EFFECT OF AQUEOUS EXTRACT OF SEEDS OF *Cassia fistula* ON THE UTERINE BIOCHEMICAL MILIEU OF FEMALE ALBINO RATS

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Summary

The development of new fertility regulating drugs from medicinal plants is an attractive preposition. In the present study an attempt has been made to analyze the possible modulatory influence of aqueous extract of *Cassia fistula* seeds on biochemical constituents (viz. glycogen, sialic acid, cholesterol, acid phosphatase and alkaline phosphatase) of uterus which may play an important role in implantation and foetal development. Post-coital administration of contragestative (pregnancy interceptory) dose (500mg/kg b.wt./day) of aqueous extract of seeds of *Cassia fistula* from day 1-5 *pc* resulted in a statistically significant decline ($p<O.001$) in the acid phosphatase activity and glycogen content ($p<O.01$) when compared with control pregnant rats while the activity of alkaline phosphatase, cholesterol and sialic acid concentration remained significantly unchanged, as compared to the controls on day 15 *pc*. Therefore, it can be suggested that the changes in the biochemical constituents of uterus may be responsible, atleast partly, for the anticonceptive / anti-implantational effect of aqueous extract of *Cassia fistula* seeds in the treated female rats.

Key words : *Cassia fistula*, glycogen, sialic acid, cholesterol, acid and alkaline phosphatase, uterus, female rats

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Introduction

The use of natural products as fertility regulating agent have been known since times immemorial and are still practiced in rural areas. A large number of plants have been reported to exhibit anti-implantation and abortifacient activity[1] but a few have been evaluated for such effects in laboratory animals. So far no single plant is available which can be developed further as a postcoital antifertility agent. Many of these plant products having inherent estrogenic or antiestrogenic effects, possibly bring about alteration in tubal transport of blastocyst or hormonal milieu of the uterus making the uterine environment hostile for implantation or foetal development.

Cassia fistula Linn (Family - Caesalpiniaceae), commonly known as " amaltas", is widely cultivated throughout India as an ornamental plant and is used for its medicinal properties. In the Indian literature different parts of this plant has been described to be useful against many diseases[2]. Pharmacologically, the plant has been investigated for its anti-diabetic[3], hypocholesterolaemic[4], antibacterial[5], antitumour[6], hepatoprotective[7], antioxidant[8] and laxative[9] effects. The plant is rich in phenolic antioxidants such as anthraquinones, flavonoids and flavan-3-ol derivatives[10].

In recent years relatively more emphasis is being laid on the examination of natural products including substances of plant origin for their postcoital antifertility activity. The mechanism of action of these substances is not known clearly. Mechanism of action of these substances can be realized by the study of the biochemical parameters of the reproductive tract. In our earlier communication[11] it has been reported that postcoital administration of aqueous extract of seeds of *Cassia fistula* at the dose 500mg/kg b.wt./day prevented pregnancy in all the treated female rats by virtue of anti-implantational property with antiestrogenic activity. With the view to elucidate the possible mode of action of the so called antifertility effect the present study attempts to analyze the possible modulatory influence of aqueous extract of *C. fistula* seeds on the biochemical constituents of uterus which may play an important role in implantation and foetal development.

Materials and Methods

Plant material and extraction :

Pods of *Cassia fistula* “Amaltas” 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 (500g) were extracted with distilled water in a Soxhlet apparatus for 36 hrs at 100ºC. The crude extract (32g) thus obtained was concentrated under reduced pressure and low temperature. The residue obtained was then utilized for evaluating antifertility efficacy by suspending in appropriate volume of olive oil.

Experimental animal :

Colony bred adult albino Wistar rats (weighing 170-200 g) for antifertility studies and bilaterally ovariectomized immature female rats (21-24 days old) for bioassay studies were used as experimental animal model. 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).

Dose and route of administration :

The animals of Group I received vehicle (olive oil, 0.2ml/rat) only and served as control. Animals of Group II, III and IV received crude aqueous extract of *Cassia fistula* at 100, 200 and 500 mg/kg b.wt./day (suspended in olive oil) doses, respectively, once a day from day 1-5 *post coitum* (*pc*). The extract was administered orally by using a curved needle and a tuberculin syringe.

Biochemical estimations :

The female rats which were in proestrous or estrous phase were left over night with males of proven fertility (in the ratio 2:1) and next morning the vaginal smears were checked for the presence of spermatozoa. The day on which the spermatozoa were observed in the vaginal smear was considered as day "Zero" of pregnancy. These females were isolated and used for further experimentation. The antinidational properties of the test substance was assessed by oral administration of 100, 200 and 500 mg/kg b.wt./day dose of aqueous extract of seeds of *Cassia* to the mated female rats from day 1-5 *postcoitum*. On day 15*pc,* autopsy was performed under light ether anesthesia and after recording their body weights, the two uterine horns were observed for the number of implantation sites, live and dead/degenerated fetuses. The fetuses were removed from the uterine horns and suitable parts of the horns of rats treated with 500mg/kg b.wt./day which showed 100% antifertility effect were frozen and used for tissue biochemical analysis.
Quantitative biochemical estimations of glycogen[12] , cholesterol[13], acid and alkaline phosphatases[14] and sialic acid[15] were made in the uterine tissue samples of control and treated female rats.

Statistical analysis :

All the results are expressed as mean ± SEM and significance was analysed statistically by students ‘t’ test and $p< 0.05$ was considered as significant level.

Results

Table 1& 2 represents the values of the uterine biochemical parameters of the control and extract treated female rats. Biochemical analysis of uterine horns of rats treated with the pregnancy interceptory dose (500 mg/kg b.wt./day) of aqueous extract of seeds of *Cassia fistula* showing cent percent antifertility effect, revealed a significant decline ($p<0.001$) in the acid phosphatase activity and glycogen content ($p<0.01$) when compared with control pregnant rats while the activity of alkaline phosphatase, cholesterol and sialic acid concentration remained significantly unchanged in comparison to controls. (Histogram1 &2).

Table 1 : Effect of aqueous extract of *Cassia fistula* seeds on the uterine biochemical parameters

Treatment Group	**Glycogen (mg/g tissue)**	**Cholesterol (mg/g tissue)**	**Sialic acid (mg/g tissue)**
Control (vehicle)	5.23 ± 0.26	6.68 ± 0.29	4.36 ± 0.30
Experimental (*C. fistula* extract)	3.60 ± 0.22**	7.18 ± 0.27ns	4.74 ± 0.32ns

[Values are mean ± SEM]

Level of Significance in relation to control :

** $p < 0.01$, ns = non-significant

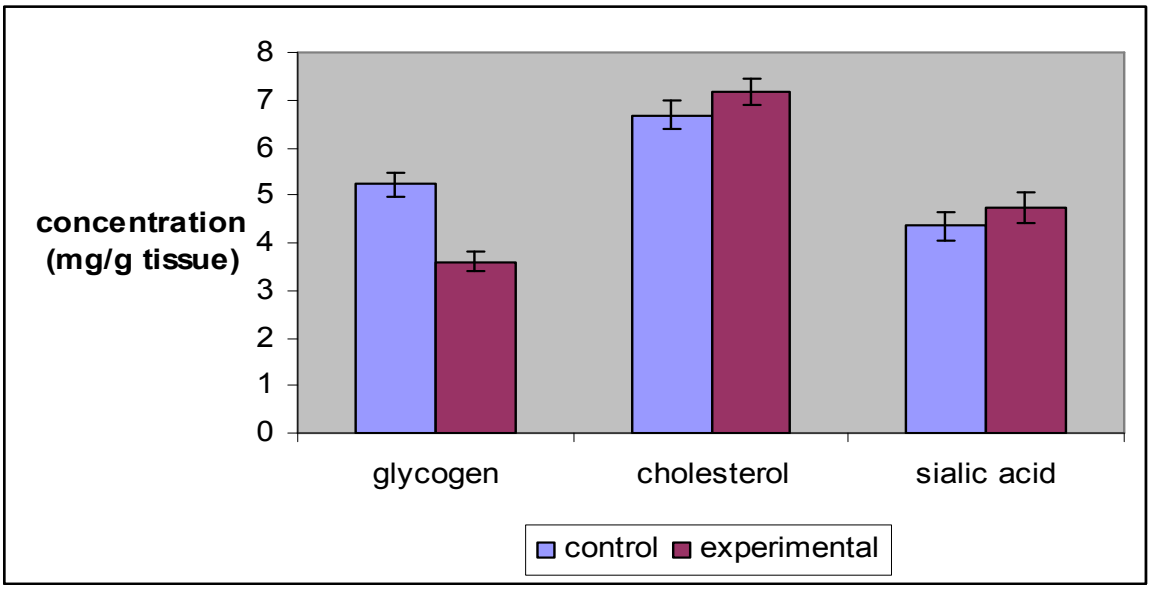
Table 2 : Effect of aqueous extract of *Cassia fistula* seeds on uterine Acid and Alkaline phosphatase activities of mated female rats

Treatment Group	**Acid phosphatase (mg Pi/g/hr)**	**Alkaline phosphatase (mg Pi/g/hr)**
Control (vehicle)	2.71± 0.15	7.41± 0.35
Experimental (*C. fistula* extract)	1.28 ± 0.07***	8.52 ± 0.43ns

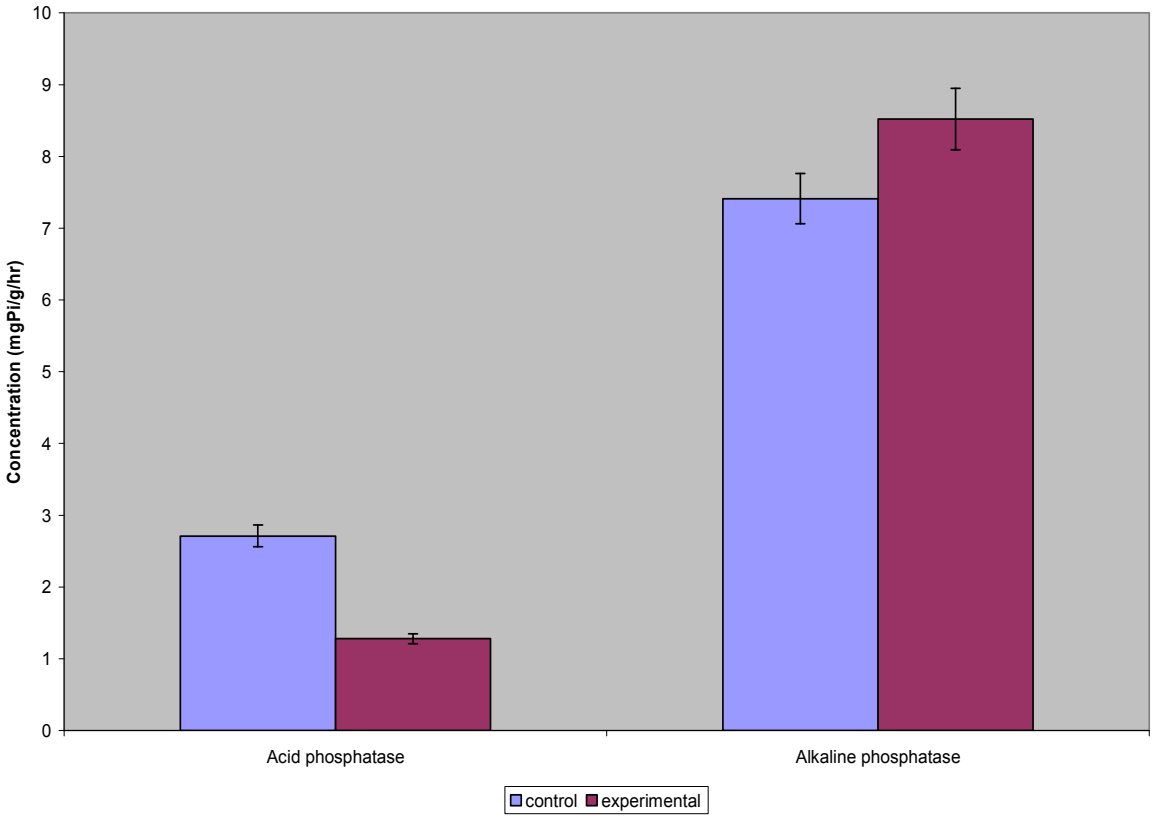
[Values are mean + SEM]

Level of Significance in relation to control :

*** $p < 0.001$, ns = non-significant



Histogram 1 : Effect of aqueous extract of seeds of *Cassia fistula* on the uterine biochemical parameters of treated female albino rats



Histogram 2 : Effect of aqueous extract of seeds of *Cassia fistula* on uterine acid and alkaline phosphatase activities of treated female albino rats

Discussion

In our earlier communication[11] it has been reported that postcoital administration of aqueous extract of seeds of *Cassia fistula* at the dose 500mg/kg b.wt./day from day 1-5*pc* prevented pregnancy in all the treated female rats by virtue of anti-implantational property with antiestrogenic activity in presence of a strong estrogen. Since the uterine biochemical milieu serves various functions. It enables the spermatozoa to ascend to the site of fertilization within the oviduct, it provides adequate nutrition for the embryo during its various developmental stages between its arrival in the uterine lumen until it has achieved implantation and maintains an appropriate environment for the physical and biochemical integrity of the blastocyst structure and it meets specific immunological requirements, which becomes increasingly important during the preimplantation phase[16]. There is possibility that estrogen agonistic or antagonistic activities of the plant substance may influence the uterine microenvironment making it hostile for implantation or for blastocyst to survive[17].

Endometrial glycogen is one of the most important factor for the development and implantation of blastocyst in early stages of gestation[18]. Saldarini and Yochim[19] have reported that mobilization of glycogen during formation of deciduma is regulated through hormonal pathways wherein the action of estrogen is dominant. An increase in glycogen mobilization provides nutritive support to the developing blastocyst for their survival. However, in the present study, a significant decline ($p<0.01$) in the uterine glycogen content in extract treated female rats indicate poor nutritive support to the developing blastocyst for their survival. These findings are correlated with many earlier reports which indicates that substances of plant origin besides exhibiting antifertility efficacy also causes a concomitant inhibition of the glycogen level of the mammalian uterus[20-21]. Inhibition of glycogen content in the uterus is due to the antiestrogenic nature of the extract and may cause a suppression in motor activity of the uterus[22], which can account for their antifertility action. It is also possible that the ciliary action is decreased by the decrease in the glycogen level and the ova is not transferred to the uterus, thus, causes antifertility action[23].

Cholesterol derived from the different sources is the precursor for the steroidogenesis of ovarian endocrine tissue[24]. A non-significant change in the uterine cholesterol content of rats treated with aqueous extract of seeds of *C. fistula* indicated no adverse effect on uterine cholesterol metabolism. No change in cholesterol level in ovary in ethanolic extract of *Allium cepa* showing antifertility was observed [25]. Sialomucoprotein, a derivative of sialic acid, forms mucous in the uterus and vagina[26] and sticks around the blastocyst fluid which ultimately helps in the attachment of the blastocyst[27]. In addition, sialic acid also acts as an immuno-barrier between the mother and embryo, thereby, has a vital role in embryo implantation in uterus. In the present study, a non-significant change in the sialic acid content of uterus in extract treated female rats was observed.

It is well established that alkaline and acid phosphatases are associated with the decidual cell reaction and play important role in implantation[28]. A high acid phosphatase activity at the time of implantation is associated with its involvement in the preparation of the implantation chamber. A significant decline in uterine acid phosphatase activity in extract treated mated female rats indicate adverse effect on uterine milieu, making it unsuitable for implantation. These results are in agreement with the findings of many workers[29-30] who also observed impairment of implantation by virtue of decline in uterine acid phosphatase activity after treatment with plant extracts in mated female rats.

Post-coital administration of the extract showed a non-significant effect on alkaline phosphatase activity suggesting no any adverse effect. These results support the earlier findings of the effect of various plant extracts on uterine alkaline phosphatase activity[31].

Therefore, it can be suggested that the pregnancy interceptory effect of aqueous extract of seeds of *Cassia fistula* might be due to the inhibition of circulating estrogen- progesterone balance which create a non receptive stage in the uterus by changing the reproductive biochemical milieu especially uterine environment which is directly involved in the implantation of eggs[32] and thus produce significant antifertility effect.

Although it would be premature to correlate the changes in the uterine biochemical constituents and the antinidational effect of the test substance but the changes in the activity of the uterine biochemical milieu could conjecturally be playing a role in the prevention of pregnancy. The antiestrogenic efficacy of the extract of the test plant in presence of a strong estrogen produced inhibitory effect which merely supports the contention that aqueous extract of seeds of *Cassia fistula* offers itself as a very promising substance for further research in pregnancy interception.

On the basis of the above observations it may be concluded that aqueous extract of seeds of *Cassia fistula* owing to its potent antiestrogenic nature alters the biochemical milieu of the reproductive tract which lead to change the normal status of the reproduction in female reproductive tract of rat and thus produce significant antifertility effect.

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