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Antifertility Activity of *Pergularia daemia*

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The ethanolic extract of *Pergularia daemia* and its steroidal fraction were studied for antifertility activity. Both the ethanol extract and the steroidal fraction showed significant antifertility activity in the pre-implantation stage in female mice. The ethanol extract also showed late abortifacient activity.

Key words: Antifertility, *Pergularia daemia*

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Introduction

During recent years, a number of papers have been published, suggesting the possible antifertility activity from the medicinal plants growing in different parts of the world (Mazumder *et al.*,1992). A great number of indigenous medicinal plants are use as antifertility agent in rural Bangladesh, a few of these plants have been studied in some detail (Chowdhury *et al.*, 1984). *P. daemia,* a perinneal twining herb belonging to the family Asclepiadaceae has a folkloric reputation as an antifertility agent and are being used by the rural people of the northern parts of Bangladesh to induce abortion. The plant is reported to contain an active constituent of glucosidic nature which exhibited oxytocic properties (Gupta *et al.*, 1946). It is also a drug of good repute in the Ayurvedic literature in uterine complaints and facilitates parturition (Kirtikar and Basu, 1994). Keeping in view, the study was undertaken to investigate the antifertility activity of *P. daemia*.

Materials and Methods

Collection of the plant: The plant, *P. daemia,* was collected from Jhenaidoh district of Bangladesh and was botanically identified by the Bangladesh National

Ethanol extract: Coarse dried powder (800 g) of stem and leaves of the plant was defatted with petroleum ether (40 - 60°C) in a soxhlet apparatus at 35°C for 48 hours. The powder was then removed, air dried and soxhletted with 95% ethanol at 40°C for 72 hours. The filtered ethanol extract was concentrated in a rotary evaporator under reduced pressure to obtain a dried greenish mass 21.4 g which was subsequently used for antifertility experiment in animals.

Steroidal fraction of the ethanol extract: The crude ethanol extract 21.4 g was treated with 1N H_2SO_4 (50 ml) for 24 hours, diluted with water 50 ml and filtered. After discarding the crude residue, the acidic aqueous phase was extracted with chloroform (40 ml X 6) to yield the chloroform extract which is termed as the steroidal fraction of the ethanol extract. This extract was evaporated to dryness *in vacuo* to yield a gummy mass 9.6 g which was used for antifertility study in animals.

Preparation of the test agent for antifertility activity: The ethanol extract and its steroidal fraction were suspended in water with the help of 1% gum accacia.

Selection of animals for the antifertility study: Adult albino mice (female 35 and male 10) of proven fertility were collected from International Centre for Diarrhoeal Diseases Research, Bangladesh (ICDDR,B). After proper marking, the female and male mice were caged separately and maintained on standard balance diet. Before initiation of antifertility activity experiments, the female mice having regular oestrous cycle and kept in cages in a ratio of 2 female; male for mating. The vaginal smear of the female mice was examined daily for the presence of thick cluster of spermatozoa. The day on which thick cluster of spermatozoa was found in the vaginal smear, it was marked as day one of pregnancy and the males were withdrawn from the cages on the day.

Drug treatment: The antifertility activity was studied according to the procedure adopted by (Choudhury, 1970) . The female mice received drug through oral route either for 1-9 or 12-16 days of pregnancy. The animals of the control group received the vehicle only. Animals receiving drug from day 1 to day 9 of pregnancy were laparotomized on the 10th day and number of implantation sites were observed in the horns of the uterus; while the animals receiving drug from day 12 to day 13 of pregnancy were allowed to continue for full term.

Results and Discussion

Results in Table 1 to 3. revealed that oral administration of the ethanol extract of *P. daemia* at a dose of 600, 400 and 200 mg/kg body weight daily was found to terminate pregnancy in the preinplantation stage in mice. But when the dose was reduced to 100 mg/kg body weight, pregnancy occured in 20% of the mice. And demonstrated that the extract was able to prevent implantation or cause resorption depending upon the dose.

To identify the bioactive fractions, the steroidal fraction was separated from the ethanol extract by standard method (Harborne, 1976) and was tested for antifertility activity in mice. The fraction at a dose of 200 mg/kg body weight exhibited antifertility activity in mice at preimplantation stage in Table 2.

The possible cause of termination of pregnancy upon oral administration of the ethanol extract and its steroidal fraction from day 1 to day 9 of pregnancy might be due to antizygotic, antiblastocytic as well as antioestrogenic property. Since the twig of the plant is used by the rural people of Bangladesh to induce abortion, late abortifacient effect of the extract was

Table 1: The antifertility activity of the ethanol extract of *P. daemia* at pre-implantation stage in mice.

Test Group n=5	Dose mg/kg body weight	Number of mice having implantation sites on day 10 of pregnancy	Number of mice having no implantation sites on day 10 of pregnancy	Mean number of implantation sites	Inhibition of fertility in %
A	600	0	5	0	100
B	400	0	5	0	100
C	200	0	5	0	100
D	100	4	1	8.5	20
Control	Vehicle	5	0	10.6	0

n = Total number of mice; The extract / vehicle (gum acacia) is administered orally from 1 to 9 day of pregnancy.

Table 2: The antifertility activity of the steroidal fraction of the ethanol extract at pre-implantation stage in mice.

Test Group n=5	Dose mg/kg body weight	Number of mice having implantation sites on day 10 of pregnancy	number of mice having no implantation sites on day 10 of pregnancy	Mean number of implantation sites	Inhibition of fertility in %
E	200	0	5	0	100
Control	Vehicle	5	0	9.8	0

n = Total number of mice; The extract / vehicle (gum acacia) is administered orally from 1 to 9 day of pregnancy.

Table: 3: Abortification activity of the ethanol extract of *P. daemia* in mice

Test Group n=4	Dose mg/kg body weight	Mice died before parturition after application	Period of abortion after application of drug	Mice died after abortion of drug (hrs)	Number of foetus delivered	Abortifacient activity in %
F	800	2	within 24 hrs	0	2, 3 – premature	50
G	600	0	Within 48 hrs	0	2, 2, 3, 4 – premature	100

n = Total number of mice; The extract is administered orally from day 12 to 13.

studied. The ethanol extract also showed late abortifacient activity in the mice. In this case pregnant mice was treated with the extract at a dose of 600 and 800 mg/kg body weight daily for any two consecutive days from 12 to 13 of pregnancy, respectively. The former dose produced 50% abortifacient activity with 50% mortality as two of the mice died after oral administrate of the extract. The extract at a dose of 600 mg/kg body weight exhibited 100% abortifacient activity without any mortality. The mice aborted all the foetuses within 48 hours of drug treatment. The present investigation clearly demonstrate that the ethanol extract and its steroidal fraction are able to prevent fertilization in the female mice.

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