Indian Journal of Experimental Biology
Vol. 37, April 1999, pp. 402-405

Progesterone potentiating effect of *Dipsacus mitis* D. Don for its contraceptive action in hamster*

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Received 4 June 1998, revised 28 December 1998

A pure compound, isolated from ethyl acetate extract (root) of *D. mitis* D. Don, prevented pregnancy by 100% in adult female hamster but partially in rat when administered orally on Days 1-7 and 1-10 post-coitum respectively. The effective dose in both species was 150 mg/kg. Using uterine wet weight in ovariectomized immature rat as bioassay method, the compound was found to be devoid of estrogenic and antiestrogenic property. On examination for progestational and antiprogestational activity, using trauma-induced deciduoma formation in immature rat uterus as end points, the compound (*per se*) did not show the former activity but in a conjoint treatment with progesterone it augmented the action of latter. The compound was assumed to act by potentiating progesterone biosynthesis, the excess of which might be the cause for interruption of pregnancy in hamster. This is the first study to report contraceptive efficacy and mode of its action at the uterine level.

Dipsacus mitis D. Don (Dipsacaceae) is an annual wild plant available in Himalayan region of Nepal at 9,000-14,000 ft. The plant is a tall herb, attaining the height of 4-10 ft[1]. Because of its yellowish root the plant is also named as "Banmula" (the wild raddish) in the region. In folklore the dried roots of another species of *Dispacus (D. asper)* is reported to be used in regulation of excessive menstrual bleeding[2].

Though a number of studies are available in literature on the other constituents of Dipsacus[3-7], none is on this species, in terms of its chemical constituents and biological activities. Therefore, an attempt was made to analyse the chemical and biological profile of a compound isolated from the ethyl acetate extract of its root. The study includes contraceptive efficacy in female rat and hamster and hormonal profile in immature rats by standardized assay procedures[8-9].

Plant material — The flowering plant was collected in October from Phulchoki, Nepal and identified at the Natural Product Development Division (NPDD), Kathmandu. The specimen was preserved by the Herbarium Section of the NPDD.

Extraction and isolation — The air dried root powder was extracted, using Soxhlet apparatus, with petroleum ether (b.p.:40-60), ethyl acetate and finally with ethanol (95%). The extracts were concentrated using rotary evaporator under reduced pressure and at 40°C temperature. The ethyl acetate extract was repeatedly subjected to vacuum liquid chromatography on silica gel using various solvent systems to get the crystallized compound. Its purification was carried out by recrystallisation with methanol. Chemically, it was found to be a triterpene glycoside.

Animal experimentation - Healthy adult (100-120g) and immature (40-50g) female rats of Sprague-Dawley strain and Syrian golden hamsters (80-100g) bred in animal facilities of the Central Drug Research Institute (CDRI) were used. They were maintained under uniform husbandry conditions (temperature 21±2°C) and photoperiod (14h light : 10h dark).

Contraceptive efficacy — The adult females (both species) at proestrous were co-caged overnight with proven fertile males (ratio 3:1). To ascertain the occurrence of mating, vaginal smear was examined for the presence of spermatozoa next morning and the day of finding them was considered as day 1 of pregnancy. The compound was administered orally from days 1-7 *post-coitum (p.c.)* to hamster and days 1-10 *p.c.* to rat through a specially fabricated stainless steel feeding needle attached to the hypodermic syringe. The animals were autopsied on day 12 *p.c.*,

*Communication No. 5781

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following inhalation of anaesthetic ether (Propyl gallate i.p.; 0.0018%; Kabra Drugs, Indore, India). The presence of implantation sites was considered as a criterion for successful mating while their absence indicated failure of implantation caused by the compound. The status of corpus luteum was also recorded simultaneously (Table 1).

Preparation of compound for bioassay — The compound was macerated with few drops of Tween-80 and then suspended in the measured quantity of distilled water for testing antiimplantation efficacy. For progestational/antiprogestational activity, the compound was first dissolved in ethanol and then made up to the required volume by adding olive oil. Ethanol was evaporated afterwards by heating on the water bath.

Estrogenic and antiestrogenic activity — The rat uterine weight bioassay method was used[8]. Immature female albino rats (40-50g) were bilaterally ovariectomized under anaesthesia and then allowed for 7 days post-operative recovery prior to the initiation of treatment. The animals of group-I (control) were injected (sc) olive oil, while those of group-II were administered the compound orally. Estradiol dipropionate (EDP) was injected (sc) to the animals of group-III, while the compound and EDP in their respective doses were given conjointly to those of group-IV (Table 2). The animals in each group were treated for three days and the autopsy was done 24 h later. The uteri were dissected out, cleared from mesenteries, blotted on filter paper and then weighed to the nearest milligram on Tortion balance.

Progestational and antiprogestational activity — This was determined by traumatizing (TR) one of the uterine horn of immature female rats, considering decidual cell reaction (DCR) as an end-point[9]. The animals were divided in six groups. Group-I served as olive oil control, while the animals of groups II-VI were initially primed with progesterone (P;2 mg/kg; sc) for 3 days prior to TR (4th day), following which the respective treatments were given for five days (Table 3). The TR was done by deeply scratching the antimesometrial luminal lining of the horn through needle. The other horn (without scratching) served as positive control. While the animals of group-II were injected (sc) P only, those of groups-III and IV were administered the compound (C) at 100 and 150 mg/kg respectively. The animals of last two groups (V and VI) were treated with the C (at 100 and 150 mg/kg) and P (2 mg/kg) conjointly. Treatments continued up to day 8 morning and the animals were autopsied in the afternoon. Each horn was cleared and weighed separately.

The study has demonstrated that the administration of pure compound causes significant reduction in number of implantations in hamster at increasing doses. For example, while at the lower doses (50 and 100 mg/kg) the efficacy was up to 50 and 80% respectively, at 150 mg/kg it was 100%. However, in case of rat the compound failed to prevent implantation completely even up to 150 mg/kg (Table 1). Out

Table 1 — Effect of compound of *Dipsacus mitis* on contraceptive efficacy in hamster and rat

Treatment (mg/kg)	Schedule (post-coitum)	No.of animals	No.of implantations (Mea±SE)	No.of corpora lutea (Mean±SE)
		Hamster		
Control	1-7	4	9.5±0.65	10.7 ± 0.25
50.00	1-7	4	4.2±2.53	8.7 ± 2.10
100.00	1-7	5	2.0±1.38	7.4 ± 1.21
150.00	1-7	3	0.0±0.00	3.3 ± 0.33
		Rat		
Control	1-10	3	8.3±0.88	8.3 ± 0.88
100.00	1-10	3	10.0±1.50	10.5 ± 0.50
150.00	1-10	3	8.0±1.00*	9.6 ± 3.05

*In two animals litter size was smaller while in the third animal foetal resorption was noticed.

of the three animals two had smaller litter size while the third had resorption of the foetuses.

In case of estrogenic and antiestrogenic activity, the compound *per se* neither demonstrated any uterotrophic activity nor it could antagonise the effect of EDP (Table 2). In progestational bioassay also, the compound *per se* (at 100 and 150 mg/kg) failed to induce deciduoma in traumatized horn (groups III and IV). Whereas, in antiprogestational bioassay the compound was found to potentiate the effect of P at both the doses (Table 3).

Results reveal that the compound possesses cent percent contraceptive efficacy in hamster but partially effective in rat. The efficacy could not be improved in rat even up to 150 mg/kg. Is this due to species difference, difficult to be explained at present? However, since pre and early postimplantation development of embryo in hamster independent on low but uniform titre of P[10] the potentiating effect of C may be responsible for better contraceptive efficacy in this species.

The mode of contraceptive action of the C is not explainable from its hormonal profile, so far as the uterotrophic/antiuterotrophic and progestational activity is concerned. But from the point of antiprogestational activity it seems C possesses the property of potentiating the effect of P at 150 mg/kg (group VI vs. group II, $P<0.01$). Possibly the potentiating effect of C is responsible for its contraceptive efficacy. The enhancement over the endogenous titre of P during early pregnancy presumably lowers the metabolic status of the endometrium, as a result of which the latter does not permit sustenance of pregnancy in a normal way. This phenomenon in the uterus has been noticed by one of us[11] as well as by others[12] with low dose of steroidal progestins (Norgestrel and Megestrol acetate).

Since the information on antifertility efficacy of *D. mitis* is not available in literature, this study is the first

Table 2 — Estrogenic and antiestrogenic activities of compound of *Dipsacus mitis* in ovariectomized rat

Treatment	Wt of uterine horns (mg; mean± SE)	*P*-values (vs. group I)
Group I (Vehicle)	27.5±12.5	—
Group II Compound (C) (100 mg/kg)	18.7±2.50	> 0.05
Group III EDP (0.5 µg/rat)	50.0±0.00	< 0.01[a]
Group IV EDP (0.5 µg/rat) + C	54.0±0.36	< 0.01[b]

[a]Gr. III vs. II, $P < 0.01$; vs. IV, $P > 0.05$
[b]Gr. IV vs. II, $P < 0.01$

Table 3 — Progestational and antiprogestational activities of compound of *Dipsacus mitis* in immature rat

Treatment	Wt of the uterine horn (mg; mean± SE)		'P' values (TH vs. CH)
	Traumatized horn (TH)	Control horn (CH)	
Group-I (Vehicle)	70.0±5.77*	36.6±6.67	< 0.05
Group II Progesterone alone (P) (2 mg/rat)	160.0±30.55	43.3±6.67	< 0.01
Group III Compound (C1) (100 mg/kg)	56.6±8.82	43.3±8.82	> 0.05
Group IV Compound (C2) (150 mg/kg)	53.3±20.28	33.3±6.67	> 0.05
Group V P+C 1	180.0±36.06	53.3±3.33	< 0.01
Group VI P+C 2	233.3±44.10	83.3±33.83	< 0.01

*Between the groups in traumatized horn;
Gr.I vs. Gr.II, $P < 0.05$; vs. Gr.III and IV, $P > 0.05$; vs. Gr. V and VI, $P < 0.01$

to report contraceptive efficacy and mode of its action at the uterine level.

The authors are grateful to RONAST-INSA and Natural Product Development Division (Nepal) for providing a grant to SS, and to Drs. C.M. Gupta, Director and S.R. Chowdhury, Head, Division of Endocrinology for providing the facilities. Recognition is afforded to Mrs. R. Pant for technical assistance and Mrs. TM. Shrestha NPDD, Nepal for collection of plants.

References

1 Flora of Phulchoki and Godawari *Bulletin of Department of Medicinal Plants No 2* (HMG Press, Kathmandu, Nepal), 1969, 58.

2 Chin W V & Keng H, *Chinese medicinal herbs* : An illustrated dictionary (1990) 71.

3 Podanyi B & Stephen Reid R, *J Nat Products*, 52 (1989) 135

4 Kouno I, Tsuboi A, Nanri M & Kawano N, *Phytochemistry*, 29 (1990) 338.

5 Abdallah O M, *Phytochemistry*, 30 (1991) 2805.

6 Tomita H & Mouri Y, *Phytochemistry*, 42 (1996) 239.

7 Ali M & Gupta J, *Oriental J Chem*, 9 (1993) 167.

8 Mehrotra P K & Karkun J N, *Indian J Exp Biol*, 13 (1975) 69.

9 Mehrotra P K & Kamboj V P, *Planta Medica*, 33 (1978) 343.

10 Gianina T, Butler M, Popick F & Steinetz B, *Contraception*, 1 (1971) 347.

11 Mehrotra P K, Srivastava A K, Dasgupta P R & Karkun,J N, *Indian J Exp Biol*, 13 (1975) 67.

12 Sanwal P C, Pande J K, Dasgupta P R, Kar A B & Setty B S, *Steroids*, 15 (1970) 711.