

# Antiimplantation activity of the ethanolic root extract of *Momordica cymbalaria* Fenzl in rats

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## ABSTRACT

**Objective:** To evaluate the antiimplantation activity of the ethanolic root extract of *Momordica cymbalaria* Fenzl.

**Materials and Methods:** The acute oral toxicity study was performed according to the OPPTS guidelines. The ethanolic root extract was investigated for antiimplantation, estrogenic and progestogenic activities at doses of 250 and 500 mg/kg body weight. Antiimplantation activity was studied on successive stages of embryogenesis. Estrogenic studies were carried out by examining uterine weight, histoarchitecture of uterus, vaginal cornification, uterine content of glucose, cholesterol and alkaline phosphatase levels in immature rats. Progestogenic activity assay was performed by pregnancy maintenance in rats and Clauberg's test (endometrial proliferation assay) in immature rabbits.

**Results:** Both doses of the ethanolic root extract exhibited highly significant ( $P < 0.001$ ) antiimplantation activity. However, an investigation of the estrogenic activity did not show any increase in uterine weight or vaginal cornification. The histoarchitecture (uterotrophic changes) such as thickness of endometrium and height of endometrial epithelium was unaltered in treated rats. There were no increases in the uterine content of glucose, cholesterol or alkaline phosphatase levels when compared with the control group. Pregnancy was not maintained in the pregnancy maintenance test for progestogenic activity. Uterine proliferation was not seen in Clauberg's test (endometrial proliferation assay) for progestogenic activity in immature rabbits.

**Conclusion:** The ethanolic root extract of *Momordica cymbalaria* Fenzl exhibited antiimplantation activity but this is not due to estrogenic or progestogenic activities.

**KEY WORDS:** Antiimplantation, estrogenic, *Momordica cymbalaria*, progestogenic

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The use of plant preparations and extracts for their antifertility properties has a long-standing history among Indian physicians. The aqueous and ethanolic extracts and dried forms of fruits and leaves of *Momordica cymbalaria* Fenzl. (Cucurbitaceae) have been shown to have antidiabetic and hypolipidemic properties.<sup>[1-3]</sup> The roots of the plant are used for menstrual irregularities, antifertility, antiovarian and abortifacient activities.<sup>[4,5]</sup> Other plants like *Momordica charantia*, *Momordica dioca* etc. of the Cucurbitaceae family also have well-established antifertility activity.<sup>[4]</sup> Our earlier study<sup>[5]</sup> has demonstrated antifertility and antiovarian potential of roots of *Momordica cymbalaria*. However, no detailed studies have been undertaken with reference to its antiimplantation property. Hence, we have undertaken this study to evaluate the antiimplantation activity of *Momordica cymbalaria* Fenzl.

## Materials and Methods

The fresh roots of *Momordica cymbalaria* Fenzl were collected from Gadag district, Karnataka and identified and authenticated by Dr. Sreenath, Department of Botany, Bangalore University, Bangalore. A specimen sample of the same was preserved in the herbarium of the Department of Botany, Bangalore University, Bangalore, with voucher no. 18122003 for future reference.

The roots of *Momordica cymbalaria* were isolated, chopped into small pieces, dried in the shade at room temperature for seven days and powdered. The powder was extracted with ethyl alcohol to get a yield of 14.1% w/w of the ethanolic root extract.

Colony-bred female albino Wistar rats (150-200 g) were

maintained under controlled standard animal house conditions with *ad libitum* access to food and water.

They were fed with standard rat feed (Amrut rat and mice feed, Pranav agro industries Ltd. Sangli, India). All the experiments were performed according to the CPCSEA norms after obtaining the approval of the institutional animal ethics committee (IAEC). The oral acute toxicity study was performed using the up and down procedure (OPPTS guidelines).<sup>[5]</sup>

**Antiimplantation activity:** Antiimplantation activity was determined as described by Khanna and Chowdary.<sup>[6]</sup> Colony-bred female albino Wistar rats of proven fertility (150-200 g) were maintained under controlled standard animal house conditions with *ad libitum* access to food and water. Vaginal smears from each rat were monitored daily. Only rats with normal estrous cycle<sup>[7]</sup> were selected for the experiment. The female rats were caged with male rats of known fertility in the ratio of 2:1 in the evening of proestrous and examined the following morning for evidence of copulation. Female rats exhibiting the copulation plug or thick clump of spermatozoa in their vaginal smears were separated and that day was designated as day one of pregnancy. Pregnant rats were divided into five groups, each group containing six animals. Group I received vehicle only (Tween 80, 1%) and Groups II and III received the ethanolic root extract at 250 and 500 mg/kg body weight doses respectively from days one to seven of pregnancy. Groups IV and V received the ethanolic root extract at 250 and 500 mg/kg body weight doses for three days from days one to three of pregnancy to assess antizygotic activity.<sup>[8]</sup> Groups VI and VII received the ethanolic extract at 250 and 500 mg/kg body weight on days four and five of pregnancy to assess blastocystotoxic activity.<sup>[8]</sup> Groups VIII and IX received the ethanolic root extract at 250 and 500 mg/kg on days six, seven, eight and nine of pregnancy to detect early abortifacient activity.<sup>[8]</sup> All these treatments were given orally. The rats were laprotomized under light ether anesthesia and semisterile conditions on day ten of pregnancy and the number of implantation sites were recorded.

**Estrogenic and antiestrogenic activities:** Estrogenic and antiestrogenic activities were determined as described by Badmi *et al.*<sup>[9]</sup> Colony-bred female albino Wistar rats, each 21-23 days old and weighing 35-45 g (immature rats) were used. Animals were randomly divided into six groups, each group consisting of six animals. Group I received vehicle only (Tween 80, 1%) and served as control. Group II received ethinyl estradiol dissolved in olive oil s.c. at a dose of 1 µg/rat. Groups III and IV received ethanolic root extract at 250 and 500 mg/kg body weight doses. Groups V and VI received ethanolic root extract at 250 and 500 mg/kg body weight doses and ethinyl estradiol (s.c, 1 µg/rat). All the above treatments were given orally for seven days. On the eighth day, the rats were sacrificed and the uteri were dissected out, surrounding tissues removed, blotted on filter paper and weighed quickly on a sensitive balance (Precisa, XB series). A portion of the uterine tissues from control and treated animals were fixed in 10% formalin buffer for 24 h, dehydrated in alcohol and then embedded in paraffin. The paraffin blocks were sectioned at 5 µm intervals and stained with haematoxylin-eosin for histological examinations. The sections were examined under digital microscope (Labomed). Diameter of uterus, thickness of endometrium and height of endometrial epithelium were measured with the help of digital

microscope software. The other portion of the uterus was homogenized in ice-cold distilled water. The homogenate was centrifuged at 3000 rpm for 15 min and the supernatant was used for the estimation of glucose, cholesterol and alkaline phosphatase levels using diagnostic kits (Autospan, Span diagnostics Ltd.).

### **Progestational and antiprogestational activities**

**Pregnancy maintenance test:** Progestational activity was assessed by the pregnancy maintenance test as described by Vogel.<sup>[10]</sup> Mature female Sprague Dawley (SD) rats were inseminated by placing them with male rats overnight in the ratio of 2:1. On the eighth day of pregnancy, the females were ovariectomized. Then, the drug was administered as follows: Group I received Tween 80, 1% (p.o. daily) from the eighth to the 19th day of pregnancy and served as the control; Group II received progesterone 3 mg/rat/day s.c. from the eighth to the 19th day and served as a reference standard; Groups III and IV received the ethanolic root extract of *Momordica cymbalaria* Fenzl at doses of 250 and 500 mg/kg (p.o. daily) from the eighth to the 19th day. Estradiol 1 µg/rat/day was administered concomitantly with the test compound. On the 20th day, the animals were autopsied and the presence or absence of implantation sites and numbers of live embryos were recorded.

**Clauberg's test:** Progestational and antiprogestational activities were assessed in rabbits using Clauberg's assay as described by Vogel.<sup>[10]</sup> Immature female rabbits each weighing 550-650g were maintained under standard experimental conditions. The animals were randomly assigned to six groups of six animals per group. All animals were injected subcutaneously with estradiol valerate at the dose of 8.3 µg/kg daily for a period of six days. After estrogen priming, they were treated as follows: Group I received 0.5% gum acacia solution 10 ml/kg (p.o. daily) for five days; Groups II and III received the ethanolic root extract of *Momordica cymbalaria* Fenzl. at doses of 250 and 500 mg/kg (p.o. daily) for five days; Group IV received norethisterone 0.75 mg/kg (p.o daily) for five days; Groups V and VI received norethisterone 0.75 mg/kg and the ethanolic root extract of *Momordica cymbalaria* Fenzl, at doses of 250 and 500 mg/kg (p.o. daily) for five days. The animals were sacrificed on the 12th day. The uterus was dissected out, adherent tissues were removed, blotted on a filter paper and was preserved in the neutral formalin buffer 10% for 24 h, then dehydrated in alcohol and embedded in paraffin wax. Sections of 5 µm were cut and stained with haematoxylin-eosin and examined under digital microscope (labomed).

**Statistical Analysis:** The data was analyzed using one way ANOVA followed by the Tukey-Kramer multiple comparison posttest. A *P* value ≤ 0.05 was considered to be statistically significant.

### **Results**

No mortality was seen in the acute toxicity test at doses up to 5000 mg/kg. Therefore, we selected 1/10<sup>th</sup> and 1/20<sup>th</sup> of this dose as doses for the study (500 and 250 mg/kg).

**Antiimplantation activity:** The ethanolic root extract at 250 and 500 mg/kg body weight doses exhibited highly significant (*P* < 0.001) antiimplantation activity. Both doses of the extract also showed significant antiimplantation activity when administered

on days 1-3 (antizygotic activity), days 4 and 5 (blastocystotoxic activity) and days 6-9 (early abortifacient activity) [Table 1].

**Estrogenic and antiestrogenic activities:** Administration of the ethanolic root extract in rats at doses of 250 and 500 mg/kg did not show any increase in the uterine weight. The ethinyl estradiol group (group II) showed a highly significant ( $P < 0.001$ ) increase in uterine weight [Table 2]. The vagina remained closed and cornification was not induced in the control or ethanolic extract-treated groups whereas the ethinyl estradiol group showed vaginal opening and cornification of cells [Table 3]. Hence, the ethanolic root extract being studied is not estrogenic in nature at either of the doses. Conjoint administration of ethinyl estradiol and the ethanolic extract at doses of 250 and 500 mg/kg body weight did not show any decrease in the uterine weight when compared with the ethinyl estradiol group [Table 2]. The histoarchitecture (uterotrophic changes) such as thickness of the endometrium and height of the endometrial epithelium in treated rats at 250 and 500 mg/kg body weight doses were similar to that of control, whereas ethinyl estradiol showed highly significant uterotrophic changes [Table 2, Figures 1-4]. Conjoint administration of ethinyl estradiol and the ethanolic root extract at doses of 250 and 500 mg/kg body weight showed no uterotrophic changes when compared with the ethinyl estradiol group [Table 2, Figures 5-6]. The ethanolic extract at doses 250 and 500 mg/kg body weight did not show any increase

in the uterine content of glucose, cholesterol and alkaline phosphatase when compared with the control group [Table 3]. However, the ethinyl estradiol group showed a significant ( $P < 0.05$ ) increase in the uterine content of glucose, cholesterol and alkaline phosphatase which was similar to the uterine contents seen with conjoint administration of the ethanolic root extract and ethinyl estradiol [Table 3].

**Progestational and antiprogestational activities:** Pregnancy was maintained by administration of estradiol 0.1  $\mu\text{g}/\text{rat}/\text{day}$  s.c. and progesterone 3 mg/rat/day s.c. for 13 days to the rats that were ovariectomized on the eighth day of pregnancy. The number of viable fetuses seen at the time of autopsy (on the 20<sup>th</sup> day) and the net success index of pregnancy maintenance was statistically significant ( $P < 0.001$ ) in the rats receiving estradiol and progesterone when compared to the animals in the control group [Table 4]. Animals in the control group had complete abortion and pregnancy was not maintained. Administration of estradiol 0.1  $\mu\text{g}/\text{rat}/\text{day}$  and the ethanolic root extract of *Momordica cymbalaria* Fenzl at doses of 250 and 500 mg/kg/day to the rats did not maintain pregnancy [Table 4].

**Clauser's assay:** Administration of estrogen and the ethanolic root extract of *Momordica cymbalaria* Fenzl at 250 and 500 mg/kg doses showed ramification of the uterus but not proliferation [Figure 8]. Administration of estrogen and norethisterone showed medium proliferation of endometrium

**Table 1**

**Effect of ethanolic root extract of *Momordica cymbalaria* on implantation in rats at different stages of pregnancy**

Treatment	Days of administration	No. of rats without implantation sites on day 10	No. of implantation sites	% of rats without implantation sites on day 10
Control (Tween 80 1%)	1 to 7	0	10.8 $\pm$ 0.94	0
Ethanolic extract 250 mg/kg	1 to 7	6	0*	100
	1 to 3	3	2.6 $\pm$ 1.35**	50
	4 and 5	6	0*	100
	6 to 9	3	3 $\pm$ 1.61**	50
Ethanolic extract 500 mg/kg	1 to 7	6	0*	100
	1 to 3	4	2.16 $\pm$ 1.51**	66.6
	4 and 5	5	1.66 $\pm$ 1.66**	83.3
	6 to 9	4	1.83 $\pm$ 1.32**	66.6

Values are mean  $\pm$  SE (standard error),  $n=6$ , \* $P < 0.001$  when compared with control, \*\* $P < 0.01$  when compared with control

**Table 2**

**Histological changes in the uterus and endometrium after treatment with the ethanolic root extract of *Momordica cymbalaria***

Treatment (dose, mg/kg body weight)	Diameter of uterus (mm) H&E 40x	Thickness of endometrium ( $\mu\text{m}$ ) H&E 100x	Height of endometrial epithelium ( $\mu\text{m}$ ) H&E 100x
Control (Tween 80, 1%)	0.50 $\pm$ 0.09	17.58 $\pm$ 0.3	165.31 $\pm$ 2.26
Ethinyl estradiol (1 $\mu\text{g}/\text{rat}$ )	1.36 $\pm$ 0.04*	37.65 $\pm$ 1.04*	352.78 $\pm$ 5.44*
Ethanolic extract (250 mg/kg)	0.49 $\pm$ 0.008	17.85 $\pm$ 0.31	166.48 $\pm$ 1.62
Ethanolic extract (500 mg/kg)	0.51 $\pm$ 0.02	17.71 $\pm$ 0.21	164.18 $\pm$ 2.82
Ethinyl estradiol (1 mcg/rat) + ethanolic extract (250 mg/kg)	1.37 $\pm$ 0.02	38.68 $\pm$ 1.31	348.35 $\pm$ 5.53
Ethinyl estradiol (1 mcg/rat)+ ethanolic extract (500 mg/kg)	1.31 $\pm$ 0.03	38.68 $\pm$ 1.31	348.35 $\pm$ 5.53

Values are mean  $\pm$  SE (standard error),  $n=6$ . \* $P < 0.001$  when compared with control

**Table 3****Estrogenic and antiestrogenic activities of the ethanolic root extract of *Momordica cymbalaria***

Treatment (dose, mg/kg body weight)	Uterine weight mg/ 100 g body weight	Vaginal cornification	Glucose mg/ 100 mg of uterus	Cholesterol mg/ 100 mg of uterus	Alkaline phosphatase IU/100 mg of uterus
Control (Tween 80, 1%)	39.31 ± 0.53	Vagina not open	0.33 ± 0.02	0.60 ± 0.09	0.26 ± 0.02
Ethinyl estradiol (1 µg/rat)	130.51 ± 9.79*	Open (+++)	0.43 ± 0.03*	0.95 ± 0.07*	0.34 ± 0.04*
Ethanolic extract (250 mg/kg)	36.66 ± 3.08	Vagina not open	0.33 ± 0.01	0.49 ± 0.24	0.21 ± 0.04
Ethanolic extract (500 mg/kg)	39.56 ± 1.95	Vagina not open	0.34 ± 0.03	0.55 ± 0.16	0.24 ± 0.01
Ethinyl estradiol (1 µg /rat) + ethanolic extract (250 mg/kg)	134.14 ± 3.41	Open (+++)	0.38 ± 0.07	0.85 ± 0.03	0.33 ± 0.02
Ethinyl estradiol (1 µg /rat) + ethanolic extract (500 mg/kg)	129.43 ± 6.68	Open (+++)	0.37 ± 0.03	0.83 ± 0.06	0.30 ± 0.03

Values are mean ± SE (standard error), n=6. +++ = cornified cells. \*P < 0.001 when compared with control

**Table 4****Effect of ethanolic root extract of *Momordica cymbalaria*, Fenzl. on the maintenance of pregnancy in the rats ovariectomized on the 8<sup>th</sup> day of pregnancy**

Treatment	Mean viable fetus	Net success index (%)
Group I: Estradiol 0.1 µg/rat/day + Vehicle (control)	0	0
Group II: Estradiol 0.1 µg/rat/day + Progesterone 3 mg/rat/day s.c. (Reference standard)	5.67 ± 0.67*	51.51 ± 6.06*
Group III: Estradiol 0.1 µg/rat/day + Ethanolic extract 250 mg/kg/day p.o	0	0
Group IV: Estradiol 0.1 µg/rat/day + Ethanolic extract 500 mg/kg/day p.o	0	0

Values are mean ± SE (standard error), n=6. \*P < 0.001 when compared to Groups I, III and IV

and endometrial glands [Figure 9]. However, administration of estrogen, norethisterone and the ethanolic root extract of *Momordica cymbalaria* Fenzl did not inhibit the proliferate changes caused by norethisterone [Figure 10].

Statistical analysis: The results have been expressed as mean ± SE (standard error). The statistical significance between groups was analyzed using one-way ANOVA test and Tukey-Kramer multiple comparison posttest and *P*value ≤ 0.05 was considered statistically significant.

## Discussion

Our earlier study<sup>[5]</sup> had demonstrated antioviulatory and abortifacient potential of the roots of *Momordica cymbalaria*. Inhibition of ovulation may be because of hormonal manipulation and may be the cause for the antifertility activity of the roots in the postcoital state of rats. Significant abortifacient potential of the roots may also be due to the antifertility activity. Hence, the present study

is designed to investigate the antifertility activity and the involvement of estrogenic and progestogenic mechanisms in the antiimplantation activity of the roots of *Momordica cymbalaria*. The extract has shown highly significant antiimplantation activity when it was administered from days 1 to 7. The extract was also given during different periods of gestation on successive stages of embryogenesis as described by Hafez.<sup>[8]</sup> From our results, it is evident that the extract inhibits implantation when given at the zygotic stage (days 1-3) and the blastocyst stage (days 3-5).<sup>[11]</sup> Similar activity is reported in many plants.<sup>[12]</sup> Many antifertility plant extracts are known to exhibit estrogenic activity in rats.<sup>[13]</sup> Estrogen causes an increase in protein synthesis, uterine weight, water uptake and retention of fluid leading to ballooning of the uterus.<sup>[14]</sup> In addition, estrogen also induces uterotrophic changes such as increase in diameter of the uterus, thickness of endometrium, height of endometrial epithelium, providing nonreceptive conditions for implantation.<sup>[15]</sup> Estrogen causes vaginal opening, which is a qualitative measure of estrogen potency. Presence of cornified cells in vaginal smears also indicates estrogen activity. Estrogen is known to increase uterine content of glucose, cholesterol, glycogen and alkaline phosphatase, thereby changing the uterine milieu and creating nonreceptive conditions in the uterus.<sup>[14]</sup> Administration of the ethanolic root extract of *Momordica cymbalaria* Fenzl to immature rats at doses of 250 and 500 mg/kg did not show any change in uterine weight or histoarchitecture of uterus (uterotrophic changes) such as diameter of the uterus, thickness of endometrium or height of endometrial epithelium. There was no vaginal opening or presence of cornified cells in the vaginal smear. The treatment also did not show any change in the uterine content of glucose, cholesterol and alkaline phosphatase when compared with control group. Conjoint administration of ethanolic extract with ethinyl estradiol did not decrease the uterine weight and histoarchitecture of uterus (uterotrophic changes) when compared with ethinyl estradiol. The ethanolic root extract itself also did not cause any decrease in the uterine content of glucose, cholesterol and alkaline phosphatase. Hence, neither dose of the ethanolic extract has any estrogenic or antiestrogenic activities.

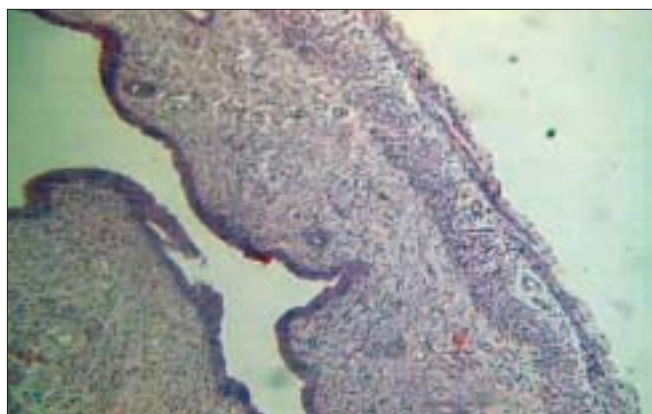
Pregnancy maintainance test and Clauberg's assay are commonly used in the bioassay for progestational activity.<sup>[10,16-18]</sup>



**Figure 1:** Section of immature rat uterus treated with Tween 80 1% (control) (H&E, 100x), n=6



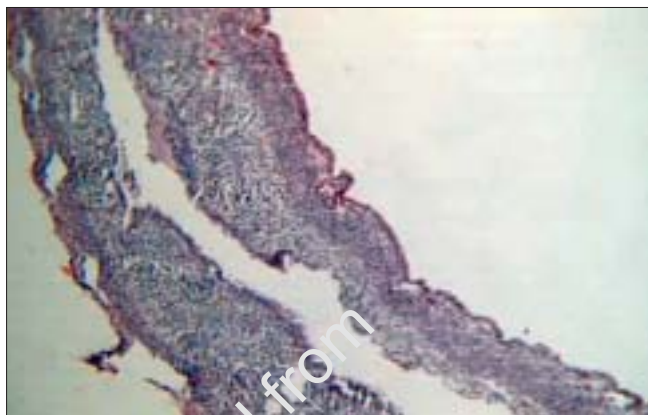
**Figure 2:** Section of immature rat uterus treated with ethinyl estradiol (s.c, 1 µg/rat). (H&E, 100x), n=6



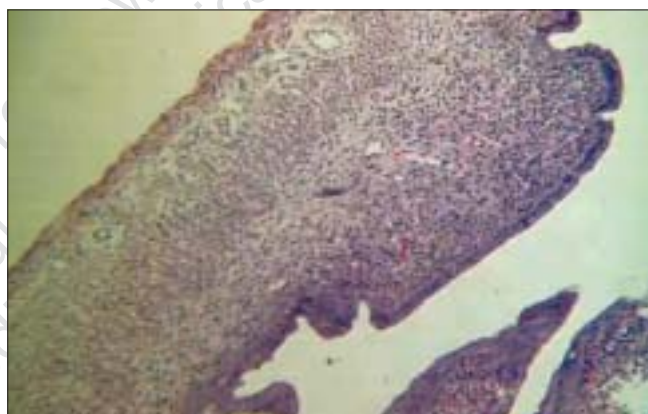
**Figure 3:** Section of immature rat uterus treated with ethanolic root extract (250 mg/kg) (H&E, 100x), n=6



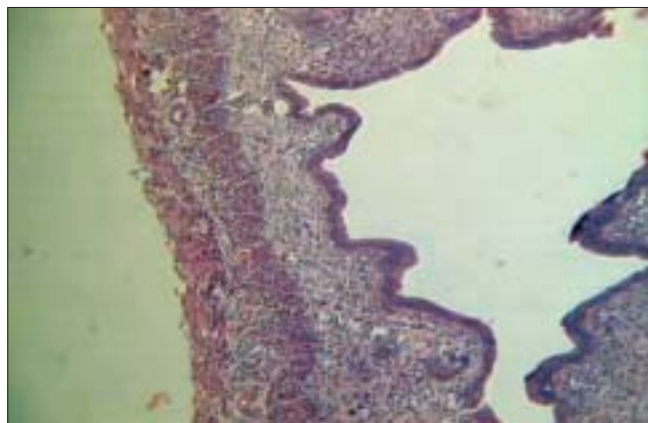
**Figure 4:** Section of immature rat uterus treated with ethanolic root extract (500 mg/kg). (H&E, 100x), n=6



**Figure 5:** Section of immature rat uterus treated with ethanolic root extract (250 mg/kg) and Ethinyl estradiol (s.c, 1 µg/rat). (H&E, 100x), n=6



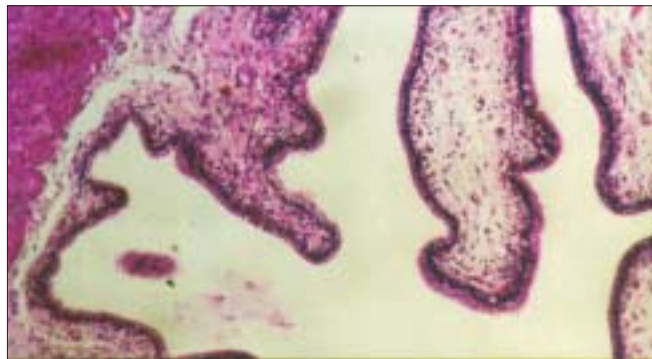
**Figure 6:** Section of immature rat uterus treated with ethanolic extract (500 mg/kg) and Ethinyl estradiol (s.c, 1 µg/rat). (H&E, 100x), n=6



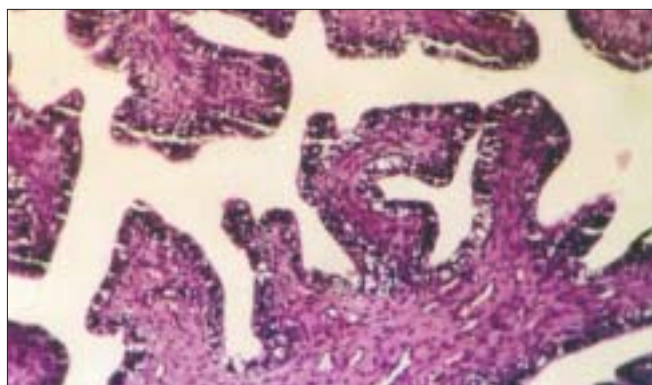
Bilateral ovariectomy performed in rats during the first half of pregnancy results in termination of gestation, but if ovariectomy is performed during the second half of pregnancy, abortion may not necessarily occur. This is due to the capacity of the placenta

to produce progesterone and estrogen. It has been shown that pregnancy can be successfully maintained in rats ovariectomized during the first half of pregnancy by administration of sufficient quantities of exogenous progesterone alone or a combination

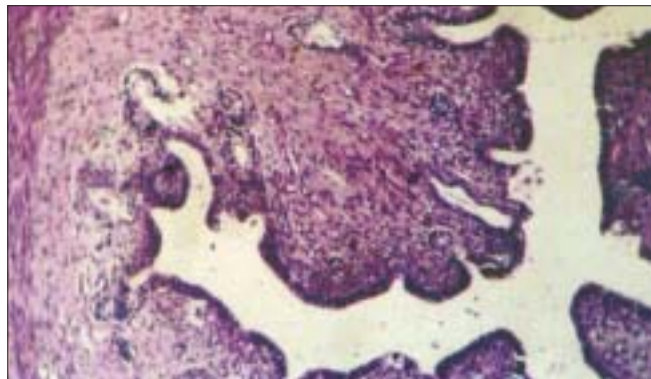
**Figure 7:** Section of immature rabbit uterus treated with 1% gum acacia powder (control) (H&E, 125x), n=6



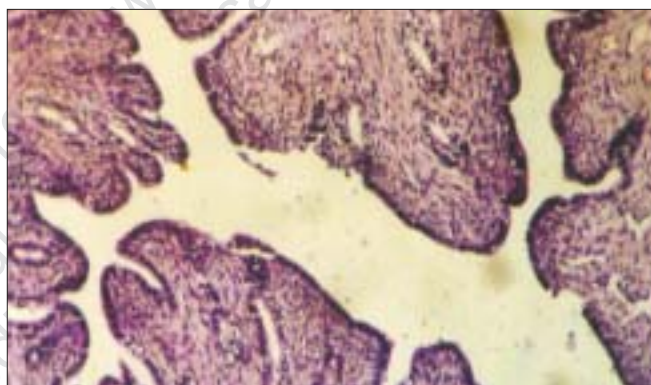
**Figure 8:** Section of immature rabbit uterus treated with ethanolic root extract of *Momordica cymbalaria*, Fenzl. (H&E, 125x), n=6



**Figure 9:** Section of immature rabbit uterus treated with norethisterone at the dose of 0.75 mg/kg (H&E, 125x), n=6



**Figure 10:** Section of immature rabbit uterus treated with norethisterone at the dose of 0.75 mg/kg and ethanolic root extract of *Momordica cymbalaria*, Fenzl at the dose of 250 mg/kg (H&E, 125x), n=6



of progesterone and estrogen. In the present study, unlike the administration of estrogen and standard progesterone, administration of estrogen and the ethanolic root extract of *Momordica cymbalaria* Fenzl. at doses of 250 and 500 mg/kg to the ovariectomised pregnant rats did not maintain pregnancy.

Clauberg's assay is another bioassay of progestational and antiprogestational activities.<sup>[13]</sup> The histological changes in the uterus (e.g., the endometrial proliferation) seen in estrogen-pretreated immature rabbits after the administration of progestational or antiprogestational compounds are assessed here in rabbits. Progestational activity of the compound was assessed by its ability to induce endometrial proliferation (increased uterine diameter and endometrial thickness) whereas its antiprogestational activity was assessed by the ability to inhibit endometrial proliferation induced by norethisterone, a progesterone analog. Administration of estrogen alone to the rabbits caused ramification of the uterus (increased endometrial epithelial cell height), but not proliferation. In the present study, administration of both estrogen and the ethanolic root extract of *Momordica cymbalaria* Fenzl. at doses of 250 and 500 mg/kg showed ramification of the uterus but not proliferation, which was similar to the effect of estrogen and vehicle alone. Hence, the ethanolic root extract of *Momordica cymbalaria* Fenzl.

at doses of 250 and 500 mg/kg may not have progestational activity. However, further investigations are needed to understand the possible antiimplantation mechanisms of *Momordica cymbalaria* Fenzl.

In conclusion, the present study indicates that the ethanolic root extract of *Momordica cymbalaria* possesses significant postcoital antiimplantation activity in rats and it may not be due to estrogenic or progestogenic activities.

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