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Antifertility potential of hydroalcoholic extract of *Cordia dichotoma* G Forst. leaves: A folklore medicine used by Meena community in Rajasthan state in India

Pallavi Sharma[1], Manjusha[1*], Sudesh Rani[1], Hitesh Malhotra[1], Nitesh[2], Sandeep Deswal[2], Surender Singh[3]

[1]*Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra-136119 Haryana, India*

[2]*R. P. Inderprastha Institute of Technology, Karnal- 132001, Haryana, India*

[3]*All India Institute of Medical Sciences, New Delhi-110029, India*

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ABSTRACT

Objective: To assess antifertility activity of hydroalcoholic extract of *Cordia dichotoma* G Forst. (*C. dichotoma*) leaves, which are traditionally used to produce sterility among the tribal women through its abortificient activity. **Methods:** Acute toxicity study was carried out in adult albino rats. The antifertility activity of extract at two dose level (200 and 400 mg/kg, orally) was evaluated in two experimental animal models *i.e.* anti-implantation and estrogenic/ antiestrogenic activity in female rats by observing number of implants, vaginal cornification, body weight, uterus weight and biochemical investigation. **Results:** The extract was found to be safe up to dose of 2 000 mg/kg body weight when administered orally. A good anti-implantation (81.22%) activity in female rats was observed at the tested dose levels (200 and 400 mg/kg, orally). The extract further showed more significant (P<0.01) increase in uterine weight and significant change in biochemical parameters in immature rats. Simultaneous administration of extract along with ethinyl estradiol showed significant estrogenic activity. **Conclusion:** The results suggest that hydro alcoholic extract of *C. dichotoma* leaves possess significant antifertility activity, which is consistent with the literature report in folk medicine of this plant in fertility regulation.

1. Introduction

The increase in population is becoming a comprehensive dilemma, causing much pressure on economic, social and natural assets. In the present scenario world's population has amplified at an alarming rate and is the main cause of poverty[1]. An incantation in the Rig-Veda had advanced the outlook, a man with many children succumbs to miseries[2]. Family planning was promoted by different government agencies through various methods of contraception. Inspite of the evidence that oral contraceptive agents have improved the rate of infertility but their unusual side effects limit the use. The adverse effects caused by oral and injectable contraceptive agents include increased blood transaminase, increased cholesterol, hormonal imbalance, hypertension, indigestion, headache, depression, fatigue, intermenorrheal bleeding, and increased risk of cancer and weight gain. Metabolism of lipid, protein and carbohydrates is also disturbed[3]. Thus, despite the availability of various contraceptives modalities, one of the most challenging pursuits in the realm of pharmaceutical and medical science is search for newer, more potent, additionally safe and less expensive agent which should have long lasting and complete reversible anti-fertility effect. For this World Health Organization has given great emphasis on folklore use

*Corresponding author: Manjusha, Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra-136119 Haryana, India.
E-mail: manjushachoudhary@gmail.com

of the anti-fertility herbs. In the recent years number of plants have been identified and evaluated for their anti-fertility activity[4].

The genus Cordia is named in the honour of German botanist and pharmacist Valetrius Cordus and established by Linnaeus in 1753. The genus comprises about 300 different species found worldwide. Members of this genus are widespread in tropical and subtropical regions of both hemispheres and strongly centered in the Neotropic ecozone[5]. The plants of this genus have served as a rich source of diverse phytoconstituents and are utilized extensively in folk medicine and pharmaceutical industries[6,7]. Certain plants of cordial species were proven to show hormonal effects[8]. Moreover, *Cordia sinensis* is utilized in birth control practices among the tribal women belonging to Meena community of Rajasthan, India[9]. The leaves of *Cordia curassavica* and *Cordia spinescens* are also used to cure menstrual pain and relieve postpartum pain respectively[10].

Cordia dichotoma (*C. dichotoma*) popularly known as gundaor tentiis a perennial tree found in tropical and subtropical regions mainly in the Sub-Himalayan tract and the outer ranges. Mostly it grows as a wild tree or planted along the sides of road and fields rarely grown for commercial purposes[11]. Compounds isolated from the plant are apigenin, cordioic acid, quercitin, linolenic acid, rutin, hesperidin, arabinose, caffeic acid, robinin and arabinoglucan. The different parts of plant such as leaves, fruits, barks and roots had been used as an anti-inflammatory, antipyretic, anti-oxidant, anticancer, expectorant, demulcent, antiarthritic, antidepressant, anti-ulcer, antifertility and cosmetic agents in system of folklore medicine[12]. *C. dichotoma* is traditionally used to produce sterility among the tribal women through its abortificient activity. The plant is reported to have an anti-inflammatory, anti-arthritic[13], antioxidant[14], antimicrobial[15, 16], ulcer protective[17] and wound healing[18] activities. The current investigation was focused on the antifertility activity of hydroalcoholic leaves extract of *C. dichotoma* in female rats.

2. Materials and methods

2.1. Procurement and identification of plant material

The leaves of plant were collected from the fields and roadside in the district of Sirsa, Haryana, India during the month of October 2012 and authentified as *C. dichotoma* G Forst. (Family: Boraginaceae) by Dr. B. D. Vashistha, Chairman, Department of Botany, Kurukshetra University, Kurukshetra, Haryana. A voucher specimen (KUK/ BOT/ IPS-05) of the plant is preserved in the herbarium of the Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra for future reference.

2.2. Preparation of crude extract

Leaves of *C. dichotoma* G. Forst were washed under running tap water and dried under shade at room temperature for two weeks. The dried leaves were ground into coarse powder and stored in air tight container. The 450 g of powdered leaves were extracted with hydroalcohol (30: 70) using Soxhlet apparatus at a temperature of 50 ℃ for 72 hr. The extract was concentrated to semi solid mass using rotary evaporator (Heidolph, model number - 4011, USA) and then lyophilized. The crude yield of the lyophilized extract was approximately 11.8 % w/w.

2.3. Preliminary phytochemical screening

The hydroalcoholic leaves extract of *C. dichotoma* (HAECD) was subjected to preliminary phytochemical screening as per reported methods[19].

2.4. Animals

Colony-bred healthy fertile male and female rats (Wistar strain) in the weight range of 200-250 g were selected for the study. The animals were obtained from National Institute of Pharmaceutical Education and Research (NIPER), Mohali and housed in animal house of Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra and maintained under laboratory condition of temperature (21.5±22 ℃), humidity (60% ±1%) and 12 h light/ dark cycle. They had free access to food and water ad libitum. They were transferred to the laboratory twelve hours prior to the experiments and given only water *ad libitum*. In all the experiments, the animals were kept in cages with raised floors of wide mesh, to prevent coprophagy. The experimental protocol and procedure used in the study were approved by the Institutional Animal Ethical Committee (Reg, No. 562/GO/02/a/CPCSEA) of Kurukshetra University, Kurukshetra (Protocol number IPS/AH/223) and and were in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Govt. of India, New Delhi.

2.5. Acute toxicity study of the extract

Adult wistar rats (200-250 g) were divided into six groups each containing six rats. The rats were fasted for 6 hours with only access to water ad libitum before experimental study. Group I, II, III, IV and V animals were administered various doses of HAECD i.e. 500, 1 000, 2 000, 3 000 and 4 000 mg/kg. Group VI received Tween 80 only. All the doses and vehicle were administered orally. The animals were observed for mortality or any sign of clinical abnormality for 72 hours by housing them individually in polypropylene cages.

2.6. Dose preparation of the test animals

Doses of the extract were selected based on the acute toxicity

studies conducted on wistar rats and administered at 200 and 400 mg/kg doses in the present study. The dose of extract was reconstituted by suspending the required quantity of HAECD in Tween 80 (5% v/v in saline) freshly before use and was injected per orally (p.o.). Vehicle control groups received equal volume of Tween 80 (5 % v/v in saline)

2.7. Antifertility study

2.7.1 Anti-implantation activity[20,21]

Female Wistar albino rats (200-250 g) of proven fertility were mated with mature male rat of proven fertility in the ratio of 2:1, in the evening of proestrous stage and examined next morning for the evidence of copulation. Vaginal smear of each rat was taken daily between 9:00 a.m. to 10:00 a.m. The rats exhibiting the copulation plug or thick clumps of spermatozoa in their vaginal smears were separated and that day was designated as day one of pregnancy. These rats were divided into three groups each containing six animals. Group I (control) animals received vehicle (Tween 80, 5% w/w in saline) only. Group II and III received HAECD at the dose levels of 200 and 400 mg/kg respectively. All the extract doses and vehicle were administered orally to the animals once daily throughout 7 days of pregnancy. On 10th day of pregnancy, the animals were laparotomized under light ether anesthesia using sterile conditions and numbers of implants present in both the uterine horns were determined.

2.7.2. Estrogenic/antiestrogenic activity[22,23]

For studies on estrogenic and anti-estrogenic activity female Wistar rats (200-250 g) were used. The bilateral ovariectomy was done under light anesthesia and sterile conditions. The uterine horns were exteriorized and ovaries excised by laparotomy incision. After one week the animals were divided into six groups containing six animals each. Group I (control) was administered with vehicle *i.e.* Tween 80 5% v/v *p.o.* 17- α - ethinylestradiol at a dose of 1 μg/rat/day suspended in olive oil, was administered to group II animals subcutaneously and which serve as positive control. Animals of group III and IV received extract at the doses of 200 and 400 mg/kg *p.o.*, respectively. Group V and VI animals received HAECD at the doses of 200 and 400 mg/kg respectively, along with ETHINYL ESTRADIOL (1 μg/rat/day) subcutaneously. All the doses and vehicle were administered orally for seven consecutive days. On the 8th day, all the animals were sacrificed under light anesthesia. The final body weight, uterine weight, vaginal opening and cornification of all the animals were observed. Blood serum was further processed for the estimation of biochemical parameters such as estrogen level, alkaline phosphates, cholesterol and total proteins.

2.8 Statistical analysis

All the values were expressed as mean ± S.E.M. The data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. The levels of significance were taken at $P<0.05$ and $P<0.01$ in relation to control and standard.

3. Results

3.1. Preliminary phytochemical screening

Preliminary phytochemical screening reveals that hydroalcoholic extract possess glycosides, alkaloids, flavonoids, tannins, volatile and fixed oils.

3.2. Acute toxicity study of extract

The result of acute toxicity study of HAECD showed no dose-dependent effect in all the treated groups upto the dose of 2 000 mg/kg when administered orally. Hence one tenth of maximum tolerated dose *i.e.* 200 mg/kg and higher dose 400 mg/kg were selected for anti-fertility activity.

3.3. Anti-implantation activity

On 10th day of pregnancy, laparotomization was done in female rats and anti-implantation effect was expressed as percentage of animals showing absence of implantation in uteri. A dose dependent anti-implantation effect of orally administered HAECD on mated female rats is shown in Table 1. The HAECD exhibits an anti-implantation activity since the inhibition of implant was observed in all treated animals and was significant ($P<0.01$) at doses 200 and 400 mg/kg with respect to control. The screening of the extract showed 59.39% and 81.22% anti-implantation activity at the doses of 200 mg/kg and 400 mg/kg body weight, respectively. Hence, maximum inhibition of

Table 1
Anti-implantation activity of HAECD in female rats.

Groups	Treatments	Dose(mg/kg)	Body weight gain (g) (mean ± S.E.M)	No. of rats without implantation sites on day 10	No. of implantation sites (mean ±SEM)	% inhibition of implantation sites on day 10
I	Control	-	49.83± 2.14	0	11.50± 0.56	Nil
II	HAECD	200	40.50± 1.94*	0	4.67± 0.49*	59.39 %
III	HAECD	400	28.83±1.90*	0	2.16±0.47*	81.22 %

HAECD: hydroalcoholic leaves extract of *C. dichotoma*; n=6 animals in each group; Values are expressed as mean ±S.E.M.;* Significant when compared to control: $P< 0.01$.

implants was seen at higher dose i.e. 400 mg/kg.

3.4. Estrogenic activity

The estrogenic effect of HAECD on mature ovariectomized rats body weight, uterine weight, vaginal opening and cornification is shown in Table 2. Oral administration of extract at doses of 200 and 400 mg/kg body weight either alone or along with ETHINYL ESTRADIOL caused a significant (P<0.01) increase in body weight and uterine weight of overiectomized rats when compared to control. At both the doses (200 and 400 mg/kg) either alone or given with ethinyl estradiol the extract evidenced vaginal opening and presence of cornified cells in the vaginal smear in all the female rats. The number of cornified cells in vaginal smear was considerably less than that of group treated with ethinyl estradiol (+++) alone but notably higher (from + to ++) than the control group. The uterotropic changes such as diameter of the uterus and thickness of the endometrium has been shown in Table 3. The extract did not produce significant effect on the diameter of endometrium at a dose of 200 mg/kg whereas height of the endometrium was significantly (P<0.001) increased when compared with the control rats. Moreover, concurrent administration of ethinyl estradiol and HAECD extract cause a significant (P<0.01, P<0.05) increase in uterine diameter and height of the endometrium when compared with control. When compared with the group treated with ethinyl estradiol there was significant increase in thickness and height of endometrium which shows that the hydroalcoholic extract at a dose of 400 mg/kg have an estrogenic activity rather than anti-estrogenic activity.

3.5. Effect on biochemical parameters

The effect of extract on biochemical parameters has been presented in Table 4. The HAECD showed significant (P<0.01) estrogenic potential by increasing the level of serum estrogen in mature ovariectomized rats in comparison to control when administered alone or along with ethinyl estradiol at the doses of 200 mg/kg and 400 mg/kg. Estrogenic activity of the extract was further confirmed by significant (P<0.01) increase in level of serum cholesterol and total protein in comparison to control when administered alone or along with ethinyl estradiol at higher dose (400 mg/kg). Further, the extract significantly (P<0.01) increased the serum alkaline phosphate level when administered alone or along with ethinyl estradiol at both doses (200 mg/kg and 400 mg/kg).

4. Discussion

Traditionally, herbal drugs are used as an attractive approach in the realm of fertility control as plants contains numerous phytoconstituents including steroidal components, flavonoids, terpenoids, alkaloids and steroidal saponins that are probably responsible for antifertility activity. Moreover, these phytoconstituents might cause certain toxic effects in the development or normal functioning of the reproductive system. Coumarin, flavonoid and sitosterol are phytoconstituents that have been related to the occurrence of certain adverse effects to the reproductive system and hence possess a potent anti-fertility activity. Flavonoids like quercitin, apigenin and luteolin and sitosterol are

Table 2
Effect of HAECD on estrogenic effect on body weight, uterine weight and vaginal cornification in female rats.

Groups	Treatments	Dose(mg/kg)	Initial body weight(g)	Final body weight (g)	Body weight gain (g)	Uterine weight (mg)	Vaginal cornification
I	Control (Tween- 80 2%v/v, p.o.)	–	226.00±5.86	229.00±5.81	3.00±0.73*	23.30±1.19	Nil
II	Ethinyl estradiol	(1 μ/rat/day)	216.00±8.08	251.17 ±5.66	35.17±2.97*	74.16±1.33*	++
III	HAECD	200	221.17±5.09	235.67±4.26	14.50±1.48*#	44.43±1.79*	+
IV	HAECD	400	225.17±8.25	245.67±6.82	20.50±1.82*#	52.09±1.82*	++
V	HAECD + Ethinyl estradiol	200 +(1 μ/rat/day)	216.17±7.97	242.50±6.94	26.33±2.51*#	69.75±1.32*	++
VI	HAECD+Ethinyl estradiol	400 +(1 μ/rat/day)	224.17±9.76	256.83±8.73	32.66±2.03*	77.04±1.76*	++

HAECD: hydroalcoholic leaves extract of *C. dichotoma*, n=6 animals in each group; Ethinylestradiol was taken as standard; Values are expressed as mean ±S.E.M; * Significant when compared to control: P<0.01; # Significant in relation to standard: P<0.01.

Table 3
Effect of HAECD on diameter, thickness and height of endometrium in ovariectomized female rats.

Groups	Treatments	Dose (mg/kg)	Diameter of endometrium (mm)	Thickness of endometrium (mm)	Height of endometrium (mm)
I	Control	_	1.42±0.01	247.32±1.98	13.70±0.30
II	Ethinyl estradiol	1 μ/rat/day	2.84±0.08*	342.44±1.10*	32.98±0.50*
III	HAECD	200	1.54±0.02#	196.66±1.32*#	21.59±0.34*#
IV	HAECD	400	2.53±0.24*	292.98±4.06*#	24.61±0.31*#
V	HAECD + Ethinyl estradiol	200 +1 μ/rat/day	1.86±0.01#	235.81±6.20	20.66±0.06*#
VI	HAECD+Ethinyl estradiol	400 + 1 μ/rat/day	3.45±0.04*	342.10±3.60*	35.38±0.10*#

HAECD: hydroalcoholic leaves extract of *C. dichotoma*; n=6 animals in each group; Values are expressed as mean ±S.E.M.; *Significant when compared to control: P<0.01; #Significant with respect standard: P<0.01.

Table 4
Effect of HAECD on biochemical parameters in ovariectomized female rats.

Groups	Treatments	Dose (mg/kg)	Estrogen (pg/mL)	Cholesterol (mg/dL)	Total protein (mg/dL)	Alkaline phosphatase (U/L)
I	Control	_	142.06±2.68	54.16±1.85	6.04±0.04	9.49±1.36
II	Ethinyl estradiol	(1 μ/rat/day)	457.69±4.03*#	77.63±0.27*	10.19±0.32*	18.16±1.33
III	HAECD	200	145.26±2.44	58.36±1.14*#	6.18±0.005#	26.09±0.52*#
IV	HAECD	400	190.17±3.12*#	90.70±2.10*#	6.43±0.57#	29.91±0.96*#
V	HAECD + Ethinyl estradiol	200 +(1 μ/rat/ day)	211.09±3.04*	42.93±1.33*	5.90±0.05#	35.22±0.49*#
VI	HAECD+Ethinyl estradiol	400 +(1 μ/rat/ day)	230.40±3.08*	34.13±0.35*	7.26±0.14*#	38.32±0.51*#

HAECD: hydroalcoholic leaves extract of *C. dichotoma*; n=6 animals in each group; Values are expressed as mean ±S.E.M.; * Significant when compared to control: $P<0.01$; # Significant with respect standard: $P<0.01$.

the active constituents of *C. dichotoma* that suggests the possible anti-fertility activity on female rats. The present study was intended to estimate the anti-fertility (anti-implantation and estrogenic/anti-estrogenic) effect of hydroalcoholic extract of plant in female rats.

For implantation, exact equilibrium of estrogen and progesterone is essential and any disturbance in the level of these hormones may cause infertility[24]. Estrogen cause an increase in uterine weight, retention of fluid leading to ballooning of the uterus, vaginal opening and cornification, therefore, creating non-receptive condition and changing the hormonal milieu in the uterus[25]. Estrogens also exert effect on lipid metabolism, serum lipoproteins and serum triglycerides (TG's) levels. In general, estrogens slightly elevate serum triglycerides, reduce cholesterol level, and increase serum estradiol level[26]. It was stated that reproductive and general metabolic effects in mature and immature rats were manipulated with the ingestion of phytoestrogenic substances and showed effects similar to those of gonadal steroids[27].

The anti-fertility activity of the extract may be due to number of possible mechanism. Moreover, one of the possible mechanisms might be anti-implantation or abortificient activity of the hydroalcoholic extract. The present study reveals that hydroalcoholic extract showed significant anti-implantation activity in female rats at both doses. The percentage inhibition of implantation may be due to anti-zygotic/blastocytotoxic activity. The biochemical serum analysis of HAECD reveals estrogenic effects by increase in cholesterol and alkaline phosphate when administered alone or along with ethinyl estradiol at higher dose. Moreover, the extract also elevates the level of alkaline phosphate and total protein in serum when administered alone or along with ethinyl estradiol at both doses. The extent of variation in biochemical parameters was somewhat less than that of only ethinyl estradiol.

The estrogenic effect of the extract was further evidenced by increased body weight, vaginal opening & number of cornified cells. Moreover, increased diameter and thickness of the endometrium shows the estrogenic activity of the extract at both doses. Estrogenic substance may cause the expulsion of ova from the fallopian tube and the disruption of functional equilibrium between the endogenous estrogen and progesterone which may result in antifertility. Post coital anti-implantation and abortificient activity will be mediated through estrogenic activity of the extract as estrogen are known to cause uterine contraction during the expulsion of fertilized egg.

Steroidal and non steroidal compounds present in the plant as pertaining to phytochemical screening can be claimed for its estrogenic potential. The HAECD shows a lesser estrogenic effect in comparison to ethinyl estradiol treated group which proves anti-estrogenic property of the extract when administered along with ethinyl estradiol at prescribed dose. The anti-estrogenic activity of the extract can be attributed to the negative feedback inhibition of the hormonal regulation. The steroidal glycosides, saponins are responsible for anti-estrogenic activity of the extract as these are reported to reduce the activity of estrogens induced enzymes in several estrogen targeted tissues. Since the cholesterol is the precursor of steroid genesis of the ovarian endocrine tissues. The decreases in cholesterol level can cause diminution of ovarian steroidogenesis. Thus, it can be concluded that fertility inhibiting effect is certainly due to multiple attributes which are dose dependent.

Present investigation gives the direction to further explore the present study for the isolation of active constituents and to determine undersigned cellular mechanism of action. Further detailed study using different animal species can be helpful in the development of suitable herbal formulation which can act as antifertility agent in population regulation.

From the present study it is concluded that hydroalcoholic extract of *C. dichotoma* leaves possess significant antifertility effect which might be due to the presence of certain estrogenic phytoconstituents in the plant that cause inhibition of implantation. Further studies on exact mechanism of antifertility action and isolation of the active components responsible for antifertility effect are in progress.

Conflict of interest statement

We declare that we have not conflict of interest.

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