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# Anti-Fertility activity of *Ficus religiosa* fruits extract on Goat Uterus *in vitro*

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## Abstract:

Use of herbal methods for fertility regulation is widely accepted alternative for the synthetic drugs containing chemical having side effects. *Ficus religiosa* is the plant having several medical properties but no report is available on the antifertility activity. Effects of 1% methanol extract of fruits extract was studied on the goat uterus *in vitro* in exposure duration dependent manner (1 hour, 4 hours and 8 hours). Effects on dimensions of uterine glands, surface epithelium, gland cell and myometrium were observed. The treatment induces a decline in uterine glands diameter which are crucial for implantation. Present observations suggest that the extract has antifertility activity and should be experimented for antifertility programme.

**Keywords:** *Ficus religiosa*, antifertility, uterine gland.





## Introduction

Exponential rise in human population in India has challenged all the development plans and has forced mankind to research on fertility regulation worldwide. The synthetic chemical agents currently being used as fertility regulating method possess the combination of hormonal and non-hormonal compounds those have several side effects. The herbal drugs of Indian origin have revealed a significant fertility regulation potential of mammalian species which can be explored for developing an antifertility drug.

The plants of Indian origin have been experimentally screened using modern techniques for identification of their anti-fertility activity [1].The screening of traditional Indian plants has revealed several pharmaceutical activities including the fertility regulation [2].

*Ficus religiosa* was selected to be screened based on the observation that 5-10 receptacles are grinded with sugar and taken before one week of menses make the women sterile indicating the effectiveness [3].

## Materials and Methods:

***Reagents:*** The reagents used during the study were of analytical grade and procured from standard laboratory suppliers.

***Methodology: Collection of Plant Material:*** The Fruits of *Ficus religiosa* were collected from the Kurukshetra University campus, Kurukshetra ($29^0$6'N, $76^0$5'E) in the month of August. The plant and sample specimen were identified by a taxonomist from Department of Botany.

***Preparations of Plant Extract (Drug):*** In order to avoid any alternation/degradation of biologically active ingredientin fresh extract, the ethanol

extract of the dried fruit was used. The 1% fraction of the methanol extract was tested for activity [4].The collected fruits were dried in the oven at $40^0$C temperature for 48 hours. The dried fruits were grinded to make fine powder. After measurement of powder it was macerated in absolute methanol i.e. 100 g / 250 ml, w/v and stirred using magnetic stirrer for one day at room temperature. Extract was then flittered through Whatman filter paper No 1. After filtration, the methanol was evaporated from the extract by heating at $55^0$C in water bath for 12 hrs. The resulting partially solid extracts were stored at -$20^0$C for future experimentation.

***Experiment Design:*** The ovary along with uterus of *Capra hircus*procured from the slaughter houses near Kurukshetra and brought to the laboratory in culture media. The uterus was dissected out, cleared off adhering adipose tissue and processed for *in vitro* experimental protocol. After washing with normal saline, the uterus was placed in culture medium (TCM-199) which was fortified with antibiotics (200 unit penicillin 10 IU/ml and streptomycin 1µg/ml) [5]. The 1% of fruit extract in culture medium is employed for antifertility assessment.

The tissues were divided into four groups. The Group A was the zero hour control and kept in Bouins fixative. The Group B was exposed to drug for 1 hour, Group C was exposed to drug for 4 hour and Group D was exposed to drug for 8 hours, all with their respective control.

***Histological Slides:*** The tissue was harvested after stipulated time and processed for histological slide preparation. For histological slides the uterus was fixed in aqueous Bouins fixative for 24hours. Then tissue was washed in running tap water for 6 hours. The specimens then were dehydrated in various grades of alcohol. After proper dehydration specimens were then embedded in paraffin wax at $58^0$-$60^0$C. The uterus was sectioned serially at 5 µm thickness and the sections were stained with the haemotoxylene for 10 minutes and allowed to develop for 5 to 15 minutes in tap water. After dehydration in 70% ethanol, the sections were stained with eosin (2% eosin in 70 % alcohol) for 1 to 2 minutes. The slide were washed in 70% ethanol and dehydrated in 90% and absolute alcohol and cleared in xylene and were mounted in DPX. Each section was examined under light microscope to study the morphological characteristics of uterine tissue.

## Results:

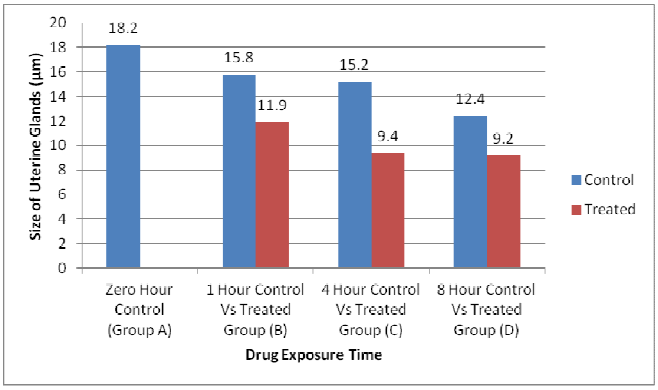
Histologically the goat uterus was comprised of endometrium(endometrial surface epithelium, endometrial glands, and epithelial cells), myometrium and perimetrium.The perimetrium is the outermost layer of uterus surroundedthe myometrium. The myometrium was comprised of the bundles muscle cells having the inner layer consisting of longitudinal and circularly arranged muscle cells and the outer layer showed irregularly arrange muscle cells, which ran longitudinally, obliquely, circularly, and transversely.The endometrium was composed of endometrial glands that secretedhormones involve in regulation of estrous cycle and implantation.

Significant changes were observed in the goat uterus treated with 1% solution of fruit extract of *Ficus religiosa*. The fruit extract induced decrease in thickness of Surface epithelium, diameter of uterine glands, diameter of gland cell and thickness of layer of myometrium according to exposure in time dependent manner (Table 1 and Graph 1).

**Table 1:** Effect of *F. religiosa* fruits extract on uterine gland diameter

| Group | Duration of Exposure | Uterine Gland Diameter (µm) | | P Value |
|---|---|---|---|---|
| | | Control | Treated | |
| A | Zero Hour | 18.2 µm | | |
| B | 1 Hour | 15.8 µm | 11.9 µm | 0.0027 (very significant) |
| C | 4 Hours | 15.2 µm | 9.4 µm | < 0.0001 (extremely significant) |
| D | 8 Hours | 12.4 µm | 9.2 µm | < 0.0001 (extremely significant) |

**Graph 1:** Showing the change in uterine gland diameter in control and treated group at different time exposures




Major changes were observed in the structure of uterine glands. There was decrease in uterine glands and gland cells diameters after fruit extract exposure in time dependent manner. The zero hour control group had the mean uterine gland diameter about 18.2 µm (Fig. 1(a) and Fig. 1(b)).

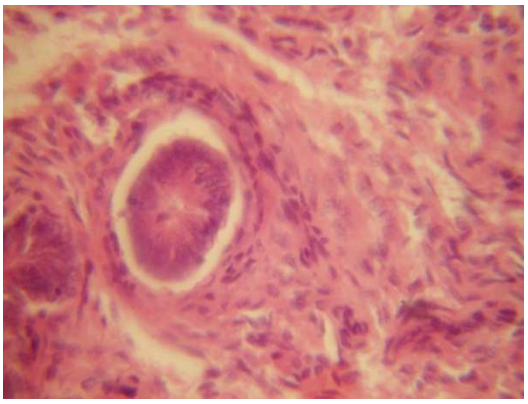


**Fig. 1(a)** Uterine gland: zero hour control Showing the normal uterine gland (400X)

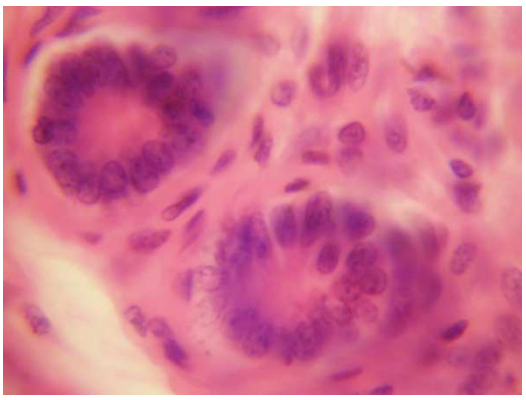


**Fig. 1(b)** Uterine gland: zero hour control Showing the normal uterine gland cells (1000X)

The 1 hour control group had the mean uterine gland diameter 15.8 µm whereas the treated group had the mean diameter about 11.9 µm (Fig. 2(a) and Fig. 2(b) respectively). The one-tailed P value is 0.0027, considered very significant.

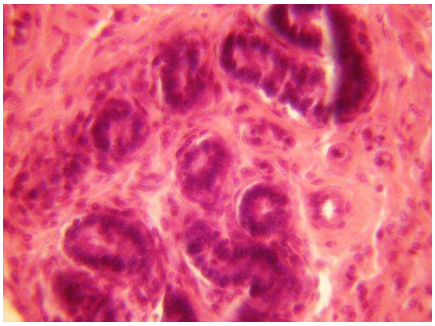


**Fig. 2(a)** Uterine gland: 1 hour control Showing small variation in diameter (400X)

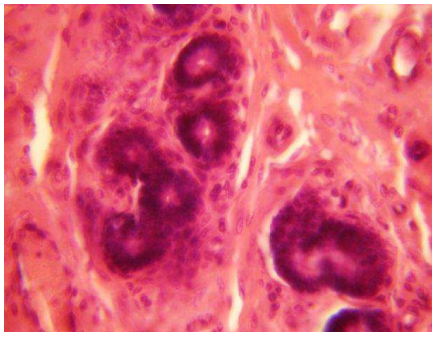


**Fig. 2(b)** Uterine gland: 1 hour treated Showing significant decrease in diameter (400X)

The alcoholic extract of *Ficus religiosa* induced hyportrophy in uterine glands and distortions of

blood vessel as the exposure duration increased. The 4 hour control group had the mean uterine gland diameter 15.2 µm whereas the treated group had the mean diameter about 9.4 µm (Fig. 3(a) and Fig. 3(b) respectively). The one-tailed P value is < 0.0001, considered extremely significant.

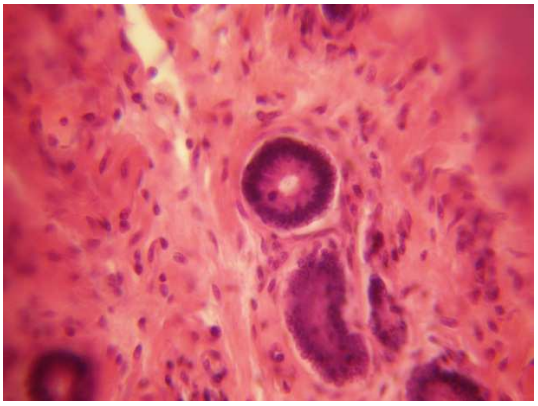


**Fig. 3(a)** Uterine gland: 4 hour control Showing small variation in diameter (400X)

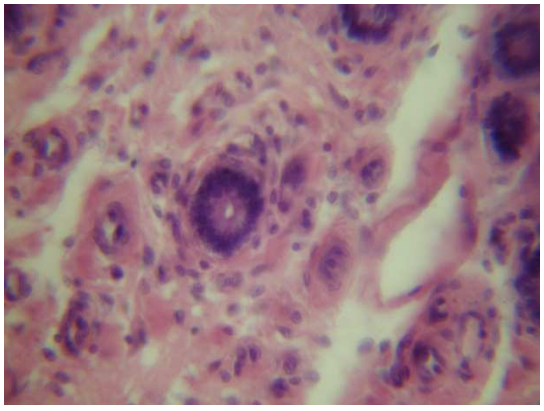


**Fig. 3(b)** Uterine gland: 4 hour treated Showing significant decrease in diameter (400X)

As the exposure duration increased from 4 to 8 hours the structure of uterine glands was distorted remarkably. The 8 hour control group had the mean uterine gland diameter 12.4 µm whereas the treated group had the mean diameter about 9.2 µm (Fig. 4(a) and Fig. 4(b) respectively).The one-tailed P value is < 0.0001, considered extremely significant.

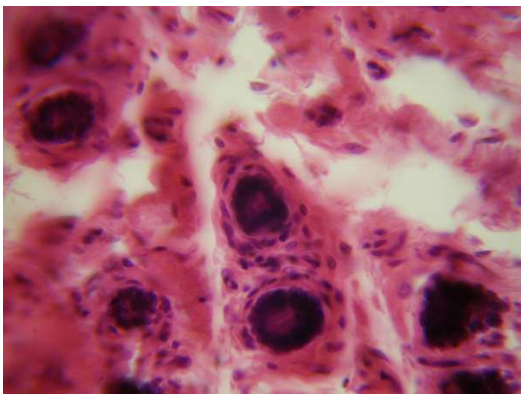


**Fig. 4(a)** Uterine gland: 8 hour control Showing intact glands (400X)

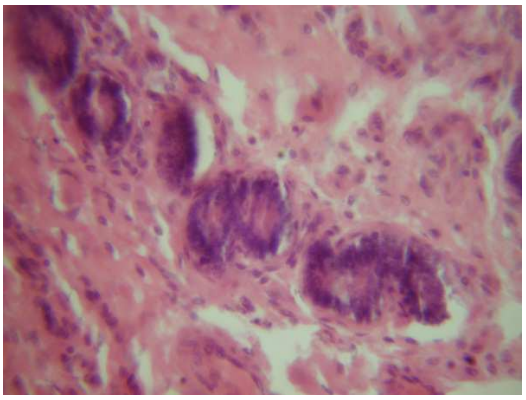


**Fig. 4(b)** Uterine gland: 8 hour treated Showing significantly ditorted glands (400X)

Small variations were observed in the thickness of layers of myometrium due to effect of plant extract. There were small changes in the size of the longitudinal and circular muscle fibres of inner and outer layer of myometrium.

## Discussion

The decrease in uterine glands diameter and myometrium thickness observed after the exposure of *Ficus religiosa* fruits extract supports the earlier findings reveled decreased myometrial volume in proportion to uterine weight and marked regression of uterine gland in female gerbils treated intraperitoneally with *Cannabis* extract [6].

Our studies also supports the earlier findings showed decreased thickness of myometrium and height of luminal epithelium in uterus of rat



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administered nicotine at 2 and 4 mg/kg body weight for 20 days, respectively [7].

Our study support the earlier work on the *Melia azedarach,* seed extract of which cause decrease in uterine glands diameter in albino rats [8]. However our studies contradict the earlier work on petroleum ether extract of *Cassia fistula* seeds which cause increase inepithelial cell height albino rats [9].

In our studies the thickness of the endometrium decreases which contradict the earlier work on *Cannabis sativa,* leaves of which cause increase in endometrium thickness in female albino rats [10]. Decrease in endometrium thickness and uterine gland diameter also contradict the earlier work on *Trianthemaportulacastrum* stem leaves and roots, alcoholic extract of which cause increase in endometrium thickness and uterine gland diameter in albino rats[11].

The GC-MS analysis of *Ficus religiosa* fruits extract marked the presence of several anti-androgenic compounds including n-Hexadecanoic acid; 9, 12-Octadecadienoic acid; 9, 12, 15-Octadecatrienoic acid and Butyl 9, 12, 15-octadecatrienoate [12].

Uterine glands are the important unit of implantation process and involve in hormonal regulation. The variations in uterine glands diameter cause hormonesrelated changes in theuterine milieu that created environment unsuitable for embryonic implantation/growth. The *Ficus religiosa* has anti-androgenic properties and so exhibit anti-implantation activity.

## Conclusion

The result of our study clearly demonstrates that *F. religiosa,* being anti-androgenic in nature, is a suitable plant for developing antifertility drug. *F. religiosa* is recommended for working out and should be experimented for antifertility programme.

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