Evaluation of Ovulation Inhibition Properties of a Phytoestrogen Isolated from *Momordica charantia* Linn. Seeds

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Abstract: *Background and objectives*: *Momordica charantia* Linn. has been used as a dietary / medicinal supplement for health care. The plant has a long history in worldwide people for the usage of vaginal discharge, to promote menstruation and to induce abortions. However, the mechanism of action for its ovulation inhibition effects is not clearly known. Therefore, the present study was carried out to evaluate the ovulation inhibition properties of phytoestrogenic molecule isolated from *M. charantia* seeds in albino rats as an experimental model.

Methods: Ovulation inhibition properties of fractions of ethanol extract and phenolphthalein a phytoestrogenic molecule isolated from seeds of *Momordica charantia* Linn. were evaluated in fertile female albino rats. In the first batch of animals having groups I, II, III, IV were treated with 15 and 25mg/100g oral doses of Fraction I and Fraction II and group V served as control. In the second batch of animals having groups I, II, III was treated with 10, 15 & 20mg/100g isolated phenolphthalein and group IV served as control.

Results: At autopsy on day 31st, decrease in ovarian weight and histological studies shows highly significant decrease in number of developing follicles, Graafian follicles, corpora lutea and significant increased in number of atreatic follicles is observed in Fraction I and phenolphthalein treatment. There are no appreciable changes are observed in the above parameters when both the doses of Fraction II administration.

Interpretation and Conclusions: The study results suggested that *M. charantia* seed fractions and phenolphthalein possess ovulation inhibition properties in experimental animals. Interestingly, in recent years, the popularity of different kinds of pharmaceutical preparations containing phytoestrogens has constantly increasing. Therefore, studies of the influence of the above mentioned plant components on female reproduction have become more and more important. This leads to discovery of newer phytoconstituents with better activities and provide source of new biomolecules for biologists to work on. The studies explore the utilization of medicinal plants for effective management of contraception. It gives a larger platform for the medicinal plant growers by providing scientific support and data to the traditional invalidated herbal drugs. Taking account of all these facts, the present study was undertaken in albino rats.

Keywords: *Momordica charantia,* phytoestrogen, phenolphthalein, ovary, ovulation, contraception.

INTRODUCTION

Many plants produce compounds that may mimic or interact with estrogen hormones in animals. Referred to as phytoestrogens, these compounds are weaker than natural estrogens. In recent studies consuming very high level of phytoestrogens may pose some health risks. Reproductive problems have been documented in laboratory animals, farm animals and wildlife. As for adverse health effects, the most likely risks associated with phytoestrogens deal with infertility and developmental problems [1]. However, it is thought that very large amounts of dietary phytoestrogens would be needed to realize these risks. Humans have used plants for medicinal and contraceptive purposes for eons. For instance, during the fourth century BC, Hippocrates noted that the wild carrot (now known as Queen Anne's lace) prevented pregnancies [2]. Its seeds, we now know, contain a chemical that blocks progesterone secretion a hormone that is necessary for establishing and maintaining pregnancy. Phytoestrogens in dry, summertime grasses reduced the number of offspring in wild populations of California quail [3] and deer [4]. Australian sheep suffered from reproductive problems and infertility after grazing in pastures with the phytoestrogen-containing clover *Trifolium subterraneum* [5]. Two phytoestrogen compounds, equol and coumestrol, were identified as the culprits. Rat pups, exposed to high doses of the plant estrogen coumestrol through their mother's milk, suffered permanent reproductive problems, female pups when grown did not ovulate, and males had altered mating behavior and fewer ejaculations [6]. *Momordica charantia* Linn. belongs to the family Cucurbitaceae growing in agricultural fields in India and different parts of the world. The plant has been used as vegetable as well as the indigenous system of medicine for the treatment of various diseases. *M. charantia* is known for its variety of biological activities like antidiabetic, antimutagenic, antioxidant, antitumour, immunomodulatory and antiulcer [7-12]. It is surprising to note that our previous results of the screening of *M. charantia* seeds have exhibited antispermatogenic and estrogenic activities at very low concentration [13, 14].

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In the present study we aimed to assess the *in vivo* ovulation inhibition properties of *M. charantia* seeds by preparing the organic fractions and resultant isolated potent naturally occurring phytoestrogen phenolphthalein using albino rat as an experimental model.

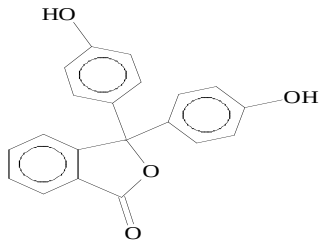
MATERIALS AND METHODS

Plant Material

Momordica charantia Linn. plants were collected from the agricultural fields of Gulbarga (Hyderabad – Karnataka region) in the year 2002 and authenticated in Department of Botany Gulbarga University, Gulbarga. The voucher specimen (HGUG-905) has been deposited in the herbarium. The ripe fruits were collected from the fields in the month of November and December 2002.

Extraction and Isolation

Seeds of *M. charantia* were powdered and subjected to soxhlet extraction with various solvents with increasing polarities (petroleum ether, chloroform and ethanol). All the extracts were concentrated to dryness in flash evaporator (Buchi) and reduced pressure and controlled temperature (40-50°c)**.** Among the three crude extracts obtained from dried seeds of *M. charantia,* the ethanol extract showed ovulation inhibition properties. To isolate the active compound from ethanol extract (80g), water was added to it and extracted repeatedly with n-butanol. The n-butanol fraction on concentration yielded a brownish semisolid which was further purified by repeated preparative thin layer chromatography over silica gel 'G' as absorbent with benzene - methanol (7:3). The Fraction I yielded brownish semisolid (48g) and Fraction II yielded dark brown semisolid (28g) when silica gel was washed with methanol. The Fraction I was then subjected to column chromatography with silica gel (60–120 mesh). The four major fractions were collected, evaporated to dryness and designated as S_1, S_2, S_3 & S_4 fractions. These fractions were rechromatographed separately. Three fractions were yielded whitish powder and one fraction was brown in colour. The preparative HPLC of the combined semi purified fractions was conducted under broadly equivalent conditions to the analytical work using a 25 x 22mm i.d sperisorb ODS 1 column at ambient temperature with the same (methanol - water 50:50 v/v) mobile phase. The compound so obtained from chromatographic separation was whitish amorphous in nature and it is identified as Phenolphthalein.



Structure of Phenolphthalein

Animals

Colony bred female albino rats of Wistar strain (140-160g) with normal estrous cycle were selected for the experiment (Animal ethics Reg. No. 34800/2001/ CPCSEA/ dated 1/9-08-2001). A maximum of six animals were housed in polycarbonate cages with soft rice husk bedding in a room controlled for light-dark cycle, ventilation (air exchange rate of 18 time/hour), temperature (23-25˚C) and relative humidity (50-60%) during the study. The cages and husk bedding was exchanged twice a week. The animal had given balanced food as prescribed by Central Food and Technological Research Institution (CFTRI) Mysore, India and water *ad libitum*.

Drug Preparation and Treatment

The doses containing Fraction I of ethanol extract of *M. charantia* seeds (15 and 25mg/100g) and Fraction II of ethanol extract of *M. charantia* seeds (15 and 25mg/100g) and phenolphthalein (10, 15 & 20mg/100g) were prepared in Tween-80 (1%) as suspension. All the groups received their respective doses orally.

Ovulation Inhibition Studies

In the first batch of animals having five groups, the groups I, II, III and IV were treated with 15 and 25mg/100g oral doses of Fraction I and Fraction II. Group V animals were maintained as control received 1% Tween-80 as a vehicle. In the second batch of animals having four groups, the groups I, II and III were treated with 10, 15 & 20mg/100g isolated phenolphthalein. Group IV animals were maintained as control received 1% Tween-80 as a vehicle. Six animals were maintained in all the groups. All the treatments were given from day 1 to 30. On day 31st all the animals were sacrificed under ether anesthesia and semisterile conditions. The ovaries and uteri were dissected out immediately and separated out from the adherent tissue and weighed to nearest mg on an electronic balance. Organs from one side of each animal were fixed in Bouin's fluid, embedded in paraffin

wax, section at 5µ, stained with Ehrlich's haematoxylene and eosine for histological studies. Number of developing follicles (size between 25 cells – 500 cells), Graafian follicles (size between 501 cells - 1000 cells), corpora lutea and atreatic follicles were counted from stained serial sections of the ovary from each rat [15]. The micrometric measurements like diameter of uterus, thickness of endometrium and myometrium, height of endometrial epithelium were made randomly chosen 20 sections from each group using ocular and stage micrometer [16]. Organs from the other side were used for biochemical studies like cholesterol [17], protein [18], glycogen [19] and DNA [20].

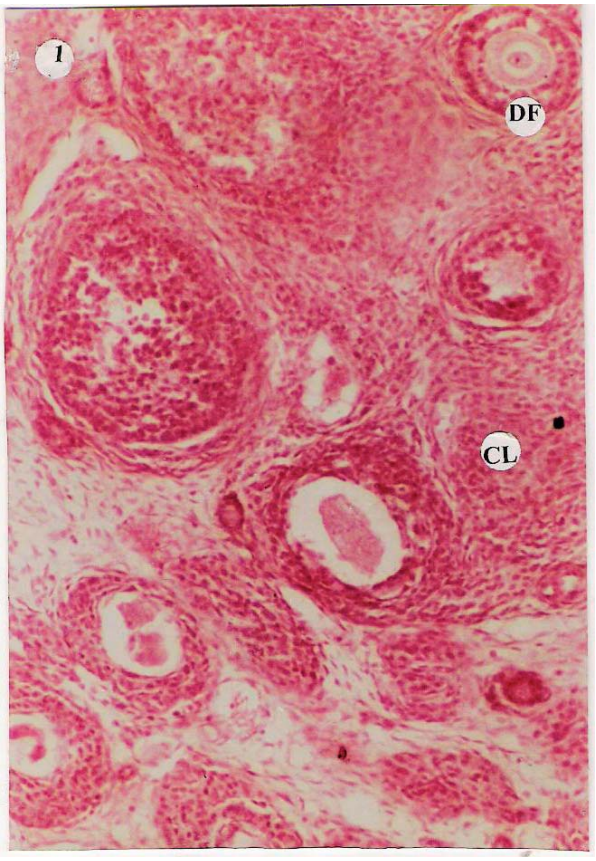


Figure 1: Cross sections of the ovary of control rat showing normal folliculogenesis. Haematoxylene - Eosine stains (x 400).

Statistical Analysis of Data

Results are expressed as Mean ± S. E. Difference between controls and the treatments were tested by the Student's *t-test* for unpaired data.

RESULTS

Results tabulated in Table **1** summarize the results of ovarian gravimetric, histological and biochemical changes due to Fraction I & II treatment for 30 days. Though the ovarian weight is decreased due to the administration of Fraction I and II at both the dose level it is non significant. The number of developing follicles, Graafian follicles, corpora lutea is decreased and that of atretic follicles is increased significantly (P<0.001) with both doses of Fraction I treatment. The results of Fraction II are parallel to that of Fraction I, but are non significant except that of number of Graafian follicles. Both the doses of Fraction I have increased the ovarian cholesterol levels (P<0.001), decreased protein (P<0.001) and glycogen (P<0.01) levels. Similarly the administration of Fraction II at both the dose levels increases the cholesterol level (P<0.01), decreases the protein level significantly (P<0.01). Though the glycogen content is decreased with both the doses of Fraction II it is non significant.

Results tabulated in Table **2** summarize the results of uterine gravimetric, histological and biochemical changes due to Fraction I & II treatment for 30 days. The administration of Fraction I at both the dose level increases the weight of the uterus significantly (P<0.001). The increase in micrometric measurements like diameter of the uterus (P<0.001) myometrium and endometrial thickness (P<0.001) and epithelial cell height (P<0.01) is also seen. Though, the results of administration of both the doses of Fraction II are parallel but they are non significant. Protein, glycogen and DNA contents of uterus are increased significantly (P<0.001) with both doses of Fraction I treatment. Both the doses of Fraction II are less effective in bringing these changes.

Results tabulated in Table **3** summarize the results of ovarian gravimetric, histological and biochemical changes due to graded doses of phenolphthalein treatment for 30 days. The weight of the ovary is decreased due to the isolated compound treatment. The number of developing follicles, Graafian follicles and corpora lutea is decreased indicating follicular growth and ovulation are inhibited. Significant increase in the number of atretic follicles is observed due to the treatment of 10mg (P<0.01), 15mg (P<0.001) and 20mg (P<0.001) isolated compound. Ovarian cholesterol level is increased significantly (P<0.001) due to the treatment of all the three doses of compound. Contrarily protein (P<0.001) and glycogen (P<0.01) contents are decreased.

Results tabulated in Table **4** summarize the results of uterine gravimetric, histological and biochemical changes due to graded doses of phenolphthalein treatment for 30 days. The weight of the uterus is

Table 1: Ovarian Gravimetric, Biochemical and Histological Changes Due to Administration of Fraction I and II of Ethanol Extract of *M. charantia* Seeds

Treatment	Dose (mg/100g body wt.)	Ovarian Weight (mg/100g body wt.)	Cholesterol (µg/mg)	Protein (µg/mg)	Glycogen (µg/mg)	Number / Ovary			
						Developing follicles	Graafian follicles	Corpora lutea	Atretic follicles
Control	Tween-80(1%)	40.16 ±1.17	15.50 ±0.34	14.33 ±0.66	4.30 ±0.42	10.00 ±0.57	7.33± 0.30	8.60 ±0.49	1.16 ±0.16
Fraction I	15	36.66 ±0.49	25.50** ±1.18	6.80** ±0.30	2.33* ±0.21	5.83** ±0.30	3.83** ±0.31	2.66** ±0.33	3.80** ±0.36
Fraction I	25	35.50 ±1.09	30.30** ±0.66	5.33** ±0.41	2.08* ±0.30	3.66** ±0.33	1.60** ±0.32	1.16** ±0.30	4.30** ±0.21
Fraction II	15	38.83 ±0.40	18.83* ±0.75	10.66* ±0.71	3.60 ±0.33	8.83 ±0.30	5.00* ±0.36	6.83 ±0.30	1.00 ±0.25
Fraction II	25	35.30 ±0.80	19.50* ±0.88	8.68* ±0.54	3.50 ±0.25	8.30 ±0.34	4.83* ±0.30	6.80 ±0.30	1.30 ±0.21

Duration: 30 days; six animals were maintained in each group.
Values are mean ± S.E.; * P < 0.01, ** P < 0.001 when compared to control.

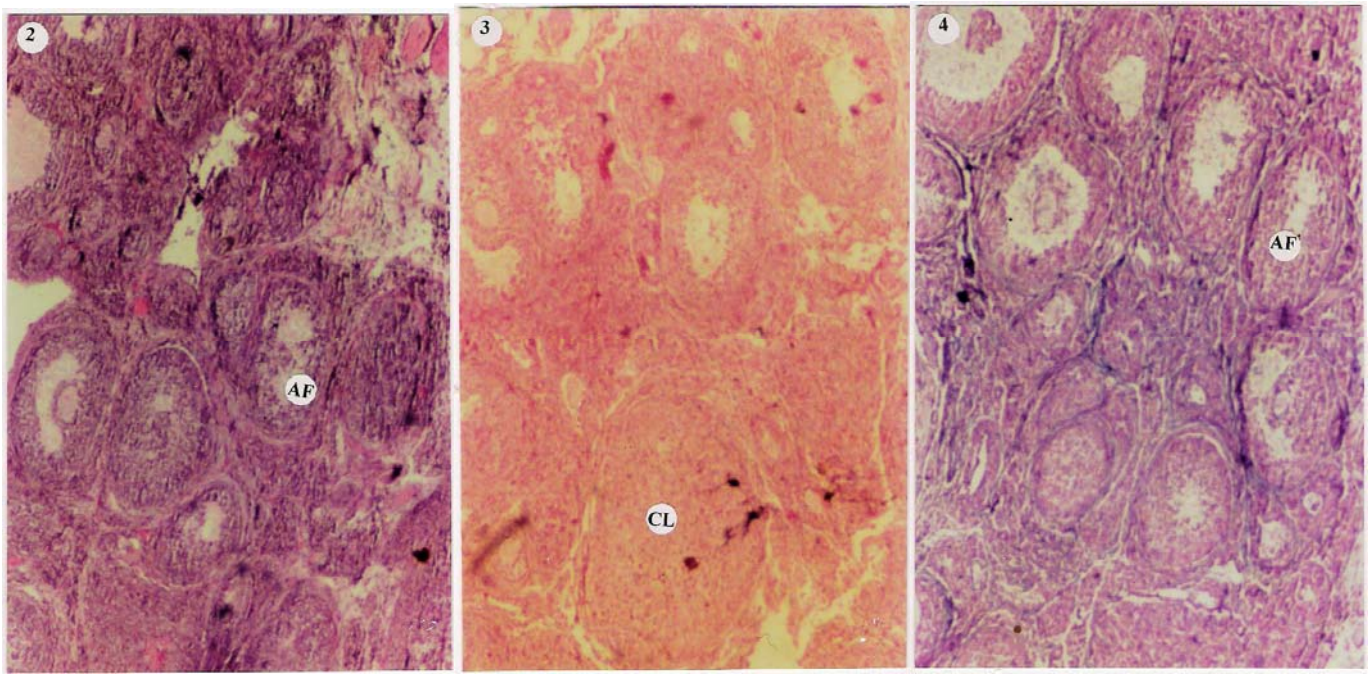


Figure 2, 3 & 4: Cross sections of the ovary of rats administered with Fraction I, Fraction II & Phenolphthalein isolated from *M. charantia* seeds respectively, showing reduced number of developing follicles (DF), Graafian follicles (GF), corpora lutea (CL) and increase in atretic follicles (AF). Haematoxylene - Eosine stains (x 400).

increased significantly (P<0.001) with all the three doses of compound treatment. Micrometric changes of uterus like diameter, myometrium, endometrial thickness, and epithelial cell height shows significant (P<0.001) increase due to the treatment of all the three doses of isolated compound indicating estrogenic activity. Biochemical contents like protein, glycogen and DNA are increased significantly (P<0.001) due to the administration of isolated compound indicating the increased growth of uterus and its metabolism.

DISCUSSION

Ovulation is estimated by the study of estrous cycle and counting the corpora lutea [21, 22]. The time sequences of events occurring during estrous cycle under controlled conditions have been extensively investigated. Much of this work has been concentrated with events occurring during critical period for the release of LH on the proestrus [23]. FSH is responsible for the initiation and differentiation of follicles towards

Table 2: Gravimetric, Biochemical and Histometric Changes of the Uterus Due to Administration of Fraction I and II of Ethanol Extract of *M. charantia* Seeds

Treatment	Dose (mg/100g body wt.)	Uterine Weight (mg/100g body wt.)	Protein (µg/mg)	Glycogen (µg/mg)	DNA (µg/mg)	Diameter (µm)	Myometrial thickness (µm)	Endometrial thickness (µm)	Epithelial cell height (µm)
Control	Tween-80 (1%)	114.16 ±3.01	5.11 ±0.27	1.26 ±0.12	2.35 ±0.26	2058.30 ±9.49	102.50 ±2.32	575.00 ±7.66	19.30 ±1.20
Fraction I	15	161.30** ±3.85	8.08** ±0.35	3.06** ±0.24	5.01** ±2.67	2222.50** ±8.37	181.30** ±4.02	808.30** ±6.03	22.66* ±0.88
Fraction I	25	173.30** ±3.58	9.16** ±0.30	4.21** ±0.23	6.98** ±2.72	2361.83** ±6.19	209.16** ±2.72	856.60** ±5.99	27.00* ±0.96
Fraction II	15	118.83 ±2.11	6.25 ±0.38	1.78 ±0.09	2.67 ±0.45	2071.30 ±7.38	115.83 ±3.01	600.60 ±5.42	20.00 ±0.57
Fraction II	25	122.16 ±1.52	6.33 ±0.37	2.00 ±0.12	3.23 ±0.67	2074.60 ±6.03	125.60 ±4.41	610.00 ±5.89	23.83 ±0.60

Duration: 30 days; six animals were maintained in each group.
Values are mean ± S.E.; * P < 0.01, ** P < 0.001 when compared to control.

Table 3: Ovarian Gravimetric, Biochemical and Histological Changes Due to Administration of Different Doses of Phenolphthalein in Albino Rats

Treatment	Dose (mg/100g body wt.)	Ovarian Weight (mg/100g body wt.)	Cholesterol (µg/mg)	Protein (µg/mg)	Glycogen (µg/mg)	Number / Ovary			
						Developing follicles	Graafian follicles	Corpora lutea	Atretic follicles
Control	Tween-80(1%)	39.83 ± 0.94	16.16 ± 0.60	12.98 ± 0.64	4.26 ± 0.45	10.83 ± 0.60	7.83 ± 0.47	8.33 ± 0.49	0.83 ± 0.30
Phenolphthalein	10	36.83 ± 0.60	25.36** ± 1.04	8.25** ± 0.32	2.38* ± 0.18	6.00** ± 0.36	4.00** ± 0.36	3.33** ± 0.21	2.83* ± 0.30
Phenolphthalein	15	36.00 ± 0.81	27.33** ± 0.66	6.28** ± 0.39	1.95* ± 0.12	3.66** ± 0.21	1.50** ± 0.22	1.00** ± 0.25	4.83** ± 0.30
Phenolphthalein	20	35.00 ± 1.12	28.50** ± 0.92	6.18** ± 0.32	1.91* ± 0.10	2.51** ± 0.34	0.83** ± 0.30	0.54** ± 0.22	5.50** ±0.42

Duration: 30 days; Six animals were maintained in each group.
Values are mean ± S.E.; *<0.01, **P<0.001 when compared to control.

Table 4: Gravimetric Biochemical and Histometric Changes of the Uterus Due to Administration of Various Doses of Phenolphthalein in Albino Rats

Treatment	Dose (mg/100g body wt.)	Uterine Weight (mg/100g body wt.)	Protein (µg/mg)	Glycogen (µg/mg)	DNA (µg/mg)	Diameter (µm)	Myometerial thickness (µm)	Endometrial thickness (µm)	Epithelial cell height (µm)
Control	Tween-80(1%)	116.33 ± 1.84	5.98 ± 0.26	1.76 ± 0.10	2.56 ±0.78	2048.31 ± 8.36	96.33 ± 2.23	541.66 ± 6.03	16.83 ± 0.79
Phenolphthalein	10	180.83** ± 3.01	8.81** ± 0.18	3.28** ± 0.19	7.98** ±2.72	2305.00**± 9.34	176.66** ± 4.42	604.16** ± 7.14	24.16** ± 0.60
Phenolphthalein	15	190.83** ± 3.52	9.18** ± 0.16	4.15** ± 0.16	8.26** ±2.65	2415.52**± 7.21	189.57** ± 4.76	658.33** ± 6.03	28.50** ± 0.76
Phenolphthalein	20	207.66** ± 3.03	10.30** ± 0.24	5.10** ± 0.11	8.76** ±3.00	2485.00**± 8.49	203.33** ± 6.69	682.50** ± 4.44	31.16** ± 0.60

Duration: 30 days; Six animals were maintained in each group.
Values are mean ± S.E.; *P<0.01, **P<0.001 when compared to control.

development of ovulatory / Graafian follicular stage and LH is essential for induction of ovulation and corpora lutea formation, which are responsible for the growth and increase in the weight of the ovary [24, 25]. Therefore, reduction in the observed weight of the ovary after the treatment of *M. charantia* seed fractions and phenolphthalein molecule may be attributed due to non-availability of gonadotrophins. In normal ovaries, estrogen secretion declines a few hours prior to the ovulatory surge of gonadotrophin [26]. More over the number of follicles that ovulate during each cycle is constant and characteristic of species. Therefore, in the present study the reduced number of developing follicles, Graafian follicle and corpora lutea which is observed in the ovaries of rats, received fractions of *M. charantia* seeds and phenolphthalein is due to the inhibition / blockade of pituitary FSH, LH and prolactin which are responsible for follicular growth and ovulation. The confirmed estrogenecity of the extract may imbalance the required hormonal environment for ovulation. Atretic follicles are degenerating pre-ovulatory follicles. The degeneration of pre-ovulatory follicles takes place when their growth and differentiation becomes disrupted [27]. The increased number of atretic follicles in the ovaries of experimental animals indicates the non-availability of gonadotrophins as FSH, LH and prolactin are essential for follicular growth and ovulation [28, 29].

A significant increase in the cholesterol of the ovary in treated animals indicates the non-availability of pituitary gonadotrophins which are necessary for conversion of cholesterol to progesterone / estrogens [30, 31]. It is evident that biosynthetic capacity of the ovary is influenced by FSH, LH and prolactin [32-34]. The lowered protein content of the ovary indicates the retarded ovarian growth, which is dependent on pituitary FSH. The ovarian glycogen is an energy source for gonadal activities [35]. Therefore, reduction in the glycogen content may be another reason for decreased ovarian activities. Uterine growth depends on the availability of ovarian steroid hormones, particularly estrogens [36, 37]. As the ovarian activities are impaired in the experimental animals the increase of the uterine weight may be due to the estrogenic nature of the fractions and phenolphthalein molecule isolated from seeds of *M. charantia.* To further sustain the specificity of the estrogenic nature of phenolphthalein, we determined other parameters previously described as being modulated by estrogens, namely the increase in uterine DNA content [38] and histological changes of uterus.

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REFERENCES

[1] Barrett J. Phytoestrogens: Friends or foes? Environmental Health Perspectives 1996; 104: 478-82.

[2] Riddle JM. Contraception and Abortion from the Ancient World to the Renaissance. Harvard University Press, Boston 1994.

[3] Leopold AS, Erwin J, Oh M, Browning B. Phytoestrogens: Adverse effects on reproduction in California quail. Science 1976; 191: 98-100. http://dx.doi.org/10.1126/science.1246602

[4] Berger PJ, Sanders EH, Gardner PD, *et al.* Phenolic plant compounds functioning as reproductive inhibitors in Microtus montanus. Science 1977; 195: 575-77. http://dx.doi.org/10.1126/science.319531

[5] Bennetts HW, Underwood EJ, Sheir FL. A specific breeding problem of sheep on subterranean clover pastures in Western Australia. Austral Vet J 1946; 22: 2-12. http://dx.doi.org/10.1111/j.1751-0813.1946.tb15473.x

[6] Whitten P, Lewis C, Naftolin F. A Phytoestrogen diet induces the premature anovulatory syndrome in lactationally exposed female rats. Biol Reprod 1993; 49: 1117-21. http://dx.doi.org/10.1095/biolreprod49.5.1117

[7] Grover JK, Gupata SR. Hypoglycemic activity of seeds of Momordica charantia. Eur J Pharmcol 1990; 183: 1026-27. http://dx.doi.org/10.1016/0014-2999(90)92880-R

[8] Amelia PG, Clara LS, Fabian D. *et al.* Antimutogens from Momordica charantia. Mut Res Fund Mol Mech Mutagenesis 1990; 230: 121-26. http://dx.doi.org/10.1016/0027-5107(90)90050-E

[9] Paolo S, Ester S. Review on some plants of Indian traditional medicine with antioxidant activity. J Ethanopharm 2000; 71: 23-43. http://dx.doi.org/10.1016/S0378-8741(00)00213-0

[10] Sylvia LH, Paul LH, Chen HC, *et al.* Anti-HIV and antitumour activities of recombinant MAP 30 from bitter melon. Gene 1995; 161: 151-56. http://dx.doi.org/10.1016/0378-1119(95)00186-A

[11] Federico S, Carmela M, Maria LM, *et al.* The immunomodulatory activity of plant proteins Momordica charantia inhibitor pokeweed antiviral protein. Int J Immunopharm 1983; 5: 335-34. http://dx.doi.org/10.1016/0192-0561(83)90037-1

[12] Ihan G, Ci G, Dem A, *et al.* Anti-ulcerogenic effect of Momordica charantia L. fruits on various ulcer models in rat. J Ethanopharmacol 2000; 71: 77-82. http://dx.doi.org/10.1016/S0378-8741(99)00178-6

[13] Naseem MZ, Patil SR, Saraswati BP, *et al.* Antispermatogenic and androgenic activities of Momordica charantia (Karela) in albino rats. J Ethnopharmacol 1998; 61: 9-16. http://dx.doi.org/10.1016/S0378-8741(98)00006-3

[14] Sharanabasappa AP, Saraswati BP. Effect of benzene extracts of Momardica charantia seeds on reproductive

activities in female Albino rats. Pro J Med Arom Plants Res 2001; 22/4A and 23/1A: 45-48.

[15] Murthy DRK, Reddy CM, Patil SB. Effect of benzene extract of Hibiscus rosa sinensis on the estrous cycle and ovarian activity in albino mice. Biol Pharm Bull 1997; 20: 756-58. http://dx.doi.org/10.1248/bpb.20.756

[16] Sharanabasappa AP, Vijaykumar B, Saraswati BP. Effect of Momordica charantia seed extracts on ovarian and uterine activities in albino rats. Pharm Biol 2002; 40: 501-507. http://dx.doi.org/10.1076/phbi.40.7.501.14688

[17] Peters JP, Vanslyke DD. Quantitative Clinical Chemistry. Vol I, Co. Baltimore: William and Wilkins 1946.

[18] Lowry OH, Rosenbrough NJ, Farr AL, *et al.* Protein measurement with follin-phenol reagent. J Biol Chem 1951; 193: 265-75.

[19] Caroll NV, Langelly RW, Row RH. Glycogen determination in liver and muscle by use of Anthrone reagent. J Biol Chem 1956; 26: 583-93.

[20] Burton K. Determination of DNA concentration with diphenylamine, In methods in Enzymology, Grossman L, Moldave K, Eds. Academic press New York 1968; 12 Part B: 163-166.

[21] Kaufmann RA, Savoy-Moore RT, Subramann MG, *et al.* Cocaine inhibits mating- induced, but not human chorionic ganadotropin-stimulated, ovulation in the rabbit. Biol Reprod 1992; 46: 641-47. http://dx.doi.org/10.1095/biolreprod46.4.641

[22] Salhab AS, Shomaf MS, Gharaibeh MN, *et al.* Effects of castor bean extract and risin a-chain on ovulation and implantation in rabbits. Contraception 1999; 59: 395-99. http://dx.doi.org/10.1016/S0010-7824(99)00045-1

[23] Kobayashi FKH, Tomotsu M. Further studies on the casual relationship between the secretion of estrogen and the release of luteinizing hormone in the rat. Endocrinology 1969; p. 501.

[24] Marsh JM. The role of cyclic AMP in gonadal function. Adv Cycl Nucl Res 1975; 6: 137-200.

[25] Richards JS. Maturation of ovarian follicles actions and interactions of pituitary and ovarian hormones on follicular cell differentiation. Physiol Rev 1980; 60: 51-84.

[26] Hori T, Makoto I, Goro K, *et al.* Relation between estrogen secretion and follicular morphology in the rat ovary under the influence of ovulating hormone or exogenous gonadtrophins. Endocrinology 1970; pp. 489-493.

[27] Peters H, McNatty KP. The ovary: A correlation of structure and function. Granada Publishing in Paul Elek Ltd. Great Britain 1980; pp. 75-79.

[28] Friedrich F, Kemeter P, Salzer H, *et al.* Ovulation inhibition with human chorionic gonadotrophin. Acta Endocrinol 1975; 78: 332-42.

[29] Richards JS, Midgley AR. Luteal cell receptor content for prolactin (PRL) and luteinizing hormone (LH), regulation by LH and PRL. Endocrinology 1976; 99: 1571-78. http://dx.doi.org/10.1210/endo-99-6-1571

[30] Mason NR, Marsh JM, Savard K. An action of gonadotrophin *In vitro*. J Biol Chem 1962; 237: 1801-806.

[31] Wang C, Hsuch AJW, Erickson GF. Induction of functional prolactin receptors by follicle stimulating hormone in rat granulosa cells *In vivo* and *In vitro*. J Biol Chem 1979; 254: 11330-336.

[32] Hardy B, Danon D, Eshkol A, *et al.* Ultra structural changes in the ovaries of infant mice deprived of endogenous gonadotrophins and after substitution with FSH. J Reprod Fert 1974; 36: 345-51. http://dx.doi.org/10.1530/jrf.0.0360345

[33] Purandare TV, Munshi SR, Rao SS. Follicular development in mice treated with antisera to FSH and LH in the neonatal period. In proceedings of the Fifth Asia and Oceania Congress of Endocrinology. (Ed. G.K. Rostogi) Endocrine society of India, Chandigarh 1974; pp. 48-54.

[34] McNatty KP, Bennie JG, Hunter WM, *et al.* The effects of antibodies to human gonadotrophins on the viability and rats of progesterone secretion by human granulosa cells in tissue culture in physiological effects of Immunity against hormones. (Eds. R.G. Edwards and M.H. Johnson) Cambridge University Press. Cambridge 1975b; pp. 41-46.

[35] Wallas O. Effect of oestrogens in the glycogen contents of the rat uterus. Acta Endocrinol 1952; 10: 175-92.

[36] Jalikhani BL. Ovarian steroids In: Text book of biochemistry and human biology. Talawar GP (Eds.) Vertical Hall Ind Pri Ltd New Delhi 1980; pp. 805-809.

[37] Findlay JK. Molecular biology of the Female reproductive system. Academic Press, California 1994; pp. 457-462.

[38] Jenson EV, De Sombre ER. In Biochemical actions of Hormones (G. Litwak, Ed.), Vol. II Academic Press New York 1972; pp. 215-255. http://dx.doi.org/10.1016/B978-0-12-452802-4.50013-5

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