from bacterial contamination, it was selected for further studies.

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CYTOTOXICITY OF METHYLISOCYANATE IN HUMAN LUNG EPITHELIAL CELL LINE (L-132)

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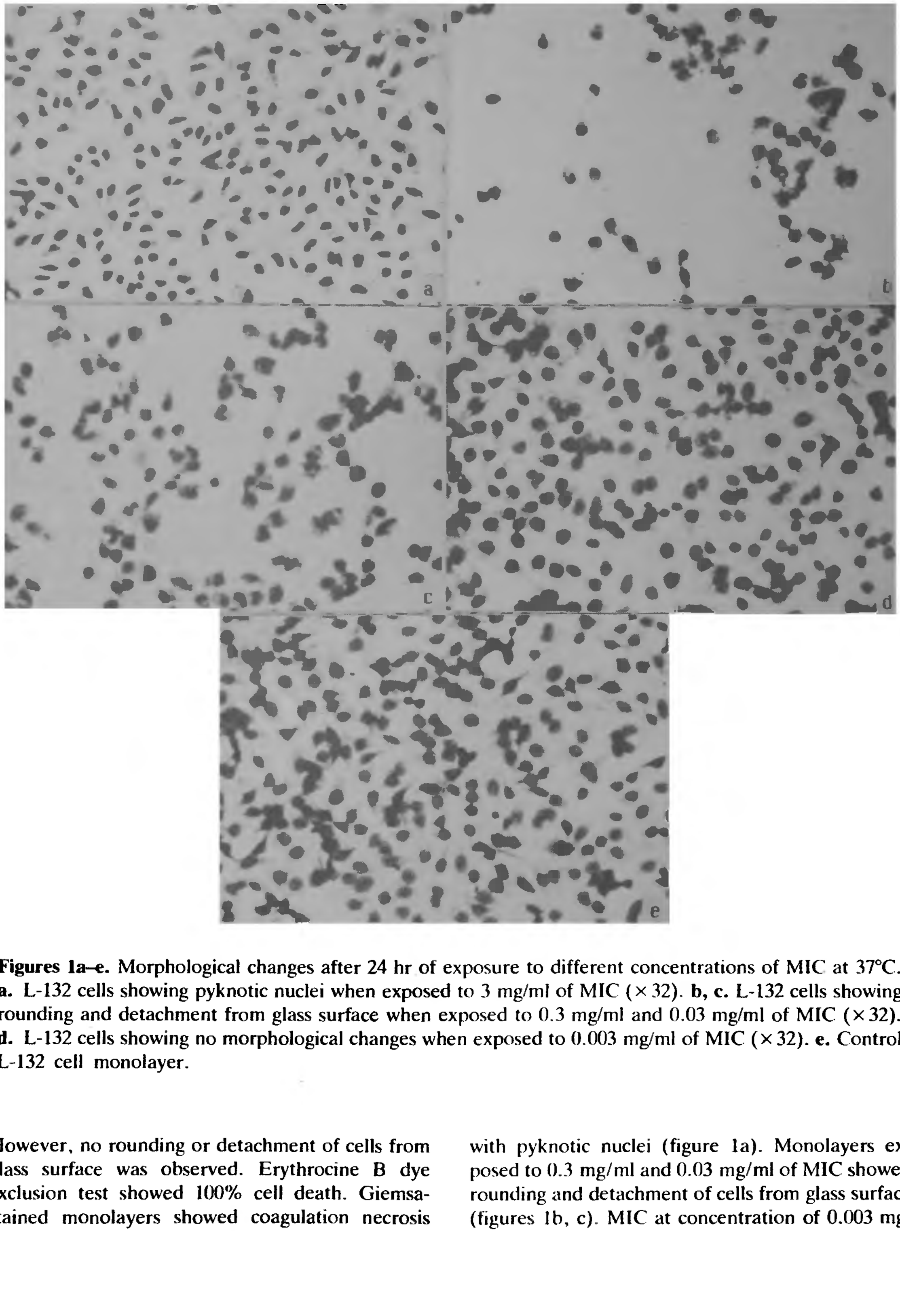
EXPOSURE of methylisocyanate (MIC) at Bhopal in December 1984 affecting large number of human and animal population prompted us to study the toxicity of MIC in a cell culture model. Different cell lines viz Hela, mammalian cell lines and human leukocytes have been used by various workers to study the cytotoxicity of chemical compounds[1–4]. However, to the best of our knowledge no such reports are available pertaining to the *in vitro* toxicity study with MIC. The present study was therefore undertaken to evaluate the toxicity of MIC on human embryonic lung epithelial cell line (L-132).

L-132 cell line was maintained in milk dilution bottles at 37°C using Eagles minimal essential media with non-essential aminoacids and Earle's salts (MEM) enriched with 7% fetal bovine serum and 3% glutamine. When the cell density of 2–3 million cells/bottle was attained they were distributed in 10×6 sets of Leighton tubes (L-tubes) containing coverslips and the tubes were then incubated at 37°C till the complete monolayers were obtained.

MIC was synthesized in the chemistry laboratory of this establishment. Ten-fold dilutions containing 3.0 to 0.0003 mg/ml of MIC were prepared in chilled MEM without serum and the pH was adjusted to 7.2. Media from all L-tubes was drained off and the tubes were rinsed twice with MEM containing no serum. Each dilution of MIC was inoculated in 0.2 ml aliquots on the monolayers and incubated for 1 hr at room temperature (25°C). A set of ten tubes was taken for each dilution. One set of 10 tubes was kept as control. After 1 hr, the tubes were rinsed with MEM without serum. They were then inoculated with serum-free MEM. The tubes were incubated at 37°C for 24–120 hr. Monolayers were observed at 24 hr intervals for cytopathic effects (CPE). Simultaneously cell viability was determined by Erythrocine B dye exclusion test. Monolayers were stained with giemsa and coverslips mounted on glass slides.

A dose-dependent CPE was observed as evident from table 1. At a concentration of 3 mg/ml of MIC, the cells showed feeble shrinkage of cell wall.



Figures 1a–e. Morphological changes after 24 hr of exposure to different concentrations of MIC at 37°C. **a.** L-132 cells showing pyknotic nuclei when exposed to 3 mg/ml of MIC (×32). **b, c.** L-132 cells showing rounding and detachment from glass surface when exposed to 0.3 mg/ml and 0.03 mg/ml of MIC (×32). **d.** L-132 cells showing no morphological changes when exposed to 0.003 mg/ml of MIC (×32). **e.** Control L-132 cell monolayer.

However, no rounding or detachment of cells from glass surface was observed. Erythrocine B dye exclusion test showed 100% cell death. Giemsa-stained monolayers showed coagulation necrosis with pyknotic nuclei (figure 1a). Monolayers exposed to 0.3 mg/ml and 0.03 mg/ml of MIC showed rounding and detachment of cells from glass surface (figures 1b, c). MIC at concentration of 0.003 mg/

Table 1 *Effect of MIC on L-132 cell monolayers*

Concentrations of MIC (mg/ml)	CPE observed at different hours				
	24	48	72	96	120
Control	—	—	—	—	—
3.0	4+	Discarded			
0.3	3+	3+	4+	Discarded	
0.03	2+	2+	3+	4+	Discarded
0.003	—	—	—	1+	1+
0.0003	—	—	—	—	—

Degree of CPE: 4+ = 75–100%, 3+ = 50–75%, 2+ = 25–50%, 1+ = 10–25%, − = No CPE.

CPE consists of coagulation necrosis, shrinkage, rounding and detachment of cells.

ml however did not reveal any toxic effect (figure 1d).

MIC at higher concentrations causes instant cell death. Gradual changes in CPE are very well correlated with increasing concentrations of MIC. The changes may be attributed either to the effect of MIC or its toxic products. Cell culture model provides a rapid and sensitive method to determine the toxic effects of MIC. Cytotoxic effects of MIC observed in this study suggests that MIC might have caused similar cell damage to lung cells of human and animal population involved in the Bhopal tragedy.

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A SIMPLE TECHNIQUE FOR ASSESSMENT OF IMMUNE-RESISTANCE OF HOSTS AGAINST GIARDIASIS

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GIARDIASIS, due to *Giardia lamblia* a common cause of malabsorption and diarrhoel illness mostly in children has become a serious global problem, especially in view of the various immuno-deficiency symptoms[1–3]. Giardiasis is a self-limiting infection in healthy humans and animals[4,5]. Both humoral[6] and cellular[7,8] immune processes are reported to play an active role during giardial infection. Peritoneal macrophages and polymorphonuclear leukocytes (PMNL) are closely associated with amoebic[9–12] infection. Phagocytic competence of these cell-types is adversely affected by virulence of the pathogen thus determining the fate of the pathogen in the host. Reduced, cellular PMNL-cytotoxicity was noticed in giardiasis[13]. Based on this principle, an *in vitro* test method has been developed to determine the immunocompetence of human-PMNL in tackling *G. lamblia* cells during giardiasis.

A drop of blood from the suspected patient was dispensed directly over a microscopic slide and incubated at 37°C for 1 hr under humidity. Blood clot was flushed-out under a jet of phosphate buffer saline (PBS) at pH 7.2. Twenty-four hour old axenically grown trophozoites of *G. lamblia* (5×10^6 cells/ml) were centrifuged at $900 \times g$ for 15 min, washed and suspended in modified complete Diamond's axenic medium[14]. Suspension was dispensed over the PMNL-monolayer and incubated at 37°C under humidity for 10–15 min. Following interaction, the monolayer was dried, fixed with methanol and stained with giemsa for 2 hr. Excess stain was removed by normal saline, dried and examined under oil immersion microscope.

The leukocyte population on the monolayer (figure 1) constituted exclusively of PMNL without any contamination with other blood cell-types. Compared to the conventional methods of preparation of peripheral PMNL, employing Ficoll Hypaque centrifugation, the present method appeared simpler. Similarly the population of *G. lamblia*-trophozoites used for interaction appeared healthy and motile (figure 2).