Antifertility and Estrogenic Activity of Seeds of *Datura alba* in Female Albino Rats

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Abstract
To evaluate the effect of acetone and ethanol of Datura alba seed on estrous cycle and to identify the estrogenic activity of active acetone and ethanol extracts in female albino rats. Plant extracts were tested for their effect on the estrous cycle of two dose levels: 200 and 400 mg/kg, respectively. The effective acetone and ethanol extracts were further studied on estrogenic activity. The acetone and ethanol extracts were most effective in interrupting the normal estrous cycle of the rats ($P<0.05$, $P<0.01$, $P<0.001$). These later exhibited prolonged diestrous stage of the estrous cycle with consequent temporary inhibition of ovulation. The antiovulatory activity was reversible on discontinuation of treatment. Both the extracts showed significant estrogenic and antiestrogenic activity.
KEY WORDS: antiovulatory activity, estrous cycle, estrogenic activity, antiestrogenic activity.

Introduction
Fertility is a biological phenomenon, which can be defined as the natural capability of producing off springs. Fertility differs from fecundity, which means the potential for reproduction influenced by gamete production, fertilization and carrying a pregnancy to them. An antifertility agent, a broader term, encompasses an agent having the capacity or tending to reduce or destroy fertility [1]. Plants have long been a source of nutrition and therapy to man [2].
The presence of vitamins, antioxidants and bioactive compounds in plant foods have been linked to systemic functions such as reproduction and immunity. The potential of managing disease using nutritional therapy has generated numerous epidemiological studies [3]. Globally, about 85,0000 plant species have been recognized as medicinally useful [4]. From ancient time to the present day, plants continue to play an indispensable role in the prevention and treatment of diseases [5,6]. Health practices of various cultures worldwide identify with the use of medicinal plants as curative and preventive medicines. The history of phytotherapy can be traced as far back as 60,000 years ago [7]. The knowledge of medicinal plant use formed the basis for traditional systems of medicine which were the only source of health care before the advent of orthodox medicines [8]

Materials and Methods

Plant Materials

The *Datura alba* were collected from the Forest research Institute Dehradun, Uttrakhand, India. The plants were identified by HOD Dr. Birendra S. Rawat Department, of Botany, University of Hemvati Nandan Bahuguna Central (Garhwal) university in Srinagar, Uttarakhand, India.

Preparation of extracts

50g of powder was weighed and the extracts of powdered material was extracted using acetone (60-70ºc) for 72h and successively extracted with ethanol for 72h each in a soxhlet apparatus. The extracts were evaporated under reduced pressure to solid masses and the percentage yield of extracts was found to be 4.0%, 7.3% respectively
Percentage yield of extract was calculated by following formula, [9]
% yield = Wt. of dry powder/ Wt. of extract× 100

ANIMALS
Female albino rats (Wistar albino rats weighing 150-220g) were used for antiovulatory activity and immature female rats (Wistar albino rats) 21-23 days were used for estrogenic activity. Animals was fed on Conventional diets and water ad libitum and they were maintained under standard conditions of humidity, temperature (20-24ºC) and light (12-h light: 12-h dark cycle). The rats were randomly assigned to control and different treatment groups, six animals per group. The institutional Animal Ethics Committee approved the experimental protocol and the conditions in the animal house approved by committee for supervision on Experiments on Animals. The animals were acclimatized for one week under laboratory conditions
Antiovulatory activity

Experiments were carried out in female wistar rats weighing (150-200g). The veginal smear of each rat was examined daily between 9-10 A.M. for 15 days to select the animals showing regular cycles (4-5days). The selected rats were divided into 5 groups of six animals each. The extracts were administered orally for five days to cover one regular estrous cycle. Group I received vehicle (1% Tween 80) and served as control. Group II-V received acetone and ethanol seed extracts of *D. alba* at 200 and 400 mg/kg bogy weight. Vaginal smear from each animal was observed every morning between 9-10 A.M for five days of treatment and subsequently for 15 days.
Estrogenic and antiestrogenic activity

The extract with antiovulatory activity was further evaluated for estrogenic activity and antiestrogenic activity. Immature female wistar strain rats, 25-30 days old, weighing between 35-45 g, were divided in to 10 groups of six rats each, The first group served as control and received the vehicle only (1% Tween 80). The second group received ethynyl estradiol (standard) in distil water at a dose of 0.02mg/kg body weight. The third, fourth, fifth and sixth group received the acetone and ethanolic extract of *D. alba* seed at two dose level 200 and 400 mg/kg body weight, respectively. The groups VII to X received ethinyl estradiol in addition to a test dose of the acetone and ethanolic extract of seed at the same dose. All the above treatment were given for three days [10].

Results

Effect of extracts of *Datura alba* seed on the estrous cycle of rats

The present study revealed that the ethanol extract of *Datura alba* seed showed an antifetility effect. Treatment of rats with ethanol extract for five days prolonged the estrous cycle significantly (P<0.05, P<0.01) as indicated in Table 7.5. It is observed that in the control group of animals treated with 1% Tween 80 which was used as a vehicle in the present experiment all the six animals manifested normal cyclical oestrus phase throughout the study period. The estrous cycle in rats with ethanolic and acetone extract at dose level of (200 mg/kg), normal cyclical oestrus phase was absent in all the six animals after 4.5 days on an average. With higher doses in 2nd group (400 mg/kg) estrous phase disappeared more quickly i. e within 3 days on an average. The estrous cycle in rats treated with acetone and ethanolic extracts showed reduced duration of estrous and metaestrous phases, characterized by a prolongation of thendiestrous phase. Withdrawal of the treatment did not indicate any significant change either in the four phases of the estrous cycle or in the duration of the cycle. Acetone extract was found to be more active.

Table 1:Effect of treatment of various extracts of *Datura alba*, on estrous cycle for 5 days in rats values are expressed in mean ±SEM, n=6

Treatment	Dose Mg/kg	Duration of cycle (days)	Duration of different phases of estrous cycle (days)			
			Proestrous (days)	Estrous (days)	Metestrous (days)	Diestrous (days)
Control	…..	4.35 ± 0.35	0.84 ± 0.11	0.83 ± 0.16	0.84 ± 0.29	1.84 ± 0.36
Da. S-A	200	5.71 ± 0.29**	0.24 ± 0.26*	0.82 ± 0.54	0.65 ± 0.33	4.11 ± 0.49**
	400	5.62±0.39***	0.31 ± 0.22*	0.68 ± 0.28	0.85 ± 0.32	3.52 ± 0.60**
Da. S-E	200	5.05 ± 0.32*	0.30 ± 0.21*	0.63 ± 0.40	0.84 ± 0.29	3.32 ± 0.49*
	400	5.15 ± 0.48*	0.40 ± 0.32*	0.67 ± 0.29	0.77 ± 0.42	3.36 ± 0.81**

Da. S – *Datura alba* seed

A- Acetone, E- ethanol

Each value represents the mean ± S.E.M. (n=6); * P<0.05. **P<0.01, ***P<0.001 vs control (Student's t test)

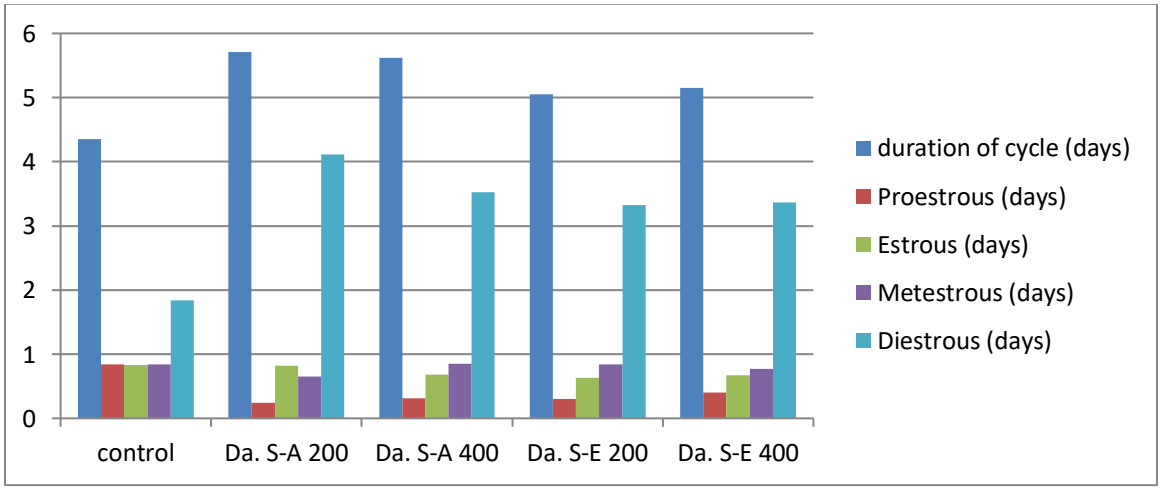

Fig. 1: Effect of treatment of various extracts of *Datura alba*, on estrous cycle for 5 days in rats values are expressed in mean ±SEM, n=6

Estrogenic activity and antiestrogenic activity

The effect of estrogenic effect of ethanol and Acetone extract is shown in Table 7.6 Oral administration of ethanol and acetone extract at 200 and 400 mg/kg body weight caused a significant increases in uterine weight in immature rats ($p< 0.05$, $p< 0.0$, $p< 0.001$). The thickness of endometrium was significantly increased when compared to the control 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 and edematous fibroblast-type cells with edema. The control rats shows closed vagina whereas the treated rats showed an open vagina. The administration of acetone and ethanol extracts aggravated a significant increase in the uterine wet weight, signifying the estrogenic activity. However, when treated with ethinyl estradiol, it lowered the effect estrogenic activity produced by ethinyl estradiol alone.

Table No. 2: Estrogenic and antiestrogenic activity of ethanol and Acetone extract of *Datura alba* values are expressed in mean ± SEM, n=6

Groups	Dose	Uterine weight (mg/100g body weight)
Control	(Tween-80, 1%)	45.51 ± 2.26
Ethinylestradiol	0.02	142 ± 4.80***
Da.S-A	200	78.44 ± 5.79**
Da.S-A	400	81.96 ± 5.16***
Da. S- E	200	68.33±3.81***
Da. S- E	400	75.25±6.12***
Ethinylestradiol+ Da.S-A	0.02+ 200	123.12±7.88***+
Ethinylestradiol+ Da.S-A	0.02+ 400	131.75±4.29***+
Ethinylestradiol+ Da.S-A	0.02+ 200	125.06±3.57***+
Ethinylestradiol+ Da.S-A	0.02+ 400	120.02±7.36***+

Da.S- *Datura alba* seed

A- Acetone, E- ethanol

B- Each value represents the mean ± S.E.M. (n=6); **P<0.01, ***P<0.001 vs control, +P<0.05, Vs Ethinyl estradiol (Student's t- test)

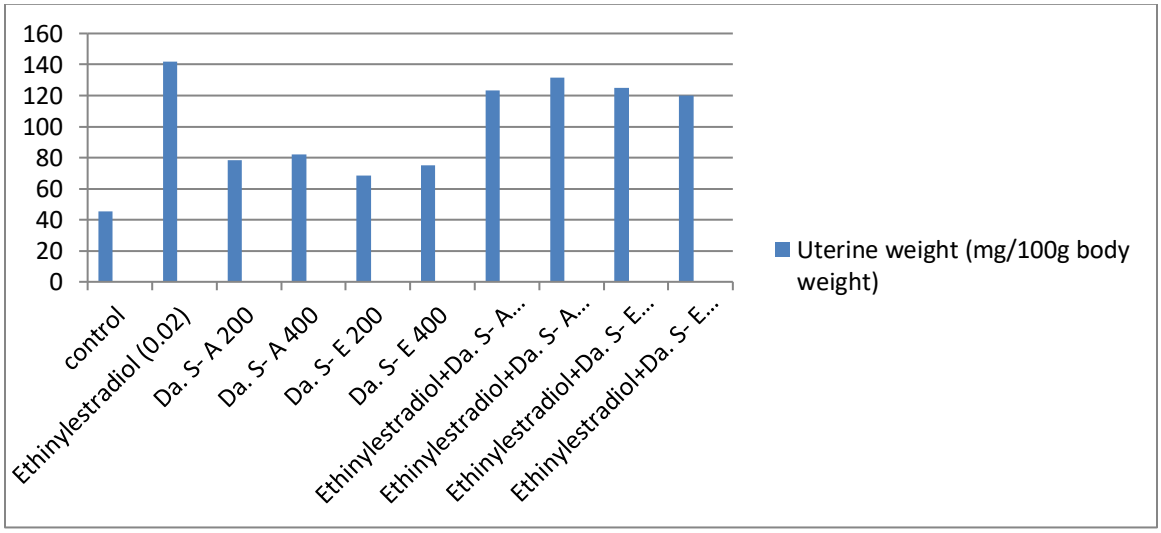

Fig. 2: Estrogenic and antiestrogenic activity of ethanol and Acetone extract of *Datura alba*

Discussion

The acetone and ethanol extracts of Datura alba seed exhibited significant ($P<0.05$, $P<0.01$, $P<0.001$) antifertility activity. The duration of estrous cycle in rats is normally 4-5 days. Three cell types are found in the vaginal smear duration a normal rat estrous cycle. The presence and absence of these cell types, and relative proportion of each cell type, determine the stages of the estrous cycle of the two extracts tested for the antiovulatory activity, acetone and ethanol extracts produced a temporary and reversible modification on the esrous cycle. The prolongation in the diestrous phase explains the remote possibility of the rats getting pregnant. The reversible nature of the antifertility activity of the extract is explained through the observation that there was no significant changes in the diestrous and the estrous cycle after withdrawing the extract from those of the control. As a result, the extracts provoked inhibition of the ovulation with consequent reduction of the cyclicity. Estrous cycle and the shift in different stages are mainly governed by the synthesis of ovarian

estrogen, which, in turn, is controlled by the secretion of pituitary gonadotropins and hypothalamic- releasing factor.

The extracts with the antiovulatory activity were further studied for their estrogenic and antiestrogenic activity. These extracts also exhibited estrogenic activity as shown by the significant increase in the diameter of the uterus, uterine weight and thickness of the endometrial epithelium when compared with the control. It was also observed that the acetone and ethanol extracts suppressed the action of ethinyl estradiol when administered together. The extracts showed a significant strogen-like activity when given alone but, with ethinyl estradiol, they exhibited a slight antiestrogenic nature. This indicates that the extract acted as a competitive antagonist to the more potent ethinyl estradiol.

Conclusion

The results of the present study indicate that the acetone and ethanol extracts of Datura alba seeds have significant antifertility activity. The seeds of this plant could be used to induce abortion. The extracts of this plant can be further explored for contraceptive use.

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