

Original Article

ANTIFERTILITY ACTIVITY OF *MOMORDICA CHARANTIA* DESCOURT PULP AND SEED HYDROALCOHOLIC EXTRACT

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ABSTRACT

Momordica charantia Descourt (Cucurbitaceae) known as Karela is an extremely bitter vegetable, commonly found in India. *M. charantia* had reported anticancer, antidiabetic, antihypertensive, anti-inflammation and antioxidant properties. It is used as antifertility agent in folklore; however no studies have been done on its antifertility action. Anti-fertility potential of the *M. charantia* hydroalcoholic extracts of seed and pulp was explored on *in vitro* spermicidal, antioviulatory, *in vivo* estrogenic and anti-estrogenic activity along with uterus histopathology. Data were presented as $M \pm SEM$ and analyzed with ANOVA followed by t-test. MCHS and MCHP are tested for its antifertility activity at doses of 200 mg/kg and 400 mg/kg, orally as it was found safe on acute dosing of 2000 mg/kg. Pulp extract at 1280 $\mu\text{g/ml}$ concentration caused significant damage to the sperm membrane integrity as evidenced by the reduction in sperm viability and tail curling. Administration of pulp extract at 400 mg/kg dose caused significant ($p < 0.001$) increases in uterine weight in immature rats while co-administration with ethinylestradiol decreased the uterus weight and increased the height of luminal epithelium with stimulated glands. Pulp extract at 400 mg/kg dose also caused significant ($p < 0.001$) prolongation estrous cycle and diestrous phase. Among the two extracts tested at two different doses, the hydroalcoholic pulp extract of *M. charantia* at 400 mg/kg was found to exhibit antifertility activity may be due to rich presence of triterpenoidal glycosides, flavonoids and alkaloids.

Keywords: Antifertility, Antioviulatory, cucurbitaceae, *Momordica charantia* Descourt; spermicidal

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INTRODUCTION

There is a growing movement of self-determined women, to regain ancient knowledge about botanical herbs once widely used for the control of pregnancy. This knowledge was once widespread; perhaps even global in nature was largely wiped out in influence of Western culture. In this direction we have aimed to identify a potent antifertility agent with minimum side effects

from an herbal source that could serve as an alternative remedy for available synthetic medicine and to formulate it into a suitable contraceptive formulation. To lead a healthy life and to get rid of diseases, herbs are being used since ancient time as Ayurvedic practice in India. In Ayurveda, the herbal substances (dravya), their potential energy and qualities (guna) and their ultimate action within the system (karma) are regarded as an inseparable biochemical combination. Each and every dravya has its own guna and karma, by which the dravya is utilised for remedial purposes. While using plant derived drugs thorough knowledge of Dravya-guna-vigyan is essential. The innate and specific property of a dravya is known as prabhav and all the herbs have their own exclusive prabhav [1].

Momordica charantia Descourt (Cucurbitaceae) is a tropical and subtropical vine widely grown for edible fruit, which is among the most bitter of all vegetables. The English names for the plant and its fruit include bitter melon or bitter gourd. The original home of the species is not known, other than that it is a native of the tropics. It is widely grown in South and Southeast Asia, China, Africa, and the Caribbean. According to Ayurvedic texts karela or bitter melon have guna - laghu (light) and ruksh (dry), rasa - katu or pungent and tikta or bitter, virya or the potency - ushana or hot and karma - mutsangrehiya. *M. charantia* have reported anticancer [2], antidiabetic [3], antihypertensive [4], anti- inflammation [5] and antioxidant [6] activity.

This plant is being widely used as an abortifacient and for birth control in Ayurveda. *M. charantia* has been traditionally used as an abortive and reducing fertility in male and female both. Naseem et al 1997 [7] reported *in vitro* antispermatogenic and androgenic activities of *M. charantia* seed alcoholic extract. Antioviulatory and estrogenic property of *M. charantia* benzene extract was reported by Sharanabasappa et al 2005 [8]. Effect of *M. charantia* seed methanolic extract on estrous cycle and histology of the ovary was reported by Ifeanyi et al (2011) [9]. Plants having similar viz. tikta (bitter), ushna (hot) and katu (pungent) Ayurvedic Energetics like *Azadirachta indica* (Neem), *Withania somnifera* (Ashwagandha), *Andrographis paniculata* (Kalmegh) and *Plumbago zeylanica* (Chitrak) having similar properties viz. tikta (bitter), ushna (hot) and katu (pungent) are known for their antifertility and abortifacient effect. Phytochemicals of this plant indicated the presence of active components like momordenol, momordicins, momordicinin, momordin, momordolol, charantin, cucurbitins, cucurbitacins, cucurbitanes, cycloartenols, erythrodiol, galacturonic acids and gentisic acid which have been isolated [10, 11].

Though fruit extract is reported to have effect on testicular function of dog, till date detailed studies on antifertility action of fruit have not been undertaken. The present study was undertaken to evaluate the anti-fertility activity of *M. charantia* hydroalcoholic seed and fruit pulp extract. The study design was directed towards *in vitro* comparative spermicidal and *in vivo* estrogenic, antiestrogenic and antioviulatory potential exploration for seed and fruit pulp.

MATERIALS AND METHODS

Plant material: Fruits of *M. charantia* were collected on August, 2008. The authentication was done by Dr. S. N. Mishra, (Sr. Scientist), K. N. K. College of Horticulture, Mandsaur, M.P. A voucher specimen (BRNCP/M/08/2008) has been deposited in the departmental museum.

Preparation of extracts: The seed and pulp of *M. charantia* from Mandsaur (Madhya Pradesh) were collected and shade dried. The dried seed and pulp were coarsely powdered and the powder was packed in to soxhlet apparatus and extracted successively with petroleum ether (60-

80°C) and ethanol (70%). The extracts were concentrated under reduced pressure. The dried extracts were stored in airtight container in refrigerator. The percentage yield of *M. charantia* hydroalcoholic seed and pulp extract (MCHS and MCHP) was 7.3% and 5.4%, respectively.

Phytochemical test: The preliminary phytochemical screening was carried out on petroleum ether and hydroalcoholic extracts of *M. charantia* seeds and pulps for the detection of various phytochemicals. Tests for common phytochemicals were performed to detect the presence of carbohydrate (Molish and Fehling's test), flavonoid (Shinoda and Alkaline reagent test), alkaloid (Mayers and Wagners test), saponin (Froth and Hemolysis test), triterpenoidal steroids (Molescott, Salkowski and Noller's test), glycoside (Borntragers and Legals test) and tannin (Lead acetate and Venillin-hydrochloride test) [12, 13].

Experimental animals: Male albino rats weighing between 150 and 200 g and immature female albino rats (25-30 days old), matured female rats weighing between 100 and 120 g were used in experiments. The animals were placed randomly and allocated to different treatments groups. The animals were housed in polypropylene cages with paddy husk bedding at a temperature of $22 \pm 2^\circ\text{C}$ and relative humidity of $65 \pm 5\%$. A 12:12 hrs, light: dark cycle was followed. Animals were allowed to free access to water and feed. All experimental procedures and protocols used in the study were reviewed by Institute animal ethical committee (IAEC) and approved (981/ac/05/cpcsea), in accordance with the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), Chennai, India.

Determination of acute toxicity (LD₅₀): The acute toxicity for MCHS and MCHP were determined in albino rats, maintained under standard conditions. The animals were fasted overnight prior to the experiment and fixed dose method was adopted as per OECD Guideline No. 420 of CPCSEA [14]. MCHS and MCHP were suspended in tween-80, 1% in distilled water and administered orally. MCHS and MCHP showed no toxicity in rat upto 2000 mg/kg dose. The dose range chosen for pharmacological studies were 200 and 400 mg/kg, orally for both MCHS and MCHP.

In vitro spermicidal activity: MCHS and MCHP were diluted with normal saline to get concentration of 40, 80, 160, 320, 640 and 1280 µg/ml. The spermatazoa were obtained from human volunteers and placed in 1 ml of modified Krebs Ringer-bicarbonate buffer (pH 7.4). One ml of each MCHS and MCHP dilution was mixed with 0.2 ml of human seminal solution and incubated at 37°C for 30 min. A drop of this mixture was placed immediately on a slide and five fields were microscopically observed under high power (400×) for assessment of sperm morphological changes and mortality [15]. The percent of motility was determined by the progressive and non-progressive movements of sperm observed under a compound microscope. The baseline viability of sperm was found to be 90% in samples treated with vehicle control showing excellent vigorous rapid forward progression

Estrogenic and antiestrogenic activity: Colony-bred (Wistar strain) immature female albino rats, 25-30 days old, weighing between 40-55 g, were selected for estrogenic and antiestrogenic activity. They were divided into ten groups consisting of five rats each. The first group served as a control and received vehicle only (Tween-80, 1%). The second group received ethinylestradiol in olive oil, 1 mg/rat per day, subcutaneously [16]. The third, fourth, fifth and sixth groups received the seed and pulp extracts at a dose of 200 and 400 mg/kg body weight, respectively. The seventh, eighth, ninth and tenth groups received, in addition to ethinylestradiol, a test dose of the seed and pulp extracts at 200 and 400 mg/kg body weight, respectively. All the above

treatments were given for 7 days. On the eighth day, the rats were sacrificed by decapitation, the uteri dissected out and surrounding tissues removed. The uteri were blotted on filter papers and weighed quickly on a sensitive balance [17].

Histopathological study: Isolated uterus slices were kept in 10% formalin solution, processed for histopathological assessment following the method of Nanji *et al* 2002 [18] briefly, uterus slices were dehydrated in ethanol (50-100%), cleared in xylene and embedded in paraffin. Sections of 4-5 μm thickness were cut using a rotary microtome and stained with heamatoxylin-eosin dye for photo microscopic observation. The sections were observed for thickness and height of endometrium along with glandular epithelium.

Antiovolatory activity: Five matured female Wister albino rats weighing 100-120 g and showing regular cycles were used for used this study. The control group consisting of five rats was treated with vehicle (tween-80, 1%) for 21 days (5 cycles) where as five rats in each of the treatment groups received the MCHS and MCHP extract orally at a dose of 200 and 400 mg/kg body weight for 21 days. Oestrous cycle of both control and treatment groups were monitored daily by vaginal lavage according to the method of Marcondes *et al* 2002 [19]). Each rat was held in a supine position. Vaginal secretion was collected after douching with 0.2 ml of normal saline (0.9% NaCl) contained in a smooth plastic pipette. A small drop of the cell suspension in the pipette was placed on a clean glass slide and swabbed by rolling with a cotton tip along the length of the slide. As soon as the smears dried out, the slides were dipped 3 to 5 times in a container of 70% alcohol in order to fix expeditiously. The slides were then stained without delay by methylene blue stain (0.5% aqueous methylene blue solution). After dipping in the methylene blue stain, the slides were rinsed in tap water, covered with cover slip and examined under a light microscope without the use of condenser lens at 40 \times magnifications. Vaginal smears were assessed once each day between 9.00 and 10.00 a.m. The proportion of characteristic cell types (leucocytes, cornified and epithelial cells) was used to determine the phases of oestrous cycle according to Mandi 1951 [20]. The length of oestrous cycles and duration of each phase of the cycle were recorded as described by Makonnen *et al* 1997 [21]. The vaginal smear from all treated rats continued to be examined under a microscope for another 7 days post administration of the extracts as described by Gebrie E 2005 [22].

Statistical analysis: The results were reported as Mean \pm SEM of five observations. Experimental data were analyzed using one-way analysis of variance (ANOVA) followed by Student's t-test to compare the difference between the control and treated values and $p < 0.05$ was considered significant. Graph Pad Prism Version 3.02 was used for statistical calculations.

RESULTS

Phytochemical estimation: Phytochemical investigation reveals presence of triterpenoids, carbohydrates, glycosides and alkaloids in both the extracts, whereas flavonoids were found only in pulp extract.

Acute toxicity study of M. charantia (2000 mg/kg): The acute toxicity studies of hydroalcoholic seed and pulp extracts of *M. charantia* showed no mortality at dose of 2000 mg/kg body weight. As per OECD Guideline No. 420 (Annexure 2d) fixed dose method, the doses selected for both the extracts were 200 and 400 mg/kg, which is 1/10 to 1/5 of limit test dose.

Table 1. Effect of *M. charantia* hydroalcoholic seed and pulp extract on *in vitro* sperm mortality

Concentration ($\mu\text{g/ml}$)	Seed Extract		Pulp Extract	
	% Viability	Grade of Motility	% Viability	Grade of Motility
1280	20	Slight wavering progression	0	Dead sperm (no motility)
640	30	Poor wavering progression	20	Slight wavering progression
320	45	Slow meandering forward progression	25	Poor wavering progression
160	60	Good fast forward progression	50	Fair slow meandering forward progression
80	65	Good fast forward progression	55	Slow forward progression
40	70	Good fast forward progression	60	Good fast forward progression
20	80	Good fast forward progression	80	Good fast forward progression
Control	90	Excellent vigorous rapid forward progression	90	Excellent vigorous rapid forward progression

Spermicidal activity: *In vitro* spermicidal activity of MCHS and MCHP showed good spermicidal effect as given in Table 1. The activities with *M. charantia* extract were dose dependent. The plant extract intoxication exerted significant decrease epididymal sperm progress motility. The spermicidal concentration of pulp extract was 1280 $\mu\text{g/ml}$, where as in the same dose seed extract showed no mortality but slight movement of sperm. Figure 1 (a, b, c) represent the mortality of sperm treated with vehicle and extract at 1280 $\mu\text{g/ml}$ concentration. At the highest concentration of MCHP (1280 $\mu\text{g/ml}$) the sperm sample showed no motility while at the concentration of (160 $\mu\text{g/ml}$) the viability of sperm decreases upto 50% with fair slow meandering forward progression. Control sample of the sperm showed 90% viability with excellent vigorous rapid forward progression. MCHS at highest concentration 1280 $\mu\text{g/ml}$ showed only 20% viability with vary faint motility. The reduction in sperm motility were significantly ($p < 0.001$) higher in pulp extract treated animals when compared to that of seed extract.

***In vivo* estrogenic and anti-estrogenic activity:** The pulp extract of *M. charantia* was found to exhibit highly significant ($p < 0.01$) estrogenic activity compared to control at both 200 and 400 mg/kg doses. The pulp extract showed better estrogenic activity compared seed extract at 400 mg/kg dose with respectively, 49.59% and 132.75%. Increase in uterine weight in contrast to 265.12% by ethinylestradiol.

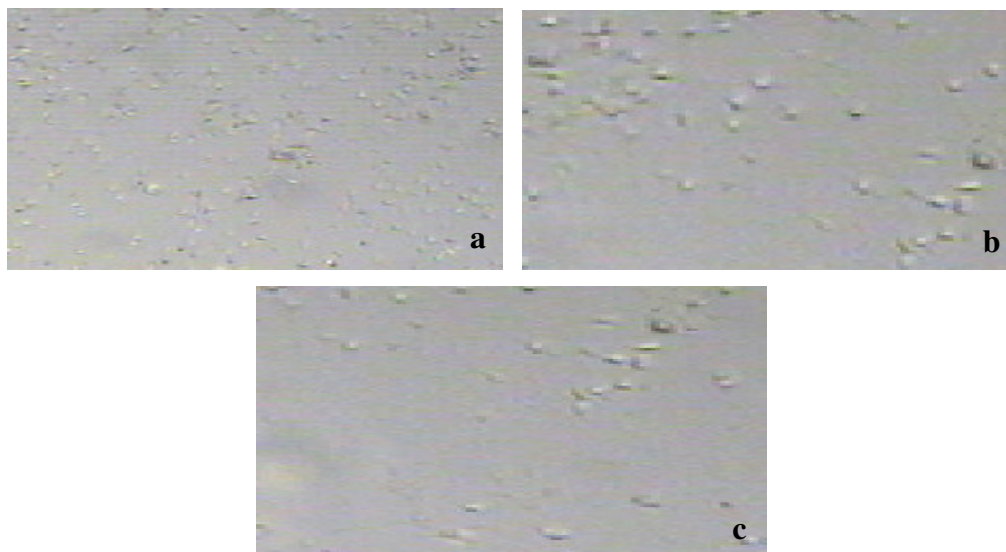


Figure 1. Photomicrographs of sperm sample treated with *M. Charantia* hydroalcoholic seed and pulp extract on *in vitro* sperm mortality of (a) Vehicle control, (b) MCHS extract and (c) MCHP extract at 1280 µg/ml concentration (400×).

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Table 2. *In vivo* estrogenic and anti- estrogenic activity of MCHP and MCHS on uterine weight of immature female rats

Treatment	Dose (mg/kg, p.o)	Uterine weight in mg/50 gm body weight
Control (vehicle)	N/A	23.45 ± 2.12
Ethinylestradiol	0.02	85.62 ± 4.33*** (265.12%)
MCHS	200	31.84 ± 2.84** (33.77%)
	400	35.08 ± 2.71*** (49.59%)
MCHP	200	50.86 ± 3.26*** (116.88%)
	400	54.58 ± 3.22*** (132.75%)
MCHS + Ethinylestradiol	200	85.42 ± 4.07 ^{ns} (264.26%)
	400	60.86 ± 2.85*** (159.53%)
MCHP + Ethinylestradiol	200	57.45 ± 2.87*** (144.98%)
	400	51.44 ± 2.18*** (119.36%)

Co-administration of *M. charantia* simultaneously with ethinylestradiol was carried out to find the antiestrogenic activity. The pulp extract at both the doses 200 and 400 mg/kg doses showed extremely significant ($p < 0.001$) inhibition of uterus weight gain (144.98% and 119.36% respectively) compared too alone where as seed extract only at 400 mg/kg showed significant effect (159.53%). Inhibition of uterus weight gain was highest with pulp extract at 400 mg/kg dose (Table 2).

Values are expressed in Mean \pm SEM, $n = 5$, $**p < 0.01$, $***p < 0.001$ compared to vehicle control. Values in parentheses signify percentage increase in uterus weight compared to vehicle control.

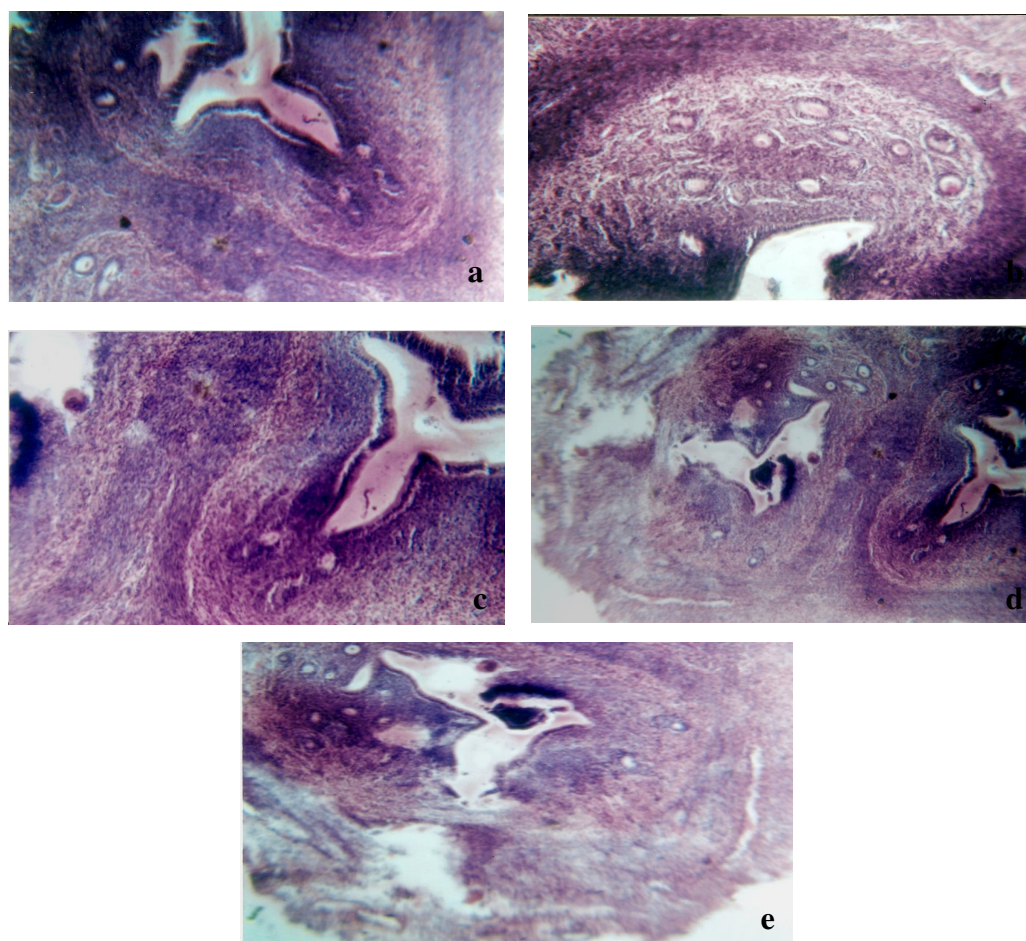


Figure 2. Photomicrographs of uterus section of female rats treated with different doses of *M. Charantia* (a) vehicle control rats indicating surface epithelium with no secretory activity; (b) MCHS 200 mg/kg treated rats showed surface epithelium with moderate secretory activity; (c) MCHS 400 mg/kg and (d) MCHP 200 mg/kg treated rats indicating increase in height of luminal epithelium with stimulated uterine glands; (e) MCHP 400 mg/kg treated rats indicating moderate increase in height of luminal epithelium and stimulated uterine glands (40 \times).

Histopathological studies: Uterus sections of animals showed the estrous cycle indicating the histopathology of surface epithelium and secretory glands. The photomicrographs of uterus treated with MCHS at 200 mg/kg and 400 mg/kg indicate moderate increase in height of luminal epithelium with normal uterine glands. Section of uterus treated with 200 and 400 mg/kg of MCHP showed moderate increase in height of luminal epithelium but stimulated uterine glands as depicted in Figure 2 (a, b, c, d, and e).

Table 3. Effect of *M. charantia* hydroalcoholic seed and pulp extract treatment over the duration and phases of the estrous cycle of immature female rats

Treatment	Dose (mg/kg, p.o)	Duration of Oestrous cycle	P.E.	E.P.	M.E.	D.E.
Control	-	5.55 ± 0.915	1.22 ± 0.20	0.80 ± 0.21	0.94 ± 0.26	2.62 ± 0.24
Seed	200	6.23 ± 0.24*	0.81 ± 0.31	0.40 ± 0.31	0.83 ± 0.21*	4.21 ± 0.37*
	400	7.20 ± 0.65**	1.05 ± 0.31	1.02 ± 0.54	1.02 ± 0.36**	4.24 ± 0.20**
Pulp	200	6.46 ± 1.16*	0.81 ± 0.22	1.16 ± 0.36	0.50 ± 0.24*	4.05 ± 0.36*
	400	8.29 ± 1.71***	1.40 ± 0.41	1.33 ± 0.42	1.16 ± 0.65***	4.42 ± 0.24***

Values are expressed in mean ± SEM, n=5, *p < 0.05, **p < 0.01, ***p < 0.001 compared to vehicle control. P.E.: Proestrous, E.P.: Estrous, M.E.: Metaestrous and D.E.: Diestrous.

Antiovolatory activity: Table 3 and Figure 3 showed the effect of *M. charantia* on different phases of oestrous cycle. The control rats exhibited regular 4 to 5 days cycle. The length of the oestrous cycle was significantly longer in seed (p < 0.01) and pulp (p < 0.001) extract treated group compared with the control. The prolongation of diestrous and metaestrous phase was highly significant (p < 0.001) in pulp extract, whereas effect on proestrous and estrous phase was non-significant with the seed extract and showed the same activity profile with moderate intensity. Table 4 complies the duration of different phases of oestrous cycle upto 7 days post-treatment, signifying normalization of cycle.

Table 4. Effect of *M. charantia* hydroalcoholic seed and pulp extract on post treatment (7 days) over the duration and phases of estrous cycle of immature female rats

Treatment	Dose (mg/kg, p.o)	Duration of Oestrous cycle	P.E.	E.P.	M.E.	D.E.
Control	-	5.92 ± 0.83	0.93 ± 0.22	0.94 ± 0.21	1.22 ± 0.26	2.81 ± 0.25
Seed	200	5.85 ± 0.37	0.86 ± 0.24	0.93 ± 0.36	1.46 ± 0.20	2.62 ± 0.20
	400	6.08 ± 0.36	1.03 ± 0.27	0.82 ± 0.37	1.46 ± 0.24	2.86 ± 0.37
Pulp	200	5.30 ± 0.21	1.02 ± 0.36	0.80 ± 0.24	1.08 ± 0.37	2.41 ± 0.31
	400	6.06 ± 0.24	0.82 ± 0.23	1.04 ± 0.27	1.32 ± 0.37	2.83 ± 0.34

Values are expressed in Mean ± SEM, n=5, All values were found to be “Non-Significant”. P.E.: Proestrous, E.P.: Estrous, M.E.: Metaestrous and D.E.: Diestrous.

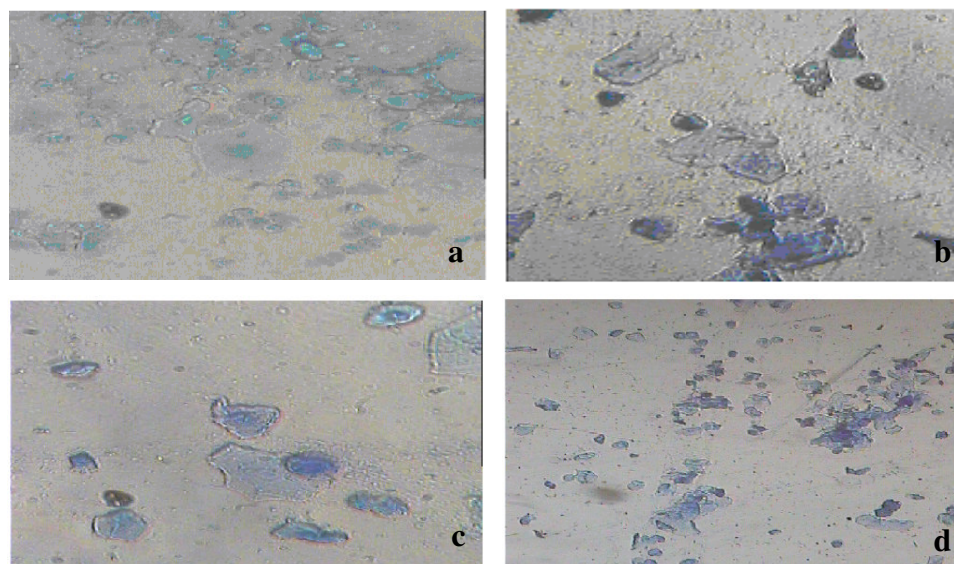


Figure 3. Photomicrographs of vaginal smear of rats treated with *M. Charantia* hydroalcoholic pulp extract at 400 mg/kg dose (a) Proestrous phase showing nucleated epithelial cell (b) Estrous phase consisting of anucleated cornified cells (c) Metaestrous phase consisting of very few leukocytes, cornified and nucleated epithelial cells (d) Diestrous phase primarily consisting of leukocytes (40×).

DISCUSSION

M. charantia has traditionally been used for family planning in various Asian and African traditional medicine. *M. charantia* contains several chemical constituents, including the glycosides mormordin, charantin cucurbitins, cucurbitacins, cucurbitanes, cycloartenols, and

alkaloid mormordicine. Its seeds contain the abortifacients α -mormorcharin and β -mormorcharin, as well as the pyrimidine nucleoside vicine [23]. Triterpene glycosides and alkaloids are reported to have antifertility effect [24, 25]. In the present study hydroalcoholic extract of *M. charantia* (pulp and seeds) was tested for its antifertility activity at doses of 200 mg/kg and 400 mg/kg. Among the two extracts tested at two different doses, the hydroalcoholic pulp extract of *M. charantia* at 400 mg/kg was found to exhibit potent antifertility activity.

Spermicidal activity of hydroalcoholic pulp and seed extracts of *M. charantia* was studied *in vitro*. Our results revealed that *in vitro* *M. Charantia* pulp extract treatment caused marked alterations in the sperm physiology in the present study. *M. Charantia* extract treatment adversely affected sperm viability and motility. The reduction in sperm motility in cauda – epididymis is of importance with regard to fertilization. Inadequate concentration and immotility of spermatazoa means they cannot penetrate the cervical mucus and thus fail to fertilize the ova. Damage to sperm membrane integrity is evidenced by the significant reduction in sperm viability and tail curling. Spermicidal compounds act on the sperm surface and disrupt the plasma membrane. Plant derivative also cause drastic inhibition in sperm membrane specific enzyme like acrocin and hyaluronidase, the most important enzyme in the process of fertilization.

Many naturally occurring polyphenolic compounds like flavonoids exercise inhibitory effect on mammalian sperm motility *in vitro*, which seems to be due to augmentation of the generation of superoxide radicals by spermatazoa involved in the inhibition of motility *in vitro* [26]. The hydroalcoholic pulps extract of *M. charantia* posses an immobilizing factor, possibly flavonoid that probably reduces motility by causing sperm nonviability by disrupting the membrane [27]. *M. charantia* has enriched presence of phenolic and flavonoid compounds like momordenol, momordolol, elaeostearic acids, erythrodilol, galacturonic acids, gentisic acid, goyaglycosides, goyasaponins and multiflorenol [28]. *In vivo* antispermatogenic activity of alcoholic seed extract has been observed by Naseem *et al* 1997 [7] along with antisteroidogenic and antiandrogenic effect.

Oral administration of hydroalcoholic pulp extract at 400 mg/kg caused significant increase in uterine weight in immature rats. Simultaneous administration of ethinylestradiol and hydroalcoholic pulp extract caused highly significant decrease in uterine weight when compared with standard. Treated rats showed open vaginas while all the control had closed vaginas. After discontinuation of treatment all the rats showed normal oestrous cycle with normalization of diestrous and metaestrous phases indicating the reversible action of extracts. Estrogen stimulates the uterus thereby changing the uterine milieu and creating non-receptive conditions [29]. The extract may have acted as estrogen in immature female rats while given alone but when given with ethinylestradiol it exhibited slight antiestrogenic activity. These results show that the extract have acted as competitive antagonist to the much more potent ethinylestradiol may be due to presence of mixed steroids like charantin [30]. Benzene extract of *M. charantia* seed was found to be highly effective in increasing the weight of uterus in adult female rats indicating estrogenic property [8].

Estrogenic substances inhibit pregnancy by suppressing the level of both follicular stimulating hormone (FSH) and luteinizing hormone (LH), which in turn prevent the implantation. According to Laurence 1993 [31] any compound possessing estrogenic activity may exhibit antifertility activity by suppressing gonadotropin secretion with consequent inhibition of ovulation. Estrogen and progesterone are the hormones responsible for histologic and functional modifications of the female genital tract.

An oestrous cycle is a rhythmic reproductive cycle in sexually-matured female mammals Hafez and Hafez 2000 [32] and is influenced by the release of gonadotropin releasing hormone from the hypothalamus, gonadotropins from the pituitary gland and sex hormones from the gonads. Female normal cyclicity characterized by vaginal changes observed in oestrous cycle is an index of good functioning of the neuroendocrine - reproductive system and ovarian activity, loss of normal oestrous cycle indicates the disruption of ovarian progesterone and estrogen balance [33]. The presence of particular cell types in each phase indicates the follicular and luteal phases of the reproductive cycle. The rat has a characteristically short oestrous cycle of 4 to 5 days in phases [20] which makes it ideal for reproductive studies [19]. Secretions of pituitary gonadotrophins are regulated by brain and neurons situated in the anterior parts of the hypothalamus that synthesize the gonadotrophin releasing hormone (GnRH). CNS depressants acts on the hypothalamus and inhibit the release of GnRH and corticotrophin releasing factor thus decreasing the circulating concentrations of LH, FSH, adrenocorticotrophic hormone and β -endorphin [34]. CNS depressant activity was observed in ethanolic fresh fruit extract of *M. charantia* [28]. According to several investigators CNS influencing drugs inhibit the release of FSH and LH from the pituitary acting through hypothalamus, blocking the neural stimulus to the gonadotrophin releasing hormone [35].

The results of this study show that *M. charantia* pulp extract significantly increases the duration of oestrous cycle along with prolongation of metaestrous and diestrous phases. The seed extract was moderately effective in increasing the duration of cycle as well as metaestrous and diestrous phases. The results of our study confirms the report of the ability of some plant extracts to prolong the oestrous cycle and diestrous phase of the cycle [36]. Estrous cycle in the rats treated with extract of *Momordica cymbalaria* (250 and 500 mg/kg b.wt) showed a decrease in the duration of estrous and the metestrous phases and prolongation of the proestrous phase [37].

Uchendu et al 2000 [38] reported that ethanolic extract of *Dalbergia saxatilis* prolonged the diestrous phase of the cycle and thus reduced fertilization in the affected experimental animals. Similar reports indicate that plant extracts adversely affected oestrous cycle in rats during diestrous phases by blocking the release of both FSH and LH may by inhibiting synthesis or hypothalamic release [39]. The sera of animals treated with the *M. charantia* seed extract showed a significant decrease in the level of assayed gonadotrophins (FSH and LH) in male Sprague-Dawley rats compared to control indicating interference of pituitary-testicular axis [40]. Ganguly et al 2007 [41] also observed a prolongation of oestrous cycle and concomitant suppression of LH following the administration of lajjalu (*Mimosa pudica*) root extract irregularity of oestrous cycle may cause distortion of endometrial function which may in turn lead to a failure of implantation and pregnancy. An irregular pattern of oestrous with a prolonged diestrous and consequently a reduced number of ova in the ovary was attributed to administration of *Garcinia kola* seed extract [42]. The present observation of irregular oestrous cycle with *M. Charantia* correlates well with antifertility effect of *Hymenocardia acida* [43]. As reported by Ifeanyi et al (2011) [9] orally administered seed methanolic extract of *M. Charantia* causes irregularities in estrous cycle. The diestrous phase was increased where as proestrous and estrous phases were also decreased, this effect on oestrous cycle was reversed after withdrawal of *M. charantia*. Anti-fertility effects of herbals reported in literature are mostly through their action on hypothalamo-pituitary-gonadal axis or direct hormonal effects on reproductive organs resulting in inhibition of ovarian steroidogenesis [25].

The prolonged oestrous cycle and estrous phase (safe period) observed in the extract treatment groups is suggestive of the antifertility effect of *M. charantia* pulp. The prolongation of diestrous phase signifies reduced probability of rats to get pregnant. Observation of oestrous cycle on post treatment period showed normalization of cycle with non-significant changes compared to normal animals. This finding substantiates that the alteration induced by MCHS and MCHP are reversible after cessation of treatment. The observation that there was no significant change in proestrous phase, after withdrawing the extract from those of control, could explain the reversible nature of antifertility effect of the extract which has also been observed from the preliminary studies as mentioned above. The results obtained in the present study on the estrous cycle and on its various phases are comparable to the studies made by Makonnen 1999 [21]. It is noted that after stopping administering the extract the normal diestrous phase and estrous cycle were resumed.

The rich presence of triterpenoids and alkaloids in fruit pulp may be responsible for its anti-fertility effect. Chen *et al* 2005 [44] reported isolation of triterpenes from *M. charantia* with cytotoxicity against HIV-1 virus. Isolation of cucurbitane triterpenoids from *M. charantia* were also reported by Harinantenaina *et al* 2005 [45]. Triterpene isolated from *Eugenia jambolana* flowers and cytotoxic alkaloids from vinca are reported to have anti-fertility potential in male rats [46, 47]. Triterpene Lupeol acetate isolated from benzene extract of *Alstonia scholarensis* has showed antifertility effect on male rats by decreasing spermatogenesis [48]. Anti-cancer effect of cucurbitane type triterpenoids from *M. charantia* fruits have been studied by Akihisa *et al* 2003 [49] on mouse skin. The concerted and sandwiched effect of triterpenes, alkaloids, flavonoids and glycosides in the hydroalcoholic extract may be enhancing the anti-fertility potential of *M. charantia*. The results of the present study substantiate the traditional and earlier anti-fertility claim of *M. charantia*, and showed comparatively higher anti-fertility efficacy of fruit pulp. Further studies are in progress for isolation and identification of bioactive phytoconstituents and establishment of mechanism of action.

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