

SHORT COMMUNICATION

CYTOTOXICITY OF THE ACETYLATED OIL OF
SEMECARPUS ANACARDIUM LINN. F.

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Summary : The cytotoxic effects of acetylated oil of *Semecarpus anacardium* nuts on the cells of P388 lymphocytic leukemia were tested *in vitro*. The product was tested at the concentrations ranging from 15–75 $\mu\text{g/ml}$. The cell kill was observed as early as three hr after the treatment. The effects of acetylated oil on the biosynthesis of DNA, RNA and protein using labelled thymidine, uridine and leucine respectively showed that the product inhibited the biosynthesis of all the three. This was indicated by the inhibition of the incorporation of their precursors. The uptake of ^3H -thymidine was inhibited 15 min after treatment; while that of ^3H -uridine and ^{14}C -leucine took 30 and 45 min respectively. Since the *S. anacardium* oil was unstable due to air-oxidation, the studies were confined to its acetylated product.

Key words : *semecarpus anacardium* oil
macromolecular biosyntheses

acetylated oil
cyt. toxicity

INTRODUCTION

Semecarpus anacardium Linn. f. (Family : *Anacardiaceae*) is a deciduous tree, distributed in the sub-Himalayan tract and in hotter parts of India (7). The nut of *S. anacardium* has been reported to possess medicinal value in the treatment of lepra nodules, warts and rheumatism (3). In the course of anti-cancer screening of indigenous plants, the extracts of *S. anacardium* nut were found to exhibit antitumour properties (2,5). The petroleum ether extract, obtained by cold percolation, was chromatographed. The benzene eluate yielded an orange-coloured oil (b.p. 200–20°/2–3 mm) possessing anti-

tumour activity against P388 lymphocytic leukaemia (6). In view of the unstable nature of oil which was evident from change in colour and loss of activity after keeping, suitable derivatives of oil were prepared. Amongst them, acetylated oil was stable and exhibited anti-tumour properties (6). The present paper reports some observations on the cytotoxic properties of the acetylated oil of *S. anacardium*.

MATERIALS AND METHODS

The crushed nuts of *Semecarpus anacardium* (1.0 kg/batch) were extracted with petroleum ether (60-80°) (5 lit) by cold percolation. The petroleum ether extract was concentrated under reduced pressure (10 mm) (yield, 280 g). The extract (67 g), on chromatography (Silica gel 60-120 mesh, BDH) and elution with benzene, yielded an oily material (yield, 34 g). On distillation under reduced pressure, the benzene eluate yielded an orange-coloured oil, (b.p. 200-20°/2-3 mm) (yield, 17 g). This oil was acetylated with acetic anhydride and pyridine. The spectral data of acetylated oil showed

UV : $\left. \begin{array}{l} \text{MeOH} \\ \lambda_{\text{max}} \end{array} \right\} 260 \text{ and } 245 \text{ nm}$; IR : $\left. \begin{array}{l} \text{Neat} \\ \nu_{\text{max}} \end{array} \right\} \sim 2900 \text{ (CH}_3 \text{ and CH}_2\text{), } 2840 \text{ (CH}_2\text{), } 1785 \text{ (C=O), } 1705 \text{ (C=O), } 1610 \text{ and } 1590 \text{ (aromatic), } 910 \text{ and } 830 \text{ Cm}^{-1}$;

while the orange-coloured oil had UV : $\left. \begin{array}{l} \text{MeOH} \\ \lambda_{\text{max}} \end{array} \right\} 280 \text{ and } 210 \text{ nm}$; IR : $\left. \begin{array}{l} \text{Neat} \\ \nu_{\text{max}} \end{array} \right\} \sim 3450 \text{ (OH), } \sim 2900 \text{ (CH}_3 \text{ and CH}_2\text{), } 2850 \text{ (CH}_2\text{), } 1625 \text{ (C=C), } 1595 \text{ (aromatic), } 1480 \text{ (CH}_2\text{) and } 1280 \text{ cm}^{-1} \text{ (benzene aromatic).}$

Since the acetylated oil was stable, a stock solution of this (1 mg/ml) was prepared by mixing 25 mg with Tween 80 (1.63 mg) and then diluting with normal saline so as to make 25 ml fine emulsion. The maintenance of P388 lymphocytic leukaemia (4) was carried out in DBA/2 mice because this is an inbred strain in which the tumour was originally induced. For antitumour testing and related studies, the tumour is transplanted in F₁ hybrid (C57BL/6 ♀♀ x DBA/2 ♂♂), since the number of offsprings in the hybrid is larger. Mice of either sex (6-weeks-old and weighing between 8-20 g) were used for the experiments.

For *in vitro* cytotoxicity studies (9), the tumour cells were withdrawn from the peritoneal cavity of BDF₁ mice bearing P388 tumour on the 7th day of tumour transplantation and were collected in chilled normal saline. The contaminating erythrocytes were lysed by treating the cell pellet with cold Tris - NH₄Cl solution (5 ml), for 10 min (1). The cells were then washed twice with normal saline and suspended in Eagle's

MEM supplemented with 5% human AB serum to give a final concentration of 1×10^6 cells/ml. The cell count was done using haemocytometer.

The cell suspension was then exposed to different concentrations of acetylated oil of *S. anacardium* at 37°C in culture tubes. The control tubes with appropriate concentrations of Tween 80 and normal saline were run simultaneously to rule out the possible toxic effects of the vehicle.

At different time intervals, aliquots were withdrawn from the tubes, mixed with Trypan blue (0.1% in saline, 2-3 drops) and immediately observed under microscope using haemocytometer. The stained and unstained cells were counted to determine the percentage of non-viable cells. The cells which take up the stain represent non-viable cells; while the colourless cells are viable cells (9).

Experiments with radiolabelled precursors : Thymidine-methyl- ^3H (Sp. activity, 13 Ci/mmole), Uridine- ^3H (Sp. activity, 3.6 Ci/mmole), and Leucine- ^{14}C (Sp. activity, 1.6 Ci/mmole) were obtained from the Isotope Division, Bhabha Atomic Research Centre, Bombay.

P388 cell suspension (1×10^6 cells/ml in Eagle's MEM, supplemented with 5% human AB serum, was treated with different concentrations of acetyl derivative at 37°C in culture tubes. At the end of one hr, the radiolabelled precursor such as ^3H -thymidine (2 $\mu\text{Ci/ml}$), ^3H -uridine (2 $\mu\text{Ci/ml}$) or ^{14}C -leucine (5 $\mu\text{Ci/ml}$) was added and the cells were further incubated for one hr. The tubes were then chilled in ice-cold water and the radioactivity incorporated in 5% TCA-insoluble material was determined using modified filter paper disc radioassay (8). Five percent TCA-insoluble material gave incorporation of radiolabelled precursors into corresponding macromolecules such as DNA, RNA and protein.

The sequence in which the inhibition of macromolecular biosynthesis took place was also studied with respect to time. P388 cell suspension (1×10^6 cells/ml) was incubated with acetyl derivative (60 $\mu\text{g/ml}$) at 37°C. At time intervals of 15, 30, 45 and 60 min., aliquots of these cell suspensions were exposed to a short pulse of either ^3H -thymidine (1 $\mu\text{Ci/ml}$), ^3H -uridine (1 $\mu\text{Ci/ml}$) or ^{14}C -leucine (2 $\mu\text{Ci/ml}$). The incorporation of radiolabelled precursors into respective macromolecules was determined as described earlier (8).

RESULTS AND DISCUSSION

The orange-coloured oil (b.p.200-20°/2-3 mm) before and after acetylation exhibited 100% cytotoxicity within 5 hr upto a dose of 60 $\mu\text{g/ml}$; while at 15 $\mu\text{g/ml}$ concentration, no appreciable cytotoxicity was observed. This was assessed by the dye exclusion method, using 0.1% Trypan blue (9). The acetyl derivative (75 $\mu\text{g/ml}$), however, exhibited 80% cytotoxicity within 3 hr (Fig. 1). The microscopic studies showed that the treatment of P388 cells with acetylated oil resulted in the shrinkage of cells and nucleus and vacuolation of cytoplasm. The nuclei in the stained preparation showed very typical pycnotic degeneration as compared to the controls. Experiments with Tween 80 or saline alone did not exhibit and cytotoxicity.

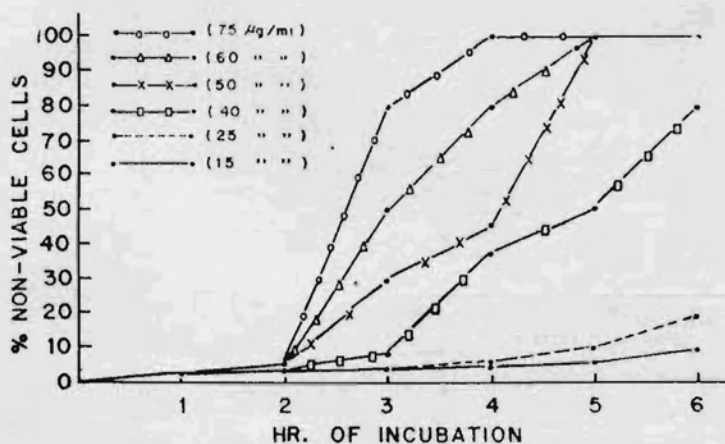


Fig. 1 : Cytotoxicity of acetylated oil of *S. anacardium* on P388 cells.

In vitro effects of acetyl derivative indicated that the incorporation of radiolabelled precursors into DNA, RNA and protein was considerably inhibited at a concentration ranging from 40-75 $\mu\text{g/ml}$ within 2 hr (Fig. 2). Moreover, at a concentration of 60 $\mu\text{g/ml}$, the inhibition of biosynthesis of DNA, RNA and protein, as compared to that of the controls was found to be significant ($P < 0.05$). Fifty percent inhibitory concentration (IC_{50}) values for DNA, RNA and protein were 27.5, 25 and 35 $\mu\text{g/ml}$ respectively. The acetyl derivative of orange-coloured oil did not show preferential inhibition of biosynthesis of any specific macromolecule.

The time course studies indicated that in the case of DNA, the inhibition appeared within 15 min; while in the case of RNA and protein, it took 30 to 45 min respectively. The acetylated oil hindered the entry of radiolabelled precursors into P388 tumour cells.

The total cellular uptakes of ^3H -thymidine, ^3H -uridine and ^{14}C -leucine were 70%, 75% and 79% respectively within 2 hr as compared to that of the untreated control tumour cells.

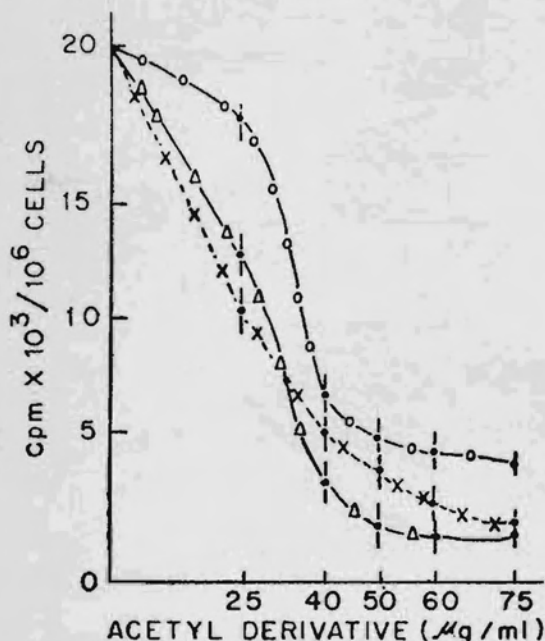


Fig. 2 : Effect of acetylated oil of *S. anacardium* on the incorporation of ^{14}C -leucine (.....○.....), ^3H -uridine (.....×.....) and ^3H -thymidine (.....△.....) into corresponding macromolecules after one hr.

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