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Effect of *Crotalaria juncea* seed extracts on the estrous cycle and ovarian activity in albino mice

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SUMMARY

Petroleum ether, benzene and alcohol extracts of seeds of *C. juncea* administered orally at the dose level of 25mg/100g body weight to adult female mice for 30 days, resulted in irregular estrous cycle with prolonged estrus and metaestrus and reduced diestrus and proestrus during the experimental period. Histological studies of the ovary indicate increases in the number of atretic follicles but decreases in the number of developing follicles, Graafian follicles and corpora lutea. The total cholesterol content of the ovary is increased, whereas ascorbic acid content is decreased. The weight of the uterus and its micrometric measurement in all experimental mice are increased significantly. The alcoholic extracts showed estrogenic activity in immature mice by early opening of the vagina, premature cornification of the vaginal epithilium and increases in uterine weight. However, alcohol extract of seeds of *C. juncea* was more effective in causing these changes compared to other extracts. After subjecting to preliminary phytochemical screenings alcohol extract showed positive; test for alkaloids, steroids, glycosides, flavones, phenols and tannins.

Key words: *Crotalaria juncea*; Mice; Estrous cycle; Ovary; Estrogenic activity

INTRODUCTION

Use of traditional medicine is not only popular in India but in other countries too that are availing the benefits of herbal remedies. The women of rural natives have used medicinal plants especially by the tribes before coitus for interception of pregnancy. A large of number of medicinal plants has been screened to explore the possibility for selecting a potential antifertility agent (Kirtikar and Basu, 1935; Nadkarni and Nadkarni, 1954; Chopra *et al.*, 1956; Bhakuni *et al.*, 1969; Kamboj, 1988) but so far no single plant is available which can safely be used to prevent pregnancy. Although few plants have shown promising results in preventing pregnancy but they have failed in the course of other investigations. With all these consequences, the research is still continued to search out potent antifertility plant. *Crotalaria juncea* Linn. (Papilionaceae) has been used in varied ailments in Indian medicine (Kirtikar and Basu, 1935; Nadkarni and Nadkarni, 1954; Kamboj, 1988). The seeds of this plant have been reported to possess antifertility activity (Chaudhury, 1966). Earlier studies of our laboratory have also shown the antispermatogenic and antiandrogenic activity of seed extracts of *C. juncea* in male albino mice (Sangamma, 2001). Hence the present study has been undertaken to evaluate the effects of various extracts of *C. juncea* seeds on estrous cycle and female reproductive organs in mice.

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MATERIALS AND METHODS

Plant material

Fresh seeds of *C. juncea* were collected from local farmers in and around Gulbarga (Karnataka, India) during October and November 2000 and identified in the Department of Botany, Gulbarga University, Gulbarga (HGUG No. 141), India, where voucher specimens were deposited.

Extraction of plant material

The seeds were shade dried, powdered and subjected to Soxhlet extraction successively and separately from non-polar to polar solvents i.e., petroleum ether (B.P. 60-80), benzene and alcohol (95%). The decoction so obtained was evaporated under reduced pressure and controlled temperature (50-60°C). The dried mass was considered as the extract and preserved at 6°C in dark and diluted as required.

Phytochemical tests

The presences of various chemical constituents in plant extracts were determined by preliminary phytochemical screening as described by Kokte (1985).

Animals

Mature, healthy, virgin female albino mice *(Mus musculus)* of Swiss strain (30-35 g) with normal estrous cycle were kept under controlled conditions of light (12 hr) and temperature (24±3°C) with free access to mice chow pellets (CFTRI, Mysore, India) and water *ad libitum.*

Study protocol

Mice showing regular cycles were randomized into 4 groups of 6 each. For administration to test animals, the extracts were macerated in 1% Tween-80 (S. D. Fine. Chem. Ltd. Bombay), resuspended in normal saline for their complete dissolution. The different extracts of *C. juncea* seeds were administered orally by using intragastric catheter at the dose level of 25 mg /100 g body weight as tested earlier in our laboratory and treated as follows.

Group-I: Received 0.1 ml Tween-80 (1%) and served as control.

Group II, III and IV received petroleum ether, benzene and alcohol extracts respectively in 0.1 ml Tween-80 (1%) for 30 days, so as to cover 6-7 cycles. Vaginal smear of all the animals were taken daily during morning and the stage of estrous cycle was identified microscopically (Asdell, 1964). The normal and treated animals were sacrificed on day 31 by cervical dislocation, 24 h after the last treatment. Ovaries and uteri were dissected out, freed from surrounding tissues, blotted on filter paper and weighed quickly to the nearest milligram on an electronic balance. Tissues from one side of each animal were fixed in Bouins fluid and processed for histological preparation. Haematoxylin-eosin stained slides were examined microscopically. Number of developing follicles, Graafian follicles, corpora lutea and arteric follicles were counted from 20 stained serial sections of the ovary (Abercrombie, 1975) from each mice. The micrometric measurements like diameter of uterus and thickness of endrometrium and myometrium were calculated by the method described by Deb *et al.*, (1964). The ovary from other side was processed for biochemical analysis such as cholesterol (Peters and Vanslyke, 1946) and ascorbic acid (Roe and Krether, 1943).

For the estrogenic/antiestrogenic test, immature mice of 25 days old were used. They were divided into four groups and treated as described in Table 4. Activity was assessed according to the method of Edgren and Calhoun (1957), taking uterine wet weight, vaginal opening and cornification as end points.

Statistical analysis

The mean and standard error of mean (SEM) were calculated and the significance of difference analyzed by applying student's '*t*' test.

RESULTS

Estrous cycle

The duration of estrus and metaestrus was increased while diestrus and proestrus was decreased in the experimental animals. However, this was significant due to the treatment with that of alcohol extract in comparison with that of control (Table 1).

Ovarian changes

Though the reduction in the ovarian weight was observed with the treatment of all the three extracts, it was significant ($P<0.01$) only with that of alcohol extract. Histological changes indicate significant decrease ($P<0.001$) in the number of developing follicles in all the treated groups. Though the reduction in the number of Graafian follicles and corpora lutea was noticed in all the treated groups, it was significant ($P<0.05$ and $P<0.01$) only with that of alcohol extract. Contrarily the number of atretic follicles was increased in all the experimental groups, but it was significant ($P<0.001$) in the

Table 1. Effect of different extracts of the seeds of *C. juncea* on duration of different phases of estrous cycle

Treatment	Estrus (d)	Metaestrus (d)	Diestrus (d)	Proestrus (d)
Control	5.60±0.24	5.08±0.03	4.01±0.31	1.40±0.24
Petroleum ether	5.80±0.37	5.09±0.02	3.61±0.23	1.21±0.20
Benzene	6.20±0.44	5.80±0.37	3.42±0.24	1.12±0.03
Alcohol	8.00±0.31***	6.28±0.03***	2.44±0.24**	0.21±0.21*

Dose: 25 mg/100 g body weight
Duration: 30 days
M±S.E.=Mean±Standard error
*P<0.05, **P<0.01, ***P<0.001 when compared to control.

Table 2. Ovarian changes due to the administration of various extracts of *C. juncea* seeds

Treatment	Weight (Mg/100 g)	Number/ovary				Cholesterol	Ascorbic acid
		Developing follicles	Graafian follicles	Corpora leutea	Atretic follicles		
Control	24.0±0.44	10.06±0.21	3.20±0.20	3.10±0.03	2.12±0.03	14.80±1.50	0.58±0.03
Petroleum ether	23.6±0.07	8.05±0.24***	3.10±0.03	2.90±0.20	2.20±0.03	16.40±0.51	0.39±0.04
Benzene	23.0±0.07	6.05±0.40***	2.90±0.24	2.61±0.23	2.60±0.05***	16.80±1.07	0.38±0.02**
Alcohol	21.8±0.06**	5.01±0.20***	2.12±0.03*	2.00±0.12**	3.00±0.01***	18.80±0.40*	0.34±0.02**

Dose: 25 mg/100 g body weight
Duration: 30 days
M±S.E.=Mean±Standard error
*P<0.05, **P<0.01, ***P<0.001 when compared to control

groups, which received benzene and alcohol extracts of the seeds of *C. juncea*. Biochemical changes indicate increased in cholesterol content due to the treatment of seed extract, which was significant (P<0.05) only with alcohol extract. Ascorbic acid content of the ovary was decreased with petroleum ether (P<0.05), benzene and alcohol (P<0.01) extracts of the seeds of *C. juncea*. These results indicate that alcohol extract of the seeds was more effective and petroleum ether extract was the least effective in causing adverse effects on the ovarian functions (Table 2).

Uterine changes

All the three seed extracts of *C. juncea* have caused increase in the weight of uterus and its diameter (P<0.001). The thickness of endometrium and myometrium also increased with the treatment of petroleum ether (P<0.01), benzene (P<0.001) and alcohol (P<0.001) extracts of seeds of *C. juncea* (Table 3).

Estrogenic/antiestrogenic activity

An administration of alcohol extract has induced vaginal opening, cornification of vaginal smear and significant (P<0.001) increase in the uterine weight. These changes which were similar to those caused by the administration of ethinyl estradiol. However, when the extract was administered

Table 3. Uterine changes due to the administration of various extracts of the seeds of *C. juncea*

Treatment	Weight (mg/100 g)	Diameter (μm)	Thickness of endometrium (μm)	Thickness of myometrium (μm)
Control	281.0±0.24	70.56±0.02	28.60±0.24	4.76±0.05
Petroleum ether	336.4±0.10***	81.41±0.23***	30.80±0.37**	5.10±0.03**
Benzene	340.8±0.37***	82.20±0.20***	32.00±0.31***	5.10±0.03**
Alcohol	356.3±0.12***	85.40±0.24***	34.81±0.58***	5.68±0.03***

Dose: 25 mg/100 g body weight
Duration: 30 days
M±S.E.=Mean±Standard error
**P<0.01, ***P<0.001 when compared to control

Table 4. Estrogenic activity of alcohol extract of *C. juncea* seeds in immature mice

Group	Treatment	Vaginal opening	Stage of estrous cycle on autopsy	Weight of uterus (mg/100 g)
I	Control (5)	Not opened	--	410.00±0.40
II	25 mg alcohol extract (5)	Opened	Estrus (only cornified cells)	804.01±2.45***
III	20 μg ethinyl estradiol (5)	Opened	Estrus (only cornified cells)	790.09±0.20***
IV	25 mg alcohol extract + 20 μg ethinyl estrdiol (5)	Opened	Estrus (only cornified cells)	910.02±8.21***

Duration: 5 days
M±S.E.=Mean±Standard error
***P<0.001 when compared to control
Number in parenthesis denotes the number of mice

Table 5. Phytochemical screening of various extracts of *C. juncea* seeds

Plant constituents	Petroleum ether	Benzene	Alcohol
Alkaloids	-	-	+
Steroids	-	-	+
Glycosides	+	+	+
Saponins	-	-	-
Flavones	-	-	+
Fixed oils	+	+	-
Phenols and tannins	-	-	+

+, Positive
-, Negative

conjointly with ethinyl estradiol the above changes were more enhanced (Table 4).

Phytochemical screening

The results of phytochemical screening of three extracts were shown in Table 5. the petroleum ether and benzene extracts have shown positive tests for glycosides and fixed oils. The alcohol extract has shown positive tests for alkaloids, steroids, glycosides, flavones, phenols and tannins.

DISCUSSION

The use of plants as such or medicaments for pregnancy interruption has been practice since ancient times in India. A large number of medicinal plants have been reported to possess antifertility activity in females (Nadkarni and Nadkarni, 1954; Kamboj, 1988; Chaudhury, 1966). The antifertility effect may be due to antiovulatory, antiimplantation or abortifacient activities. Extracts of flowers of *Hibiscus rosa sinensis* (Murthy *et al.*, 1997), seeds of *Randia dumetorum* (Singh *et al.*, 2000), seeds of *Momardica charantia* (Sharanabasappa *et al.*, 2002) and many other plants have been reported for this antiovulatory activity. Similarly the stem bark of *Alangium salvifolium* (Murugan *et al.*, 2000), the roots *Calotropis procera* (Kamath and Rana, 2002) and aerial parts of *Rivea hypocrateriformis* (Shivalingappa *et al.*, 2001). The abortifacient activity of the stem bark of *Ailanthus excelsa* Linn (Dhanasekaran et al., 1993) and leaves of various Rubus species (Dhanbal *et al.*, 1999) have also been investigated.

In this investigation, petroleum ether, benzene and alcohol extracts of seeds of *C. juncea* administered for 30 days increased the duration of estrus and metaestrus and decreased the duration of diestrus and proestrus significantly during experimental period indicating the mild estrogenicity of the extracts, as estrogens are necessary for cornification of vaginal epithelial cells (Murthy *et al.*, 1997). It is well documented that FSH is essential for follicular growth and LH is necessary for ovulation and corpora lutea formation (Richards, 1980), which are responsible for the growth and increase in the weight of ovary. Therefore observed reduction in the ovarian weight after the treatment of *C. juncea* seed extracts may be due to reduction in the follicular growth and ovulation, which are dependent on availability of gonadotrophins. A significant increase in cholesterol in the ovaries of treated animals may be due to non-availability of pituitary FSH, LH and prolactin, which are essential for steroidogenesis (Mason *et al.*, 1962). Decrease in the ovarian ascorbic acid also indicates the depletion of pituitary LH release (Krum, 1964). Though the follicular atresia is common in rat ovary, the increased number of atretic follicles of experimental animals indicates

the non-availability of required amount of gonadotrophins for follicular growth and ovulation (Friedrich *et al.*, 1975). Uterine growth depends on the availability of ovarian steroid hormones, particularly estrogens (Jalikhani, 1980). In the ovary developing and Graafian follicles, are the major source of estrogens (McNatty *et al.*, 1976). Therefore, the reduction in the number of healthy follicles in the experimental animals, the increase in the uterine weight may be due to the direct effect of *C. juncea* seed extract.

In the present experiment the estrogenic effect of *C. juncea* has been confirmed by its administration in immature rats, which has caused early opening of vagina, cornification of vaginal epithelial cells and increase in uterine weight. Therefore it may be concluded that *C. juncea* seed extracts have potent antiovulatory and estrogenic activity.

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