PUBLICATIONS

Research Papers arising from the present study presented at various conferences

Author	Conference	Title of the paper
Raju Koneri, Saraswati CD, Balaraman R	38th Annual Indian Pharmacological Society Conference	Antiimplantation activity of ethanolic extracts of roots of *Momordica cymbalaria*
Raju Koneri, Kamal Modh , Balaraman R	38th Annual Indian Pharmacological Society Conference	Antidiabetic activity of ethanolic extracts of roots of *Momordica cymbalaria*
Raju Koneri, Ajeesha, Balaraman R	38th Annual Indian Pharmacological Society Conference	Male antifertility activity of ethanolic extracts of roots of *Momordica cymbalaria*
Raju Koneri, Sujauddin, Balaraman R	38th Annual Indian Pharmacological Society Conference	Antiovultory activity of ethanolic extracts of roots of *Momordica cymbalaria*
Raju Koneri, Hariprasad, Balaraman R	38th Annual Indian Pharmacological Society Conference	Cardio protective activity of ethanolic extracts of roots of *Momordica cymbalaria* in isoproterenol induced myocardial infarction
Raju Koneri, Aruna,Vinoth Kumar, Balaraman R	38th Annual Indian Pharmacological Society Conference	Hapatoprotective activity of ethanolic extracts of roots of *Momordica cymbalaria*

Research papers arising from the present study published in journals

1. **Koneri R**, Saraswati CD, Balaraman R, Ajeesha EA. Antiimplantation activity of the ethanolic root extract of *Momordica cymbalaria* Fenzl in rats. Indian J Pharmacol 2007;39:90-96

2. **Koneri R**, Balaraman R, Saraswati CD. Antiovulatory and abortifacient potential of the ethanolic extract of roots of *Momordica cymbalaria* Fenzl in rats. Indian J Pharmacol 2006; 38:111-4.

Research Paper

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

Raju Koneri, C.D. Saraswati*, R. Balaraman*, E.A. Ajeesha*

Visveswarapura Institute of Pharmaceutical Sciences, 25th main, Banashankari 2nd Stage Bangalore - 560070, *Department of Pharmacy, Faculty of Technology and Engineering, M. S. University of Baroda, Baroda, India

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Correspondence to:
Raju Koneri
E-mail: rajukoneri@rediffmail.com

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 progestrogenic 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. Progestrogenic 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 progestrogenic activity. Uterine proliferation was not seen in Clauberg's test (endometrial proliferation assay) for progestrogenic activity in immature rabbits.

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

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

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.[1-3] The roots of the plant are used for menstrual irregularities, antifertility, antiovulatory and abortifacient activities.[4,5] Other plants like *Momordica charantia*, *Momordica dioca* etc. of the Cucurbitaceae family also have well-established antifertility activity.[4] Our earlier study[5] has demonstrated antifertility and antiovulatory 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).[5]

Antiimplantation activity: Antiimplantation activity was determined as described by Khanna and Chowdary.[6] 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[7] 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.[8] 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.[8] 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.[8] All these treatments were given orally. The rats were laprotamized 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.*[9] 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 Vogal.[10] 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 Vogal.[10] 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 norethesterone 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 1/10th and 1/20th 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 ueterine 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 μg/rat/day s.c. and progesterone 3 mg/rat/day s.c. for 13 days to the rats that were ovarietomized on the eighth day of pregnancy. The number of viable fetuses seen at the time of autopsy (on the 20th 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 μg/rat/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].

Claugerg'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 ± 0.94	0
Ethanolic extract 250 mg/kg	1 to 7	6	0*	100
	1 to 3	3	2.6 ± 1.35**	50
	4 and 5	6	0*	100
	6 to 9	3	3 ± 1.61**	50
Ethanolic extract 500 mg/kg	1 to 7	6	0*	100
	1 to 3	4	2.16 ± 1.51**	66.6
	4 and 5	5	1.66 ± 1.66**	83.3
	6 to 9	4	1.83 ± 1.32**	66.6

Values are mean ± 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 (μm) H&E 100x*	*Height of endometrial epithelium (μm) H&E 100x*
Control (Tween 80, 1%)	0.50 ± 0.09	17.58 ± 0.3	165.31 ± 2.26
Ethinyl estradiol (1 μg/rat)	1.36 ± 0.04*	37.65 ± 1.04*	352.78 ± 5.44*
Ethanolic extract (250 mg/kg)	0.49 ± 0.008	17.85 ± 0.31	166.48 ± 1.62
Ethanolic extract (500 mg/kg)	0.51 ± 0.02	17.71 ± 0.21	164.18 ± 2.82
Ethinyl estradiol (1 mcg/rat) + ethanolic extract (250 mg/kg)	1.37 ± 0.02	38.68 ± 1.31	348.35 ± 5.53
Ethinyl estradiol (1 mcg/rat)+ ethanolic extract (500 mg/kg)	1.31 ± 0.03	38.68 ± 1.31	348.35 ± 5.53

Values are mean ± 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 8th 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 postest and P value ≤ 0.05 was considered statistically significant.

Discussion

Our earlier study[5] had demonstrated antiovulatory 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 progestrogenic 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.[8] 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).[11] Similar activity is reported in many plants.[12] Many antifertility plant extracts are known to exhibit estrogenic activity in rats.[13] Estrogen causes an increase in protein synthesis, uterine weight, water uptake and retention of fluid leading to ballooning of the uterus.[14] 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.[15] 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.[14] 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 maintaince test and Clauberg's assay are commonly used in the bioassay for progestational activity.[10,16-18]

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

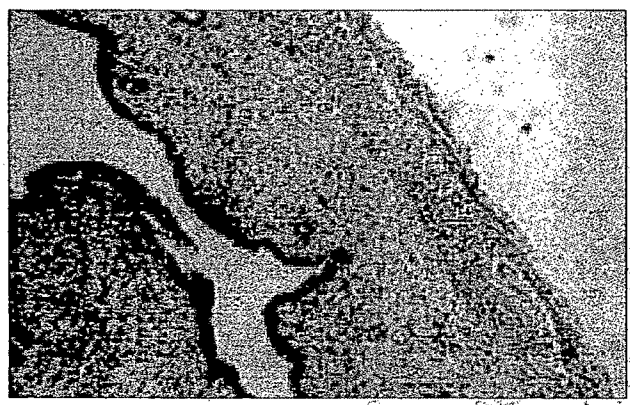

Figure 3: Section of immature rat uterus treated with ethanolic root extract (250 mg/kg) (H&E, 100x), n=6

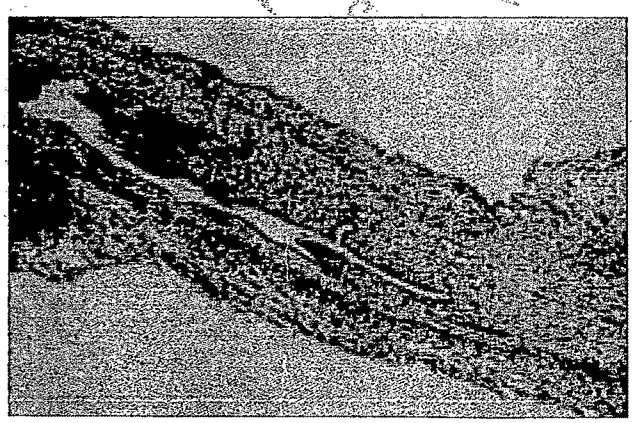

Figure 4: Section of immature rat uterus treated with ethanolic root extract (500 mg/kg). (H&E, 100x), n=6

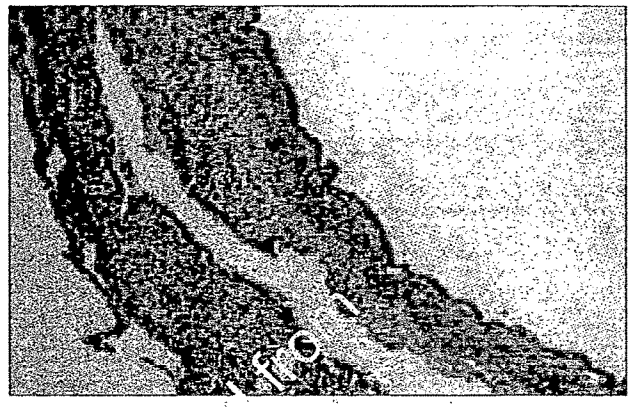

Figure 5: Section of immature rat uterus treated with ethanolic root extract (250 mg/kg) and Ethinyl estradiol (s.c, 1 µg/rat). (H&E, 100x), n=6

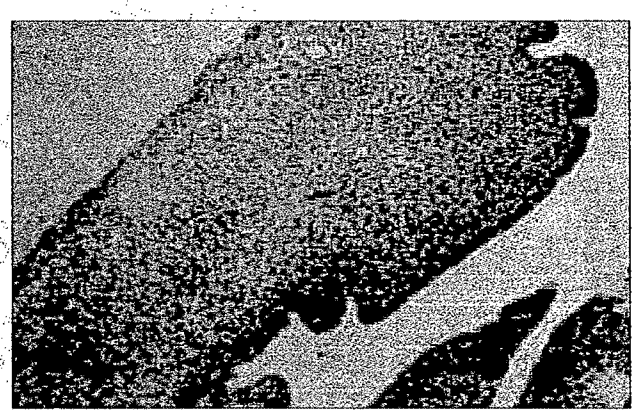

Figure 6: Section of immature rat uterus treated with ethanolic extract (500 mg/kg) and Ethinyl estradiol (s.c, 1 µg/rat).(H&E, 100x), n=6

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

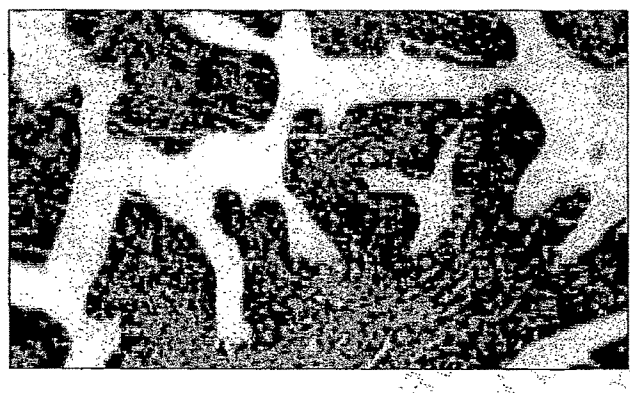

Figure 9: Section of immature rabbit uterus treated with norethisterone at the dose of 0.75 mg/kg (H&E, 125x), n=6

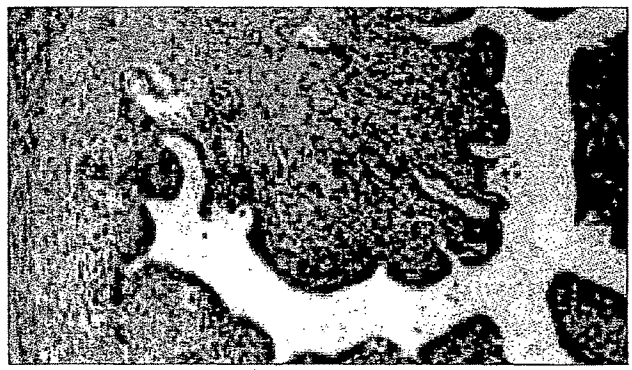

Figure 10: Section of immature rabbit uterus treated with norethisterone at the dose of 0.75 mg/kg and ethanolic root extract of *Momordica cymbalaria*, Fenzl at the dose of 250 mg/kg (H&E, 125x), n=6

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.[13] 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 progestrogenic activities.

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Research Paper

Antiovulatory and abortifacient potential of the ethanolic extract of roots of *Momordica cymbalaria* Fenzl in rats

R. Koneri, R. Balaraman*, Saraswati CD

Visveswarapura Institute of Pharmaceutical Sciences, Vokkaligara Sangha Campus,K R Road, Bangalore 560004
*Department of Pharmacy, Faculty of Science and Technology, M.S. University of Baroda, Baroda.

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Correspondence to:
Raju Koneri
E mail:
rajukoneri@rediffmail.com

ABSTRACT

Objective: To study antiovulatory and abortifacient activities of the ethanolic extract of roots of *Momordica cymbalaria* Fenzl.

Materials and Method: Female Wistar albino rats (150 to 200 g) with at least three regular estrous cycles were administered ethanolic extract of roots of *Momordica cymbalaria* orally (250 or 500 mg/kg) through gastric gavage from the 6th to the15th day of pregnancy (the period of organogenesis). Control group received the vehicle (1%Tween 80, p.o. daily). Daily morning vaginal smears were taken. The animals were laparotomised under light ether anesthesia on the 19th day of pregnancy. The uterus was observed for the number of implantation sites, resorptions, and dead and alive fetuses. The ovaries were dissected and subjected to histopathological and biochemical studies. The fetuses were weighed and examined for anatomic malformations. Fetuses with obvious or suspected malformations were processed for skeletal studies and the rest for visceral studies.

Results: A highly significant (P<.001) decrease in the duration of the estrous cycle and the metaestrous phase and increase in the duration of the proestrous phase was seen. However, the diestrous phase was unchanged at both 250 and 500 mg doses in the treated group, compared with the untreated group. A significant decrease in ovarian weight and a highly significant increase in serum cholesterol with 250 mg/kg dose were seen. Histology of ovary showed an increase in preovulatory and atretic follicles. Ethanolic extract showed an abortifacient effect that was dependent on dose, in pregnant rats, during organogenesis. At 250 mg /kg, ethanolic extract did not show any abortifacient activity, but reduced the number of viable fetuses and resorptions. There was no change in the fetal weight, compared with the control group. At 500 mg/kg, ethanolic extract showed a highly significant (P< 0.001) abortifacient activity.

Conclusion: The ethanolic extract, at both doses (250 and 500 mg/kg), showed antiovulatory activity. It was abortifacient at 500 mg/kg, but not at 250 mg/kg.

KEY WORDS: Estrous phase, ovarian follicles, proestrous.

Introduction

Synthetic estrogens and progesterones, in combination or alone, are extensively used as contraceptives. Although they are highly effective, they are associated with high incidence of side effects. Therefore, the search for new antifertility molecules with minimal side effects continues. Many plant preparations are used to control fertility.[1, 2] The extracts and the dried fruits and leaves of *Momordica cymbalaria Fenzl* were shown to have antidiabetic and hypolipidemic properties.[3-5] Roots are used by the natives of north interior Karnataka and Andhra Pradesh to treat gynecological ailments and to induce abortions. However, scientific study has not been conducted to validate these effects. Therefore, this study examined antiovulatory and abortifacient activities of the ethanolic extract of roots of *Momordica cymbalaria Fenzl.*

Material and Methods

Plant collection and preparation of the extract

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

The roots were isolated and chopped into small pieces. Next, they were dried under shade at room temperature for seven days. The dried roots were powdered, passed through sieve (coarse 10/44), and extracted with 95% v/v ethanol using Soxhlet extractor.[6] The combined extracts were concentrated at 40° C to obtain dark brownish yellow residue. The yield obtained from this process was found to be 21.5 % w/w

Animal

Colony bred female adult albino rats of Wistar strain (150-200 gm) were maintained under controlled standard animal house conditions. They had access to standard rat feed and water *ad libitum*. All the animal procedures were performed according to the CPCSEA norms. The Institutional Animal Ethics Committee approved the experimental procedures. The acute oral toxicity study was performed using the up and down procedure (OPPT guidelines).

Antiovulatory activity

Vaginal smear from each rat was examined daily for 15 days, and those rats exhibited three regular cycles[7] were included in the study. The selected rats were divided into three groups of six animals each. Drugs and vehicle were started in the estrous phase and administered orally, daily for 15 days. Group I received vehicle only (1%Tween 80, p.o. daily) and served as control. Groups II and III received ethanolic extract at 250 and 500 mg/kg, respectively. The 15-day treatment was to cover three regular estrous cycles. Vaginal smear from each animal was observed every morning between 9-10 A.M. On the 16th day, 24 hours after the last treatment, the animals from each group were sacrificed. Ovaries and uteri were dissected out, freed from extra deposition, and weighed on a sensitive balance (Precisa, XB series). One ovary from each animal was processed for biochemical analysis of cholesterol. The other ovary was fixed in 10% formalin buffer for histological study.

Abortifacient activity

The female rats were caged with male rats of known fertility in the ratio of 2:1 in the evening of proestrous. They were examined the following morning for the presence of sperms. Rats exhibiting thick clumps of spermatozoa in the vaginal smear were separated, and that day was designated as day one of pregnancy. The pregnant rats were divided into three groups of six animals each. Group I received vehicle only (1%Tween 80, p.o. daily) and served as control. Group II and group III received ethanolic extract at 250 and 500 mg/kg, p.o. daily, respectively. The extracts were administered orally through gastric gavage from the 6th to the 15th day of pregnancy (period of organogenesis). The animals were laparotomised under light ether anesthesia on the 19th day of pregnancy. Both horns of the uterus were observed for the number of implantation sites, resorptions, and dead and alive fetuses.[9]-[11]

The results are expressed as mean ± SEM. The statistical significance between groups was analysed using the one-way ANOVA test and the Tukey Kramer multiple comparison post-test. $P < 0.05$ was considered significant.

Results

Mortality in the acute toxicity test was not seen in the limit test at a dose of 5000 mg/kg. Therefore, 1/10th & 1/20th of the dose were selected for this study.

Ethanolic extract at 250 and 500 mg/kg caused a significant ($P < 0.001$) decrease in the duration of the estrous and metestrous phases, no change in the duration of the diestrous phase, and a significant ($P < 0.001$) increase in the duration of the proestrous phase, compared with the control group. [Table 1]

Table1

Effect of the ethanolic extracts of *Momordica cymbalaria* on the duration of the different phases of the estrous cycle in rats.

Group	Treatment	Dose (mg/kg)	No. of days in proestrous	No. of days in estrous	No. of days in metestrous	No. of days in diestrous
I	Control (1%Tween 80)	—	2.08 ± 0.53	3.33 ± 0.33	4 ±0.28	5.58 ± 0.37
II	Ethanolic extract	250	7.08 ± 0.5*	1.25 ± 0.28*	1.41 ±0.2*	5.16 ± 0.47
III	Ethanolic extract	500	7.41 ± 0.8*	0.75 ± 0.4*	0.75 ±0.35*	6.16 ± 1.23
One-way ANOVA	F		20.853	15.969	34.973	
	df		15,2	15,2	15,2	15,2
	P		<0.0001	0.0002	0.0001	

Values are mean±SEM. n=6 in each group. *P<0.001 when compared with control.

Table2

Effect of the ethanolic extracts of *Momordica cymbalaria* on ovarian weight.

Group	Treatment	Dose (mg/kg)	Ovarian weight in mg/100 g body weight	Cholesterol level in ovary (mg/50 mg)
I	Control (Tween 80, 1%)	—	40.80±1.13	0.33±0.05
II	Ethanolic extract	250	31.88±0.9*	1.23±0.15**
III	Ethanolic extract	500	34.26±1.16*	0.55±0.03
One-way ANOVA	F		19.131	17.671
	df		15,2	15,2
	P		<0.0001	0.0001

Values are mean±SEM. n=6 in each group. *P< 0.01, ** P< 0.001 when compared with control.

Table 3

Abortifacient effects of the ethanolic and the aqueous extracts of *Momordica cymbalaria* in rats, when fed orally between days 6 and 15 of pregnancy.

Treatment		*Dose (mg/kg)*	*No. of foetus in individual rat*	*No of resorptions in individual rat*	*No of foetus*	*Foetus weight*	*No. of rats aborted*	*Abortion in %*
Control (Tween 80, 1%)		—	13,11,9,12,8,11	0,0,0,0,0,0	10.66±0.16	1.35 ±0.03	0/6	0.0%
Ethanolic extract		250	10,4,12,2,10,8	3,0,3,1,2,0	6.16±1.24**	1.32 ±0.04	0/6	0.0%
		500	0,0,0,0,0,7	0,0,0,0,0,7	—	0*	6/6	100%
One-way	F				15.899			
ANOVA	df				15,2			
	P				<0.0002			

Values are mean±SEM. n=6 in each group. *P<0.001, **P<0.05 when compared with control.

Figure 1. Section of rat ovary treated with Tween 80 1%(control) shows matured graffian follicle(GF), corpus luteum(CL) and developing follicles(DF). H x E 100x

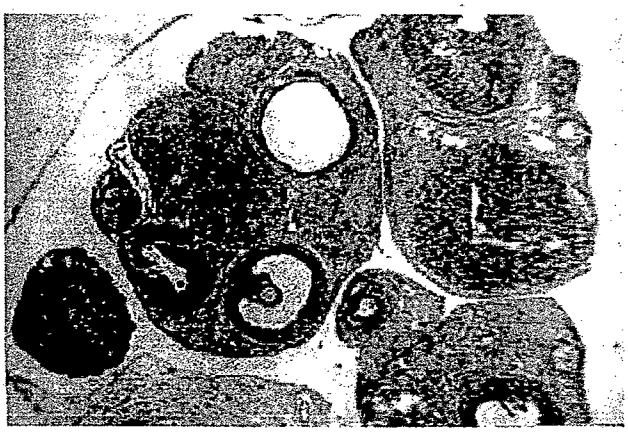


Figure 2. Section of rat ovary treated with ethanolic extract (250mg/kg body weight) shows many primary developing follicles (DF), atretic follicles(AF), and disorganised stroma cells. H x E 100x

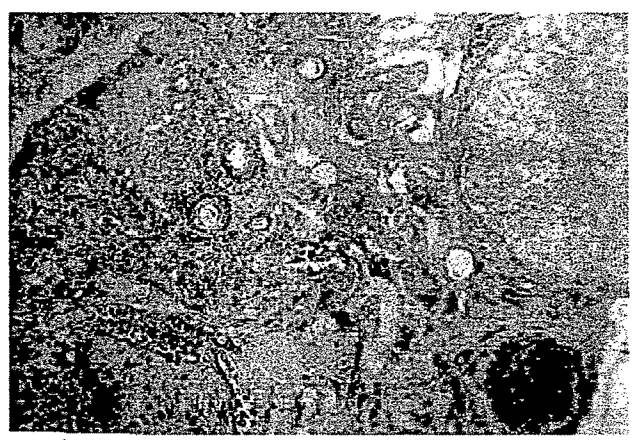
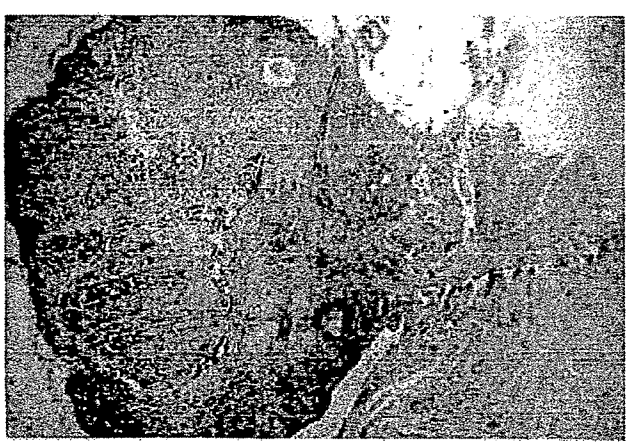


Figure 3. Section of rat ovary treated with ethanolic extract (500mg/body weight) shows many primary developing follicles(DF), atretic follicles(AF) and disorganized stroma cells. H x E 100x



Ethanolic extract at 250 and 500 mg/kg significantly ($P<0.01$) decreased the weight of ovaries, compared with the control group. [Table 2] An increase in preovulatory follicles and atretic follicles was seen in the treated groups (Figure 2 and 3). A highly significant ($P<0.001$) increase in ovarian cholesterol level was observed at 250 mg/kg dose, compared with the control group. [Table 3]

At 500 mg/kg, ethanolic extract showed a highly significant ($P<0.001$) abortifacient effect. At 250 mg /kg, ethanolic extract did not show any abortifacient activity, but reduced the number of viable fetuses and resorptions (6.16 ± 1.24). There was no change in the fetal weight, compared with the control group. [Table 4]

Discussion

Many morphological, histological, physiological, and biochemical changes occur in the ovary during the estrous cycle. During the maturation of preovulatory follicles, ovulation takes place under the combined and balanced influence of ovarian and extra ovarian hormones. Imbalance in these

mones leads to irregularity in the ovarian functions and tion of the estrous cycle.[12]-[14]

he estrous cycle in the rats treated with extract (250 and mg/kg) showed a decrease in the duration of estrous and metestrous phases. It was also characterised by a longation of the proestrous phase. The prolongation of the estrous phase indicates that maturation of the follicle in preovulatory phase was delayed, leading to non-maturation graffian follicle. Non-availability of matured graffian follicle as indicated by reduction in the estrous and the metastrous ases. Therefore, ovulation was inhibited. This result was further supported by our histopathological studies [Figure 2 and 3] in which the transverse section of the ovary showed the presence of primary or developing follicles.

Ovary can be considered an aggregate of three endocrine tissues, the stroma, the follicle and the corpus luteum. The weights of these tissues constitute the net weight of the ovary. During the estrous cycle the weight of the ovarian tissue increases under the influence of gonadotrophic and steroidal hormones. The decrease in the weight of ovaries of the rats treated with extract indicates a decrease in the activity of the stroma, the follicle, and the corpus luteum in the ovary. This decrease may be due to the non-availability of gonadotrophic steroidal hormones or both.[13]

Atretic follicles are degenerating preovulatory follicles. The generation of preovulatory follicles takes place due to non-ilability of steroidal hormones (essential for their maturation d differentiation), non-availability of local estrogen produced granulosa cells, or imbalance in endogenous steroid, protein hormones. The presence of increased atretic follicles in rats treated with ethanolic extract, compared with control indicates that the extract promotes the degeneration of ovulatory follicles. Cholesterol is the precursor for the roidogenesis of ovarian endocrine tissues. The significant rease in ovarian cholesterol in the treated group (250 mg/ dicates that cholesterol is not used for steroidogenesis.[13] 500 mg dose did not show a similar effect indicating that it rs only at a lower dose.

Abortion refers to the premature expulsion of the products nception from the uterus. Abortion may be due to maternal osure to chemicals, which can disrupt pregnancy and cause tachment of the embryo.[10] Ethanolic extract at 500 mg/kg owed 100% abortifacient activity, while 250 mg/kg did not w abortifacient activity. However, it reduced the number of le fetuses.

To conclude a highly significant decrease in the duration of the estrous and the metestrous phases and increase in the duration the proestrous phase was seen. In addition, a highly significant decrease in ovarian weight and increase in cholesterol level, compared with the control group, was noted. These findings indicate that extract *Momordica cymbalaria* produces temporary inhibition of ovulation. The result of administration of extract to the pregnant rats during organogenesis shows that the extract is abortifacient only at the higher dose of 500 mg. These findings could explain the traditional use of *Momordica cymbalaria* as abortifacient and antiovulatory.

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