

Antifertility Activity of the Alkaloidal Fraction of *Pergularia daemia*

Md. Golam Sadik, M. A. Gafur, M. Shah Alam Bhuiyan,
M. Motiur Rahman and Helal U. Biswas

The ethanol extract of *Pergularia daemia* and its steroidal fraction are reported to have antifertility activity. In this work we studied the alkaloidal fraction of the ethanol extract to observe its antifertility activity. Oral administration of the alkaloidal fraction at a dose of 200mg kg⁻¹ body weight showed a significant activity in preimplantation stage of female mice. The activity of the alkaloidal fraction, when compared with the steroidal fraction, was found to be more pronounced since the former inhibited not only the fertility of the female mice but also took short period to return the oestrous cycle to normal, with in 4 to 6 days of drug treatment while steroidal fraction treated mice returned to normal within 6 to 8 days.

Key words: Antifertility activity, *Pergularia daemia*, alkaloidal fraction

Md. Golam Sadik
Department of Pharmacy,
Rajshahi University,
Rajshahi-6205,
Bangladesh

Fax: 88 0721 750064
E-mail: gsadik2@yahoo.com
rajucc@citechco.net

Introduction

In recent years, there has been considerable interest on plants to explore the possibility of selecting a potent antifertility agent (Mazumder *et al.*, 1993). It is a well known that a good number of medicinal plants are in use as traditional contraceptive pill in rural Bangladesh, a few of these plants have been studied in some detail (Chowdhury *et al.*, 1982 and 1984). *Pergularia daemia*, a perinneeal 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). Recent report (Sadik *et al.*, 2000) from our laboratory has shown that the C₂H₅OH extract of *p. daemia* and its steroidal fraction possess significant anti-implantation and abortifacient activity. The present investigation is an attempt to evaluate the antifertility activity of the alkaloidal fraction and to compare its activity with that of the steroidal fraction.

Materials and Methods

Collection of the plant: The plant, *Pergularia daemia*, was collected from Jhenaidoh district of Bangladesh and was botanically identified by the Bangladesh National Herbarium, Dhaka, Bangladesh where a voucher specimen was deposited.

Ethanol extract: The C₂H₅OH extract was prepared by extraction of dried powder of stems and leaves of the plant Sadik *et al.* (2000).

Steroidal and alkaloidal fraction of the ethanol extract: The crude extract 21.4g was treated with 1N H₂SO₄ 50ml for 24hr., diluted with water 50ml and filtered. After discarding the crude residues the acidic aqueous phase was extracted with chloroform 40ml X 6 to yield the chloroform extract (CHCl₃) which is termed as the steroidal fraction of the C₂H₅OH extract. This extract was evaporated to dryness *in vacuo* to yield a gummy mass (9.6g), which was used for antifertility study in animal. The remaining acidic aqueous layer was then made basic by adding concentrated ammonia and was extracted with CHCl₃ (40ml x 3) which is termed the alkaloidal fraction of the extract. This extract was evaporated to dryness *in vacuo* to yield a brownish gummy material (1.5g), which was subsequently used for antifertility study in animal.

Tests for alkaloids: Presence of alkaloid in the alkaloidal fraction obtained from the C₂H₅OH extract was investigated by Dragendorff, Mayer, Wagner and Hager's tests as described by Harborne (1976).

Preparation of the test agent for antifertility activity: The alkaloidal and steroidal fractions were suspended in water with the help of gum acacia (1%).

Selection of animals for the antifertility study: Adult albino mice (20 female and 10 male) of proven fertility were collected from International Center 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 were selected and kept in cages in a ratio of two female: one 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, was marked as day one of pregnancy and males were withdrawn from the cages on the day.

Drug treatment: The antifertility activity was studied according to the procedure adopted from the literature with modification in drug treatment schedule (Choudhury, 1970). The female mice received drug through oral route for 1 to 9 days of pregnancy at a dose level mentioned in the Table 2. 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 numbers of implantation sites were observed in the horns of the uterus.

Study of oestrous cycle of mice: The oestrous cycle in mice was studied based on cytological morphology of the vaginal smear as described by Choudhury (1970).

Results and Discussion

In the previous investigation it was proved that the C₂H₅OH extract of *p. daemia* and its steroidal fraction possess significant antifertility activity in the preimplantation stage in female mice (Sadik *et al.*, 2000). As the extract also contain a significant amount of alkaloid it was interesting to observe the antifertility activity of the alkaloidal fraction.

Oral administration of the alkaloidal fraction at a dose of 200mg kg⁻¹ body weight daily was found to terminate pregnancy in the preimplantation stage of the experimental mice as no implantation site in the uterine horn was observed after laparotomy on the 10th day of pregnancy (Table 1). While mice of the control group treated with vehicle (gum acacia) showed implantation sites when examined after sacrificing the animal. The observations clearly demonstrated that the alkaloidal fraction was able to prevent implantation in female mice. The possible cause of termination of pregnancy upon oral administration of the drug from day 1 to day 9 of pregnancy might be due to antizygotic, antiblastocytic as well as antioestrogenic property.

Table 1: The antifertility activity of the alkaloidal and steroidal fraction of C₂H₅OH extract of *P. daemia* at pre-implantation stage in mice

Test Group n=5	Dose mg kg ⁻¹ body weight	No. of mice having implantation sites on day 10 of pregnancy	No. of mice having no implantation sites on day 10 of pregnancy	Mean number of implantation sites	Inhibition of fertility in %
A	200	0	5	0	100
B	200	0	5	0	100
Control	Vehicle	5	0	10.6	0

n = Total number of mice, A = Alkaloidal fraction treated group, B = Steroidal fraction treated group; The extract vehicle (gum acacia) is administered orally from 1 to 9 day of pregnancy

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Table 2: Study of oestrous cycle of female mice treated with steroidal and alkaloidal fraction of C₂H₅OH extract or the vehicle after insemination

Group n=5	Mouse No.	Days								
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th
A	1	S	L	L	L	O	D	D	M	P
	2	S	D	D	O	M	D	D	D	P
	3	S	L	L	D	D	O	M	D	D
	4	S	L	D	D	O	M	D	D	O
	5	S	L	L	O	D	D	D	M	O
B	1	S	L	L	L	L	D	D	O	P
	2	S	L	L	D	M	P	O	D	D
	3	S	L	L	L	D	D	O	M	D
	4	S	D	D	D	D	D	M	O	D
	5	S	L	M	O	D	D	L	O	M
Control Group	1	S	L	L	L	L	L	L	L	L
	2	S	L	L	L	L	L	L	L	L
	3	S	L	L	L	L	L	L	L	L
	4	S	L	L	L	L	L	L	L	L
	5	S	L	L	L	L	L	L	L	L

A = Alkaloidal fraction treated group, B = Steroidal fraction treated group;

S = Cluster of spermatozoa in smear, L = Leucocytic smear, O = Oestrous-phase, D = Di-oestrous phase, P = Pro-oestrous phase

Since both the fractions of the C₂H₅OH extract at a dose of 200mg kg⁻¹ body weight inhibited the implantation of the fertilized ova (Table 1), the activity of the alkaloidal fraction was compared with that of the steroidal fraction in terms of their effect on the oestrous cycle. The oestrous cycle of the alkaloidal fraction treated mice returned to normal from pregnancy within 4 to 6 days of drug treatment (Table 2). On the other hand the oestrous cycle of the steroidal fraction treated mice returned to normal within 6 to 8 days of drug treatment. This suggests that the alkaloidal fraction is more active and possesses no effect of prolonging the oestrous cycle compared to that of steroidal fraction. The dioestrous phase of the oestrous cycle is prolonged by the steroidal fraction.

This investigations clearly demonstrate that the alkaloidal fraction of the ethanol extract has great potential to prevent fertilization in the female mice. A detail study is therefore, warranted for identifying and evaluation of its active principle(s) and mode of action.

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