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EFFECT OF METHANOLIC EXTRACT OF *ABRUS PRECATORIUS* SEEDS ON FERTILITY OF FEMALE RATS

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Abstract

This study examines the post-coital contraceptive potential of a methanolic extract of Abrus precatorius *seeds using albino rats. The major constituent of this extract was found to be abrin, an amino acid derivative* (68% *by weight). Furthermore, the extract had no steroidal constituents. Oral administration of the extract using various dose and time regimens (single dose of* 150*mg/kg on day* 1 *of pregnancy; twice daily dose of* 150*mg/kg on day* 1 *of pregnancy; a single dose of* 300*mg/kg, on day* 1 *of pregnancy; and* 300*mg/kg dose from day* 1-7 *of pregnancy) did not suppress fertility significantly. However, a tendency towards foetal aggregation was evident. It is concluded that the polar fractions of this seed (presumably abrin), has very little potential as a post-coital contraceptive agent.*

Key words : methanolic extract, *Abrus precatorius* seeds, fertility, rats

1. Introduction

Oestrogens in high doses, oestrogen-gestagen combinations and intra uterine devices are the only effective methods of post-coital contraception available to women(1). Long term use of steroids are dangerous and therefore an unmet need remains for the development of safe, orally active, post-coital agents of non steroidal origin (1). The development of such an agent from medicinal plants is an attractive proposition and several studies have been launched with numerous plant products with this aim in mind (2, 3, 4). In this respect, Dasai et al. (5) and Zia-Ul-Haque et al. (6) have demonstrated post-coital contraceptive activity in rats with non-polar fractions of seeds of *Abrus precatorius* L (family Leguminoceae). This antifertility action has been attributed to a C-25 sterol, abridine (6), and thus it receives low priority as a potential post coital contraceptive in view of the untoward reactions associated with steroids (1). Of special interest to note is the claim of Sri Lankan Ayurvedic Physicians that the powdered seed of *Abrus precatorius* when taken orally inhibit human conception (7), although this has not been validated clinically.

Recently, polar fractions of *Abrus precatorius* seeds have been shown to suppress fertility of male rats (8) and to inhibit motility of ejaculated human sperm (9). These effects are presumed to be due to abrin, an amino acid derivative (10). To the best of our knowledge, the effects of polar fractions of *Abrus precatorius* seeds on female fertility have not been reported.

The present study was initiated to investigate whether polar fraction of *Abrus precatorius* seed is able to suppress fertility of female (post-coitally) as it does with the male. This was tested using a methanolic extract and albino rats.

2.0 Materials and Methods

2.1 Preparation of the extract: *Abrus precatorius* seeds (dried) were obtained from a local medicinal plant drug outlet. Identity of these seeds was authenticated by Professor B. A. Abeywickrema, Department of Botany, University of Colombo, Sri Lanka.

The finely powdered seeds (460g) were left in 2L of redistilled methanol (Petroleum Corporation. Sri Lanka) at room temperature (28-30° C) for 6 days and filtered. The filterate was concentrated under reduced pressure to give a solvent-free crude extract, a brown semi solid (yield: 22.2g). This solid was dissolved in distilled water for administration to rats. This extract was stored at 4° C and used within two weeks of preparation.

2.2 Animals: Cross bred albino rats from our own colony were used (3-4 months old and weighing between 200-225g). The animals were housed under standard conditions with free access to pelleted food (Oils and Fats Company Ltd., Seeduwa, Sri Lanka), green leaves and tap water.

2.3 Assessment of post-coital contraceptive action: Female rats showing regular vaginal smear cycles, were individually caged overnight with a male of proven fertility. The next morning females showing sperm positive vaginal smears were selected and was considered to be on day 1 of gestation. These rats were randomly divided into 7 groups and were either administered orally with the extract or distilled water (vehicle): group I, (N = 12) received 150mg/kg dose of extract on day 1 of pregnancy (at 10.00): group II, (N = 6) received 1ml of distilled water on day 1 of pregnancy (at 10.00); group III (N = 7) received 150mg/kg dose of extract at 10.00 and another 150mg/kg of extract at 17.00 on day 1 of pregnancy; group IV (N = 6) received 1ml of distilled water at 10.00 and another 1ml at 17.00 on day 1 of pregnancy; group V (N = 9) received 300mg/kg dose of extract at 12.00 on day 1 of pregnancy; group VI (N = 6) received 1ml of distilled water at 12.00 on day 1 of pregnancy; group VII (N = 3) received 300mg/kg dose of extract as 12.00 from day 1-7 of pregnancy; group VIII (N = 8) received 1ml of distilled water at 12.00 from day 1-7 of pregnancy.

These treated rats were examined daily for overt signs of clinical toxicity. On day 14, these rats were laporotomized under ether (BDH Chemicals Ltd., Poole, U.K.), anaesthesia using aseptic precautions and the number of implants in each uterine horn was counted and their external appearance and the spacing were noted.

Table

The effect of methanolic extract of *Abrus precatorius* seeds on fertility of female rats Fertility is expressed as mean numbers of foetuses ± SEM

Group number	Treatment	N	Number of foetuses in the utertine horns (mean — SEM)
I	150mg/kg/extract (at 10.00) on day 1 of pregnancy	12	6.58 ± 1.14
II	1ml distilled water (at 10.00) on day 1 of pregnancy	6	7.66 ± 0.42
III	2x150mg/kg/extract (at 10.00 and 17.00) on day 1 of pregnancy	7	4.85 ± 1.94
IV	2x1ml distilled water (at 10.00 and 17.00) on day 1 of pregnancy	6	7.16 ± 0.65
V	300mg/kg/extract (at 12.00) on day 1 of pregnancy	9	5.33 ± 0.64
VI	1ml of distilled water (at 12.00) on day 1 of pregnancy	6	9.33 ± 0.42
VII	300mg/kg/extract (at 12.00)/day from day 1 to 7 of pregnancy	3	11.33 ± 1.45
VIII	1ml of distilled water (at 12.00)/day from day 1 to 7 of pregnancy	6	8.66 ± 1.05

2.4 Statistics: The results were analysed using Student's t-test and differences were judged significant when p was 0.05 or less.

2.5 Isolation of Abrin: The crude extract (0.5g) was subjected to column chromatography on silica gel eluting with 20% methanol in methylene chloride (10). This yielded pure Abrin as a pale yellow powder (0.12g) Mpt-293°C, UV spectrum in methanol ymax 220nm (Emax 5.59×10^5), 280 nm.

2.6 Estimation of the Abrin content in the crude extract: 4.5mg of the crude extract was dissolved in 100ml of methanol and 1.0ml of this solution was diluted to 10ml in methanol and transferred to the UV spectrometer cell (Varian DMS 90. Double-beam Spectrophotometer). UV spectrum of the crude extract was identical in shape to the UV spectrum of Abrin. The Abrin content was estimated by using Beer-Lamberts Law(A = Ecl) (11). The UV peak at 220nm of absorption 0.792 was used for the calculation.

2.7 Test for steroids: Libermann-Burchard test (12) was performed; 50mg of the crude extract was dissolved in 2ml of dry chloroform and to this was added 0.5ml of Libermann-Burchard reagent (acetic anhydride-sulphuric acid 19.1 v/v). Development of a red colour in the Libermann Burchard test indicates the presence of steroidal compounds in the crude extract.

3. Results

The food and water intake of the extract treated animals were seemingly normal. Moreover, all treated rats appeared healthy and active upto the day of laporotomy: no treatment related overt clinical signs of toxicity were evident.

None of the treatment regimens with the extract reduced fertility significantly (see Table). Nevertheless, 3 out of 7 rats (42%) were found to be completely sterile in the group III and 2 out of 12 (16.6%) in group I. In all treatment groups the size of the foetuses also appeared normal with respect to age of pregnancy. However, the spacing of the foetus in the uterine horns seemed to be reduced, especially at the cervical end, in the rats treated with 150mg/kg dose of extract twice a day on day 7 of pregnancy.

4. Discussion

The abrin content of the crude extract was found to be $68.0 \pm 4\%$ (by weight). Absence of a red colour in the Libermann Burchardt test indicated that the crude extract was devoid of steroidal compounds.

In this study methanol was used as the extraction solvent; methanol is known to yield polar compounds like amino acids and their derivatives such as abrin (13). Indeed, the major constituent of our crude extract was found to be abrin (68% by weight). Furthermore, the extract was devoid of steroidal compounds as evident from the Libermann-Burchardt test. Therefore, if this extract were to interrupt fertility, it is likely to be mediated through a non-steroidal constituent such as abrin which is unlikely to have undesirable side effects.

The results of this study show that the methanolic extract of *Abrus precatorius* seeds (abrin) failed to suppress fertility of female rats when given post-coitally. In contrast, such polar extracts of this seed is known to impair fertility of male rats when given orally(8). Therefore, the inability of abrin to suppress fertility in this study cannot be attributed to a disruption of its structural integrity by gastrointestinal enzymes or to any interference encountered with its absorption in the intestines. Destruction by liver or enhanced

clearance of abrin is also unlikely as it is able to reduce fertility of male rats when administered in a similar fashion(8). The foetal aggregations evident in this study provides support for the aforementioned contentions.

In polytocons species like rat the foetuses are usually evenly placed in the uterus(13). The localization and spacing of the foetuses in the rat uterus is claimed to be dependent on uterine prostaglandin and progesterone levels (14) and on ruffled surfaces of the endothelial cells of the antimesometrial side of the uterus (13) The disturbances in the distribution of the foetuses seen in this study may have been caused by subtle changes in one or more of these parameters induced by the extract (abrin). The extract appears to be non-embryotoxic since no marked elevation of non viable foetuses were evident. However, it remains to be seen whether polar extracts of *Abrus precatorius* seeds has an abortifacient action in mid or full term pregnant rats or an effect on the onset of parturition.

In conclusion, the results of this study show that methanolic extract of *Abrus precatorius* seeds (abrin) has no post-coital contraceptive activity in female rats, but has a tendency to cause foetal aggregation. Further studies are needed to ascertain whether this effect on foetuses interfears with late pregnancy and on viability of offspring at birth.

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