

Biological Evaluation of Euphorbia Latexes in IRAN:

I. Tumour Producing Effect in Mice

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ABSTRACT

Eight euphorbia latexes of IRAN have been studied. All species had tumour promoting effect and four of them produced papillomas. No malignant tumours were observed.

INTRODUCTION

The Euphorbia or spurges are the largest genus of the family Euphorbiaceae. Most of these plants produce toxic latex which has a blistering, irritant, emetic and purgative action (1, 2). The latex causes dermatitis and severe irritation to mucous membranes and eyes. It has been proved that the skin irritant latexes of several species of Euphorbia exhibit carcinogenic activity when applied repeatedly on mouse skin with an initial dose of carcinogen agent (DMBA) (3). In this respect Euphorbia consists of a group of plants with considerable toxicological hazards, especially that over 75 different species of this genus has been identified in IRAN and they grow in large population in different part of country.

In this report 8 Euphorbia latexes of IRAN have been studied: Euphorbia bungi Boiss., Euphorbia lanata Sieb., Euphorbia tinctoria Boiss., Euphorbia virgata L., Euphorbia microsciadia Boiss. et Buhse., Euphorbia esula L., Euphorbia gerardiana Jacq., and Euphorbia larica Boiss.

MATERIALS AND METHODS

MICE: Male and female mice of the IRAN KZ (albino) outbred strain (availability: Taj Pahlavi Cancer Institute, IRAN) were used for all experiments. They were housed in groups of five in plastic cages. White wood shavings were used as bedding.

The mice were fed on cubed diet (obtained from Razi Institute IRAN) and water ad libitum.

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TECHNIQUE OF APPLICATION TO THE SKIN: The hair of the entire dorsal area from the root of the tail to nape of the neck was removed with electric clippers from all mice before the beginning of the experiments and thereafter at 2 to 3-weeks intervals. All test substances were applied to the skin from calibrated pipettes, care being taken to ensure that the solution spread evenly over the entire clipped area.

RECORDING OF TUMOURS: All mice were examined at weekly intervals for tumours of the skin and other organs. The skins of killed mice, together with those found dead, examined postmortem.

HISTOLOGY: Specimens of skin, skin tumours, and other tissues were examined histologically. Formol saline was used as a fixative and the sections were stained with hematoxylin and eosin.

CHEMICALS: The Euphorbia latices were collected from nonsucculent plants by cutting the stems, each stem provided no more than two drops of latex. Rubber gloves were worn to protect the hands during collection. The samples were deposited in a freezer prior to its use. Solution of 10% of latices in acetone was prepared and the solution was kept in dark at 4°C. All the latices on deposit form a semi-solid material and the whole samples were used for preparation of solution. Samples of the latices in acetone did not show any deterioration or colour changes during storage in dark and at 4°C. 9, 10-Dimethyl-1, 2-benzanthracene (DMBA) was obtained from Fluka AG., Switzerland and acetone from E. Merck.

RESULT

Three experiments have been undertaken; for each experiment 80 mice of both sexes in 8 groups were used. Each group consisted of five males and five females.

EXPERIMENT 1.

Test of the latex of 8 species of euphorbia for tumour producing activity.

Mice were clipped and 24 hours later they began to receive once-weekly applications of 0.5 ml acetone extract of Euphorbia latex. The concentration of the latex was held at 10% for the period of experiment (26 weeks). In first 3 weeks there was no macroscopic damage. During the 10 weeks period the first papillomas appeared. Papillomas began to appear on the backs of mice. The incidence of papillomas steadily increased, and by the 26th week 22 of 76 survivors were with papillomas (Figs. 1, 2, 3, 4). In 8 mice ulceration and crusting of the skin occurred. Eventually all the skin lesions healed, their place being taken by persistent hairless scars. Although animals kept



Fig. 1. Showing multiple papillomas during 8th week of treatment with extract of *Euphorbia virgata* L.

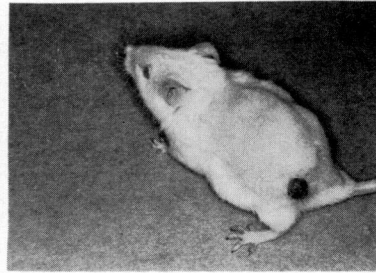


Fig. 2. Same group as Fig. 1 Showing single papilloma.

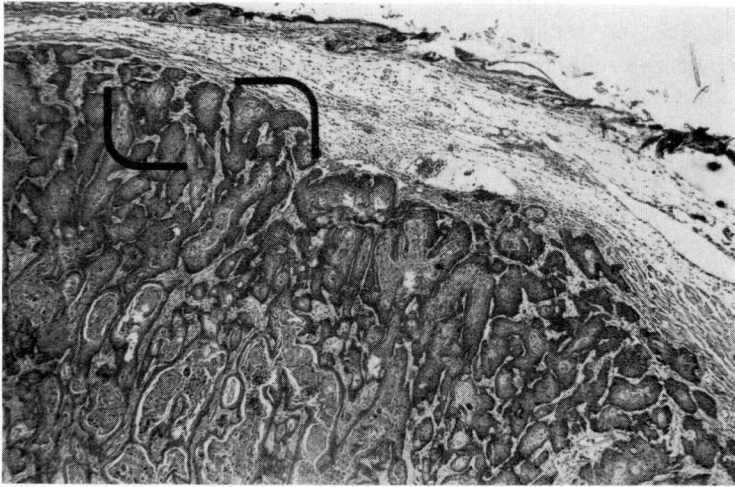


Fig. 3. Micrograph shows skin papilloma on a mouse, same animal as Fig. 1 H & E. $\times 75$

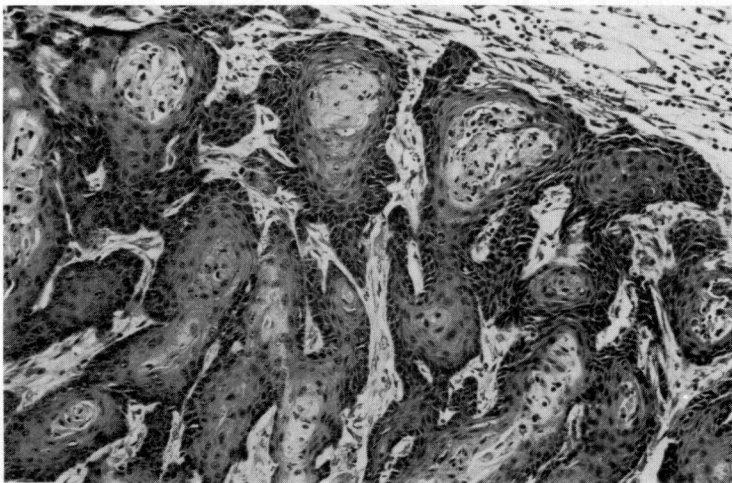


Fig. 4. Enlargement of the marked area in Fig. 3. H & E. $\times 300$

Table 1. *Tumour production by latex of 4 species of euphorbia*

Euphorbia	No. of animals with tumours				
	10th week	14th week	18th week	22nd week	26th week
tinctoria Boiss	1	2	2	3	3
virgata L.	5	8	9	9	9
microsciadia Boiss	2	4	4	4	4
geradiana Jacq	1	3	3	5	6

Once weekly applications of 0.5 ml each. For details see text

untill 40 weeks, no malignant skin tumours were seen in this experiment. The result of this experiment indicated clearly that the latex of Euphorbia (species: Euphorbia tinctoria Boiss., Euphorbia virgata L., Euphorbia microsciadia Boiss., and Euphorbia geradiana Jacq.) contain a tumour-producing substance (Table 1).

EXPERIMENT II

Test of the latex of 8 species of Euphorbia for tumour promoting activity.

Mice were clipped and 24 hours later they received a single application of 300 μ g DMBA in 0.5 ml acetone to the clipped dorsal skin on the first day of the experiment. Three weeks later they began to receive once weekly application of 0.5 ml of the extracts of the latices. Papillomas appeared on the backs of mice during the 8th week of treatment with extract. The incidence of papillomas (mainly multiples) steadily increased and by the 26th week it was 60 of the 74 survivors. The results of this experiment have proved the work of Roe and Peirce (1960) and indicated clearly that the latex of Euphorbia contains a potent tumour-promoting substance.

EXPERIMENT III

Test of a single dose (300 μ g) DMBA for tumour producing activity.

Mice were clipped and 24 hours later they received a single application of 300 μ g DMBA in 0.5 ml acetone to the clipped dorsal skin on the first day of the experiment. There was seen only one animal with papillomas of the 79 survivors during the 26th weeks.

CONTROL BATCH

Eighty mice in 8 groups were used. Each group consisted of five males and five females. Mice were only shaved at the beginning of the experiments and they were examined thereafter at 2 to 3-weeks intervals. No papillomas

were seen in this batch of animals.

DISCUSSION

The latices of genus Euphorbia contain irritant and tumor promoting substances. These compounds are chemically related to tetracyclic diterpenes (4-8). A wide range of tumor producing and promoting activity of acetone extracts of 8 Euphorbia latices of Iran was observed. Acetone extracts of 4 out of 8 species produced 22 papillomas in 80 mice for up to 26 weeks. Acetone extracts of all 8 Euphorbia latices elicited 60 skin tumors in 80 mice pretreated with DMBA, whereas 80 mice treated with the same dose of DMBA only developed only one papilloma.

No malignant tumors were seen within 40 weeks in the experiments reported. For biological testing of different active principle of latices of 4 producing tumor species of Euphorbia a series of experiments have been undertaken, the results will be reported in the near future.

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