Oestrogenic activity of enoxolone in rodents

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In searching for an active plant oestrogen Sharaf et al (1975) claimed that they were able to separate glycyrrhetic acid from liquorice (*Glycyrrhiza glabra*) by mineral acid hydrolysis of the natural glycyrrhizin from liquorice root and to identify glycyrrhetic acid as an active oestrogen. However, material so obtained, and also commercially available glycyrrhetic acid may contain up to 30% of impurities. We have examined the effects of a purified preparation on the testes and on the accessory sexual structures of both female mice and male rats. All experiments were performed on female albino mice and Biorex Wistar male rats of a single strain bred in these laboratories, ten or more animals being used in each vehicle control, positive control and test group. The animals, housed under animal house conditions about 60% relative humidity and at 22° C in rooms providing alternating 14 h light and 10 h darkness, had free access to Dixon's FFG (M) diet and water. Enoxolone (glycyrrhetic acid, mol. wt 470·67) was obtained by hydrolysis of carbenoxolone sodium (enoxolone hemisuccinate disodium salt) using molar aqueous sodium hydroxide solution at 50° C for 2 h followed by recrystallization from aqueous acetone until purity of better than 99% was achieved (t.l.c., g.l.c.). The resultant enoxolone was crystallized from aqueous ethanol m.p. 283–287° C *lit.* m.p. 283–303° C and was better than 99% pure by t.l.c. The methyl ester, formed quantitatively by the action of diazomethane, also was >99% purity (g.l.c.).

Reference oestrogens (oestradiol-17β mol. wt 272·27, ethinyloestradiol, mol. wt 296·39) and enoxolone were dissolved in a vehicle of 8 parts of redistilled isopropyl myristate and 2 parts of ethanol and were administered subcutaneously or orally by a stainless steel stomach tube in a volume of 0·1 ml (mice) or 0·2 ml (rats).

The uterine weight response in mice was not effected by enoxolone given to immature albino mice, 8–10 g (fed by one foster mother per group of 10) for 3 consecutive days by either subcutaneous (s.c.) or oral routes at doses up to 0·1 g kg^{-1}, s.c. and 0·2 g kg^{-1}, orally which were 40 000 and 80 000 times the doses of ethinyloestradiol required to produce an appreciable (200–400%) dose-related increase in uterine weight (2·5 and 5·0 μg kg^{-1}).

Enoxolone, in doses of up to 64 mg kg^{-1}, s.c. (8000 times the ED50 of oestradiol-17β) was without oestrogenic activity in ovariectomized mice each 25 g in groups of 12 in the more specific vaginal cornification test made after priming the animals with 1 μg oestradiol -17β one week before being given the test compound in a single subcutaneous injection.

In male rats (each 150 g, groups of 10) given enoxolone at oral doses (16 and 32 mg kg^{-1}), which were 10 and 20 times, the effective dose of ethinyloestradiol (1·6 mg kg^{-1}) showed no suppressive or stimulating effects on the weights and sizes of accessory sexual structures and testes which remained similar to those of vehicle control animals. Thus enoxolone has no oestrogenic, androgenic or anti-androgenic activity in three standard tests. The uterotrophic test is not necessarily specific for oestrogens as both androgens and progesterone may also increase uterine weight but the more specific vaginal cornification test in ovariectomized mice was confirmatory and in intact male animals there was no androgenic or anti-androgenic activity.

The reason for the discrepancy of these results with those of other authors would seem to be due at least, in part, to the purity of the glycyrrhetic acid used.

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