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Original Research Article

Antifertility activity of *Momordica dioica* and *Lagerstroemia speciosa* in experimental ratsKrishna Patil¹, Manojkumar Mahajan¹, Aman Upaganlawar^{1,*},
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ABSTRACT

Agents that control fertility are termed as antifertility agents or oral contraceptives. Many plant-based products have the antifertility potential which need to be explored. The present study therefore was undertaken to evaluate possible antifertility effects of hydroalcoholic extract of fruits of *Momordica dioica* (HAEMD) and aqueous extract of leaves of *Lagerstroemia speciosa* (AELS) in experimental rats. Experiments were carried using wistar rats of either sex. Animals were administered with different doses of HAEMD and AELS (250, and 350 mg/kg, p. o) for 30 days. The body weight and reproductive organs weights for male (Testis) and for female (Uterus) were analyzed. Sperm count and Sperm motility was determined in male rats by manually. Testosterone in male and progesterone levels in female rats were checked. Effect of both extracts on Estrous cycle in female rats were checked. Section of testis were taken for histopathological study. The effect of the extract on male rats showed decreased testis weight, reduced sperm count and motility as compared to control animals. The blood testosterone level in male rats was reduced in treatment groups. The testicular histology was altered as compared to control male rats. In female animals, extracts showed antioviulatory activity, alteration in estrous cycle, decreased uterus weight and serum progesterone levels. It is concluded that HAEMD and AELS are effective as antifertility plants for both male and female rats.

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1. Introduction

The world population is growing at an alarming rate of 176 people per minute, with a current estimate of 6 billion people predicted to exceed 10.8 billion by the year 2050. India is the world's second most populous nation. Such population explosion may adversely affect country's economic development, imposes burden on health services and therefore is serving as the major threat to families and societies. Also, growing population has impact on available resources which is linked to the variety of problems including unemployment, starvation, etc. There

is an urgent need to provide the world's population with secure and long-term reliable fertility control.^{1,2} Research on oral contraceptives for controlling human fertility is as old as recorded history. Despite the availability of wide range of synthetic contraceptives, these cannot be used regularly due to their negative health effects. Thus, the quest for antifertility agents in natural sources is one method being used to find newer remedies. In the ancient Indian literature like Ayurveda, Siddha, and Unani, many plant based preparations claimed to possess fertility-regulating properties. Many plants and plant-based formulations have been used to safely control fertility, which has led to the creation of new antifertility molecules derived from natural products. Some active constituents were isolated and are

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being used in clinical studies as antifertility agents.³

Lagerstroemia speciosa (Lythraceae) has commonly known as Banaba, queen of flower, crepe myrtle, and pride of India. Banaba is a rich source of active chemical constituents i.e. corosolic acid. Corosolic acid is reported to possess anti-diabetic, antioxidant, neuroprotective, and anti-inflammatory properties.⁴⁻⁶

Momordica dioica is a dioeciously climbing herb belonging to the family Cucurbitaceae.⁶ It is a rich source of active chemical constituents Momordica ursenol (flavonol), Momordica foetida, alkaloid (momordicin), flavonoids, glycosides, i.e. amino acids, tannins and phenolic compounds. It has been known to have many medicinal properties namely anti-tumorigenic, analgesic, anti-diabetic, anti-inflammatory, and anti-allergic activity.⁷⁻¹¹

Aqueous extract of *Momordica dioica*¹² and leaf extract of *Lagerstroemia speciosa*¹³ has been reported to possess antifertility activity. Based on the reported data, the present study was designed to carry out the antifertility activity of an aqueous extract of *Lagerstroemia speciosa* (AELS) leaves and hydro-alcoholic extract of fruits of *Momordica dioica* (HAEMD).

2. Materials and Methods

2.1. Plant material

The aqueous leaves extract of *Lagerstroemia speciosa* (AELS) was obtained as a gift sample from Kuber Impex Pvt Ltd. Indore. The fruits of *Momordica dioica* were collected from the local market of the Nandurbar district, India. The extract of *Momordica dioica* fruit was prepared in the laboratory using the hydro-alcoholic maceration technique.¹⁴

2.2. Preparation of extract

Fruits of *Momordica dioica* were air-dried in the shade, pulverised by a mechanical grinder, passed through a 40 mesh sieve before being stored in airtight containers. Using the Hydro-alcoholic maceration process, the powdered fruits (250 g) were extracted with ethanol (95% w/v) and distilled water for 8 days. Using a rotary evaporator [Rotavapour – BUCHI 011], these extracts were concentrated to dryness and temperature control was used to produce solid masses that were fully free from solvents. The yield obtained from the above process was found to be 6.5gm.

2.3. Animals

Adult albino rats of Wistar strain (150-350g) were procured from the Wockhardt Research Centre, Aurangabad, India. The animals were kept on a standard laboratory environment under controlled circumstances, with free access to a pellet

meal and ad libitum water. The experimental procedures and protocols used in this study was reviewed and approved by institutional Animal Ethics Committee (IAEC) of SNJB's SSDJ College of Pharmacy, Neminagar, Chandwad.

2.4. Experimental design

Group 1: received only normal water and feed for 30 days and served as control.

Group 2 & 3: were treated with HAEMD (250 & 350 mg/kg, p.o) for 30 days in male rats.

Group 4 & 5: received HAEMD (250 & 350 mg/kg, p.o) for 30 days in female rats.

Group 6 & 7: received AELS (250 & 350 mg/kg, p.o) for 30 days in male rats.

Group 8 & 9: received AELS (250 & 350 mg/kg, p.o) for 30 days in female rats.

The HAEMD and AELS were dissolved in water and administered orally by gastric intubation. Body weight were assessed every alternate day and dose was adjusted accordingly.

2.5. Pharmacological screening

On the 31st day i.e. 24 hrs. after the last dose of the test extracts in respective groups, animals were weighed, blood was collected from retro-orbital plexus under anesthesia. Serum was separated and animals were sacrificed with light ether anesthesia to dissect out the reproductive organs.

2.6. Parameters evaluated in male rats

2.6.1. Determination of body and reproductive organ weight

The initial and final body weights of the animals were recorded. The testes were dissected out, freed from adhering tissue, blotted on filter paper and weighted on a sensitive balance to the nearest milligram.

2.7. Sperm count

At a temperature of 36°C, the cauda regions of the epididymis were chopped individually in two Petri dishes with 1.0 mL of normal saline, the semen mixture was then sucked into a red blood pipette to the 0.5 mark, diluted with warm normal saline, then sucked up to the 101 mark. A drop of the semen mixture was placed on the Neubauer counting chamber and spread by capillary action under the cover glass. The chamber was mounted on the microscope stage, observed at a magnification of 45X, and the concentrations were tallied and expressed in million per mL.¹⁵

Sperm count/ml was calculated as follows:

2.8. $X \times 20 \times 10^4 / \text{cm}^2 / \text{ml} / \text{epididymis}$

Where X is the average mean of spermatozoa of all 4 squares.

2.8.1. Sperm motility

On a separate glass slide, a drop of the sperm-saline mixture was taken. One slide was examined under the microscope after being covered with a cover slip. The motility of spermatozoa per unit area was then calculated to quantify sperm motility at the caudal epididymis. A smear was made on another slide and total morphological abnormalities were observed.¹⁶

2.8.2. Testicular histopathology

At the time of dissection of rats, one (right) of the testes of each animal was collected. A small puncture of the capsule was made with the tip of the scalpel. Subsequently, the testes were preserved in neutral formalin buffer 10% for 24 hrs. dehydrated in alcohol and embedded in paraffin wax. The sections of 5µm was cut and stained with hematoxylin – eosin and examined under digital microscope.¹⁷

2.8.3. Estimation of sex hormones

Blood samples were collected from rats for estimation of serum levels of sex hormones.^{17,18}

2.9. Parameters evaluation in female rats

2.9.1. Determination of body and reproductive organ weight

The initial and final body weights of the animals were recorded. The testes were dissected out, freed from adhering tissue, blotted on filter paper and weighted on a sensitive balance to the nearest milligram.

2.9.2. Anti – ovulatory activity

Female albino rats of Wistar strain (150 – 200g) were maintained under standard conditions with access to food and water libitum. The vaginal smear of each rat was examined daily for 15 days to select animals showing regular estrous cycles.^{19,20} The selected rats were divided into 3 groups of 6 animals each. The treatment was given for 15 days to cover 3 regular estrous cycles. Vaginal smear from each animal was observed every morning at 9 – 10 A.M. On the 16th day, 24 hrs. After the last treatment the animals from each group were sacrificed, ovaries and uteri were dissected out freed from extra disposition and weighted on a sensitive balance. One ovary from each animal was fixed in formalin buffer for histological studies.

2.9.3. Estimation of sex hormones

Blood samples were collected from rats for estimation of serum levels of sex hormones. Sera generated from collected blood samples by allowing them stand for 3hrs. to ensure

complete clotting, centrifuging the clotted samples at 3000 rpm for 10 min and aspirating off the serum with sterile needles and syringes were used for hormonal analysis.^{21,22}

2.9.4. Statistical analysis

Statistical analysis was carried out by one way ANOVA followed by Dunnett's test. Results were expressed as mean \pm SEM from six rats in each group.

3. Results

3.1. Reproductive organs weight

The effect of hydro-alcoholic extract of fruits of *Momordica dioica* and aqueous extract of leaves of *Lagerstroemia speciosa* at the dose of 250 mg/kg and 350 mg/kg body weight shows slight decrease in reproductive organ weight i. e. testis in male rats and uterus in female rats was observed in all treated groups at the end of the treatment.

3.2. Sperm analysis

A significant reduction in ($p < 0.001$) is sperm count number was noticed in high and medium dose treated groups as compared to control. The mean \pm SEM of sperm motility rate of control and treated group are shown in Table 2. A significant reduction ($p < 0.001$) in sperm motility rate was seen in dose-dependent manner.

3.3. Hormonal analysis

The concentration of plasma testosterone (ng/ml) hormone in adult male rats and plasma progesterone (ng/ml) hormone in adult female rats following 30 days of treatment has been given in Table 3. Plasma testosterone levels and Plasma progesterone levels were reduced significantly ($p < 0.0001$) in treated groups when compared with control group.

3.4. Anti – ovulatory activity: (Estimation of estrous cycle)

After administration of hydro-alcoholic extract of fruits of *Momordica dioica* and aqueous extract of leaves of *Lagerstroemia speciosa*, at the dose of 250mg/kg and 350mg/kg for 30 days, the rats of all treatment groups showed the change in duration of estrous cycle than that of the normal estrous cycle of control grouped animals.

3.5. Testicular histopathology

Histology of testicular tissues are given in Figure 1. Section of testis of rat testis indicates normal control, 250mg/kg and 350mg/kg hydro-alcoholic extract of fruits of *Momordica dioica* and aqueous extract of leaves of *Lagerstroemia speciosa* HAEMD and AELS. Observation like cytolytic lesions in the germinal layer and extensive lytic activity was noted.

Table 1: Effect of hydro-alcoholic extract of fruits of *Momordica dioica* and aqueous extract of leaves of *Lagerstroemia speciosa* on Reproductive organ weights in male (Testis) and female (Uterus) rats.

S.No.	Groups	Dose	Testis	Uterus
1.	Control	–	4.416 ± 0.18	5.91 ± 0.36
2.	HAEMD	250 mg/kg	3.916 ± 0.07*	4.36 ± 0.21*
		350mg/kg	3.833 ± 0.05**	4.21 ± 0.21*
3.	AELS	250 mg/kg	3.821 ± 0.14***	4.18 ± 0.20*
		350mg/kg	3.566 ± 0.04***	4.00 ± 0.21*

All values are Mean ± SEM, and statistically analyzed using One Way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test. n=6, **p<0.05, *p<0.01, ***p<0.001

Table 2: Effect of hydro-alcoholic extract of fruits of *Momordica dioica* and aqueous extract of leaves of *Lagerstroemia speciosa* on epididymal sperm density and sperm motility.

S.No.	Groups	Dose	Sperm density (count/ml)	Sperm motility (%)
1.	Control	–	64.35 ± 0.85	65.66 ± 1.11
2.	HAEMD	250 mg/kg	54.16 ± 1.01**	54.32 ± 0.85**
		350mg/kg	50.33 ± 0.80***	52.18 ± 0.57***
3.	AELS	250 mg/kg	51.33 ± 0.61***	52.23 ± 0.83***
		350mg/kg	48.00 ± 0.96****	47.46 ± 0.57***

All values are Mean ± SEM, and statistically analyzed using One Way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test. n=6, **p<0.05, *p<0.01, ***p<0.001

Table 3: Effect of hydro-alcoholic extract of fruits of *Momordica dioica* and aqueous extract of leaves of *Lagerstroemia speciosa* on Serum testosterone level in ng/ml in the male rats and Serum Progesterone level in ng/ml in the female rat.

S.No.	Groups	Dose	Testosterone (ng/ml)	Progesterone(ng/ml)
1.	Control	–	3.22 ± 0.02	52.36 ± 8.73
2.	HAEMD	250 mg/kg	2.93 ± 0.02**	17.46 ± 2.91***
		350mg/kg	2.77 ± 0.46**	15.80 ± 2.64***
3.	AELS	250 mg/kg	2.91 ± 0.02***	17.38 ± 0.18***
		350mg/kg	2.74 ± 0.13***	15.77 ± 0.22***

All values are Mean ± SEM, and statistically analyzed using One Way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test. n=6, **p<0.05, *p<0.01, ***p<0.001

Table 4: Effect of hydro-alcoholic extract of fruits of *Momordica dioica* and aqueous extract of leaves of *Lagerstroemia speciosa* on Estrous cycle in female rats.

S.No.	Groups	Dose	Duration of Estrous cycle (Days)
1.	Control	–	4.30 ± 0.02
2.	HAEMD	250 mg/kg	3.37 ± 0.36*
		350mg/kg	3.21 ± 0.21*
3.	AELS	250 mg/kg	3.23 ± .055*
		350mg/kg	3.04 ± 0.16*

All values are Mean ± SEM, and statistically analyzed using One Way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test. n=6, **p<0.05, *p<0.01, ***p<0.001

4. Discussion

In a developing country, family planning is restricted due to lack of literacy, education and access to healthcare facilities. One primary objective of study is to develop herbal contraceptive medications for both males and females that are more suitable for both protection and monetary reasons.²³ Several plants containing flavonoids, tannins, terpenes, quinines, and diterpenoid lactones have been demonstrated to produce male antifertility via various mechanisms.²⁴ The phytochemical screening indicated the

existence of flavonoids, steroids, and other compounds.^{4,25} According to the literature, flavonoids and phytosteroids can alter hormone levels in the body as well as antifertility in both animals and humans.^{25,26}

Momordica dioica is widely used in many parts of the world, and multiple studies have shown that it has nephroprotective, antibacterial, analgesic, antidiabetic, hepatoprotective and antioxidant activities.²⁷ *Lagerstroemia speciosa* multiple studies have shown that it has antihyperglycemic, antihyperlipidemic, antioxidant,

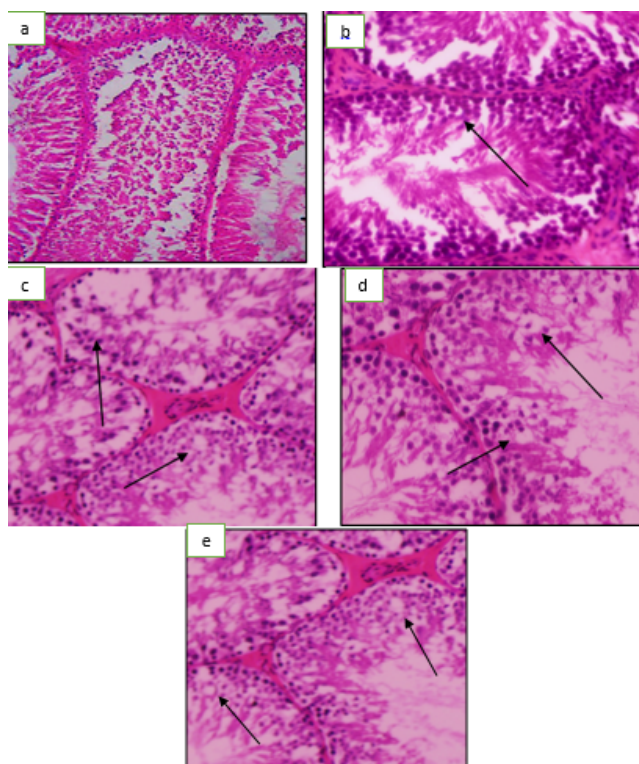


Fig. 1: Section of testis of rat testis where; **A:** Control group; with normal morphology of caput epididymal cells; **B:** and **C:** low dose *Momordica dioica* treatment group; cytolitic lesions in germinal layer; **D:** and **E:** high dose of aqueous extract of leaves of *Lagerstroemia speciosa* treatment group; observation like cytolitic lesions in the germinal layer and extensive lytic activity observed.

anti-inflammatory, antineoplastic activities.²⁸ Previously, no study has been carried out to reveal the contraceptive potential of this plant. Therefore, the present study was conducted and the results showed that aqueous leaf extract of *Lagerstroemia speciosa* exerts antifertility effects on male and female reproductive system of adult rats as depicted by significant reduction in fertility rate. In present study, administration of HAEMD & AELS, at the dose of 250mg/kg and 350mg/kg for 30 days in adult male and female rats induced highly significant *antifertility activity* among all the extract treated groups as compared to control groups.

HAEMD & AELS was a growth inhibitor, as there was a substantial difference in the initial and final body weight of the treated group. Complete spermatogenic arrest is not necessary for the male contraception; fertility can be eliminated by altering structure or function of spermatozoa.²⁸ HAEMD & AELS treated rats reduced sperm motility and density in the caudal and caput epididymal segments. The current investigation found a decrease in testosterone levels in the blood. This observation was comparable to the previous findings.²⁹ Reduced testosterone levels in males may affect spermatogenesis and

result in male infertility.

Treatment with the HAEMD & AELS was very efficient in generating reversible functional sterility. Histopathological observation like cytolitic lesions in the germinal layer, invasion of genial element in to the lumen of seminiferous tubule, disintegration of luminal gonial element and sperm resulting in the accumulation of an edematous fluid, the absence of intact sperm in seminiferous tubules and epididymis.^{30,31} In all dose treated groups, HAEMD & AELS caused a dramatic and dose dependent reduction in the quantity of spermatogonia, spermatocytes, and mature spermatozoa, as well as sloughing off of germinal epithelium.

The HAEMD & AELS was examined for antioviulatory and estrogenic activities in this study. The rat has a brief estrus cycle of 4 to 5 days in stages, making them ideal for reproductive studies.³² The administration of HAEMD & AELS was linked to an irregular pattern of estrous with a prolonged diestrous and, as a result, a lower number of ova in the ovary. As a result, the extracts inhibited ovulation, resulting in a reduction in cyclicity. HAEMD & AELS was discovered to have substantial estrogenic activity, as evidenced by decrease in uterine weight.

The hormone progesterone is responsible for the histology and functional alterations of the female genital tract.³² During the estrous cycle, preovulatory follicles mature and ovulate under the combined and balanced effect of ovarian and extra ovarian hormones. Any imbalance in these hormones causes irregular ovarian function and irregular variations in the duration of the estrous cycle.^{33,34} All groups of treated rats at doses 250mg/kg and 350 mg/kg of HAEMD & AELS demonstrated temporary modification of estrous cycle characterized by decrease in estrus and metestrous phases and prolongation of proestrous phase. Therefore, the extracts in all treated groups provoked inhibition of ovulation. Progesterone is necessary for pregnancy maintenance; any variations in progesterone levels result in embryo separation.³⁵ The treated groups' progesterone levels were compared to the control group. the HAEMD & AELS at 250mg/kg and 350 mg/kg showed highly significant decrease in progesterone level. Decreases the normal hormone level had some effect on the implantation activity.

5. Conclusion

It may be concluded that HAEMD and AELS shows anti-fertility activity and further in depth studies are needed to determine the phytoconstituents responsive for anti-fertility activity of the test extracts.

6. Conflict of Interest

The authors declare no relevant conflicts of interest.

7. Source of Funding


None.


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