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Antifertility and hormonal properties of flavones of *Striga orobanchioides*

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Abstract

The two flavones, apigenin and luteolin, isolated from *Striga orobanchioides*, were investigated for endocrine and contraceptive properties. Graded doses of these compounds (5–25 mg/kg body weight/day) when administered from day 1 to day 4 of pregnancy showed dose-dependent and significant anti-implantation activity. The mean effective Dose 100% (MED_{100}) for both compounds was found to be 25 mg/kg body weight. Oral administration of these compounds caused a significant increase in uterine weight in immature ovariectomised rats. It also caused a significant increase in uterine diameter, thickness of the endometrium and its epithelial cell height when compared with those of control rats. The uterotrophic potency was less than that of ethinyl estradiol. Simultaneous administration of these compounds with ethinyl estradiol caused a significant increase in uterine weight, uterine diameter, thickness of the endometrium and height of endometrial epithelium. The extent of these changes was also less than that in only ethinyl estradiol-treated rats. Hence the compounds exhibited estrogenic properties at their contraceptive dose level when given alone. However, along with ethinyl estradiol, they exhibited slight anti-estrogenic activity.

Keywords: Apigenin; Luteolin; Anti-implantation activity; Estrogenic activity

1. Introduction

Medicinal plants have been used by the women of rural communities and especially by tribals to prevent conception. *Striga lutea* is one such plant used by Ayurvedic physicians in and around Gulbarga. Earlier studies in our laboratories have shown a significant antifertility effect of this plant (Hiremath and Hanumantharao, 1990; Hiremath et al., 1990) and of *S. densiflora* (Hiremath et al., 1996a). There are about thirty species belonging to the genus *Striga* distributed in the warmer regions of Asia, Africa and Australia. Chemical analysis of *S. lutea* (Hanumantharao, 1988; Hiremath and Hanumantharao, 1990), *S. asiatica* (Nakanishi et al., 1985) and *S. aspera* (Khan, 1993) has been carried out. No other chemical or biological investigations have been carried out on any species belonging to the genus *Striga*. Hence, we were interested to submit *S. orobanchioides* to detailed chemical and biological investigation as an antifertility agent. The crude ethanolic extract of *S. orobanchioides* showed significant antifertility and estrogenic activity (Hiremath et al., 1994). It has also shown significant antiandrogenic activity in male rats (Hiremath et al., 1997). The ethanolic extract, after column chromatography, yielded two compounds, apigenin (5,7,4′-trihydroxy flavone) and luteolin (5,7,3′,4′-tetrahydroxy flavone) (Hiremath et al., 1996b). The present study was carried out in order to explore the anti-implantation, estrogenic and/or anti-estrogenic action of these compounds.

2. Materials and methods

The animals used in these experiments were colony-bred Wistar strain, female albino rats (150–200 g) of proven

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fertility. All the animals were maintained under controlled standard husbandry conditions with food and water ad libitum. All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Gulbarga University, Gulbarga. Vaginal smears from each rat were monitored daily. Only the rats with normal estrous cycles were selected for the experiments. The rats found in the proestrus phase were caged with males of proven fertility in a ratio of 2:1. The females were examined the following morning for evidence of copulation. The animals, which showed thick clumps of spermatozoa in the vaginal smears were separated and that day was designated as day 1 of pregnancy. The test compounds were synthesized in the laboratory, using known procedures (Harborne et al., 1975) and purified by recrystallization three to four times, and finally by column chromatography until a single spot was obtained on thin layer chromatography (TLC) (benzene:ethyl acetate, 8:2). These compounds were identified on the basis of various physical (mixed melting point, co-TLC, etc.) and spectral data (Ultra Violet, Infra Red, Nuclear Magnetic Resonance and Mass) and found identical to those isolated from *S. orobanchioides*. These test compounds were suspended in distilled water with Tween-80 (1%) and were administered orally by intragastric catheter at the doses desired. Appropriate vehicle-treated controls were run.

2.1. Anti-implantation studies

Graded doses of apigenin and luteolin (5–25 mg/kg body weight) were administered orally to separate sets of rats for each dose of each compound from day 1 to day 4 of pregnancy. Control animals received the vehicle (Tween-80, 1%) only. The animals were laparotomised on day 10 of the pregnancy, under light ether anesthesia and semi-sterile conditions. The uteri were examined to determine the number of implantation sites.

2.2. Estrogenic and anti-estrogenic activity

The uterine weight and vaginal cornification method was employed for this assay (Edgren and Calhoun, 1957). Colony-bred immature ovariectomised female albino rats (Wistar strain), 21–23 days old and weighing between 30 and 40 g were used. They were divided into experimental and control groups consisting of 8 animals in each group. The two compounds, apigenin and luteolin, were suspended in distilled water with Tween-80 (1%) and administered orally for 7 days at the dose level of 5, 10, 15, 20 and 25 mg/kg body weight to separate groups of rats for each dose of each compound. Ethinyl estradiol in olive oil 0.03 mg/kg body weight was injected subcutaneously for 7 days in another group to induce estrous. Tween-80 (1%) was administered orally to the control animals. Apigenin and luteolin at the dose levels of 5, 10, 15, 20 and 25 mg/kg body weight were also administered orally along with ethinyl estradiol in olive oil at 0.03 mg/kg body weight subcutaneously to different groups of rats for the same period.

On the 8th day of the experiment, all the animals were killed by decapitation under ether anaesthesia and the uteri were dissected out, cleared of their surrounding tissue, blotted on filter paper and weighed quickly on a sensitive balance. Vaginal smears were recorded daily. Positive smears were those containing nucleated or cornified epithelial cells and not more than a few leukocytes.

The uterine tissues from the control group and the groups treated with ethinyl estradiol, apigenin and luteolin at 25 mg/kg body weight and ethinyl estradiol along with apigenin and luteolin at 25 mg/kg body weight were fixed in Bouin's fluid for 24 h, dehydrated in alcohol and then

Table 1
Post-coital antifertility activity of apigenin and luteolin in rats (8 animals were used in each group, laparotomy was done on day 10)

Treatment	Dose mg/kg body weight	No. of rats having no implantation sites	Mean no. of implantation sites ± S.E.	Antifertility activity %
Control	–	–	8.25 ± 0.72	–
Apigenin	5	1	7.12 ± 1.10	12.5
	10	2	5.62 ± 1.37[a]	25.0
	15	5	2.87 ± 1.43[b]	62.5
	20	6	1.75 ± 1.16[c]	75.0
	25	8	–	100.0
Luteolin	5	1	6.75 ± 1.03	12.5
	10	3	4.62 ± 1.42[a]	37.5
	15	5	2.25 ± 1.11[c]	62.5
	20	7	0.87 ± 0.87[c]	87.5
	25	8	–	100.0

[a] $P < 0.05$, when compared with control.
[b] $P < 0.01$, when compared with control.
[c] $P < 0.001$, when compared with control.

Table 2
Estrogenic activity of apigenin and luteolin (values are Means ± S.E., + = nucleated epithelial cells, + + = nucleated and cornified cells, + + + = cornified cells only, ethinyl estradiol = s.c., apigenin and luteolin = oral)

Treatment	Dose mg/kg body weight	Uterine weight mg/ 100 g body weight	Vaginal cornification
Control	–	32.68 ± 1.66	Vagina not open
Ethinyl estradiol	0.03	271.62 ± 3.56[a]	+ + +
Apigenin	5	41.96 ± 1.28[b]	+
	10	50.45 ± 1.93[b]	+
	15	59.46 ± 2.02[c]	+ to + +
	20	62.98 ± 2.91[c]	+ to + +
	25	70.72 ± 3.33[c]	+ to + +
Luteolin	5	43.29 ± 1.55[c]	+
	10	53.49 ± 1.80[c]	+
	15	60.44 ± 2.32[c]	+ to + +
	20	68.91 ± 2.29[c]	+ to + +
	25	72.14 ± 4.98[c]	+ to + +

[a] $P < 0.001$, when compared with control.
[b] $P < 0.05$, when compared with control.
[c] $P < 0.01$, when compared with control.

embedded in paraffin. The paraffin blocks were sectioned at 6-μm intervals and stained with haematoxylin–eosin for histological examination. Statistical analysis was carried out using Student's *t*-test. The results were judged significant if $P < 0.05$.

3. Results

3.1. Anti-implantation studies

A dose-dependent anti-implantational response was evident (Table 1). With an increase in the dose of the compounds, the percentage of implantation failure increased and was significant at doses of 10–25 mg/kg body weight ($P < 0.05$ to $P < 0.001$). At the dose level of 25 mg/kg body weight, 100% of the treated rats had no implantation sites when laparotomised on day 10 of pregnancy. The MED_{100} for both compounds was found to be 25 mg/kg body weight in the day 1–4 regimen.

3.2. Estrogenic and anti-estrogenic activity

The effect of the compounds on the immature rat uterus is shown in Tables 2–4. Oral administration of the test compounds (5–25 mg/kg body weight) caused a significant increase in uterine weight in immature ovariectomised rats (versus control, $P < 0.05$ and $P < 0.01$). The uterotrophic potency was less than that of ethinyl estradiol. Both compounds at their contraceptive dose level significantly increased the uterotrophic response, including diameter of the uterus ($P < 0.001$), thickness of the endometrium ($P < 0.001$) and height of the endometrial epithelium ($P < 0.01$) when compared to those of control

Table 3
Anti-estrogenic activity of apigenin and luteolin (values are means ± S.E., + = nucleated epithelial cells, + + = nucleated and cornified cells, + + + = cornified cells only, Ethinyl estradiol = s.c, Apigenin and Luteolin = oral)

Treatment	Dose mg/kg body weight	Uterine weight mg/ 100 g body weight	Vaginal cornification
Control	–	32.68 ± 1.66	Vagina not open
Ethinyl estradiol	0.03	271.62 ± 3.56[a,b]	+ + +
Ethinyl estradiol + apigenin	0.03 + 05	257.85 ± 3.27[a,b]	+ + +
	0.03 + 10	253.68 ± 3.40[a,b]	+ + +
	0.03 + 15	241.44 ± 3.98[a,c]	+ + +
	0.03 + 20	240.92 ± 3.85[a,c]	+ + +
	0.03 + 25	237.57 ± 5.16[a,c]	+ + +
Ethinyl estradiol + luteolin	0.03 + 05	254.40 ± 4.66[a,b]	+ + +
	0.03 + 10	246.42 ± 4.62[a,b]	+ + +
	0.03 + 15	240.58 ± 3.43[a,c]	+ + +
	0.03 + 20	238.86 ± 4.54[a,c]	+ + +
	0.03 + 25	236.47 ± 4.07[a,c]	+ + +

[a] $P < 0.001$ when compared with control.
[b] $P < 0.05$ when compared with ethinyl estradiol.
[c] $P < 0.01$ when compared with ethinyl estradiol.

Table 4
Histological changes in the uterus and endometrium after treatment with apigenin and luteolin (values are means ± S.E., ethinyl estradiol = s.c., apigenin and luteolin = oral)

Treatment	Dose mg/kg body weight	Diameter of uterus (μm)	Thickness of endometrium (μm)	Height of endometrial epithelium (μm)
Control	–	632.40 ± 4.24	147.40 ± 2.62	15.30 ± 0.69
Ethinyl estradiol	0.03	1496.00 ± 11.04[a]	564.82 ± 9.76[a]	56.52 ± 1.79[a]
Apigenin	25	1011.50 ± 7.71[a]	300.47 ± 3.37[a]	18.70 ± 0.69[b]
Luteolin	25	1052.30 ± 7.37[a,c]	321.30 ± 3.12[a,d]	19.55 ± 0.74[b]
Ethinyl estradiol + Apigenin	0.03 + 25	1349.80 ± 8.09[a,e]	484.50 ± 4.34[a,e]	48.45 ± 1.44[b,e]
Ethinyl Estradiol + luteolin	0.03 + 25	1293.30 ± 8.19[a,e]	456.45 ± 2.78[a,e]	45.05 ± 1.13[a,e]

[a] $P < 0.001$, when compared with control.
[b] $P < 0.01$, when compared with control.
[c] $P < 0.01$, when compared with apigenin.
[d] $P < 0.001$, when compared with apigenin.
[e] $P < 0.01$, when compared with ethinyl estradiol.

rats. The uteri of these rats were inflated and full of fluid, resembling the proestrous/estrous uterus. The epithelium of the endometrium consisted of spindle-shaped cells with basal nuclei. The stroma consisted of loose, edematous and fibroblast-type cells.

Both compounds at the doses of 15, 20 and 25 mg/kg body weight induced vaginal opening and the smear showed proestrous or estrous conditions. The number of cornified cells in vaginal smears was considerably higher (+ to + +) than that of the controls (0 to +), but notably less than that of ethinyl estradiol-treated rats (+ +).

Simultaneous administration of apigenin or luteolin with ethinyl estradiol caused a significant increase in uterine weight (versus control $P < 0.001$), but the extent of the uterotrophic response was less than that produced by ethinyl estradiol alone ($P < 0.05$ and $P < 0.01$). There was also a significant increase in uterine diameter ($P < 0.001$), thickness of the endometrium ($P < 0.001$) and height of endometrial epithelium ($P < 0.001$) when compared with those of control rats. The extent of these changes was also less than that of only ethinyl estradiol-treated rats ($P < 0.01$). The compounds therefore have weak estrogenic activity at their contraceptive dose level when given alone. However, along with ethinyl estradiol, they exhibited slight anti-estrogenic property. Luteolin was the more estrogenic of the two test compounds ($P < 0.01$ and $P < 0.001$, Table 4).

4. Discussion

Many workers, at different centers, are carrying out isolation and characterization of the active principles from plants exhibiting antifertility activity. Butin, a flavonoid isolated from *Butea monosperma* (Bhargava, 1986), 5,7,3′-trihydroxy 6,8,4-trimethoxy flavone from *Vitex negundo* (Bhargava, 1984), acacetin and 3′,4′-dimethoxy luteolin from *S. lutea* (Hiremath and Hanumantharao, 1990), puerarin and diadzein from *Pueraria tuberosa* (Shukla, 1993) and a number of isoflavones (Pathak et al., 1993) have been studied for their antifertility activity. The present investigation showed that the antifertility principles present in the ethanolic extract of *S. orobanchioides* are also flavonoids.

A dose-dependent anti-implantation response was evident for both the compounds. The loss of implantation caused by the compounds may be due to their anti-zygotic, blastocytotoxic or anti-implantation activity as described by Hafez (1970).

In immature female rats, both compounds exhibited definite estrogenic activity at their contraceptive dose. Hence, the anti-implantation activity of these compounds may be due to an imbalance in endogenous estrogen and

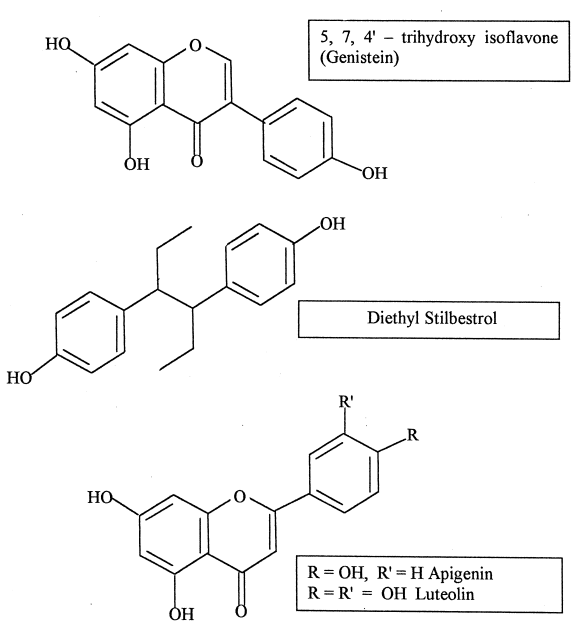

Fig. 1. Chemical structures of genistein, diethyl stilbestrol, apigenin and luteolin.

progesterone levels. As supported by histological evidence, the antifertility effect may be due to an unfavorable uterine milieu and failure to form deciduoma in the endometrium, which is essential for blastocyst implantation.

Kellis and Vickery (1984) reported that several flavones, including agipenin, inhibit human estrogen synthetase (aromatase) and may thus compete with steroids in their interaction with certain mono-oxygenases and thereby alter steroid hormone metabolism. Miksicek (1993) also reported that several commonly occurring flavonoids, including apigenin and luteolin, mimic the biological effects of 17β-estradiol by virtue of their ability to bind to and activate the nuclear estrogen receptor. In the present investigation also, the compounds while showing weak estrogenic activity, acted as competitive antagonists of the much more potent ethinyl estradiol. Several isoflavones, including genistein, diadzein and formononetin, have also been reported to be weak estrogens (Farmakalidis et al. 1985; Pathak et al., 1993; Shukla, 1993).

The estrogenicity of these two flavones can be understood in view of the superficial and conformational similarity between these compounds and the dihydroxystilbene estrogens (diethyl stilbestrol and hexestrol). Structural parallels have been drawn between the estrogenic flavones (genistein and daidzein) and the principal physiological estrogen (17β-estradiol). These similarities include a planar ring system that contains a parahydroxy substituted A-ring and a second in-plane hydroxy group located at a distance of approximately 12 Å from the first (Thomas and Keenam, 1986). All three features are also conserved in the two flavones studied (Fig. 1).

Therefore, based on the present results, the anti-implantation activity of apigenin and luteolin may also be mainly due to their estrogenic activity. It is interesting to note that the administration of these compounds did not lead to permanent sterility in rats, since discontinuation of the treatment allowed a prompt return to normal fertility.

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