SHORT COMMUNICATIONS

THE OESTROGENIC ACTIVITY OF POMEGRANATE SEED OIL

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(Received 30 September 1963)

In view of the widespread use of pomegranate seeds for therapeutic purposes it was decided to study the oestrogenic activity of pomegranate seed oil, which has not been investigated previously.

The oestrogenic effect of the oil was tested in immature rabbits by the uterine weight method and in ovariectomized mice by the vaginal smear technique. The results were evaluated by the method of Robson (1938). Immature rabbits were injected with the pomegranate seed oil daily for 10 days, killed without anaesthesia and their uteri weighed. Mature albino mice, weighing about 35 g, were ovariectomized, injected with the oil in the morning and evening of two consecutive days. Four smears were taken: the first in the evening of the day after the last injection, the second and third in the morning and evening of the day after, and the 4th in the morning of the following day. Smears were regarded as positive when epithelial cornification and a decrease in the number of leucocytes could be demonstrated. The oestrogenic action of the pomegranate oil was compared with that of a standard dose of oestradiol previously assayed in different groups of mice and rabbits.

The oil was prepared by exhaustive extraction of the powdered pomegranate seeds with petroleum ether (50–75°). The petroleum ether extract was evaporated to dryness under reduced pressure and the residual yellowish oil was heated in a vacuum oven at a temperature not exceeding 60° until a constant weight was obtained. The yield of the yellowish fixed oil obtained amounted to about 20% of the dry weight of the seeds. To obtain the unsaponifiable fraction, 50 g. of the oil were dissolved in 300 ml. ethanol. This solution was mixed with a solution of 50 g. of potassium hydroxide in 50 ml. water and the mixture was refluxed on a boiling water bath for about 3 hr. The solution was then evaporated to two-thirds of its bulk and water was added in excess. The soap solution was repeatedly shaken in a separating funnel with fresh portions of ether until complete extraction was effected. The combined ethereal extracts and washings were washed with water until free from alkalinity as indicated by litmus paper, dried over anhydrous sodium sulphate and finally evaporated to dryness in vacuo. This procedure yielded 1.5 g. of unsaponifiable matter as a yellow oil mixed with crystals.

When pomegranate seed oil was injected in doses of 0.2–0.4 ml. into ovariectomized mice twice daily for 2 days, an oestrogenic effect, demonstrated by cornification of vaginal cells, was obtained. To verify this effect, the oestrogenic action of the
Robson, Sharaf, Cheng, drinum studied soya contents of that i.e. granate Uterine controls oil Oestrogenic Table 0-5 Rabbits Rabbits Rabbits Rabbits Rabbits Control oil was also tested in immature rabbits injected daily with 0.5 ml. of the oil for 10 days. Uterine development in the injected rabbits was greater than in the non-treated controls (Tables 1 and 2). When the oestrogenic activity of various doses of pomegranate seed oil was compared with that of 0.1 µg. of the naturally occurring hormone, i.e. oestradiol in ovariectomized mice, the effect of 0.4 ml. of the oil corresponded to that of 0.1 µg. of oestradiol. However, in immature rabbits the oestrogenic activity of 0.5 ml. of the oil was almost the same as that of 10 µg. oestradiol. Further experiments showed a synergism between oestradiol and the pomegranate seed oil.

Table 2 shows that only the unsaponifiable fraction had oestrogenic activity.

Oestrogenic activity has also been reported for other plants, for example alfalfa and soya beans (Cheng, Story, Payne, Yoder & Burrough, 1953) and Trifolium alexandrinum (Sharaf & Fadle, 1962).

The properties of the unsaponifiable fractions of pomegranate seed oil are being studied with a view to oral administration in clinical tests.

REFERENCES