Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Online International Journal Available at http://www.cibtech.org/jls.htm
2013 Vol. 3 (1) January-March, pp.167-174/Yadav and Jain.
Research Article

EFFECT OF PETROLEUM ETHER EXTRACT OF *CASSIA FISTULA* SEEDS ON UTERINE HISTOARCHITECTURE OF OVARIECTOMIZED FEMALE RATS

***R. Yadav[1] and G. C. Jain[2]**
[1]Department of Zoology, Mahatma Gandhi Institute of Applied Sciences, JECRC Campus, Jaipur (India) -302022
[2]Reproductive Physiology Laboratory, Centre for Advanced Studies in Zoology, University of Rajasthan, Jaipur (India)
**Author for Correspondence*

ABSTRACT
In the present investigation, the uterine histoarchitecture of immature bilaterally ovariectomized female albino rats treated with petroleum ether extract of *Cassia fistula* seeds administered in the presence and absence of estradiol valerate (EDV) have been studied. Administration of petroleum ether extract of *Cassia fistula* seeds alone at the dose 100mg/kg b.wt. Resulted in increased uterine wet weights ($p<0.05$) and luminal epithelial cell height ($p<0.001$) but did not induce premature opening of the vagina. This suggests that the extract exhibited mild estrogenic activity when given alone, but when the extract was administered cojointly with EDV (0.1mg/kg b.wt.), it significantly ($p<0.001$) prevented the estrogen-induced uterotrophic effect, thus showing an antiestrogenic nature of the extract in the presence of a strong estrogen. Thus, the results of the present study indicate that the extract possess antiestrogenic property which may be responsible, at least partly, for the anticonceptive effect.

Key Words: *Cassia Fistula, Uterus, Female Rats*

INTRODUCTION
The development of new fertility regulating drug from medicinal plants is an attractive preposition. Although a variety of synthetic contraceptive agents are available, but these cannot be used continuously because of their side effects. So, natural plant substances possessing mild inherent estrogenic and antiestrogenic properties offer themselves as an effective non-conventional source of contraception with less deleterious side effects. Many of these plant products having inherent estrogenic or antiestrogenic activity possibly bring about alteration in tubal transport of blastocyst or hormonal milieu of the uterus making the uterine environment hostile for implantation or fetal development. On the basis of this inherent virtue, the estrogenic and antiestrogenic nature of many contraceptive agents has been assessed.
Cassia fistula Linn (Hindi-Amaltas; English-Golden Shower or Indian Laburnum), a medium-sized tree belonging to the Family–Caesalpiniaceae, is widely cultivated throughout India as an ornamental plant and is used for its medicinal properties. Different parts of this plant have been used extensively in the folklore medicine for the treatment of a variety of diseases (Chopra *et al.,* 1992 and Barthakur *et al.,* 1995). Pharmacologically, the plant has been investigated for its anti-diabetic (Esposito *et al.,*1991; Einstein *et al.*, 2013) hypocholesterolaemic (El-Saadany *et al.,*1991), antifertility (Yadav and Jain, 1999) antitumour (Gupta *et al.,* 2000) hepatoprotective (Bhakta *et al.,* 2001 and Das *et al*.,2008) antioxidant (Luximon-Ramma *et al.,* 2002) laxative (Akanmu *et al.,* 2004) anti-inflammatory (Raju *et al.,* 2005) antibacterial and antifungal activity (Duraipandivan and Ignacimuthu (2007) anthelminthic (Moshahid *et al.,*2010) antimicrobial (Aneja *et al.,*2011) antifeedant and larvicidal (Duraipandiyan *et al.,*2011) activity. The plant is rich in phenolic antioxidants such as anthraquinones, flavonoids and flavan-3-ol derivatives (Bahorun *et al.,* 2005).
In our earlier communication (Yadav and Jain, 2009), it has been reported that post-coital administration of petroleum ether extract of *Cassia fistula* seeds possess significant antifertility activity as it interfered

with steroidal conditioning of the uterus and renders it hostile to ovum implantation. In the view of its remarkable antiimplantation activity and in order to gain insight into its possible mechanism of action, the present study attempts to analyze the hormonal profile of petroleum ether extract of *C. fistula* seeds in immature bilaterally ovariectomized female rats which may play an important role in implantation and fetal development and this may be an important step in studying the contraceptive nature of the extract.

MATERIALS AND METHODS

Plant Material and Extraction

Cassia fistula "Amaltas" pods were collected during the season and were thoroughly dried in the shade. The plant was authenticated at the Department of Botany, University of Rajasthan, Jaipur (India). The seeds separated from the shade dried pods were ground to coarse powder. The powdered seeds (1000g) were subjected to Soxhlet extraction with petroleum ether (B.P. 60^0-80^0C). The crude extract (12%) so obtained was concentrated under reduced pressure and low temperature. The residue thus, obtained was then utilized for evaluating the estrogenic/antiestrogenic activity by suspending in appropriate volume of olive oil.

Experimental Animal

Colony bred bilaterally ovariectomized immature female rats (21-24 days old) were used as an experimental animal model for bioassay studies. All the animals were housed in standard laboratory conditions (temperature 22 ±3^{o}C and 14hr light/10hr dark cycle) with free access of food (Lipton India Ltd) and tap water *ad libitum*. All the experimental procedures were performed according to the guidelines for the care and use of experimental animals and approved by the Institutional Ethical Committee for Animals Care and Use, University of Rajasthan, Jaipur (India).

Hormonal Profile (Estrogenic/Antiestrogenic Activity)

Crude petroleum ether extract of the test substance was subjected to standard bioassay procedures for assessment of estrogenic or antiestrogenic activity in terms of the rat uterotrophic assay (Dorfman and Kincl, 1966, WHO protocol). Estrogenic or antiestrogenic activity of the extract was assessed by uterine wet weight, vaginal cornification and premature vaginal opening in sexually immature bilaterally ovariectomized female rats. Colony-bred immature female albino rats (21-24 days old) were bilaterally ovariectomized by dorsolateral approach, under light ether anaesthesia and semisterile conditions. After a rest period of seven days, these were randomly divided into four groups of five animals each and treated as follows:

Group I: Control group, receiving olive oil only (0.2ml/rat/day), orally.

Group II: Estradiol valerate (EDV, 0.1 mg/kg b.wt./day), intramuscularly (i.m.).

Group III: Extract alone (100 mg/kg b.wt./day), orally.

Group IV: Extract (100 mg/kg b.wt./day, orally) + EDV (0.1 mg/kg b.wt./day, i.m.), conjointly.

All these rats received treatment twice daily for 3 consecutive days. These treated rats were sacrificed 24 hours after the last dose administration. Their body weights were recorded. Uteri were carefully dissected out, freed from adherent tissues, blotted on filter paper and were weighed quickly to nearest milligrams on digital pan-balance. Condition of vaginal opening was also recorded. A part of the uterine horn of rats from each group was fixed in Bouin's fixative for histological/histometrical evaluations and the remaining halves of the uterine horns were frozen for tissue biochemical analysis.

Histometric Analysis (Uterine Luminal Epithelial Cell Height)

Haematoxylin-eosin stained slides were observed microscopically for changes in tissues and cellular organization, luminal epithelial cell height. One hundred luminal epithelial cells from 25 sections were measured randomly with an ocular micrometer at x400. Two diagonal and one medial length were

measured, averaged and expressed as mean epithelial cell height and were then calibrated with a stage micrometer.

Statistical Analysis

All the values are expressed as the mean ± SEM and significance was analyzed statistically by Students't' test and $p < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Hormonal Profile* (*Uterine Weight and Vaginal Opening)

Table 1 shows the results of uterine bioassay test carried out in bilaterally ovariectomized immature female rats. Oral administration of the extract (100mg/kg b.wt./twice daily) to ovariectomized immature female rats produced a slightly significant ($p<0.05$) increase in the uterine wet weight. However, the extract did not induce premature opening of the vagina, thus, suggesting a mild estrogenic activity of the extract. But, when the extract was administered conjointly with estradiol valerate (EDV, 0.1mg/kg b.wt./twice daily)), it significantly ($p<0.001$) prevented the estrogen-induced uterotrophic effect, thus, reflecting antiestrogenic nature of the extract in the presence of a strong estrogen.

Table 1: Showing changes in the wet uterine weight, vaginal opening and luminal epithelial cell height of bilaterally ovariectomized immature female rats after treatment with estradiol valerate and / or petroleum ether extract of *cassia fistula* seeds

Group	Treatment group	Dose	Uterine weight (mg/100 gm b. wt.)	vaginal opening	Luminal epithelial cell height (µm)
1	Control	0.2ml olive oil	38.36 ± 1.63	closed	10.45 ± 0.32
2	Estradiol valerate	0.1mg/kg b.wt.	529.36 ± 23.91aaa	open	42.50 ± 0.29aaa
3	Pet. ether extract	100mg/kg b.wt.	45.50 ± 1.41*	closed	16.72 ± 0.37***
4	Pet. ether extract +Estradiol valerate	100mg/kg b.wt. + 0.1mg/kg b.wt.	308.50 ± 13.99###	open	35.64 ± 0.24###

(Tabular values represent the mean ± SEM of 5 animals)

1. *a Estradiol treated rats (group 2) were compared with controls of group 1.*
   aaa p<0.001 (Highly significant)
2. ** Extract treated groups were compared with controls of group 1.*
   **p<0.05 (slightly significant) *** p<0.001 (Highly significant)*
3. *# (Extract + Estradiol valerate both) treated groups were compared with controls of group 2*
   ### p< 0.001 (Highly significant)

Following surgery, in the ovariectomized controls which lack estrogens, the uterine weight was reduced. Treatment of estradiol valerate (EDV) to these ovariectomized immature female rats induced a significant increase in uterine weight and accelerated premature vaginal opening in all these animals confirming well known sensitive bioassays of estrogenicity (Jordan *et al*., 1985; Clark and Markaverich, 1988). Estradiol valerate treatment to ovariectomized rat produced significant hypertrophy and hyperaemia of the uterine tissue and accumulation of uterine luminal fluid which may cause increase in uterine weight. Furthermore, the gain in uterine weight may be due to biosynthesis of RNA and protein actively (Turner and Bagnara, 1976).

The results obtained during this study were in accordance with several earlier reports describing estrogenic and/or antiestrogenic properties of a number of anticonceptive plant preparations in uterine bioassay test (Sharma *et al*., 1976; Chandhoke and Gupta, 1978; Jacob *et al*., 1986 and 1988; Dhar *et al*., 1992; Sisodia and Prakash, 1994 and Sarita and Bhagya, 2012).

Thus, the hormonal profile (estrogenic / antiestrogenic) of the test plant substance, like other such agents support the conjecture that estrogenic / antiestrogenic substances act as pregnancy interceptory agents.

Uterine Histoarchitecture (Uterine Luminal Epithelial Cell Height)

The mammalian uterus appears to be an ideal organ for measuring estrogenicity of a substance as after estrogen administration; the atrophic uterus of the ovariectomized rat is rapidly converted into a growing structure (Muller *et al*., 1958).

The extract when administered alone to ovariectomized rats induced a mild stimulation of all the uterine constituent elements and a highly significant ($p<0.001$) increase in uterine luminal epithelial cell height when compared with ovariectomized controls (Group I) only, thus, showing the estrogenic nature. But when the extract was administered along with a strong estrogen (ie. EDV), it showed antagonism of EDV-induced hypertrophy of the uterine constituent elements and a highly significant ($p<0.001$) decline in uterine luminal epithelial cell height in comparison to EDV alone treated rats (Group II).

In the present investigation, uterus of ovariectomized control rats showed an infantile appearance. The myometrium and endometrium layers were reduced in thickness. The lumen was slit - like and lined with small atrophic cuboidal cells. The stroma appeared dense and compact having few, small, inactive uterine glands (Figure1). Thus, confirmed well known estrogen deprivation effect (Sud and Setty, 1974). However, estradiol valerate (EDV) hormone replacement therapy to the ovariectomized immature rats induced significant hypertrophy and hyperplasia of endometrial tissue. The uterine lumen was enlarged and lined with tall columnar epithelial cells. The stroma was edematous and loose and exhibit well developed uterine glands (Figure 2). These results were in accordance with the well known effect of estrogens on the uterus of ovariectomized animals (Ljungkvist, 1971 and Weitlauf, 1988).

Oral administration of petroleum ether extract of *Cassia fistula* seeds alone to ovariectomized rats resulted in mild stimulation of all uterine constituent elements (ie. few endometrial glands in compact stroma, Figure 3), whereas, conjoint treatment with EDV showed impairment of EDV induced uterine stimulation. The luminal epithelial cell height was reduced significantly. The stroma was comparatively less edematous with less active endometrial glands (Figure 4). Thus, histological picture also confirmed antiestrogenic activity of the extract. Thus, administration of petroleum ether extract of *Cassia fistula* seeds alone to ovariectomized rats, in general, resulted in slight increase in the size of uterine lumen, number of uterine glands and the luminal epithelial cell height. Whereas administration of the extract conjointly with EDV to ovariectomized rats produced a reduction in the size of uterine lumen, number of uterine glands and the luminal epithelial cell height, thus, suggesting antiestrogenic nature of the extract. This indicates that the extract and EDV are antagonistic in their action, as their combination decreased all the parameters of the uterus more than their individual administration.

There are many reports which highlight similar antiestrogenic effect on uterine histology of ovariectomized female rats after administration of various plant extracts (Pakrashi and Chakraborty, 1978; Chandhoke and Gupta, 1978 and Shukla *et al*., 1988).

Thus, beside uterine wet weight bioassy, uterine histoarchitecture of ovariectomized immature rats receiving extracts alone or in combination with estradiol valerate also supported estrogenic and / or antiestrogenic property of the extract.

This antiestrogenic nature of the extract may be responsible for the pregnancy interceptory effect of the test plant (Yadav and Jain, 2009). Thus, like other relatively weak estrogenic substances it behaved as an antiestrogen in the presence of relatively more potent estrogen by possibly affecting the uterine estrogen receptor binding. A number of plants possessing antiestrogenic activity have also been reported to interrupt pregnancy (Dao *et al.*, 1996; Badami *et al.*, 2003; Sharma and Bhinda, 2005 and Ravichandran

et al., 2007). Antiestrogens with weak estrogenic activity administered early in pregnancy may interfere with implantation by altering the normal pattern of hormonal conditioning of uterus required for conception (Psychoyos and Prepas, 1987).

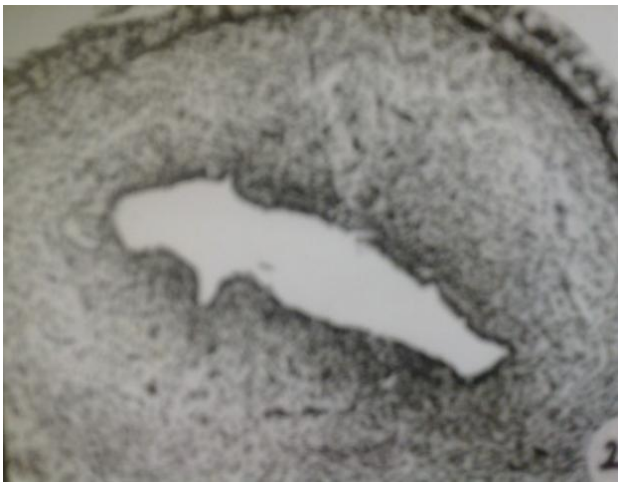

Figure 1: T.S. of uterus of bilaterally ovariectomized control rats showing infantile appearance with reduced luminal epithelial cell height, compact stroma and atrophic endometrium.

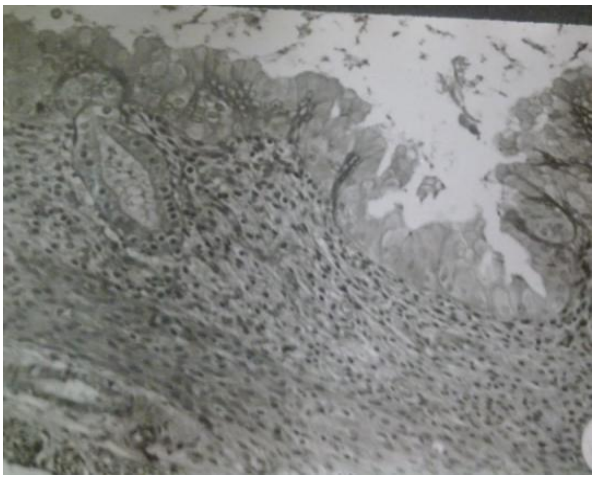

Figure 2: T.S. of uterus of bilaterally ovariectomized rats treated with EDV alone, illustrating the hypertrophy of the endometrial epithelium, uterine lumen lined with tall columnar epithelial cells and few enlarged uterine glands lined with columnar cells in the loose edematous stroma.

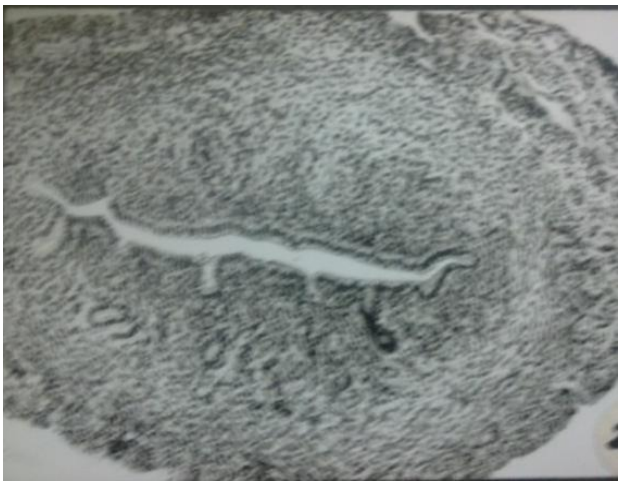

Figure 3: T.S. of uterus of bilaterally ovariectomized rats treated with petroleum ether extract of *cassia fistula* seeds illustrating the mild stimulation of all the constituent elements, increased thickness of luminal epithelium and presence of few small endometrial glands.

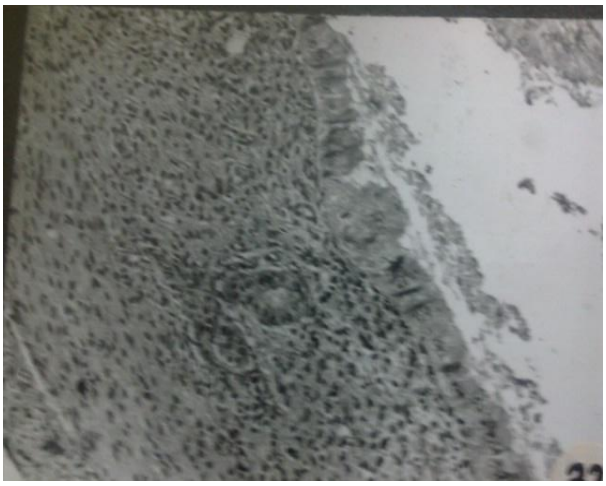

Figure 4: T.S. of uterus of bilaterally ovariectomized rats treated with petroleum ether extract of *cassia fistula* seeds along with EDV, displaying antagonism of EDV induced hypertrophy of the uterine constituents, lumen lined with less tall columnar epithelial cells, regressing endometrial glands and atrophic endometrium.

On the basis of the above observations it may be concluded that petroleum ether extract of *Cassia fistula* seeds owing to its antiestrogenic nature alters the biochemical milieu of the reproductive tract especially uterine environment (Rao *et al.*, 2005) which lead to change the normal status of reproduction in female reproductive tract of rat and thus produce significant antifertility effect.

The antiestrogenic efficacy of the extract in presence of a strong estrogen produced inhibitory effect which merely supports the contention that petroleum ether extract of *Cassia fistula* seeds offer itself as a very promising substance for further research in pregnancy interception.

ACKNOWLEDGEMENT

The authors are thankful to the Head of Department and Coordinator, Centre for Advanced Studies in Zoology for providing necessary laboratory facilities and also the University of Rajasthan for financial support to Ms Rajesh Yadav.

REFERENCES

Akanmu MA, Iwalewa EO, Elujoba AA and Adelusola KA (2004). Toxicity potentials of *Cassia fistula* fruits as laxative with reference to Senna. *African Journal of Bomedical Research* **7**(1) 23-26.

Aneja KR, Sharma C and Joshi R (2011). *In vitro* efficacy of amaltas (*Cassia fistula* L.) against the pathogens causing otitis externa. *JJM* **4**(3) 175-183.

Badami S, Aneesh R, Sankar S, Sathishkumar M N, Suresh B and Rajan S (2003). Antifertility activity of *Derris brevipes* variety coriacea. *Journal of Ethnopharmacology* **84**(1) 99-104.

Bahorun T, Neergheen VS and Aruoma OI (2005). Phytochemical constituents of *Cassia fistula*. *African Journal of Biotechnology* **4** 1530-1540.

Barthakur NN, Arnold NP and Alli I (1995). The Indian laburnum (*Cassia fistula* L.) fruit: an analysis of its chemical constituents. *Plants Food Human Nutrition* **47** 55-62.

Bhakta T, Banerjee S and Subhash C (2001). Hepatoprotective activity of *Cassia fistula* leaf extract. *Phytomedicine* **8**(3) 220-224.

Chandhoke, N and Gupta S (1978). Estrogenic activity of DQ_1-A steroidal lactone - Isolated from *Datura quercifolia. Indian Journal of Experimental Biology* **16** 648-652.

Chopra RN, Nayar SL and Chopra IC (1992). Glossary of Indian medicinal plants, *Publication and information Directorate, CSIR,* New Delhi 54.

Clark JH and Markaverich BM (1988). Action of ovarian steroid hormones. In The Physiology of Reproduction *E Knobil and J Neill Raven Press,* New York **17** 675-724.

Dao B, Vanage G, Marshall A, Bardin CW and Koide SS (1996). Antiimplantation activity of antiestrogens and mifepristone. *Contraception* **54** 253-258.

Das S, Sharma G and Barman S (2008). Hepatoprotective activity of aqueous extract of fruit Pulp of *Cassia fistula* (AFCF) against carbon tetrachloride (CCL_4) induced liver damage in albino rats. *Journal of Clinical Diagnosis and Research* **2** 1133-1138.

Dhar JD, Setty BS, Lakshmi V and Bhakuni DS (1992). Postcoital antifertility activity of the marine plant *Achrostichum aureum* L in rat. *Indian Journal of Medical Research* **96**(B) 150-152.

Dorfman RI and Kincl FA (1966). Uterotrophic activity of various phenolic steroids. *Acta Endocr* (Kobenhavn) **52** 619.

Duraipandivan V and Ignacimuthu S (2007). Antibacterial and antifungal activity of *Cassia fistula* L.: An ethnomedicinal plant. *Journal of Ethnopharmacology* **112** 590-594.

Duraipandiyan V, Ignacimuthu S, and Gabriel Paulraj M (2011). Antifeedant and larvicidal activities of Rhein isolated from the flowers of *Cassia fistula* L. *Saudi Journal of Biological Sciences* **18** 129-133.

Einstein JW, Rais MM and Mohd MA (2013). Comparative evaluation of the antidiabetic effects of different parts of *Cassia fistula* Linn, a Southeast Asian plant. *Journal of Chemistry* 10.

El-Saadany SS, el-Massry RA, Labib SM and Sitohy MZ (1991). The biochemical role and hypocholesterolaemic potential of the legume *Cassia fistula* in hypercholesterolaemic rats. *Nahrung* **35** 807-815.

Esposito Avella M, Diaz A, deGracia I, de Tello R and Gupta MP (1991). Evaluation of traditional medicine: effects of *Cajanus cajan* L and of *Cassia fistula* L on carbohydrate metabolism in mice. *Review Medicine of Panama* **16** 39-45.

Gupta M, Mazumder UK, Rath N and Mukhopadhyay DK (2000). Antitumour activity of methanolic extract of *Cassia fistula* L seeds against Ehrlich ascites carcinoma. *Journal of Ethnopharmacol*ogy **72** 151-156.

Jacob D, Dhir RN, Vyas DK and Bhatt S (1988). The possible mode of pregnancy interceptory action of *Carica papaya* seeds extract in the rat. *The Indian Zoologist* **12** 99-102.

Jacob D, Sharma S and Vyas DK (1986). Estrogenic and Postcoital antifertility effect of *Carica papaya* seed extract in the rat. *The Indian Zoologist*, **10** 127-129.

Jordan VC, Mittal S, Gosden B, Koch R and Liberman ME (1985). Structure- activity relationship of estrogen. *Enviro. Health Perspect*, **61** 97-110.

Ljungkvist L (1971). Attachment reaction of rat uterine luminal epithelium- The effect of estradiol, estrone and oestriol on the morphology of the luminal epithelium of spayed virgin rats. *Acta Society of Medicines*, Uppsala **76** 139-157.

Luximon- Ramma A, Behorun T, Soobrattee MA and Aruoma OI (2002). Antioxidant activities of phenolic, proanthocyanidins and flavonoid components in extracts of *Cassia fistula*. *Journal of Agriculture, Food and Chemistry* **50** 5042-5047.

Moshahid AR, Irshad M and Singh M (2010). Assessment of anthelmintic activity of *Cassia fistula* L. *Middle-East Journal of Scientific Research* **5**(5) 346-349.

Muller GC, Ailene MH and Kristian FJ (1958). Studies on the mechanism of action of oestrogens. *Recent program in Hormone Research* **14** 95-139.

Pakrashi A and Chakraborti B (1978). Antiestrogenic and antimplantation effect of aristolic acid form *Aristolochia indica* (Linn). *Indian Journal of Experimental Biology* **16** 1283-1285.

Psychoyos A and Prepas I (1987). Inhibition of egg development and implantation in rats after post-coital administration of the progesterone antagonist RU 486. *Journal of Reproduction and Fertility* **80** 487-491.

Raju I, Moni M and Subramanian V (2005). Anti-Inflammatory and Antioxidant activities of *Cassia fistula* Linn Bark Extracts. *African Journal of Traditional Chenistry* **2**(1) 70-85.

Rao MV, Sunder RS and Chawla SL (2005). Reproductive toxicity of a fungicide combination (Metalaxyl + Mancozeb) in adult rats. *Journal of Cell Tissue Research* **5** 299-302.

Ravichandran V, Suresh B, Satishkumar MN, Elango K and Srinivasan R (2007). Antifertility activity of hydroalcoholic extract of *Ailanthus excelsa* (Roxb): An ethnomedicine used by tribals of Nilgiris region in Tamilnadu. *Ethnopharmacology* **112** 189-191.

Sarita M and Bhagya M (2012). Antiimplantation and anti-estrogenic activity of *Eugenia jambolana* lam Seed. *Journal of Pharmacy Research* **5**(5) 2607-2609.

Sharma JD and Bhinda A (2005). Antifertility activity of steroidal extract of *Trigonella foenum graecum* (seeds) in female rats. *Asian Journal of Experimental Science* **19**(1) 115-120.

Sharma MM, Lal G and Jacob D (1976). Estrogenic and pregnacy interceptory affects of *Daucus carota* Seeds. *Indian Journal of Experimental Biology* **14** 506-508.

Shukla S, Mathur R and Prakash AO (1988). Antifertility profile of aqueous extract of *Moringa oleifera* roots. *Journal of Ethnopharmacology* **22** 51-62.

Sisodia B and Prakash AO (1994). Anitfertility profile of *Zingiber rosemum* in rats. Abstract, in National Symposium on Reproductive health Care, Jaipur, India 46.

Sud NK and Setty BS (1974). Histochemical evaluation of estrogenic and antiestrogenic properties of some non-steroidal postcoital antifertility agents. *Indian Journal of Medical Research* **62** 1437-1442.
Turner CD and Bagnara JT (1976). General Endocrinology, *W.B. Saunder Company, Philadelphia - London* 225-257.
Weitlauf HM (1988). Biology of implantation: In "The Physiology of Reproduction" edition E Knobil and J Neil *et al., Revan Press, Ltd.* New York 231-254.
Yadav R and Jain GC (1999). Antifertility effect of aqueous extract of seeds of *Cassia fistula* in female rats. *Advances in Contraception* **15** 292-301.
Yadav R and Jain GC (2009). Antifertility effect and hormonal profile of petroleum ether extract of seeds of *Cassia fistula* in female rats. *International Journal of PharmTech Research* **1**(3) 438-444.