Wogonin, Wogonin, 5,7-dihydroxy-8-methoxyflavone as Oestrogenic and Anti-implantational Agent in the Rat

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Wogonin, 5,7-dihydroxy-8-methoxyflavone, extracted from Holmskioldia sanguinen Retz. has been tested for oestrogenic and anti-implantational activities in the rat. Wogonin displayed mild oestrogenic but effective anti-implantational activities. In the three days uterotrophic bioassay, at a dose level of 10 mg/kg body weight per day, intramuscularly, the wet uterine and vaginal weights increased significantly. Wogonin at the xame dose level when given intramuscularly from days 1-3, 4-6, 1-7 post coitum and orally from days 1-7 pou coitum effectively inhibited implantation. However, when the drug was administered from days 7-9 post coitum, it did not affect the implantation but interfered with normal development of the embryos which resulted in the resorption of foetuses. Wogonin. mild oestrogenic compound, exhibited interceptory activity at all the stages of pregnancy.

Keywords: Holmskioldia; flavone; wogonin; antifertility; anti-implantation in the rat.

INTRODUCTION

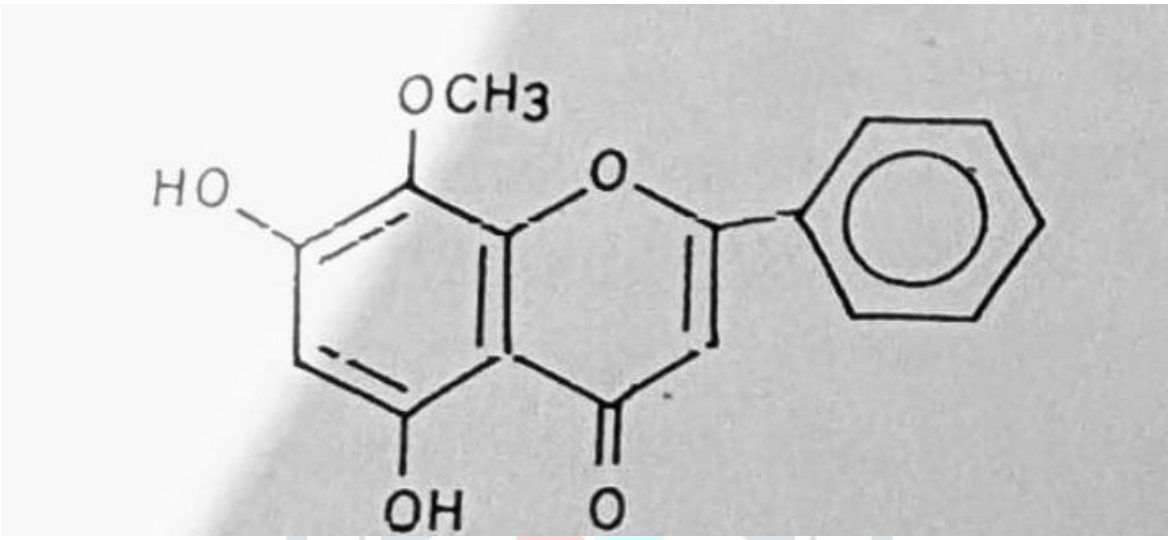
Holmskioldia sanguinen Retz, a struggling shrub, 10-30 feet in height, is distributed in subtropical and Himalayan regions from Kumaon to Bhutan. It belongs to the family Verbenaceae. This is the only species of the genus Holmskioldia growing in India. A survey of the literature indicated that no chemical and biological work has been reported on this plant. We have isolated a number of compounds from this plant including "wogonin' (Joshi et al., 1983).

Many flavonoids and oestrogens which display antifertility activity are similar in their basic structures, i.e., presence of benzopyrone ring chromone and an bydroxy group on C-7 (Blye and Homm, 1967; Greenwald, 1966; Jones and Pope, 1961: Nagarajan and Parmar, 1977; Sanyal, 1960, Seshadri et al., 1981 and Sharma et al., 1971). In view of wogonin being a flavonoid the present investigation was undertaken to assess its oestrogenic potential and post coital contraceptive activity in the rat.

MATERIALS AND METHODS

Extraction and isolation of test material. Shrub Holmskioldia sanguinea Retz. was obtained from United Chemicals and Allied Products, Calcutta, India. Air dried and coarsely powdered plant material (2.5 kg) was extracted with benzene over a steam bath for 3x12h. The whole plant was used for this extraction. The solvent was removed under reduced pressure, and the concentrated extract was column.

chromatographed over deactivated silica gel. Elution was carried out with solvents of increasing polarity. Eluants were collected in fractions of 70-80 mL. cach and evaporated to dryness under reduced pressure. Fractions exhibiting similar TLC behavior were mixed. The fraction collected by cluting with ethyl acetate (100%) afforded a yellow mass of 7g after evaporation of the solvent. It was found to be homogeneous on TLC and was crystallized from ethyl acetate benzene (1:1) mixture giving yellow needle-shaped crystals (Geissman, 1962), melting point (mp) 203-204 "C (reported mp 203 °C) of wogonin.



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Animal model. Colony bred adult female rats of the Sprague Dawley strain were used. They were maintained in uniform husbandry conditions te.. temperature 25±2°C and a relative humidity of 50-60%. Animals were kept in plastic cages measuring 12"x10"x8", with three animals per cage. Animals were ted with rat feed pellets (Hindustan Lever Ltd., India) and water was provided ad libitum.

Hormone bioassay. For the oestrogen bioassay (Emmens. 1950), bilaterally ovariectomized rats were used on the 8th day following the operation. They were randomized into three groups of five animals each and treated daily for three days as follows: group 1 vehicle, olive oil (0.2mL/animal); group 2 conjugated oestrogen (0.10 µg/kg body wt); group 3 wogonin (10 mg/kgbody wt). Animals were killed 24 h after the last administration of the drug. Uterus and vagina were dissected out, freed from adherent tissue, and weighed to the nearest milligram. Uterine tissues were fixed in Bouin's fluid for histology.

Post coital antifertility activity. For assessment of post coital antifertility efficacy, six groups of five females each were taken and mated with coveal male rats of proven fertility. The day on which the vaginal smear showed the presence of spermatozoa was considered to be day 1 of pregnancy. Wogonin at 10 mg/kg body wt per day, with 0.2 mL olive oil as vehicle, was given as follows:

Group 1, control (vehicle alone),
Group 2, wogonin from days 1–3 post coitum i.m.,
Group 3, wogonin from days 4–6 post coitum, i.m.,
Group 4, wogonin from days 1–7 post coitum i.m.,

Group 5, wogonin from days 1–7 post coitum, oral.

The animals were laparotomized on day 10 of gestation and the number of implantation sites was recorded. The rats were then allowed to deliver normally in term and the morphology and number of litters delivered were observed (Morris et al., 1967a).

Statistical analysis. Data are expressed as mean ± SEM and were analyzed for statistical difference by using Student's t-test (Swinscow, 1985).

RESULTS

Weight response. Wogonin (10 mg/kg body wt per day for 3 days) caused a significant ($p < 0.001$) increase in wet uterine weight, while the increase is less significant in the case of the vagina ($p < 0.02$) of ovariectomized rats, when compared with vehicle treated ovariectomized animals. The wet weight of the uterus of conjugated oestrogen treated (0.10 µg/kg body wt per day for 3 days) animals was more than that of wogonin treated animals ($p < 0.01$). The weight of the vagina of oestrogen treated animals remained the same as that of wogonin treated animals. (Table 1)

Table 1 **Change in uterine and vaginal weights following the admistration of a conjugated oestrogen and wogonin in ovariectomized rats**

Teatment	Weight of uterus	Weight of vagina
Control (vehicle[a] alone)	120 ± 2.96	47 ± 0.88
Conjugated oestrogen (0.10mg/kg body wt per day i.m. for 3 days	251 ± 3.71	57 ± 2.02
Wogonin oestrogen (10mg/kg body wt per day i.m. for 3 days)	232 ± 3.71 [b,c]	56 ± 0.88[d]

a	Olive oil was used as a vehicle.
b	p <0.001 compared with control group.
c	p < 0.01 compared with conjugated oestrogen treated animals.
d	p < 0.02 compared with control group. (All values expressed as mean ± SE)

Post-coital antifertility activity. Table 2 shows that wogonin possessed maximum anti-implantation activity (100%) in the animals treated from days 1-7 post coitum im. or oral. Treatment from days 1-3 and 4-6 post coitum also inhibited implantation (80%). Treatment from the post implantation period (days 7-9 post costum) also inhibited the fertility of female rats (60%) by interfering with the development of embryos and resulted in the resorption of foetuses by the uterine wall.

DISCUSSION

Hyperemia of the uterus has been reported after oestrogen administration (Cole, 1950) Oestrogen therapy to ovariectomized rats induces an increase in the weight of the uterus due to an increase in both water and total solid content (Astwood, 1938). Administration of wogonin, a flavone, induced an increase in the weight of the uterus of ovariectomized rats, which indicated an oestrogenic effect, histological observations also support this conclusion because oestrogen stimulates and enlarges the endometrial and myometrial elements of the uterus (Lerner et al., 1966), Wogonin was found to exhibit mild oestrogenic effects when compared with conjugated oestrogen.

The hypothalamus, anterior pituitary, ovary, oviduct, uterus and vagina are the areas where substances exhibiting antifertility activity may exert their action(s). A drug may exert its antifertility effect in more than one of these areas and not necessarily by the same pharmacological mechanism (Farnsworth et al., 1975). The post coital antifertility effects of wogonin during different stages of gestation appear to be due to its action on several such areas to be due to its action on several such areas and due to its oestrogenic nature, because oestrogenic nature, because oestrogen is known to accelerate the movement of ova through the oviduct (Morris and van Wagenen, 1966). In our study, treatment from days 1-3 post coitum which resulted in inhibition of implantation may be due to the acceleration of ovum leading to asynchronization with sperm availability or due to the development of zygote and endometrium. According to Morris et al., (1967b), oestrogen directly affects the endometrium. Wogonin inhibited implantation when administered at implantation duration, but this effect may also be due to wogonin's effect on the endometrium by disrupting the normal environment essential for implantation of the blastocyst. The corpus luteum is responsible for the increase in peripheral plasma progesterone during early as well as late pregnancy (Deanesly,1966). Oestrogens suppress the luteal function which disturb the normal development of the embryos (Pincus, 1965). Wogonin interferes with the development of the embryo. This effect may be due to its actions on the corpus luteum.

Table 2. Post coital antifertility effects on wogonin (10mg/kg body wt per day) in rats

Treatment given on days of Pregnancy	Route of drug amini-station	No. of females treated	Lapatotomy on day 10 of gestation		No. of females delivered	No. of litters	Inhibitition of pregnancy
			Foetus	Resorption			
Control	i.m.	5	31	1	5	30	0
1-3	i.m.	5	2	0	1	2	80
4-6	i.m.	5	0	4	0	0	80
7-9	i.m.	5	2	12	2	2	60
1-7	i.m.	5	0	0	0	0	100
1-7	oral	5	0	0	0	0	100

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