Oestrogenic and Pregnancy Interceptory Efficacy of a Flavonois Mixture from Grangea maderaspatana Poir (Artemisia maderaspatana) in the Mouse

Dr. Sunita Jain

(Department of Chemistry, S P N K S (P.G) College, Dausa, 303303

A mixture of flavonoids extracted from the plant Grangea maderaspatana possessed oestrogenicity and antiimplantational activities in the mouse. In the **3** day uterotrophic bioassay, administration of the drug at a dose of 20mg/kg body weight per day, intramuscularly to ovariectomized females, resulted in a highly significant $(p < 0.001)$ increase in the wet uterine and vaginal weights. However, in comparison with conjugated oestrogen, the extract proved to be mildly oestrogenic. Flavonoids, if administered orally at the same dose level effectively interfere with all stages of pregnancy. Maximum interceptory efficacy was recorded when the drug was administered from days 4-6 post coitum. However, there was a reduction in antinidational activity only if the drug was administered from days $1-3$ and $7-9$ post coitum.

Keywords: Grangea; oestrogenicity; pregnancy interception; flavonoids

INTRODUCTION

Grangea maderaspatana Poir is a small herb of the family Compositae. The extract of aerial parts of the plant contains a mixture of flavones, which are reported to possess antifertility activity (Nagarajan and Parmar, 1977). Flavonoids are similar to oestrogen in their basic structure, i.e. presence of benzopyrone ring (Sharma et al., 1971). The present investigation of *Grangea maderaspatana*, which contains a mixture of flavones, attempts to examine the hormonal profile and the post-coital antifertility activity during various stages of pregnancy of the mouse.

MATERIAL AND METHODS

Extraction of test material. The air-dried aerial parts (1 kg, supplied by United Chemical and Allied Products, Calcutta, India) were extracted with Et_2O -petrol-MeOH ($1:1:1$) at room temperature for 24 h . Evaporation of the solvent at reduced pressure furnished a greenish semi-solid mass which was dissolved in 200 mL MeOH and left overnight in the refrigerator. It was then filtered, and the filtrate was concentrated and chromatographed over silica gel. The elution was carried out with solvents in order of their increasing polarity. Eluants were collected in fractions of $70-80$ mL each and evaporated to dryness under vacuum. Fractions which showed similar TLC behaviour were pooled. A fraction collected by eluting $\text{C}_6\text{H}_6-\text{CH}_2\text{Cl}_2-\text{Et}_2\text{O}\ (1:1:1)$ yielded 65 mg of a yellow mass which contained a

mixture of highly oxygenated flavonoids. This mixture was then subjected to oestrogenicity and antifertility tests.

Animal model. Adult parous nonpregnant female Swiss albino mice were bilaterally ovariectomized and used 10 days after operation. Animals were fed with mouse food pellets (Lipton India Ltd., India) and water was provided *ad libitum.*

Oestrogenic and antioestrogenic bioassay. An oestrogenic bioassay (Rubin et al., 1951) was performed. In this assay, 10 -day bilaterally ovariectomized mice were used. The extract was orally administered at a dose of 20mg/kg body weight/mouse for 3 consecutive days. Conjugated oestrogen was injected at a dose of $0.20\mu\ \text{g/kg}$ body weight/mouse for 3 consecutive days to another group of animals.

In the antioestrogenic bioassay animals were treated simultaneously with 20mg/kg body weight/mouse of flavinoids and $0.20\mu\ \text{g/kg}$ body weight/mouse of conjugated oestrogen for 3 consecutive days. Suitable controls receiving olive oil were maintained throughout. For both the above assays animals were killed 24 h after the last dose administration. The wet uterine and vaginal weights were recorded and statistically evaluated by Student's t-test (Swinscow, 1985). The uteri were fixed in Bouin's fluid and processed for histological study.

Post-coital antifertility activity. In this test five groups of adult parous female mice were taken and mated with males of proven fertility. Mating was confirmed by the presence of a vaginal plug or spermatozoa in the vaginal lavage. The mixture of flavonoids at a dose of 20mg/kg body weight/day with 0.2 mL olive oil as vehicle, was given orally as follows: Group I: control (0.2 mL vehicle alone); Group II: flavonoids from days 1-13 post coitum; Group III: flavonoids from days 4-6 post coitum; Group IV: flavonoids from days $7-9$ post coitum; Group V: flavonoids from days $1-7$ post coitum.

The animals of control and experimental groups were autopsied on day 12 post coitum and the number of implantation and resorption sites, if any, were recorded.

TABLE 1: Effect on uterine and vaginal weights of ovariectomized mice treated daily with flavonoids of *Grangea maderaspatana* Poir, conjugated oestrogen and their combination for 3 days.

Treatment	Mean weight of the uterus (mg/100 g body weight ± SEM)	Mean weight of the vagina (mg/100 g body weight ± SEM)
Control	56.00 ± 1.73	12.00 ± 0.57
Conjugated oestrogen (0.20 µg/kg body weight/day)	226.25 ± 2.91 [a]	29.00 ± 1.72 [a]
Flavonoids	92.33 ± 2.02 [a]	16.00 ± 1.52

(20 mg/kg body weight/day)		
Flavonoids (20 mg/kg body weight/day) + Conjugated oestrogen (0.20 µg/kg body weight/day)	216.16 ± 3.65 [a]	21.33 ± 1.33 [a]

Significance in relation to control: [a] $p<0.001$

RESULTS

Organ weights

Administration of conjugated oestrogen caused a highly significant ($p < 0.001$) increase in uterine and vaginal weights, this increase was four-fold compared with the vehicle treated ovariectomized animals. Administration of flavonoids also resulted in a highly significant increase $(p < 0.001)$ in the mean uterine weight. However, the increase in vaginal weight was not statistically significant. This increase was three times less than the increase in organ weights of animals treated with oestrogen alone. When flavonoids were administered in combination with conjugated oestrogen, there was a statistically insignificant decrease in the mean uterine and vaginal weight of the treated mice compared with the organ values of the conjugated oestrogen alone treated animals (Table 1).

Histology

Administration of flavonoids alone caused a marked stimulatory change in the atrophic uterus and vagina of the ovariectomized mouse. There was a general increase in the diameter of the uterine horns. The number of uterine glands increased and the endometrial vascularity moderately enhanced. Treatment with conjugated oestrogen also caused hypertrophy of the uterus. The muscularis of the uterine tissue was greatly increased. The vagina was also markedly stimulated, and the vaginal lumen was found to be filled with cornified cells. Oestrogen and flavonoids combined therapy does not appreciably alter the histological profile of the uterus and vagina. The histoarchitecture of both the organs seemed comparable to that of the group treated with oestrogen alone.

Post-coital antifertility activity

As is evident from Table 2 oral administration of flavonoids from days 4-6 post coitum and days 1-7 post coitum proved to be most potent (80%) in preventing nidation. Treatment from days $1-3$ post coitum resulted in 60% antiimplantational activity, whereas only 40% of antiimplantational activity was recorded at the postimplantational stage of pregnancy, i.e. from days $7-9$ of pregnancy.

Table 2. Post-coital antifertility efficacy of flavonoids of *Grangea maderaspatana* Poir at a dose of 20mg/kg body weight/day

Treatment given orally on days of pregnancy (post coitum)	Number of animals	Autopsy on day 12 post coitum		Number of females delivered	Number of litters	Inhibition of pregnancy (%)
		Fetus	Resorption sites			
Control	5	28	2	5	28	0
1-3	5	3	0	2	3	60
4-6	5	1	0	1	1	80
7-9	5	7	11	3	7	40
1-7	5	1	3	1	1	80

DISCUSSION

It is well known that the mammalian uterus is an ideal target organ for the measurement of oestrogenicity of a substance. After administration of oestrogen the atrophic uterus of the ovariectomized female was rapidly converted into an actively growing structure (Muller *et al*., 1958). Thus, after the administration of conjugated oestrogen to an ovariectomized mouse in the present investigation there was a marked hypertrophy and hyperemia of the uterine tissue as evidenced by an increase in the uterine weight and histology.

Simultaneous administration of flavonoids and the conjugated oestrogen resulted in a decline in the uterine weight compared with the organ weight of females treated with conjugated oestrogen alone. This observation essentially simulates the effect produced by concurrent administration of flavonoids, a potent post-coital antifertility substance, and a standard oestrogen in mice. Thus the so called “antioestrogenic” effect produced is explained on the basis of competitive inhibition between a weak and strong oestrogen at the level of receptor sites of oestrogen in the uterus (Jacob and Morris, 1969) which resulted in inhibition of the uterine response as observed by organ weight depression and histology of the uterine tissue.

Administration of flavonoids from days 4-6 post coitum produced a maximum post-coital antifertility effect. An identical maximal response was produced from days 1-7 of pregnancy. Thus, the 20 mg/kg body weight dose when administered either during days 4-6 post coitum or days 1-7 post coitum resulted in an 80% antinidational effect. These results more or less concur with the findings of Singh et al. (1990) who also reported 80% antiimplantational activity in the rat after treatment with wogonin at a dose of 10 mg/kg body weight from days 4-6 post coitum. The post-coital antifertility effect of flavonoids during different stages of pregnancy appeared to be due to its inherent oestrogenic nature. Thus, in the present investigation inhibition of nidation during various stages of pregnancy may be due either to retention of ova in the oviducts (Burdick and Pincus, 1935) or due to the hastening of the passage of ova through uterine tubes and the uterus (Greenwals, 1959).

Administration of flavonoids at the post-implantational stage resulted in only 40% inhibition of implantation. This result may be due to the interference of flavonoids at the developmental stage of the embryo, resulting in resorption of fetuses by the uterine wall (Pincus, 1965). The oestrogen antagonistic effect produced by flavonoids thus further supports the contention that this plant preparation has a definite but mild oestrogenic nature and, therefore, offers itself as a promising material for further research in pregnancy interception.

REFERENCES

Burdick, H. E. and Pincus, G. (1935). The effect of oestrin injections upon the developing ova of mice and rabbits. *Am. J. Physiol.* **111**, 201-207.

Greenwald, G. S. (1959). The comparative effectiveness of estrogens in interrupting pregnancy in the rabbit. *Fertil. Steril.* **10**, 155-161.

Jacob, D., and Morris, J. M. (1969). The oestrogenic activity of post coital antifertility compounds. *Fertil. Steril.* **20**, 211-220.

Mueller, G. C., Ailene, M. H., and Kristian, H. J. (1958). Mechanism of action of estrogen. *Recent Prog. Horm. Res.* **14**, 95-139.

Nagarajan, G. R., and Parmer, V. S. (1977). Flavonoids of *Prunus cerasus. Planta Med.* **32**,50-53.