

Effect of *Lithospermum ruderale* on Oestrous Cycle of the Rat.* (17666)

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In 1941 a treatise by the United States Department of Agriculture on the medicinal uses of plants by Indian tribes of Nevada(1), contained the following annotation on *Lithospermum ruderale*. "In one settlement, it is believed that the plant has contraceptive properties, and that a cold water infusion of the roots, taken daily for a period of 6 months, will induce sterility thereafter." Alcohol extracts of lithospermum administered to mice, in their diet, inhibit or abolish the normal oestrous cycle and reduce the incidence of birth.(2-4) Further work has shown that the gonadotrophic content of pituitaries of mice on a lithosperm diet was reduced, and the conclusion was drawn that the primary action of the drug was a depression of pituitary activity(5,6). However, more extensive experiments have not supported this conclusion(7,8). When work on lithosperm began in our laboratory 2 years ago, the roots and remainder of the plant (tops) were dried and

Effect of Diet Containing 20% Lithosperm Root on Estrous Cycle of Rat

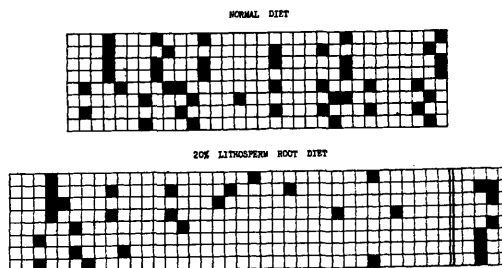


Fig. 1

Double line indicates return to normal diet; black squares represent vaginal cornification.

ground separately. Experiments were then carried out as follows.

Oral administration to rats. Eight adult Sprague Dawley female rats were placed on an *ad lib.* diet of Purina fox chow for 36 days. Following this the diet was changed to one consisting of 20% dry lithosperm roots and 80% Purina chow, for an additional 36 days. The oestrous cycle of these animals was followed throughout by daily vaginal smears and the results of this experiment may be seen in Fig. 1. The experiment was then repeated using lithosperm tops, in concentrations of 20% and 25% of the diet. However, the effect of tops on the oestrous cycle was negligible.

About one year later part of the experiment was repeated, using a water extract of the original dried root. This extract was prepared by extracting twice with cold water, and finally with water at 90°C. An amount of extract equivalent to 200 mg of dried root, was fed daily by stomach tube for 10 days to 5 adult female rats, and no effect was noted on the oestrous cycles. The dose was then doubled to an equivalent of 400 mg/day, and a questionable inhibition of oestrous cycles was obtained.

Other feeding experiments have been carried out, using a lyophilized water extract of lithosperm, kindly supplied by Ayerst, Mc-

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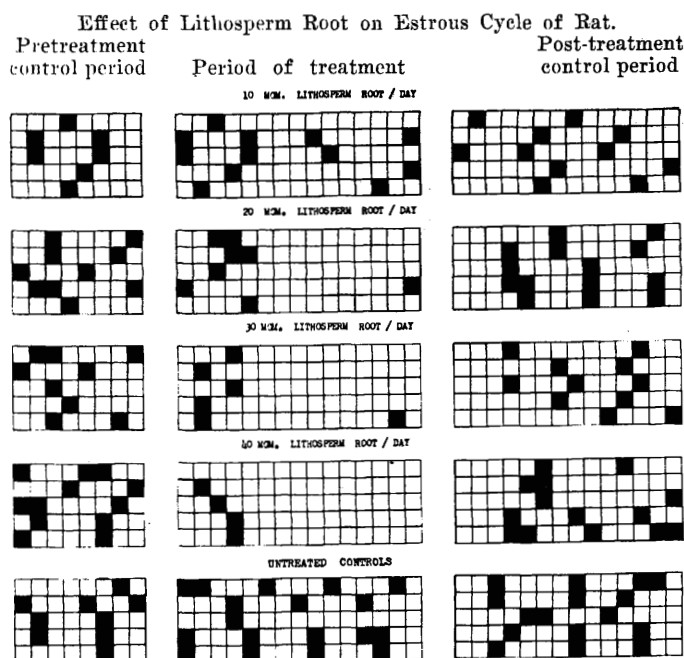


Fig. 2

Kenna and Harrison of Montreal. This extract again was roots and tops, and represented about a ten-fold concentration of the crude root, and a 3-fold concentration of the tops of the plant. Daily tube feeding of 400 mg of lyophilized root extract did not appear to produce any inhibition of the oestrous cycles, nor did doubling this amount have any effect. In various feeding experiments some reduction in weight gain may be noted. This is usually transitory and of a small magnitude. In control experiments considerably greater weight loss may be induced without interruption of the oestrous cycle.

Subcutaneous injection of extracts. Extensive work has now been done which indicates that the effects of subcutaneous injection of lithosperm extracts are much greater than those following oral administration. A typical experiment is as follows.

Daily vaginal smears were done on 5 groups of 5 Sprague Dawley female rats for 37 days. During the first 8 days no treatment was given. For the 2nd period of 15 days, the first 4 groups of rats received 10 mg, 20 mg, 30 mg and 40 mg respectively of lyophilized extract of lithosperm roots. The 5th groups received

no treatment. During the remaining period of 14 days no injections were given. It may be seen in Fig. 2 that 10 mg had only slight effect on the oestrous cycle, but that the larger doses produced almost complete anoes-trous, this effect appearing after 3 or 4 days. Extracts of tops, in comparable experiments, though somewhat less active, showed the same inhibitory effect.

Effect of Oestradiol on Lithosperm Treated Rats. Sixteen bilaterally ovariectomized adults were injected daily for 3 days with a total dose of 1 μ g of oestradiol. Eight of these rats were injected subcutaneously daily with 80 mg of lithosperm root, starting the day prior to oestrogen and continuing throughout the experiment. Daily vaginal smears showed that 7 of the 8 lithosperm treated animals came into oestrous, while all the controls showed vaginal cornification. This experiment supports Cranston's original observation, that lithosperm does not inhibit the action of oestrogen.

Oestrogenic Activity of Lithosperm. The presence of a water soluble oestrogen has been noted during the course of various experiments with lithosperm extracts. It is more abun-

dant in the tops than the roots, but both preparations may be shown to be oestrogenic by direct application to the vaginas of ovariectomized rats. The oestrogen content varied from one extract to another, but 200 mg of lyophilized tops may contain up to 1 γ equivalent of stilboestrol, when assayed on the uterine weights of immature rats. The oestrogen content of lithosperm is a factor which may influence the gonadotrophic content of the pituitaries of treated animals and throws some doubt on the interpretation of previously published assays.

Summary. It has been shown that *Lithospermum ruderale* has an anti-oestrous effect on rats, the roots containing more active material than the tops. The subcutaneous injection of extracts of the plant is at least 10 times more effective than oral administration. Lithosperm does not inhibit the action of administered oestradiol. Experiments indicate that lithosperm contains a water soluble oestrogen which is found primarily in the tops.

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Value of Daphnia as an Assay Organism for Veratrum Alkaloids. (17667)

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Veratrum plants contain an unknown number of alkaloids of apparently similar but not identical physiological action, which are not amenable to chemical assay. The availability of a biological testing method would, therefore, be of decided advantage. Recently, *Daphnia magna* has been extensively publicized as a testing object. Daphnia have been recommended before for the assay of cardiotonic drugs(1). Concerning the adaptation of the test for Veratrum, the only information available is the statement that the criterion of potency consists in the arrest of the heart of *Daphnia magna*. It is evident that this possibly measures a toxic effect not necessarily proportional to the therapeutic value of the material. Another point is that any analogy between Daphnia and human being cannot be postulated. For the present study we prepared jervine, veratrosine, pseudojervine and veratramine following the methods of Jacobs and Craig(2). Having difficulty in obtaining *Daphnia magna*, our first experiments were performed with a non-identified Daphnia strain obtained from a New Jersey pond; later we repeated the work with *Daph-*

nia magna.

To make these tests, a capillary tube, bent slightly downward at the end, is fixed with Scotch tape to an object carrier with a ground hollow in such a way that the tip rests at the edge of the hollow. A small piece of absorbent cotton is placed into the hollow without touching the capillary. The latter is connected to a suction pump. Daphnias are caught with a wide-tipped medicinal dropper and placed in the center of the cotton bed. The excess of water is promptly removed by the suction but sufficient liquid remains in the cotton to cover the animals caught between the fibers, provided the capillary does not touch the cotton. It is feasible to observe 4 or 5 small Daphnias at a time but only one or 2 of the *magna* species. The medication, in proper dilution, is then dropped from a pipette on the cotton until the water is replaced by the solution. From this moment on the time of action is counted. Every 3 to 4 minutes one-half cc of the solution is flown through the cotton to prevent a change in concentration by evaporation. The normal heart rate of the Daphnia ranges from 250 to 600 beats per minute(1), which is beyond the possibility of counting. Under the influence of the alkaloids the rate

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