

# Phytochemistry and in vitro Studies on Anti-Fertility Effect of *Ficus religiosa* Fruits Extract on Uterine Morphology of Goat (*Capra hircus*)

Goyal A. K.

Reproductive Physiology  
Laboratory, Department of  
Zoology, Kurukshetra University,  
Kurukshetra-136119, India.

## Corresponding Authors:

Atul Kumar Goyal  
Ph.D. Research scholar,  
Department of Zoology,  
Kurukshetra University,  
Kurukshetra-136119,  
Email: atlgyl@gmail.com

## Abstract:

Use of herbal methods for fertility regulation is widely accepted alternative for the synthetic drugs containing chemical having side effects. *Ficus religiosa* is a large evergreen tree found throughout India, wild as well as cultivated. It is a highly apparent plant in Ayurvedic system of medicine for the treatment of various ailments. No report is available on the antifertility activity of plant extract hence; the present study was carried out to investigate the same. The phytochemical analysis was done using GC-MS. The compound detection employed the NIST Ver. 2.0 - Year 2008 library. The results of the GC-MS analysis provide different peaks determining the presence of many phytochemical compounds with different therapeutic activities. The major phytoconstituents were n-Hexadecanoic acid; 9, 12-Octadecadienoic acid; 9, 12, 15-Octadecatrienoic acid, and Butyl 9, 12, 15-octadecatrienoate. 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:** GC-MS analysis, Phytochemistry, Pharmacology, Hexadecanoic acid, Octadecanoic Acid, *F. 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* Linn (Moraceae) commonly known as 'Peepal tree' is a large widely branched tree with leathery, heart shaped, long tipped leaves on long slender petioles and purple fruits growing in pairs. The tree is regarded as a sacred tree to both Hindus as well as Buddhists. It has got mythological, religious and medicinal importance in Indian culture since ancient times (3-5). The tree grows throughout India and widely cultivated in south-east Asia especially in vicinity of temples. In Ayurveda, *F. religiosa* belongs to a class of drugs called rasayana. Rasayana are rejuvenators, antioxidants and relieve stress in the body (6-7). In India it is known by several vernacular names, the most commonly used ones being Asvatthah (Sanskrit), Sacred fig (Bengali), Peepal (Hindi), Arayal (Malayalam), Ravi (Telgu) and Arasu (Tamil) (8).

*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 (9). Our list objective was 1) To devise a method for prepare *F. religiosa* fruits extract 2) GC-MS analysis of *F. religiosa* fruits extract 3) To screen plant extracts for antifertility activity. Different methods of testing based on literature, as is or modified, using goat as experimental animal were tried.

### Botanical Description of *F. religiosa*

#### Taxonomy/Botanical Classification

**Domain:** Eukaryota

**Kingdom:** Plantae

**Subkingdom:** Viridiplantae

**Phylum:** Tracheophyta

**Subphylum:** Spermatophytina

**Infraphylum:** Angiospermae

**Class:** Magnoliopsida Brongniart

**Subclass:** Dilleniidae

**Super order:** Urticales

**Order:** Urticales

**Family:** Moraceae

**Division:** Magnoliophyta

**Tribe:** Ficeae

**Genus:** *Ficus Linnaeus*

**Specific epithet:** *religiosa L.*

#### Morphology

*F. religiosa* is a large deciduous tree with few or no aerial roots. It is often epiphytic with the drooping branches bearing long petioled, ovate, cordate shiny leaves. Leaves are bright green, the apex produced into a linear-lanceolate tail about half as long as the main portion of the blade. The receptacles occurring in pairs and are axillary, depressed globose, smooth and purplish when ripe. The bark is flat or slightly curved, varying from 5 to 8 mm in thickness, outer surface is grey or ash with thin or membranous flakes and is often

covered with crustose lichen brown or ash coloured, surface has shallow irregular vertical fissures and uneven due to exfoliation of cork, inner surface smooth, yellowish to orange brown and fibrous (10-11).

#### Microscopy

An external features of bark of *F. religiosa* showed that bark differentiated into outer thick periderm and inner secondary phloem. Periderm is differentiated into phellem and phelloderm. Phellem zone is 360 mm thick and it is wavy and uneven in transection. Phellem cells are organized into thin tangential membranous layers and the older layers exfoliate in the form of thin membranes. The phelloderm zone is broad and distinct. Phelloderm cells are turned into lignified sclereids. Secondary phloem differentiated into inner narrow non-collapsed zone and outer broad collapsed zone. Non-collapsed zone consists of radial files of sieve tube members, axial parenchyma, and gelatinous fibres. Outer collapsed phloem has dilated rays, crushed obliterated sieve tube members, thick walled and lignified fibres, and abundant tannin filled parenchyma cells. Laticifers are fairly abundant in the outer secondary phloem zone. Phloem rays are both uniseriate and multiseriate. Multiseriate rays are homocellular and uniseriate rays are either homocellular or heterocellular (10).



**Figure 1 and 2:** *Ficus religiosa* (General Morphology of Plant and Fruits)

### Physical Constants

Total ash 7.86 % w/w, acid insoluble ash 0.41 % w/w, alcohol soluble extract 7.21 % w/w and water soluble extractive 15.76 % w/w (10).

### Fruits

The tree fruits in May/June and bears a small flat-topped figs (12-13 mm or ½ inch in diameter), which appears in pairs in the angles of the leaves on the twigs (or above the scars in the bark left by fallen leaves). They have 3 basal bracts, are green at first and ripen to a blackish purple (may have reddish dots). The fruiting tree becomes a treat for many different birds and animals (10).

### Phytochemistry of *F. religiosa*

Preliminary phytochemical screening of *F. religiosa* barks, showed the presence tannins, saponins, flavonoids, steroids, terpenoids and cardiac glycosides (10, 12).

The barks of *F. religiosa* showed the presence of bergapten, bergaptol, lanosterol,  $\beta$ -sitosterol, stigmasterol, lupen-3-one,  $\beta$ -sitosterol-d-glucoside (phytosterolin), vitamin k<sub>1</sub>(13-16). The bark also contains tannin, wax, saponin,  $\beta$ -sitosterol, leucocyanidin-3-O- $\beta$ -D-glucopyranoside, leucopelargonidin-3-O- $\beta$ -D-glucopyranoside, leucopelargonidin-3-O- $\alpha$ -L-rhamnopyranoside, lupeol, ceryl behenate, lupeol acetate,  $\alpha$ -amyrin acetate, leucoanthocyanidin and leucoanthocyanin (17).

Leaves yield campesterol, stigmasterol, isofucosterol,  $\alpha$ -amyrin, lupeol, tannic acid, arginine, serine, aspartic acid, glycine, threonine, alanine, proline, tryptophan, tyrosine, methionine, valine, isoleucine, leucine, n-nonacosane, n-hentricontanen, hexa-cosanol and n-octacosan (18-20).

The fruit of *F. religiosa* contains asgaragine, tyrosine, undecane, tridecane, tetradecane, (e)- $\beta$ -ocimene,  $\alpha$ -thujene,  $\alpha$ -pinene,  $\beta$ -pinene,  $\alpha$ -

terpinene, limonene, dendrolasine, dendrolasine  $\alpha$ -ylangene,  $\alpha$ -copaene,  $\beta$ -bourbonene,  $\beta$ -caryophyllene,  $\alpha$ -trans bergamotene, aromadendrene,  $\alpha$ -humulene, alloaromadendrene, germacrene, bicyclogermacrene,  $\gamma$ -cadinene and  $\delta$ -cadinene (21). Alanine, threonine, tyrosine have been reported in seeds of *F. religiosa* (22).

It roots contains tannin, wax, saponin,  $\beta$ -sitosterol, leucocyanidin-3-O- $\beta$ -D-glucopyranoside, leucopelargonidin-3-O- $\beta$ -D-glucopyranoside, leucopelargonidin-3-O- $\alpha$ -L-rhamnopyranoside, lupeol, ceryl behenate, lupeol acetate,  $\alpha$ -amyrin acetate, leucoanthocyanidin and leucoanthocyanin(23).

### Potential Use of *F. religiosa*

#### Analgesic

Methanolic extract of stem bark of *F. religiosa* were administrated orally to rats at a dose level of 250mg/kg body weight was shown strong analgesic activity against acetic acid induced analgesic response (24).

#### Neuroprotective

Methanolic extract of stem bark of *F. religiosa* inhibited the acetylcholinesterase activity and results in increase generation of acetylcholine (Ach), as decreased level of Ach are marker of various neurodegenerative disorders (ND) thus provides the evidence of Neuroprotective drug (25).

#### Antiulcer

Ethanollic extract of stem bark of *F. religiosa* was given to rats at dose level 100, 200 and 400 mg/kg, significantly increased the pH of gastric acid while at the same time reduced the volume of gastric juice. It also works against gastric ulcer and pylorus ligation (26).

**Hepatoprotective**

*F. religiosa* stem bark powder was given to rats at a dose level of 200 mg/kg orally induce significant hepatoprotective activity against Paracetamol (2g/kg) induced hepatotoxicity (27). Paracetamol intoxication in normal rats elevated the levels of SGPT, SGOT, ALP, total bilirubin significantly and histologically showed the disarrangement and degeneration of normal hepatic cells indicating acute centrilobular necrosis. The rat treated with alcoholic extract and aqueous extract showed a significant reduction in all the biochemical parameter elevated by paracetamol. Ethyl acetate and pet ether extract showed moderate reduction in biochemical parameters (27).

**Nephroprotective**

Alcohol extract of *F. religiosa* stem bark reduced the blood urea nitrogen level close to normal value against the toxic effects induced by anti-Tuberculosis drugs rifampicin and isoniazid in rabbits (28). The histopathological studies of kidney of normal rabbit indicated that in the proximal convoluted tubules nuclei were normal in appearance, renal parenchyma was also normal in structure. Kidney of treated rabbits showed severe degree of infiltration in the glomerulus without renal tubular space between the glomerulus, congestion in the renal parenchyma, necrosis and condensed nucleus. The rabbits treated with extract show normal appearance of the nuclei with no condensed nucleus without any necrosis but at some places there was mild congestion. Kidney tubular cells structure was normal in appearance (28).

**Anti-Diarrhoeal**

Acetone extract of stem bark of *F. religiosa* was administered to castor-oil-induced rats at a dose level of 200 mg/kg showed marked reduction in the number of diarrhea stools and total weight of

diarrhoeal faeces and, frequency and consistency of diarrhoea (29).

**Antimicrobial**

Aqueous extract of the *F. religiosa* stem bark show antimicrobial activity at 100 mg/ml concentration against the *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Aspergillus niger* and *Candida albicans* (30). Comparatively acetone extract inhibit growth of *Bacillus subtilis* at lower concentration whereas *E. coli* show sensitivity at higher concentrations of the same extract (31). Methanol extract was very active against *Escherichia coli*, *Proteus vulgaris*, *Bacillus subtilis* and *Staphylococcus aureus* but showed no activity against *P. aeruginosa* (31). The antibacterial activity of *F. religiosa* leaves had tested against various bacteria like *P. vulgaris*, *E. coli*, *B. Subtilis*, *S.aureus*, *Pseudomonas aeruginosa* and *K. Pneumonia*. Chloroform extract inhibited the growth of various Salmonella species, *P. vulgaris*, *E. coli*, *B. Subtilis* and *K. Pneumonia* (32, 33). Diethyl ether and methanol extracts were showed maximum inhibition on *S.aureus* followed by *E.coli* and *Pseudomonas aeruginosa* (34).

**Anti-diabetic**

Methanolic extract of *F. religiosa* bark administered orally at a dose of 100 mg/kg to streptozotocin induced diabetic rats showed significant anti-hyperglycemic activities. The extract showed asignificant reduction in blood glucose level and showed improved in body weight (35). Ethanol extracts of *F. religiosa* leaves were administered orally to alloxan induced diabetic albino rats at a dose level of 250 mg/kg and 100mg/kg body weight. The extract was found effective to lower the blood glucose level significantly in diabetic rats (36). *F. religiosa* root

bark when given at a dose of 25 mg/kg orally to fasting rabbits produced a maximum fall of the blood sugar level, equivalent to 81% of the tolbutamide standard, after 4 hrs, while with *i.v.* injections of 5-7.5 mg/kg a maximum effect was achieved after 2 h (37).

#### **Anti-inflammatory**

Aqueous extract of *Ficus religiosa* bark administered to Carrageenan induced albino rats at a dose level 25, 50 and 100 mg/kg; *s.c.*, significantly reduced the volume of paw edema in a dose dependent manner (38). The leaves extract of *F. religiosa* was showed the anti-inflammatory activity in albino rats against carrageenan induced pedal oedema (39). Methanolic extract inhibited the production of nitric oxide (NO), pro-inflammatory cytokines (tumor necrosis factor-alpha (TNF-alpha), interleukin beta (IL-1beta) and IL-6) and inflammatory mediators through the down regulation of extracellular signal-regulated kinase (ERK), c- Jun N-terminal kinase (JNK) & p38 mitogen-activated protein kinase (MAPK) signaling pathway (40).

#### **Antioxidant**

Ethanollic extract of leaves of *F. religiosa* was tested at different dilutions from 200µg/ml to 1000µg/ml shows antioxidant activity (41).

#### **Wound-Healing**

Hydroalcoholic extract of *F. religiosa* leaves applied on wounded Wistar albino rats result in high rate of wound contraction, decrease in the period for epithelialisation, high skin breaking strength leading to wound healing (42).

#### **Anti-ulcer**

Ethanollic extract of leaves of *F. religiosa* given to rats at a dose level 250 mg/kg and 500 mg/kg significantly decreased the volume of gastric acid secretions, free acidity and total acidity and ulcer

index in aspirin induced ulcer and pylorus ligation in dose dependent manner (43).

#### **Memory Enhancing**

Ethanollic extract of *Ficus religiosa* leaves was studied for memory enhancing activities in Wistar albino rats and Swiss albino mice by five models: Elevated-Plus Maze, Step through passive avoidance test, Sodium nitrite intoxication, Hebb-Williams Maze and Radial Arm Maze (44). Scopolamine (1mg/kg, *i.p.*) and sodium nitrite (95mg/kg, *s.c.*) was used as inducing agent and Piracetam (200mg/kg, *i.p.*) was used as standard nootropic agent. The extract significantly improved memory and reversed the amnesia induced by scopolamine and hypoxia induced by sodium nitrite (44).

#### **Laxative**

Aqueous extract of *F. religiosa* leaves extract was administered to albino wistar rats at different doses (100, 200, 400 mg/kg, *p.o.*) showed significant laxative activity and reduced loperamide induced constipation in dose dependent manner. The extract induced a significant enter pooling and excretion of Cl<sup>-</sup>, Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup> in the intestinal fluid (45).

#### **Anticancer**

Pet ether extract of *F. religiosa* leaves was tested for *in vitro* anticancer activity using human MCF 7 cell line by trypan blue exclusion method. The viable tumor cell count decreased in dose dependent manner and more predominantly beyond concentration of 200 µg/mL where the extract inhibits the entire viable tumor MCF-7 cell (46).

#### **Antiamnesic**

Methanol extract of fruits of *F. religiosa* was tested on scopolamine-induced anterograde and retrograde amnesia in mice at a dose level 10, 50 and 100 mg/kg, *i.p.* showed a significant

improvement of memory in dose dependent manner (47).

### **Anticonvulsant**

Aqueous aerial root extract of *F. religiosa* at dose level 25, 50 and 100 mg/kg was investigated in strychnine, pentylenetrazole, picrotoxin and isoniazid induced seizures in mice. The extract showed no toxicity and protected the animals in the strychnine, pentylenetrazole tests in a dose dependent manner (48). The extract possesses anticonvulsant activity through the glyceric pathway as it increased the inhibitory effect of glycine at all glycine receptors. Anticonvulsant effect of *F. religiosa* also involve additional Gama amino butyric acid (GABA) ergic pathway (48).

### **Female Reproductive System of Goat**

The female reproductive tract of goat consists of the vulva labia, vagina (copulatory organ), cervix, body of the uterus, uterine horns, oviduct (also called Fallopian tube) and the ovary.

### **Ovaries**

Ovaries are almond-shaped, paired, and located on each side of the pelvic cavity. The ovaries contain the ova (eggs), and secrete female reproductive hormones (progesterone and estrogens). Estrogens are responsible for the development of the secondary sex characteristics and the physical and behavioral changes that does display during heat. Progesterone is responsible for changes in the uterine environment for embryo implantation, as well as for maintaining pregnancy and promoting mammary gland growth and development during pregnancy (49-55).

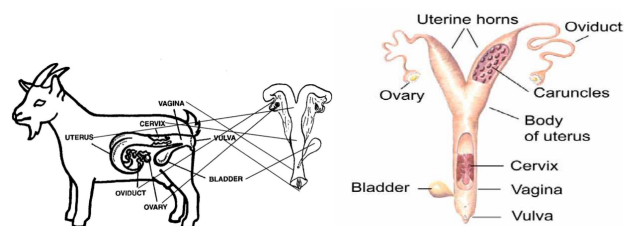
### **Oviduct**

Oviducts are tiny, convoluted tubes located on each side of the uterus that connects the ovary to the uterine horns. The oviducts are divided into three distinct segments that transport the ova and

spermatozoids in opposite directions. Once the ova are released from the ovary during ovulation, they are captured in the oviduct. The oviduct is the site where the ova are fertilized, in a segment known as the ampulla. The oviduct is the site where further capacitation of the spermatozoa occurs. The oviduct opens like a funnel (the infundibulum) near the ovary. The infundibulum receives ova released from the ovary and transports them to the site of fertilization in the oviduct (49-55).

### **Uterus**

Goat uterus is bicornuate, which means that it has two long cornus or horns that connect the uterine body to the oviducts. In animals with multiple births, each horn can contain one or more fetuses. The uterus is a smooth, muscular organ that stretches during the pregnancy along with the growth of a fetus or fetuses. The uterus is the site where the embryo migrates and develops throughout the pregnancy until parturition. The uterus provides a proper environment for embryo development, supports development of the fetus (supplying nutrients, removing waste, and protecting the fetus), and transports the fetus out of the maternal body during birth. The endometrium, or internal layer of the uterus, is formed by glands that secrete endometrial milk that nourishes the embryo (49-55).



**Figure 3 and 4:** Parts of Female Reproductive System of Goat

The endometrial glands also secrete prostaglandin F2 $\alpha$  or PGF2 $\alpha$ , a hormone responsible for the

luteolysis or degradation of the corpus luteum (CL) at the end of the estrous cycle or days before parturition. The uterus separates itself from the vagina through a cartilaginous structure named the cervix (49-55).

### **Cervix**

The cervix is the gateway to the uterus and is a muscular canal consisting of several folds of tissue referred to as "rings." The cervix has relatively little smooth musculature. It participates in sperm transport, and during pregnancy, blocks bacterial invasion. The mucus produced during pregnancy (also during the luteal phase) forms a plug that makes the opening through the cervix impermeable for micro-organisms and spermatozoa (49-55).

### **Vagina**

The vagina is a large and tubular elastic structure. It is located between the cervix and vulva. The vagina is the copulation organ of the female, receiving the penis during mating, and it expands during birth (49-55).

### **Vulva**

The vulva is the external genitalia consisting of the vestibule and the labia. The vestibule is common to the urinary and genital tract. The vestibule joins the vagina with the urethral orifice. The labia consist of the labia majora and minora, the outer and inner folds of skin outside the vagina. The labia majora is homologous to the scrotum in males, and it is the visible external portion of the female tract (49-55).

### **Clitoris**

The clitoris is located in the lower portion of the vulva. It is the excitatory organ of a goat reproductive tract (49-55).

### **Reproductive Physiology of Goat**

Goats are seasonally polyestrous. The season usually comes from about September through

January with the strongest heat occurring in October and November. Sexual maturity or puberty occurs at 5–8 months of age. Estrus heat signs last from few hours to few days including flagging tail; red, swollen vulva; excessive urination; mucus discharge from vulva; excessive vulcanization; a drop in milk production and most importantly standing to be mounted. Reproduction depends on successful deposition of the buck's semen in the vagina of the goat at the correct time in her oestrus cycle (49-55).

Every 18–25 days a fluid-filled follicle containing oestrogen develops in the ovary. The oestrogen is carried throughout the body by the bloodstream. It brings the goat on heat by affecting certain tissues that play a part in fertilization: for example, the glands lining the cervix, which produces clear stringy mucus and an attractive odour. Heat usually lasts for 24–36 hours (49-55).

Ovulation usually occurs towards the end of heat. The egg is released from the follicle to enter the fallopian tube, with the assistance of the funnel-shaped infundibulum. For successful fertilization, the sperm must travel from the cervix through the uterus and along the uterine horn to the fallopian tube into which the egg is extruded (49-55).

If the heat periods are not regular, ovulation is unlikely to coincide with them and conception becomes difficult. The function of the ovary is to produce an egg every 20 days (normal range 19–21 days), and the regular onset of heat to prepare the doe at this interval is essential. One part of this preparation is the action of oestrogen on the muscle fibres in the uterus, making them sensitive to the hormone oxytocin, which in turn makes the muscles contract in a wave-like action. This helps the sperm to reach the egg (49-55).

This assistance for the sperm is essential, since its average life span in the female genital tract is

about 24 hours. The sperm travels against the flow of the uterine mucus, and would certainly perish without reaching the fallopian tubes if its only method of propulsion was the wriggle of its tail. So the wavelike action moves the sperm up the genital tract against the flow of mucus and towards the ovary. Once released, the egg perishes within 12 hours if it is not fertilized (49-55).

After ovulation the cells in the ovary produce a different hormone, progesterone. This takes the goat out of heat, stops the wave-like muscle contractions and helps to prepare the uterine wall for implantation of the fertilized zygote. Progesterone is produced in the cavity left by the ruptured follicle, which fills with cells to form the yellow body (corpus luteum). This continues to secrete progesterone for 13-14 days. If the egg is fertilized, the corpus luteum goes on to play a part in early gestation (49-55).

However, if fertilization does not take place the uterine wall secretes prostaglandin, a substance which causes the corpus luteum to regress. This occurs between day 16 and day 18, thus completing a full cycle. A new follicle begins to develop and produce oestrogen over these days to bring the goat into heat again at about day twenty (49-55).

### General Histology of Goat Uterus

The goat uterus was histologically comprised of endometrium (endometrial surface epithelium, endometrial glands, and epithelial cells), myometrium and perimetrium. These three structural entities showed variations in their dimensions at different phases of estrous cycle. The uterus was internally comprised of endometrium with characteristic endometrium surface epithelial cells; epithelial cells and the uterine glands were laid on the basal layer. The surface epithelium (functional layer) extended

upto lumen of uterus and showed cyclic changes during different phases of the estrous cycle (49-55).

Histologically, the endometrium comprised of ciliated and secretory cells. The luminal epithelium formed a densely organized stromal zone (stratum compactum) while the glandular epithelium formed loosely organized stromal zone (stratum spongiosum). These layers were richly vascularized. The stroma resembled mesenchymal tissue and consisted of loosely arranged stellate cells with large, round or ovoid nuclei supported by a network of fine connective tissue in which lymphocytes, granular leukocytes, and macrophages were scattered (49-55).

The shape of uterine glands varied from tubular to spherical. Endometrium was differentiated into stratum basale (basal layer) and a stratum functionale (functional layer), which differ in their structure, function, and blood supply. The stratum basale was laid directly on the myometrium and was more narrower, cellular, and fibrous layer than components of stratum functionale (49-55).

The myometrium was largely comprised of the smooth muscle component of the uterine wall. Myometrium was comprised of bundles of smooth muscle cells separated by thin strands of connective tissue containing fibroblasts, collagenous and reticular fibers, mast cells, and macrophages. The layers of the muscle were not sharply defined because of the intermingling of smooth muscle cells. Generally, inner and outer layers of smooth muscle were distinguished according to direction and deposition of bundles. The inner layer was thin and consisted of longitudinal and circularly arranged smooth muscle cells. The outer layer was the thickest and showed irregularity in the arrangement of the smooth muscle cells, which ran longitudinally,



obliquely, circularly, and transversely. The uterus was surrounded by perimetrium (49-55).

#### **Antifertility Botanical Agents Targeting Uterus**

##### **Hibiscus rosa-sinensis (China Rose/Guhmohr) (Malvaceae)**

Ethanol extract of *Hibiscus rosa-sinensis* given orally to the rats at a dose of 400mg/kg exhibited a very potent anti-implantation (56). Phytochemical analysis indicated the presence of Steroid, tannins, saponins and flavonoids (57). Administration of extract caused a significant increase in uterine weight, diameter of the uterus and thickness of the endometrium. It appears that the extract has estrogenic activity, but no antiestrogenic activity (56).

##### **Ocimum sanctum (Basil/Tulsi) (Lamiaceae)**

*Ocimum sanctum* leaves have been shown to possess anti-implantation activity in experimental albino rats (58). Phytochemical analysis revealed the presence of alkaloids, saponins, tannins, steroids, terpenoids, flavonoids, glycosides, carbohydrates, proteins and coumarins (59). *Ocimum sanctum* leaves disrupt the estrus cycle and estrus stage is prolonged. It also causes decrease in number of endometrium glands (58).

##### **Striga orobanchioides (Missi) (Scrophulariaceae)**

Ethanol extract of *Striga orobanchioides* given to albino rats induced significant anti-implantation activity (60). Antioxidant activity of the ethanol extract suggests the presence of flavonoids in *Striga orobanchioides* (61).

##### **Calotropis procera (Sodom apple/Aak) (Asclepiadaceae)**

Ethanol extract of *Calotropis procera* roots administered to female albino rats at the dose level of 250 mg/kg showed strong anti-implantation activity (62). The phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, and cardiac

glycosides with a very high content in water extracts (63). However no antiestrogenic activity could be detected from extract treatment (62).

##### **Lawsonia inermis (Hina/Mehndi) (Lythraceae)**

*Lawsonia inermis* root extract was given to female Wistar rats caused loss of implantation sites (64). Phytochemical screening of the extracts showed the presence of glycosides, phytosterol, steroids, saponins, tannins and flavonoids (65).

##### **Ricinus communis (Castor Bean/Arand) (Euphorbiaceae)**

Methanol extract of *Ricinus communis* seeds was given to albino mice at a dose level of 200 mg/kg body weight induced anti-implantation activities (55). Phytochemical screening indicated the presence of alkaloids, saponins, phenols, flavonoids and tannins (67).

##### **Terminalia belerica (Baheda) (Combretaceae)**

Ethanol extract of *Terminalia belerica* bark was given to female albino rats at the dose level of 25mg/100g body weight cause loss of implantation (68). The phytochemical showed the presence of phytosterols, carbohydrates, flavonoids, phenolic compounds and tannins (69). The loss of implantation caused by the extract may be due to antizygotic, blastocytotoxic or anti-implantation activity (68).

##### **Physalis alkekengi (Bladder cherry/Kakraj) (Solanaceae)**

*Physalis alkekengi* plant extract was given to female albino rats at the dose level of 150 mg/kg induced significantly decreased the number of implantation sites (70). Phytochemical screening revealed the presence of tannins, saponins, alkaloids, flavonoids and glycosides (71). According to the importance of progesterone and estrogen hormones in the maintenance of implanted embryo, the anti-fertility activity of this plant seems to be due to this fact that P.

*alkekengi* is an antagonist for this hormones and can interfere with fertility (70).

#### **Allium cepa (wild onion/Piyaz) (Liliaceae)**

Ethanol extract of *Allium cepa* given to female Wistar rats at a dose level of 300 mg/kg showed significant inhibition of number of implant sites (72). Phytochemical screening revealed the presence of alkaloids, flavonoides, cardiac glycosides, terpenes and resins (73).

#### **Asparagus africanus (Climbing asparagus) (Asparagaceae)**

Ethanol extract of *Asparagus africanus* leaves and roots given to rats at a dose level of 300 mg/kg of body weight resulted in significant reduction in the number of implants (74). Phytochemical screening showed the presence of saponins, carbohydrates, glycosides and mucilages (75).

#### **Caesalpinia pulcherrima (Peacock Flower/Guletura) (Caesalpinaceae)**

Ethanol extract of *Caesalpinia pulcherrima* leaves given to albino mice at a dose level of 400 mg/kg body weight caused inhibition of implantation (76). Phytochemical screening showed the presence of alkaloids, steroids, flavonoids, saponins, gums and tannins (76-77).

#### **Curcuma aromatica (Wild Turmeric/Jangli Haldi) (Zingiberaceae)**

Ethanol extract of *Curcuma aromatica* rhizomes given to female rats show strong anti-implantation activity (78). Phytochemical screening showed the presence of alkaloids, carbohydrates, phytosterols, fixed oils, fats, proteins, amino acids, glycosides, flavonoids, saponins and tannins (78-79).

#### **Leonotis ocymifolia (Sun-Bird Flower) (Lamiaceae)**

Ethanol extract of *Leonotis ocymifolia* leaves given to female rats reduced the number of implants significantly (80). Phytochemical screening showed the presence of phenols,

flavonoids, alkaloids, saponin, glycoside and tannins (81).

#### **Gloriosa superba (Glory Lily) (Liliaceae)**

Hydroalcoholic extract of *Gloriosa superba* tuber given to female albino rats showed significant anti-implantation activity (82). Phytochemical screening revealed the presence of alkaloids, glycosides, steriods, terpenoids and tannins (83).

#### **Citrus limonum (Lemon) (Rutaceae)**

Alcoholic extract of *Citrus limonum* seeds given to female albino mice showed significant anti-implantation activity (84). Phytochemical screening revealed the presence of steriods, glycosides, flavonoids, fats and oils (85).

#### **Achyranthes aspera (Devil's Horsewhip/Phutkanda) (Amaranthaceae)**

Methanol extract of *Achyranthes aspera* leaves given to female Wistar rats reduced significantly the number implantation sites (86). Phytochemical screening revealed the presence of alkaloids, carbohydrates, saponins, tannins, phenolic compounds, proteins, amino acids, flavanoids and volatile oil (87).

### **Material and Methods**

#### **Collection of Plant Material**

The Fruits of *Ficus religiosa* were collected from the Kurukshetra University campus, Kurukshetra (29°06'N, 76°05'E) in the month of August. The plant and sample specimen are identified by a taxonomist from department of Botany.

#### **Preparation of Plant Extract**

In order to avoid any alternation/degradation of biologically active ingredient in fresh extract, the ethanol extract of the dried fruit was used. The 1% fraction of the methanol extract was tested for activity.

The collected fruits were dried in the oven at 40°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 filtered through Whatman filter paper No 1. After filtration, the methanol was evaporated from the extract by heating at 55°C in water bath for 12 hrs. The resulting partially solid extracts were stored at -20°C for future experimentation.

### **GC-MS Analysis**

GC-MS analysis of these extracts was carried out by following the method of Hema *et al.* (88) GC-MS analysis were performed using a Perkin-Elmer GC clauses 500 system and Gas Chromatograph interfaced to a mass spectrometer (GC-MS) equipped with an Elite-1, fused silica capillary column (30 m X 0.25 mm ID X 1  $\mu$  df, composed of 100% Dimethyl poly siloxane).

For GC/MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml/min and an injection volume of 2 ml was employed (Split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min) with an increase of 10°C/min to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da.

### **Identification of Components**

The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The detection employed the NIST (National Institute of Standards and Technology) Ver.2.0-Year 2008 library. The spectrum of the unknown component was compared with the spectrum of the known

components stored in the NIST library. The name of the components of the test materials was ascertained.

### **Experiment Design for in vitro Treatment**

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 1 $\mu$ g/ml). 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 24 hours. 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<sup>o</sup>-60<sup>o</sup>C.

The uterus was sectioned serially at 5  $\mu$ m thickness and the sections were stained with the haematoxyline 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

### GC-MS Analysis of Fruits of *F. religiosa*

The results pertaining to GC-MS analysis leads to the identification of number of compounds from the GC fractions of the methanolic extract of *F. religiosa*. These compounds were identified through mass spectrometry attached with GC. These observations may be due to the nature of biological active components and the stronger extraction capacity of methanol could have been produced number of active constituents. The GC-MS analysis of the extracts showed the presence of phytochemicals enlisted in Table.

**Table:** Phytochemicals identified in methanolic fruit extract of *F. religiosa* using GC-MS

Peak	RT	Area %	Name of the compound	Molecular Formula	MW
120	16.806	11.23	n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256
122	16.954	4.35	Hexadecanoic acid, ethyl ester	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284
133	18.562	36.45	9, 12-Octadecadienoic acid	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280
134	18.660	8.87	9,12,15-Octadecatrienoic acid	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	278
135	18.785	7.05	Octadecanoic acid	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284
160	25.698	2.29	Butyl 9,12,15-octadecatrienoate	C <sub>22</sub> H <sub>38</sub> O <sub>2</sub>	334

RT=Retention Time, MW=Molecular Weight.

### Effect of Plant Extract on Uterine Morphology of Goat

Histologically the goat uterus was comprised of endometrium (endometrial surface epithelium, endometrial glands, and epithelial cells), myometrium and perimetrium. The endometrium was composed of endometrial glands that

secreted hormones 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).

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 (Figure 6(a) and Figure 6(b)).

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 (Figure 7(a) and Figure 7(b) respectively). The one-tailed P value is 0.0027, considered very significant.

The alcoholic extract of *Ficus religiosa* induced hypertrophy 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 (Figure 8(a) and Figure 8(b) respectively). The one-tailed P value is < 0.0001, considered extremely significant.

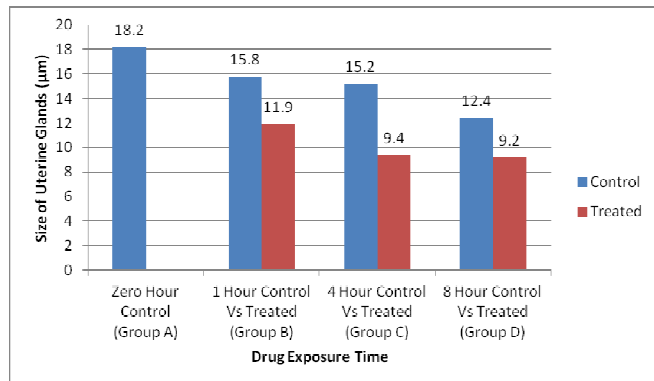
As the exposure duration increased from 4 to 8 hours the structure of uterine glands was distorted remarkably. The 8 hour control group had the mean uterine gland diameter 12.4 µm whereas the treated group had the mean diameter about 9.2 µm (Figure 9(a) and Figure 9(b))

respectively).The one-tailed P value is < 0.0001, considered extremely significant.

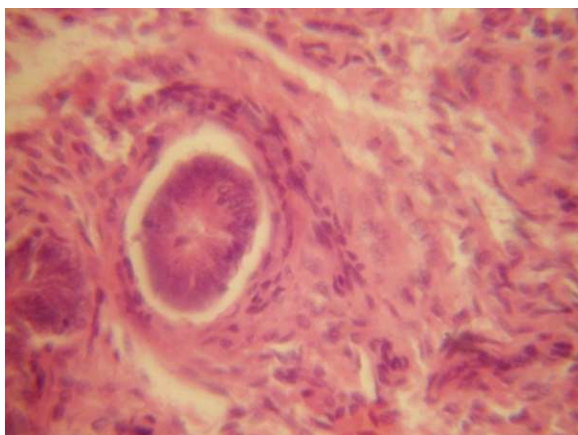
Small variations were observed in the thickness of layers of myometrium due to effect of plant extract. There were small changes in the size of the longitudinal and circular muscle fibres of inner and outer layer of myometrium.

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)

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



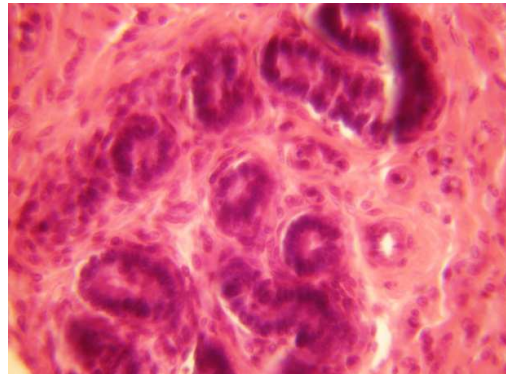
**Figure 5:** Showing the change in uterine gland diameter in control and treated group at different time exposures



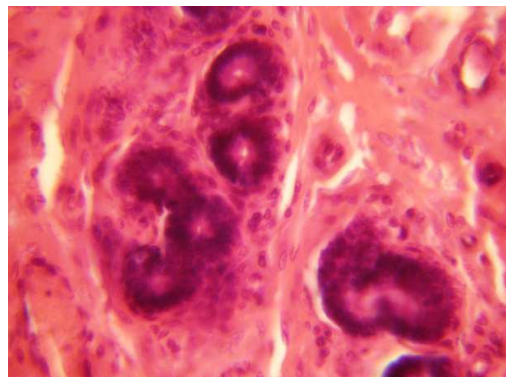
**Figure 6(a):** Uterine gland: zero hour control showing the normal uterine gland (400X)



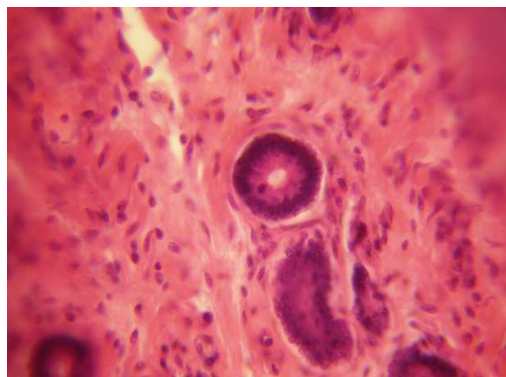
**Figure 6 (b):** Uterine gland: zero hour control showing the normal uterine gland (1000X)



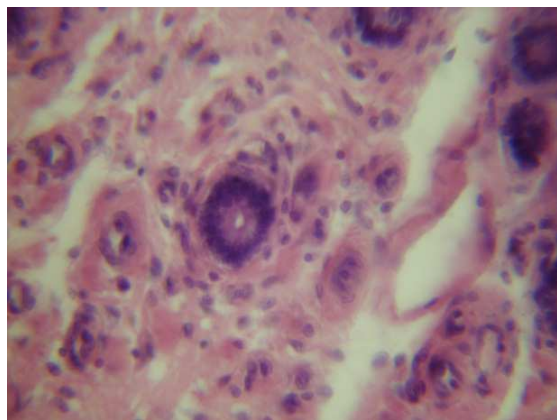
**Figure 7 (a):** Uterine gland: 1 hour control showing small variation in diameter (400X)



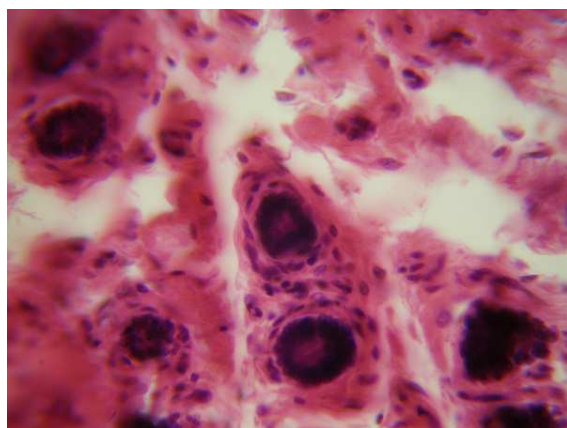
**Figure 7 (b):** Uterine gland: 1 hour treated showing significant decrease in diameter (400X)



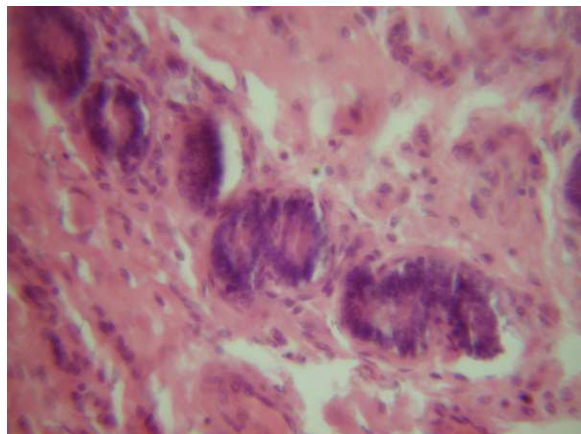
**Figure 8 (a):** Uterine gland: 4 hour control showing small variation in diameter (400X)



**Figure 8 (b):** Uterine gland: 4 hour treated Showing significant decrease in diameter (400X)



**Figure 9 (a):** Uterine gland: 8 hour control Showing intact glands(400X)



**Figure 9 (b):** Uterine gland: 8 hour treated Showing significantly distorted glands (400X)

### Discussion

The medical properties of many compounds found in GC-MS of methanolic extract of *F. religiosa* fruits can be related to the earlier studies

on the *Vitex altissima* L. The n-Hexadecanoic acid is found possess the Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor, Antioxidant, Hypocholesterolemic activities. The Hexadecanoic acid, ethyl ester is known to possess the Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor activities. The 9,12-Octadecadienoic acid is found to be have Hypocholesterolemic, 5-Alpha reductase inhibitor, Antihistaminic, Insectifuge, Antieczemic, Antiacne activities. (89)

The medical properties of other compounds can be related to earlier studies on the *cassia italic*. The 9,12,15-Octadecatrienoic acid possess the Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge Antihistaminic, Antiarthritic, Anticoronary, Antieczemic Antiacne, 5-Alpha reductase inhibitor Antiandrogenic properties. (89) No medical activity of the Octadecanoic acid is known (89). Also medical activity of Butyl 9,12,15-octadecatrienoate is unknown.

The decrease in uterine glands diameter and myometrium thickness observed after the exposure of *Ficus religiosa* fruits extract supports the earlier findings revealed decreased myometrial volume in proportion to uterine weight and marked regression of uterine gland in female gerbils treated intraperitoneally with *Cannabis* extract (90).

Our studies also supports the earlier findings showed decreased thickness of myometrium and height of luminal epithelium in uterus of rat administered nicotine at 2 and 4 mg/kg body weight for 20 days, respectively (91).

Our study support the earlier work on the *Melia azedarach*, seed extract of which cause

decrease in uterine glands diameter in albino rats (92). However our studies contradict the earlier work on petroleum ether extract of *Cassia fistula* seeds which cause increase in epithelial cell height albino rats (93).

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 (94). Decrease in endometrium thickness and uterine gland diameter also contradict the earlier work on *Trianthema portulacastrum* stem leaves and roots, alcoholic extract of which cause increase in endometrium thickness and uterine gland diameter in albino rats (95).

## Conclusion

Uterine glands are the important unit of implantation process and involve in hormonal regulation. The variations in uterine glands diameter cause hormone-related changes in the uterine milieu that created environment unsuitable for embryonic implantation/growth. The *Ficus religiosa* has anti-androgenic properties and so exhibit anti-implantation activity. The GC-MS method is a direct and fast analytical approach for identification of phytochemicals. 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. *F. religiosa* is recommended for further working out to find out the potential phytochemical causing anti-implantation activity and should be experimented for underlying mechanism of action.

## Acknowledgements

I wish to avail the opportunity to express my heartfelt gratitude to my esteemed teachers, Professor Rajneesh K. Sharma and Dr. Satendra K. Yadav, for their perpetual energy and enthusiasm in research has motivated me, they were always accessible and willing to help in research.

## References

- 1) Sharma RK, Chopra G, Bhushan G, Munjal K: Use of Indian plants in fertility regulation in mammals. *J. Exp. Zool. India* 2003; 6: 57-74.
- 2) Maurya R, Srivastava S, Kulshreshta DK, Gupta CM: Traditional Remedies for Fertility Regulation. *Current Medicinal Chemistry*. 2004; 11:1431-1450.
- 3) Ghani A: Medicinal plants of Bangladesh with chemical constituents and uses. Asiatic Society of Bangladesh. Dhaka 1998; 236.
- 4) Singh D, Goel RK: Anticonvulsant effect of *Ficus religiosa*: role of serotonergic pathways. *J. Ethnopharmacol.* 2009; 123: 330-334.
- 5) Prasad PV, Subhaktha PK, Narayana A, Rao MM: *Bull. Indian Inst. Hist. Med Hyderabad* 2006; 36:1-20.
- 6) Govindarajan R, Pushpangadan P, Vijayakumar M: Antioxidant approach to disease management and the role of 'Rasayana' herbs of Ayurveda. *J. Ethnopharmacol* 2005; 99: 165.
- 7) Arora D, Dubey SD, Ojha JK: Role of rasayana in preventing the complications of diabetes mellitus. *J. Diab. Assoc* 1999; 39:47.
- 8) Warriar PK: Indian medicinal plants-A compendium of 500 species. Orient Longman Ltd., Chennai 1996; 3:38-39.
- 9) Shah GM, Khan MA, Ahmad M, Zafar M, Khan AA: Observations on antifertility and abortifacient herbal drugs, *African Journal of Biotechnology*. 2009; 8(9):19
- 10) Babu K, Shankar SG, Rai S: *Turk. J. Bot.* 2010; 34:215-224.
- 11) Warriar PK: Indian medicinal plants-A compendium of 500 species, Orient Longman Ltd., Chennai, Vol. III, 1996, 38-39.
- 12) Jiwala SA, Bagul MS, Parabia M, Rajani M: *Indian J. Pharm. Sci.* 2008; 70:31-35.
- 13) Swami KD, Bisht NPS: *J. Indian Chem. Soc.* 1996; 73: 631.
- 14) Swami KD, Malik GS, Bisht NPS: *J. Indian Chem. Soc.* 1989; 66: 288-289.
- 15) Varshney IP, Bhatnagar SP: *Indian J. Pharmacol.* 1965; 27: 235.
- 16) Ambike S, Rao MR. Studies on a phytosterol from the bark of *Ficus religiosa*. *Indian J. Pharmacol.* 1967; 29:91-92

- 17) Husain A, Virmani OP, Popli SP, Misra LN, Gupta MM, Srivastava GN, Abraham Z, Singh AK: Dictionary of Indian Medicinal Plants, CIMAP, Lucknow, India. 1992; 546.
- 18) Panda SK, Panda NC, Sahue BK: Indian Vet. J. 1976; 60: 660-664.
- 19) Verma RS, Bhatia KS: Indian J. Hosp. Pharm. 1986; 23: 231-232.
- 20) Behari M, Rani K, Usha MT, Shimiazu N: Curr. Agric. 1984; 8: 73.
- 21) Grison L, Hossaert M, Greeff JM, Bessiere JM: Phytochemistry. 2002; 61: 61-71.
- 22) Ali M, Qadry JS: J. Indian Chem. Soc. 1987; 64: 230-231.
- 23) Husain A, Virmani OP, Popli SP, Misra LN, Gupta MM, Srivastava GN, Abraham Z, Singh AK: Dictionary of Indian Medicinal Plants, CIMAP, Lucknow, India. 1992; 546.
- 24) Verma N, Chaudhary S, Garg VK, Sachin: Anti-Inflammatory and Analgesic Activity of Methanolic Extract of Stem Bark of *F. religiosa*. International journal of Pharma Professional's Research. 2010; 1(2):145-147
- 25) Vinutha B, Prashanth D, Salma K, Sreeja SL *et al*. Screening of selected Indian medicinal plants for acetylcholinesterase inhibitory activity. J Ethnopharmacol. 2007; 109: 359-363.
- 26) Khan MSA, Hussain SA, Jais AMM, Zakaria ZA, Khan M. Antiulcer activity of *F. religiosa* stem bark ethanolic extract in rats. J Medicinal Plants Research. 2011; 5(3):354-359.
- 27) Suryawanshi K, Khakre S, Chourasia A, Chaurasiya PK, Pawar RS, Jhade D. Hepato-Protective Activity of Stem Bark Extracts of *F. religiosa* Linn in Rats. IJBR. 2011; 2(8):466-475.
- 28) Hashmi N, Muhammad F, Javed I, Khan JA, Khan MZ, Khaliq T, B Aslam. Nephroprotective effects of *F. religiosa* linn (peepal plant) stem bark against isoniazid and rifampicin induced nephrotoxicity in albino rabbits. Pak Vet J. 2013; 33(3): 330-334.
- 29) Panchawat S, Sisodia SS. Evaluation of Anti-Diarrhoeal Activity of Stem Bark Extracts of *F. religiosa* Prepared by Different Methods of Extraction. International Journal of Pharmaceutical & Biological Archives. 2012; 3(1):218-222.
- 30) Rajiv P, Sivaraj R. Screening for Phytochemicals and Antimicrobial Activity of Aqueous Extract of *F. religiosa* Linn. Int J Pharm Pharm Sci. 2010; 4(5): 207-209.
- 31) Manimozhi DM, Sankaranarayanan S, Kumar GS. Effect of different extracts of stem bark of *Ficus* sp. on multidrug resistant pathogenic bacteria. Int J Pharm Sci Res. 2012; 3(7):2122-2129.
- 32) Hemaiswarya S, Poonkothai M, Raja R, Anbazhagan C. Comparative study on the antimicrobial activities of three Indian medicinal plants. Egypt J Biol. 2009; 1: 52-57.
- 33) Rajiv P, Sivaraj R. Screening for Phytochemicals and Antimicrobial Activity of Aqueous Extract of *Ficus religiosa* Linn. Int J Pharm Pharm Sci. 2012; 4(5): 207-209.
- 34) Ramakrishnaiah G, Hariprasad T. *In vitro* antimicrobial activity of leaves and bark extracts of *Ficus religiosa* (Linn.). Indian Journal of Pharmaceutical & Biological Research. 2013; 1(1): 38-43.
- 35) Verma N, Kumar D, Chaudhary AK, Singh R, Tyagi S. Antidiabetic Potential of Methanolic Bark Extract of *Ficus Religiosa* L. In Streptozotocin-Induced Diabetic Rats. International Journal of Biopharmaceutical & Toxicological Research. 2011; 2(1): 261-265.
- 36) Choudhary S, Pathak AK, Khare S, Kushwah S. Evaluation of antidiabetic activity of leaves and fruits of *F. religiosa* Linn. Int. J. of Pharm. & Life Sci. 2011; 2(12): 1325-1327.
- 37) Ambike SH, Rao MR. Indian J. Pharmcol. 1967; 29:91-92
- 38) Viswanathan S, Thirugnanasambantham P, Reddy MK, Narasimhan S, Subramaniam GA. Anti-Inflammatory and Mast Cell Protective Effect of *Ficus Religiosa*. 1990; 10(2): 122-125.
- 39) Gulecha VS, Sivakumar T, Mahajan MS, Khandare RA, Upasani CD. *Ficus Religiosa* and *Ficus Glomerata* Extracts Exhibits Anti-inflammatory Activity in the Rat. Pharmacologyonline. 2010; 1: 324-331.
- 40) Jung HW, Son HY, Minh CV, Kim YH, Park YK. Methanol extract of *Ficus* leaf inhibits the production of nitric oxide and proinflammatory cytokines in LPS-stimulated microglia via the MAPK pathway. Phytother Res. 2008; 22(8): 1064.
- 41) Charde RM, Dhongade HJ, Charde MS, Kasture AV. Int J Pharm Sci Resear. 2010; 1(5): 73- 82.
- 42) Roy K, Shivkumar H, Sarkar S. Wound healing potential of leaf extracts of *F. religiosa* on Wistar albino strain rats. Int J Pharm Tech Resear. 2009; 1(3):506-508.
- 43) Kumar H, Srivastava SK, Rao CV, Yadav S. Anti-Ulcer Activity of *F. religiosa* L. Leaves on Experimental Animals. International Journal of Pharmaceutical Sciences Review and Research. 2011; 11(2): 83-86.
- 44) Devi WB, Sengottuvelu S, HaJaShrief S, Lalitha V, Sivakumar T. Memory Enhancing Activities of *Ficus religiosa* Leaves in Rodents. IJRAP. 2011; 2 (3) 834-838.
- 45) Singh D, Mukhija M, Sundriyal A, Mangla V. Evaluation of Laxative Activity of *Ficus Religiosa* Linn. (Moraceae) Leaves Aqueous Extract In Albino Wistar Rats. World Journal of Pharmacy and Pharmaceutical Sciences. 2013; 2(6): 5384-5395.
- 46) Gulecha V, Sivakuma T. Anticancer activity of *Tephrosiapurpurea* and *Ficus religiosa* using MCF 7 cell lines. Asian Pacific Journal of Tropical Medicine. 2011; 526-529
- 47) Kaur D, Singh B, Goel RK. Antiamnesic effect of *F. religiosa* in scopolamine induced anterograde and retrograde amnesia. Pharmaceutical Biology. 2010; 48(2): 234-240.
- 48) Patil MS, Patil CR, Patil SW, Jadhav RB. Anticonvulsant activity of aqueous root extract of *F. religiosa*. J Ethnopharmacol. 2011; 133: 92-96.



- 49) Chemineau P, Berthelot X, Daveau A, Maurice F, Viguié C, Malpoux B, Canmelatonin be used in out-of-season reproduction in domestic mammals? *Contraception, Fertility, Sexuality*, 1993; 21(10): 733–738.
- 50) Chemineau P, Guillaume D, Migaud M, Thiéry JC, Pellicer-Rubio MT, Malpoux B. Seasonality of reproduction in mammals: Intimate regulatory mechanisms and practical implications. *Reproduction in Domestic Animals*. 2008; 43(2): 40–47.
- 51) Chemineau P, Gauthier D, Thimonier J. *Reproduction des Ruminants en Zone Tropicale France: Institut National de la Recherche Agronomique*. pp: 1–80.
- 52) Hafez ESE, Hafez B: *Reproduction in Farm Animals (7th ed)*. New Jersey: Blackwell. 2000.
- 53) Hawken PA, Beard AP, Esmaili T, Kadokawa H, Evans AC, Blache D, Martin GB. The introduction of rams induces an increase in pulsatile LH secretion in cyclic ewes during the breeding season. *Theriogenology*. 2007; 68(1): 56–66.
- 54) Morrow DA. *Current therapy in theriogenology*. Toronto: W. B. Saunders. 1986.
- 55) Maria L, Leite B. *Biology of reproduction of goats*. Alabama A&M and Auburn Universities. 2009; UNP-1007: 1–7.
- 56) Vasudeva N, Sharma SK: Post-Coital Antifertility Activity of *Hibiscus rosa-sinensis* Linn. roots. *Evid Based Complement Alternat Med*. 2008; 5(1):91–94.
- 57) Soni D, Gupta A, Solanki R, Jana GK: Anti-implantation and pregnancy interruption activity of japakusuma (*Hibiscus rosa-sinensis*) in albino rats. *Int. J. Res. Ayurveda Pharm*. 2013; 4(3).
- 58) Ahmed M, Khan MY, Khan AA: Effects of *Ocimum sanctum* (Tulsi) on the reproductive system: An updated review. *Biomedical Research* 2002; 13(2/3):63–67.
- 59) Kumari SPK, Sri Devi V, Lakshmi MVVC: Studies on Phytochemical screening of aqueous extract collected from fertilizers affected two medicinal plants. *J. Chem. Bio. Phy. Sci. Sec. B*. 2012; 2(3):1326–1332.
- 60) Hiremath SP, Badami S, Swamy HK, Patil SB, Londonkar RL: Antifertility activity of *Striga orobanchioides*. *Biol Pharm Bull* 1994; 17(8):1029–31.
- 61) Badami S, Gupta MK, Suresh B: Antioxidant activity of the ethanolic extract of *Striga orobanchioides*. *J Ethnopharmacol* 2003; 85(2–3):227–30
- 62) Ranab AC, Kamatha, Jagadish V: Preliminary study on antifertility activity of *Calotropis procera* roots in female rats. *Fitoterapia*. 2002; 73(1):111–115.
- 63) Mainasara MM, Aliero BL, Aliero AA, Dahiru SS: Phytochemical and Antibacterial Properties of *Calotropis Procera* (Ait) R. Br. (Sodom Apple) Fruit and Bark Extracts. *International Journal of Modern Botany* 2011; 1(1):8–11.
- 64) Agunu A, Samagoro C, Nuhu H: Evaluation of anti-fertility of *Lawsonia inermis* L. (Lythraceae) roots found in Kaduna State, Nigeria. *Planta Med*. 2011; 77:PF30.
- 65) Raja W, Ovais M, Dubey A: Phytochemical Screening and Antibacterial Activity of *Lawsonia inermis* Leaf Extract. *International Journal of Microbiological Research* 2013; 4(1):33–36.
- 66) Sani UM, Sule MI: Anti-fertility activity of methanol extracts of three different seed varieties of *Ricinus communis* Linn (euphorbiaceae). *Nig. Journ. Pharm. Sci*. 2007; 6(2):78 – 83.
- 67) Obumselu FO, Okerulu IO, Onwukeme VI, Onuegbu TU, Eze RC: Phytochemical and antibacterial analysis of the leaf extracts of *Ricinus communis*. *Journal of Basic Physical Research* 2011; 2(2):68 – 72.
- 68) Vishwanatha T, Satishagouda S, Patil JS, Patil BS: Anti-implantation activity of *Terminalia bellirica* bark extracts in female albino rats. *BTAJ* 2009; 3(4).
- 69) Manohar VR, Chandrashekar R, Rao SN: Phytochemical analysis of *Terminalia bellerica* fruit pulp extracts. *World Journal of Pharmacy and Pharmaceutical Sciences* 2012; 1(4):1376–1383.
- 70) Montaserti A, Pourheydar M, Khazaei M, Ghorbani R: Anti-fertility effects of *Physalis alkekengi* alcoholic extract in female rat. *Iranian Journal of Reproductive Medicine* 2007; 5(1):13–16.
- 71) Estakhr J, Javdan N: Preliminary Study of Phytochemical Screening and Antibacterial Activity of *Physalis alkekengi* against *Staphylococcus aureus*. *Pharmacologyonline*. 2011; 3: 97–103.
- 72) Thakare VN, Kothavade PS, Dhote VV, Deshpande AD: Antifertility Activity of Ethanolic Extract of *Allium cepa* Linn in Rats. *International Journal of PharmTech Research* 2009; 1(1):73–78.
- 73) Gazuva SY, Makanjuola ER, Jaryum KH, Kutshik JR, Mafulul SG: The phytochemical composition of *Allium capa Allium sativum* and effect of their aqueous extract (cooked and raw form) on the lipid profile and other hepatic biochemical parameters in female albino Wistar rats. *Asian J. Exp. Biol. Sci*. 2013; 4(3).
- 74) Tafesse G, Mekonnen Y, Makonnen E: Antifertility effect of aqueous and ethanol extracts of the leaves and roots of *Asparagus africanus* in rats. *Afr Health Sci*. 2006; 6(2):81–85.
- 75) Wani JA, Achur RN, Nema RK: Phytochemical Screening and Aphrodisiac Activity of *Asparagus racemosus*. *International Journal of Pharmaceutical Sciences and Drug Research* 2011; 3(2):112–115.
- 76) Kumar S, Singh J, Baghotia A, Mehta V, Thakur V, Choudhary M, Verma S, Kumar D: Antifertility potential of the ethanolic extract of *Caesalpinia pulcherrima* Linn. leaves. *Asian Pacific Journal of Reproduction* 2013; 2(2): 90–92.
- 77) Bose U, Hossain L, Bala V, Shill AK, Rahman AA: Anti-inflammatory and neuropharmacological activities of *Caesalpinia pulcherrima* bark. *RJPBCS* 2011; 2(1):77–84.

- 78) Trishna D, Bhushan MS, Mrinmoy B, Mohanty JP, Dibyendu S: Evaluation of phytochemical screening and anti-fertility activity of *Curcuma aromatica* casalisb. IJPSR 2010; 1(1).
- 79) Srividya AR, Dhanabal P, Bavadia P, Vishnuvarthan VJ, Kumar MNS: Antioxidant and antidiabetic activity of *Curcuma aromatica*. IJRAP 2012; 3(3).
- 80) Tafesse G, Mekonnen Y, Makonnen E: *in vivo* and *in vitro* Anti-Fertility and Anti-Implantation Properties of *Leonotis ocymifolia* in Rats. Afr. J. Trad. CAM. 2005; 2(2):103 – 112.
- 81) Oyedemi SO, Yakubu MT, Afolayan AJ: Antidiabetic activities of aqueous leaves extract of *Leonotis leonurus* in streptozotocin induced diabetic rats. Journal of Medicinal Plants Research 2011; 5(1):119-125.
- 82) Latha KP, Kirana H, Girish HN: Anti- Implantation Activity of the Hydroalcoholic Tuber Extract of *Gloriosa superba* Linn in Female Albino Rats. IJAPBC 2013; 2(3).
- 83) Senthilkumar M: Phytochemical Screening and Antibacterial Activity of *Gloriosa superba* Linn. International Journal of Pharmacognosy and Phytochemical Research 2013; 5(1):31-36.
- 84) Kulkarni TR, Kothekar MA, Mateenuddin M: Study of Anti-Fertility Effect of Lemon Seeds (*Citrus limonum*) in Female Albino Mice. Indian J Physiol Pharmacol. 2005; 49(3):305–312.
- 85) Kulkarni TR, Mateenuddin M, Sahasrabudhe RA, Pandit VA: Estrogenic Activity of Alcoholic Extract of Lemon Seeds (*Citrus limonum*) on Immature Albino Rats. International Journal of Research in Pharmaceutical and Biomedical Sciences 2012; 3(3):1231-1235.
- 86) Shibeshi W, Makonnen E, Debella A, Zerihun L: Phytochemical, Contraceptive Efficacy and Safety Evaluations of the Methanolic Leaves Extract of *Achyranthes aspera* L. in Rats. Pharmacologyonline. 2006; 3:217-224.
- 87) Kokila AP, Sarju NP, Vaishali VC, Chetan RP: Preliminary Phytochemical, Pharmacognostical and Microbial Screening of *Achyranthes aspera* (Amaranthaceae). J. Nat. Prod. Plant Resour. 2013; 3(1):15-17.
- 88) Hema R, Kumaravel S, Gomathi S. and Sivasubramaniam C. Gas chromatography-Mass spectroscopic analysis of *Lasonia inermis* leaves. New York Sci. J. 2010; 3(11): 141-143.
- 89) Sahaya SS, Janakiraman N. and Johnson M. Phytochemical Analysis of *Vitex altissima* L. using UV VIS, FTIR and GC-MS. International Journal of Pharmaceutical Sciences and Drug Research 2012; 4(1): 56-62.
- 90) Dixit VP, Arya M, Lohiya NK. Mechanism of action of chronically administrated cannabis extract on the female genital tract of gerbils. Indian Physiol. Pharmacol. 1976; 20-38
- 91) Patil RS, Patil S, Bhaktaraj B, Patil SB. Effect graded doses of nicotine on ovarian and uterine activities albino rats. Indian J- Exp- Biol. 1999; 37-184.
- 92) Mandal R, Dhaliwal PK. Antifertility effect of *Melia azedarach* Linn. seed extract in female albino rats. Indian Journal of Experimental Biology. 2007; 45:853-860.
- 93) Yadav R, Jain GC. Effect of petroleum ether extract of *Cassia fistula* seeds on uterine histoarchitecture of ovariectomized female rats. Indian Journal of Fundamental and Applied Life Sciences. 2013; 3(1):167-174.
- 94) Zade V, Wikhe M, Dabhadkar D, Dawada S, Patil U. Antifertility efficacy of *Cannabis sativa* leaves on female albino rats. IJSIT. 2013; 2(2):107-117
- 95) Pare S, Zade V, Dabhadkar D. Evaluation of potential antifertility activity of plant *Trianthema portulacastrum* in female albino rat. Int.J.A.PS.BMS. 2013; 2(1):007-011.

**Article History:** -----

Date of Submission: 05-06-2014

Date of Acceptance: 19-06-2014

Conflict of Interest: NIL

Source of Support: NONE

