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Estrogenic Activity of Fruit Extract of *Artocarpus lakoocha* in female Sprague-Dawley Rats

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ABSTRACT

Estrogenic activity of the fruit extract of Artocarpus lakoocha (Moraceae) in female sprague-dawley rats. The powdered of the fruit were extracted with hydro alcoholic and the resultant extracts were subjected for phytochemical analysis to identify different phyto constituents. Preliminary Phytochemical investigation showed the presence of flavonoids, tannins, steroids, and saponins, as major secondary metabolites. Some of the physiochemical parameters such as ash value, extractive value and loss on drying were also been studied. Estrogenic activity of extract were studied by removing it both ovaries, and then given the different dose of extract as per body weight of animals by the oral route. Treatment of animal with Hydro-alcoholic extract of fruit of Artocarpus lakoocha shows significantly ($p<0.05$) value. It is observed that Hydro-alcoholic extract of fruit of Artocarpus lakoocha posses estrogenic property which was evident by biochemical parameters and histopathological reports.

Key words: Estrogenic activity, Estrogen, Fertility, Overectomy*, Artocarpus lakoocha* vaginal smear.

INTRODUCTION

A vast majority of population particularly those living in villages depend largely on herbal medicines. Scientific data on a good number of medicinal plants investigated has been well documented. However, only very few drugs of plant origin could reach clinical use and the National Formulary could not adopt even a dozen of plant medicines. This could be possible with the concerted efforts of team of phytochemists and pharmacologists working together in a few well organized laboratories for multi-dimensional chemical and pharmacological screening of the active fraction of the plants followed by the tests for specific activity such as anti-allergic, anti-inflammatory, hypolipidaemic, hypoglycaemic, hepatoprotective, adaptogenic, anti-fertility etc.[1]

The popular usage of phytoestrogens as an alternative or complimentary herbal treatment in particular has grown in recent years as they are thought to protect against cardiovascular disease, osteoporosis, and a range of hormone dependent cancers and to alleviate menopausal symptoms.[2],[3] Epidemiological evidence furthermore supports the usage of phytoestrogens for hormone related conditions.[4] The incidence of breast, endometrial, and prostate cancer, as well as cardiovascular disease, is notably lower in Asian countries as compared to Western populations.[5]

The two Women's Health Initiative (WHI) studies, involving clinical trials of menopausal women using HRT, had to be terminated prematurely due to a number of risks associated with HRT.[6] The use of traditional medicine and

medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed.[7]

Natural products as contraceptives:
Population explosion is an imminent hurdle for a country's development as the natural resources are limited. The population of India is multiplying at an alarming rate and has crossed one billion. Fertility regulation has therefore become the major concern of people of all walks of life. The regulations of human fertility have global consequences in terms of resources depletion, population and poverty. Now, it has become one of the priorities of the National Family Programs and therefore, there is an urgent need to improve the access and the quality of contraceptive service in the country.[8]

Chemical constituents:
The various chemical constituents present in *Artocarpus lakoocha* are β-sitosterol, cycloartenol, cycloartenone, α-amyrin acetate and lupeal acetate. It is a good source of soluble tannins. It also contain artocarpin, norartocarpin, cycloartocarpin, resorcinol and oxyresveratrol. Also contain pentosans, lignin, holocellulose and α-cellulose.

Artocarpus lokoocha contains β-sitosterol, cycloartenol, cycloartenone, nor-cyclartenone, α-amyrin, lupeol, cycloartenyl acetare.

The plant also contains artocarpin, nor-artocarpin, nor-cycloartocarpin, cycloartocarpin, resorcinol and lectin.The stem bark contains oxyresveratrol. The root contains lakoochins A and B.Root bark contains prenylated 2-arylbenzofurans-artolakoochol, 4-hydroxy artolakoochol and cycloartolakoochol. [9-12]

MATERIALS AND METHODS

Plant material
The fruit of *Artocarpus lakoocha* used in this study were obtained from the local market of Lucknow Uttar Pradesh (India) and were identified based on its morphological characteristics with reference number NBRI/CIF/260/2011 from NBRI, Lucknow. The fruit were dried and crushed to small pieces using pestle and mortar and powered in an electric grinder.

Preparation of Hydroalcohlic extract:
The powdered plant materials (180.0 g each of pulp, pericarp, seeds and leaf) were extracted in different Soxhlet apparatus at elevated temperature (65°C) using 0.5 liter of a mixture of methanol: water (7:3, v/v). All four extracts were filtered individually through fresh cotton bed. The solvent from the filtrates was then removed separately at reduced pressure to have gummy concentrate of the crude extracts which were used in the present study (% yields for pulp, pericarp, seed and leaf were 12.3, 10.8, 14.9 and 3.6 respectively).[13]

Selection of animals, caring and handling:
The Estrogenic activity carries out on female Sprague–Dawley rats of (50-60gm), Purchase by animal house of Central Drug Research Institute Lucknow, or any other registered animal house. They are maintaining in 12 h light/dark cycle at 23 ± 20^0C. They are allowing to standard pellet diet and water, adlibitium. The study is approving by the Institutional Animal Ethics Committee (IAEC) according to the regulation of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and ethical norms are strictly, following during all experimental procedure.

CPCSEA approval no. - Hygia/M. Pharm/05/2011-12

Experimental Design:
Estrogenic activity of the extract will assess in bilaterally ovariectomized female Sprague-Dawley rats. Vaginal smears will carry out to monitor cellular differentiation and to evaluate the presence of leukocytes, nucleated epithelial cells, or cornified cells. Vaginal smear samples will collect between 08.00 and 10.00 am daily. The vaginal smears will prepare by washing with 10 µl of normal saline (NaCl 0.9%) and then thinly spread on a glass slide.

They will allow to dry at room temperature and then stained using Methylene blue dripping. The slides will rinse in distilled water after 30 min and allow drying.

Overectomy model in immature female rats:
Female Sprague-Dawley rat (60-70gm) in individual cages with no access to drinking water. Animals are divided into five group each contain 5 animals in, control, test and standard. The experimental protocol comprises as follows:

Table no. 1 Overectomy model

GROUP	TREATMENT	DOSE TREATMENT
Group 1	Control	Receive 0.6%w/v Sod.CMC suspension at a dose of 10ml/kg
Group 2	Standard	Receive aqueous suspension of diethylstilbestrol in 0.6%(w/v) Sod.CMC at a dose of 2mg/kg
Group 3	Test 1	Receive extract of *Artocarpus lakoocha* fruit 200mg/kg
Group 4	Test 2	Receive extract of *Artocarpus lakoocha* fruit 400mg/kg
Group 5	Test 3	Receive extract of *Artocarpus lakoocha* fruit 600mg/kg

Uterotropic activity:
The estrogenic activity of *Artocarpus lakoocha* extract in-vitro will examine by uterotropic assay of immature rats. I ovariectomized mature female rats and administer them orally once daily with extract of *Artocarpus lakoocha* for 7 days, beginning on the day of the surgery. Additional rats will administer orally with vehicle as a control and other group with standard β-estradiol as a positive control. At 24 h after the last treatment, rats will weigh and euthanized by decapitation and take weight of uterus and liver.

Table no. 2 Uterotropic activity

GROUP	TREATMENT	DOSE TREATMENT
Group 1	Control	Receive 0.6%w/v Sod.CMC suspension at a dose of 10ml/kg
Group 2	Standard	Receive aqueous suspension of diethylstilbestrol in 0.6%(w/v) Sod.CMC at a dose of 2mg/kg
Group 3	Test 1	Receive extract of *Artocarpus lakoocha* fruit 200mg/kg
Group 4	Test 2	Receive extract of *Artocarpus lakoocha* fruit 400mg/kg
Group 5	Test 3	Receive extract of *Artocarpus lakoocha* fruit 600mg/kg

Vaginal cytology:
This was done by vaginal smear method .Vaginal smear was taken by introducing a few drops of saline in to vagina with the help of eye dropper. The saline was expelled into the vagina and withdrawn two or three times. The contents of the eye dropper was placed and spread on a glass slide, the smear was immediately fixed with 1%w/v aqueous methylene blue for 5-6 min. The smear was examined under microscope to check the presence or absence of leukocytes and nucleated epithelial cells.

Vaginal opening:
The vaginal opening was observed and noted daily. Increase in vaginal opening is indicative of estrogenic activity.

RESULTS

HISTOPATHOLOGICAL STUDIES (Vaginal cytology):
All the animal was euthanized and the principal vital organ were removed and macroscopically analyzed. After macroscopic analysis, representative fragments of uterus was fixed in a 90% solution of saline-formalin (pH 7.4) and enclosed in paraffin. Five- micrometer section were obtained and colored with Methylene blue- eosin for evaluation under an optical microscope.

Control:

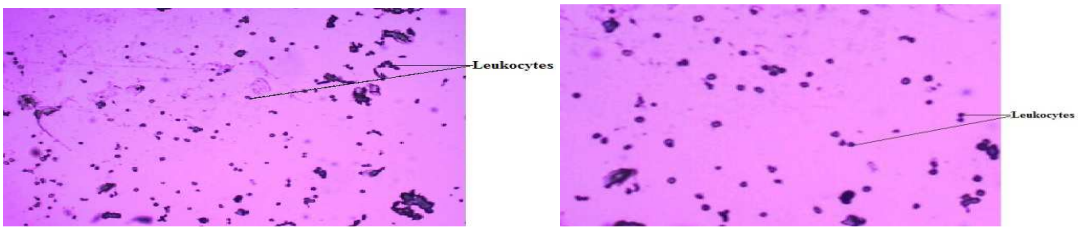

Figure no. 1 [Methylene blue stained vaginal smear of control group treated rat, showing only leukocytes cells (i.e., in diestrous stage)]

Standard:

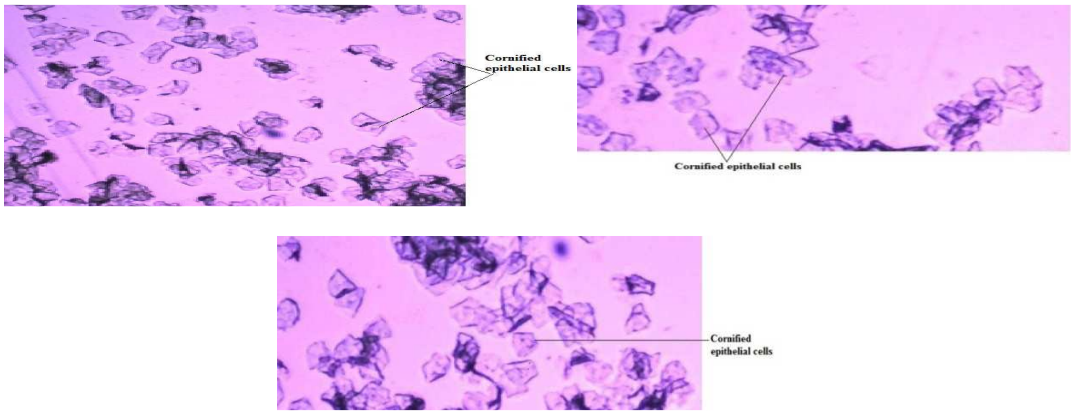

Figure no. 2 [Methylene blue stained vaginal smear of diethylstilbestrol (2 mg/kg, p.o) treated rat, showing only cornified epithelial cells (i.e., in estrous stage)

Extract: 200mg/kg:

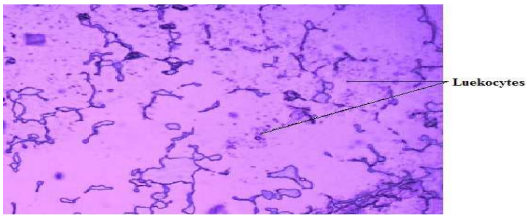

Figure no. 3 [Methylene blue stained vaginal smear of control group treated rat, showing only leukocytes cells (i.e., in diestrous stage)

Extract: 400mg/kg:

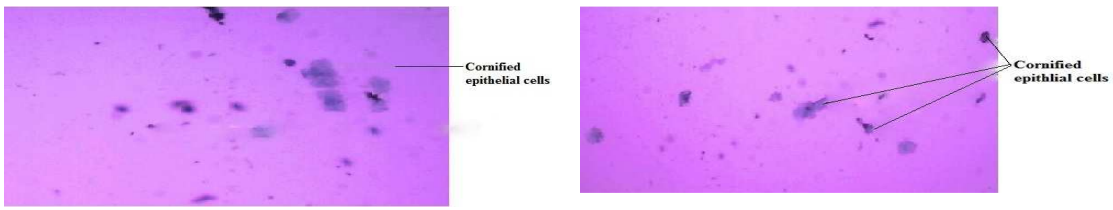

Figure no. 4 [Methylene blue stained vaginal smear of *Artocarpus lakoocha* fruit extract (400 mg/kg, p.o) treated rat, showing only few cornified epithelial cells (i.e., in between estrous diestrous stage)

Extract: 600mg/kg:

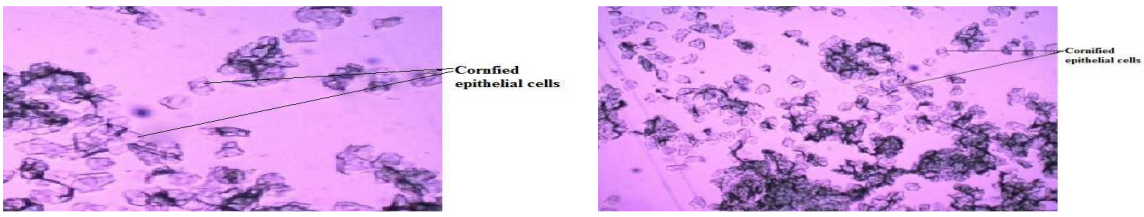

Figure no. 5 [Methylene blue stained vaginal smear of *Artocarpus lakoocha* fruit extract (600 mg/kg, p.o) treated rat, showing only cornified epithelial cells

Estrogenic activity on immature rats

Table no. 3 Effect of hydroalcohlic extract of *Artocarpus lakoocha* in immature female rats

Group No.	Drug treatment	Number of animals	Dose	Uterus weight (Mean ± Sem)
1.	Control	5	10ml/kg	110.55 ± 1.1674
2.	Standard	5	2mg/kg	236.22 ± 7.1559 ***
3.	Extract of *Artocarpus lakoocha* fruit	5	200mg/kg	117.45 ± 1.3472 **
4.	Extract of *Artocarpus lakoocha* fruit	5	400mg/kg	163.83 ± 7.0053 ***
5.	Extract of *Artocarpus lakoocha* fruit	5	600mg/kg	219.60 ± 8.7119***

Data was analyzed by one-way ANOVA followed by Dunnet test

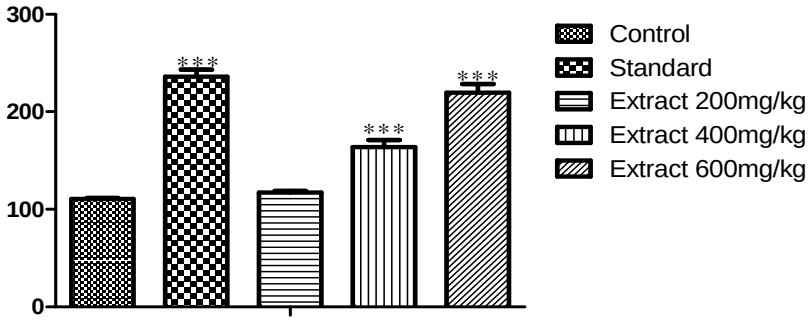

Figure no. 6 (Values are Expressed as Mean ± S.E.M., (n=5) one way ANOVA followed by Dennett's test* P value<0.05, **P value <0.01, *<P value<0.001 compared with control group)**

Estrogenic activity on mature rats

Table no. 4 Effect of hydroalcohlic extract of *Artocarpus lakoocha* in immature female rats

Group No.	Drug treatment	Number of animals	Dose	Uterus weight (Mean ± Sem)
1.	Control	5	10ml/kg	505.66 ± 7.3860
2.	Standard	5	2mg/kg	925.358 ± 7.7837 ***
3.	Extract of *Artocarpus lakoocha* fruit	5	200mg/kg	536.224 ± 5.6239 **
4.	Extract of *Artocarpus lakoocha* fruit	5	400mg/kg	550.946 ± 8.2247 ***
5.	Extract of *Artocarpus lakoocha* fruit	5	600mg/kg	906.19± 6.9134***

Data was analyzed by one-way ANOVA followed by Dunnet test

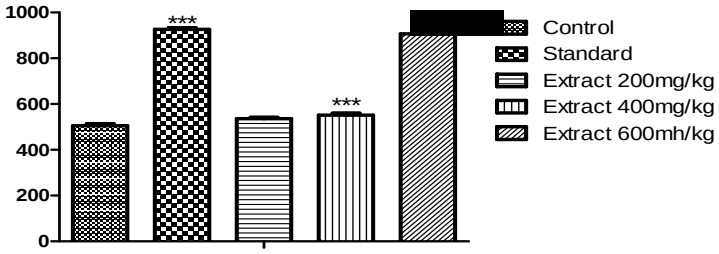

Figure no. 7 (Values are Expressed as Mean ± S.E.M., (n=5) one way ANOVA followed by Dennett's test* P value<0.05, **P value <0.01, *<P value<0.001 compared with control group)**

Table no.5 Vaginal opening in immature female Sprague Dawley rats

Group	Treatment	Dose mg/kg	Vaginal opening (%)						
			Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1.	Control (sod. c m c)	10	0	0	0	0	0	0	0
2.	Standard (Diethylstilbestrol)	2	0	0	0	70	100	90	80
3.	Extract of *Artocarpus lakoocha* fruit	200	0	0	0	5	5	6	8
4.	Extract of *Artocarpus lakoocha* fruit	400	0	0	0	45	55	50	60
5.	Extract of *Artocarpus lakoocha* fruit	600	0	0	0	60	85	75	70

The hydro-alcoholic extract showed a dose dependent increase in vaginal opening from day fourth onward compared to control.

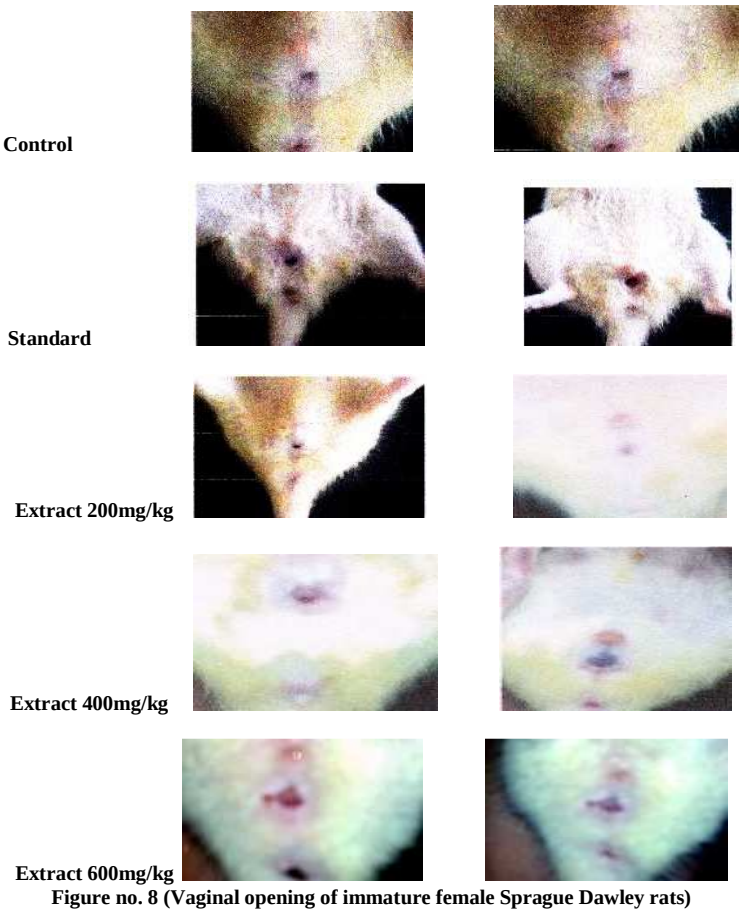

Figure no. 8 (Vaginal opening of immature female Sprague Dawley rats)

DISCUSSION

Uterus and the female reproductive tract undergo innumerable physiologic and biochemical changes under the influence of ovarian hormones such as estrogen.[14] If female rats are ovariectomized, the resultant lack of estrogen causes atrophy of the uterus and the reproductive tract; administration of estrogenic substances to ovariectomized rats leads to vaginal cornification and vaginal opening and proliferative changes in uterine endometrium.[15] Estrogenic compounds are known to cause the keratinization and cornification of the vaginal epithelium, causing the superficial cells to be shed into the lumen to form large squamous cells.[16]

Flavonoids and phenolic compounds are known to possess estrogenic activity.[17] It clearly indicated that both the doses contain estrogen like compound. Estrogen levels are lowest during estrus phase and increased gradually during diestrus phase and reaches to peaks at the proestrus phase.[18], [19] Thus the dose dependent increase in percentage vaginal cornification shown by the extract of *Artocarpus lakoocha* can be attributed to its estrogenic activity.

Thus in the near future *Artocarpus lakoocha* may play a very important role as potential estrogenic activity, anthelmintic agent and other pharmacological activities due to the presence of steroids, tannins and flavonoids.

With a further study on the efficacy and safety aspect, the drug in future might be recommended for preventing the development of estrogen related cancers, blunting the symptoms of menopause, and provide in cardioprotective effect.
In view of literature finding, sufficient studies were reported on the bark and heartwood and lesser attention was paid towards fruits. Therefore, the present study was planned to concentrate upon fruits and was selected for the study, which is a rich source of phytoconstituents.

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