

formulation of choice for *in vivo*<sup>6</sup> studies, which confirmed the ability of the formulation to release the drug in a concentration-independent manner in the biological environment with excellent *in vivo-in vitro* correlation ( $r=0.9995$ ). Comparative results obtained from IR spectral analysis at pre and post sterilization conditions revealed that the drug remained chemically unchanged in sterilization conditions. Validity and authenticity of the sterilization procedure was confirmed by test for sterility, which yielded negative results for the presence of aerobic and fungal organisms.

Accelerated stability study performed at three different temperatures, 30, 40, and 50° for 4 w and the graphical data treatment using method proposed by Free and Blythe<sup>15</sup> indicated that the shelf life of the formulation will be 72 d if stored at ambient conditions. The shelf life predictions were made at 90% confidence levels.

In conclusion, these results indicate that formulation F6 (drug reservoir with 2 % HPMC and 4 % EC as rate controlling membrane) has achieved the targets of the present study such as increased residence time, prolong zero order release, reduction in frequency of administration, and thus may improve patient compliance.

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## Postcoital Antifertility Activity of the Root of *Momordica dioica* Roxb

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Accepted 5 September 2001

Revised 14 August 2001

Received 8 January 2001

**Aqueous and ethanol extracts from the root of *Momordica dioica* Roxb. (Cucurbitaceae) were tested for postcoital antifertility activity in female rats. Both the extracts were found to be most effective in causing significant abortifacient activity. The extracts showed moderate estrogenic activity. Histological studies of the uterus were carried out to confirm estrogenic activity.**

*Momordica dioica* Roxb. Ex. Willd. (Cucurbitaceae) is a perennial dioecious climber with tuberous roots found

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throughout India<sup>1</sup>. The fruits are used to prevent inflammation caused by lizard excretion<sup>2</sup>. The whole plant is used for treatment of eye diseases, poisoning and

fever<sup>3</sup>, while the leaves and fruits are used for mental and digestive disorders and the roots for bleeding piles, bowel affections and urinary complaints<sup>4</sup>. The aqueous extract of the root has spermicidal activity and anthelmintic activity<sup>4</sup>. The root of the plant is being used by Ayurvedic physicians in various parts of North Karnataka to induce abortion. However no antifertility testing has been reported on this plant. Any imbalance in the equilibrium of estrogen and progesterone can cause antiimplantation. Hence the present study is an effort to explore postcoital antifertility activity and also estrogenic and antiestrogenic activity of the root. Phytochemical investigations<sup>5-8</sup> have revealed the presence of traces of alkaloids and ascorbic acid in fruits. Also lectins,  $\beta$ -sitosterol, saponin glycosides, steroidal glycosides, triterpenes, ursolic acid and other steroids are reported to be present in this plant.

Shade dried roots were powdered coarsely and successively extracted in a Soxhlet extractor for 25 h using 21 each of petroleum ether [60-80°], chloroform and ethanol. The remaining material was extracted by maceration with water at room temperature for 7 d. Each extract was evaporated in a rotary flash evaporator. The yield, colour and consistency of the residue of the various extracts is shown in Table 1. Aqueous and ethanolic extracts were subjected to phytochemical testing<sup>9</sup>, which showed the presence of saponin glycoside of triterpenoid type.

To study antiimplantation activity, female Wistar rats weighing between 120-180 g and young female rats weighing between 35-40 g (21-23 d) old were used. The method described by Khanna and Choudhury<sup>10</sup> was employed. Only those rats with normal estrous cycles were selected and antifertility activity was determined. Rats found in proestrous phase of the estrous cycle were caged with males of proven fertility, in the ratio of 2:1

and were observed the next morning for evidence of copulation. Rats showing lumps of spermatozoa in their vaginal smears were separated and that day was noted as pregnancy day 1 and those rats were divided into 4 groups containing 6 rats in each group. The extracts were administered at 200 mg/kg p.o. from day 1 to day 7 of pregnancy. Control rats received the vehicle (water) only. On day 10, laprotomy was performed under light ether anaesthesia and semi sterile conditions. The uteri were examined to determine the number of implantation sites along with corpora lutea. The rats were allowed to recover and deliver after full term.

For studying estrogenic and antiestrogenic activity, young female Wistar rats, (21-23 d old) weighing between 35-40 g were bilaterally ovariectomised under light ether anaesthesia and sterile conditions. They were divided into 8 groups consisting of 6 rats each. The first group served as a control and received vehicle only (Tweën-80, 2%). The second group received ethinyl estradiol in olive oil, 1  $\mu$ g/rat/day, subcutaneously. The third, fourth and fifth groups correspondingly received aqueous, ethanol extract soluble part and ethanol extract insoluble part at a dose of 200 mg/kg. The sixth, seventh and eighth groups received, in addition to ethinyl estradiol, aqueous extract, ethanol extract – soluble part and ethanol extract insoluble part at the dose of 200 mg/kg, respectively. All the above treatments were given for 7 d. On day 8, the rats were sacrificed by decapitation, the uteri dissected out, weighed and fixed in Bouin's fluid for 24 h. The diameter of uterus, thickness of endometrium and the height of the endometrial epithelium were measured in 10 randomly selected sections using a calibrated ocular micrometer. Statistical analysis was carried out using student's t test. The results were expressed as mean  $\pm$ SEM.

TABLE 1: DETAILS OF VARIOUS EXTRACTS

| Extract                          | Colour and consistency    | Yield (g) | % w/w |
|----------------------------------|---------------------------|-----------|-------|
| Petroleum ether, extract         | Dark brown semisolid      | 3         | 0.3   |
| Chloroform extract               | Dark brown semisolid      | 2         | 0.2   |
| Ethanol extract (soluble part)   | Dark brown viscous liquid | 30        | 3     |
| Ethanol extract (insoluble part) | Cream coloured powder     | 10        | 1     |
| Aqueous extract                  | Light brown semisolid     | 25        | 2.5   |

TABLE 2: ABORTIFACIENT ACTIVITY OF THE ROOT OF *MOMORDICA DIOICA* ROXB.

| Group | Treatment (mg/kg)                    | No. of rats | No. of rats with implantation on day 10 | No. of implantation sites in individual rats on day 10 | No. of rats delivered | Abortifacient activity (%) |
|-------|--------------------------------------|-------------|---|--|-----------------------|----------------------------|
| I     | Control                              | 6           | 6                                       | 10,11,9,9,9,10   | 6                     | Nil                        |
| II    | Aqueous extract 200                  | 6           | 6                                       | 9,11*,7,10,11,9  | 1                     | 83.33                      |
| III   | Ethanol extract (soluble part) 200   | 6           | 6                                       | 7,7,6,8,9,9  | Nil                   | 100                        |
| IV    | Ethanol extract (insoluble part) 200 | 6           | 6                                       | 8,9,7,10,9,6   | Nil                   | 100                        |

\*out of 11 implants, only 5 litters were born.

TABLE 3: ESTROGENIC AND ANTIESTROGENIC ACTIVITY OF VARIOUS EXTRACTS

| Group | Treatment (Dose)   | Uterine weight mg/100 g body wt. Mean $\pm$ SEM | Vaginal Histology             |
|-------|--|---|-------------------------------|
| I     | Control  | 78.5 $\pm$ 2.72                                 |                               |
| II    | Ethinyl estradiol (1 $\mu$ g/rat/day)  | 179 $\pm$ 2.15                                  | nucleated and cornified cells |
| III   | Aqueous extract(200 mg/kg)   | 104.83 $\pm$ 2.09*                              | cornified cells               |
| IV    | Ethanol extract – soluble part (200 mg/kg)   | 111.85 $\pm$ 3.48*                              | cornified cells               |
| V     | Ethanol extract – insoluble part (200 mg/kg)   | 95.66 $\pm$ 2.11                                | cornified cells               |
| VI    | Ethinyl estradiol (1 $\mu$ g/rat/day) + Aqueous extract (200 mg/kg)                    | 217.33 $\pm$ 2.996*                             | nucleated and cornified cells |
| VII   | Ethinyl estradiol (1 $\mu$ g/rat/day) + Ethanol extract – soluble part (200 mg/kg)     | 218.35 $\pm$ 6.55*                              | nucleated and cornified cells |
| VIII  | Ethanol extract – insoluble part (200 mg/kg) and Ethinyl estradiol – 1 $\mu$ g/rat/day | 182.66 $\pm$ 5.75*                              | nucleated and cornified cells |

Estrogenic activity \*p <0.001 vs control, student's t-test, n=6

All the extracts were evaluated for post coital antifertility activity at the dose of 200 mg/kg (Table 2). Aqueous extract showed 83% and ethanol extracts 100% abortifacient activity, when compared with control. The estrogenic and antiestrogenic activity of the extracts is

shown in Tables 3 and 4. Oral administration of these extracts caused significant increase in uterine weight. The uterotrophic potency, as shown by the increase in weight of uterus is about 59% in the case of aqueous extract, 63% in the case of ethanol extract (soluble part)

TABLE 4: HISTOLOGICAL CHANGES IN THE UTERUS AND ENDOMETRIUM OBSERVED FOR VARIOUS TREATMENTS

| Treatment Dose  | Height of endometrial epithelium ( $\mu\text{m}$ ) $\pm$ SEM | Thickness of endometrium ( $\mu\text{m}$ ) $\pm$ SEM | Diameter of uterus ( $\mu\text{m}$ ) $\pm$ SEM |
|---|--|--|--|
| Control   | 28.9 $\pm$ 1.83  | 61.7 $\pm$ 2.24                                      | 329.46 $\pm$ 7.08                              |
| Ethinyl estradiol (1 $\mu\text{g}$ /rat/day)  | 50.58 $\pm$ 2.67   | 351.14 $\pm$ 6.62                                    | 745.62 $\pm$ 10.01                             |
| Aqueous extract (200 mg/kg)   | 39.02 $\pm$ 1.94   | 121.38 $\pm$ 7.11*                                   | 492.72 $\pm$ 14.91*                            |
| Ethanol extract – soluble part (200 mg/kg)  | 44.8 $\pm$ 1.45  | 86.7 $\pm$ 4.32*                                     | 445.03 $\pm$ 8.27*                             |
| Ethanol extract– insoluble part (200 mg/kg)   | 31.79 $\pm$ 2.204  | 144.5 $\pm$ 7*                                       | 533.7 $\pm$ 14.4*                              |
| Ethinyl estradiol (1 $\mu\text{g}$ /rat/day) + aqueous extract (200 mg/kg)                  | 52.02 $\pm$ 2.25*  | 484.05 $\pm$ 21.68*                                  | 1030.3 $\pm$ 16.99*                            |
| Ethinyl estradiol (1 $\mu\text{g}$ /rat/day) + ethanol extract – soluble part (200 mg/kg)   | 58.96 $\pm$ 1.07   | 420.5 $\pm$ 3.71*                                    | 926.2 $\pm$ 6.86*                              |
| Ethinyl estradiol (1 $\mu\text{g}$ /rat/day) + ethanol extract – insoluble part (200 mg/kg) | 44.8 $\pm$ 2.405   | 368.5 $\pm$ 16.86*                                   | 835.2 $\pm$ 15.93*                             |

\* p <0.001 vs control, student's t-test, n=6

and 54% in the case of ethanol extract (insoluble part), as compared with that of ethinyl estradiol, which shows mild estrogenic activity of these extracts. This fact is well supported by other parameters such as increase in diameter of the uterus, thickness of endometrium and height of endometrial epithelium. Therefore, the abortifacient activity may be due to mild estrogenic effect of the extracts, causing the reabsorption of the implants by the uterus.

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