

Research Article

Effect of Contragestative Dose of Aqueous Extract of *Curcuma Longa* Rhizome on Uterine Biochemical Milieu of Female Rats

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ABSTRACT

The use of natural products as fertility regulating agent especially among woman have been known since times immemorial and are still practiced in rural areas. In the present study, an attempt has been made to analyze the possible modulatory influence of aqueous extract of *Curcuma longa* rhizome 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 body weight/day) of aqueous extract of rhizome of *Curcuma longa* from day 1-5 *post-coitum* (*pc*) resulted in a statistically highly significant decline ($p < 0.001$) in the acid phosphatase activity, a statistically significant decline ($p < 0.01$) in glycogen content and a statistically non-significant decline in alkaline phosphatase activity and sialic acid concentration when compared with control pregnant rats while the cholesterol concentration remained significantly unchanged, in comparison 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 *Curcuma longa* rhizome in the treated female rats.

Key Words: *Curcuma longa*, glycogen, sialic acid, cholesterol, acid and alkaline phosphatase, uterus, female rats

INTRODUCTION

The development of new fertility regulating drugs from medicinal plants is an attractive proposition. Natural plant substances possessing mild inherent estrogenic or anti-estrogenic properties offer themselves as effective non-conventional source of contraception with less deleterious side effects. Many plants and herbs have been reported to have potential antifertility properties (Casey, 1960). Many of these plant products having inherent estrogenic or anti-estrogenic 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.

Curcuma longa (English-Turmeric) a member of family Zingiberaceae has been extensively used as a colouring agent, condiment and in the treatment of inflammatory conditions and other diseases (Ammon & Wahl, 1991; Srimal, 1997). Curcumin, (diferuloyl methane) bis (4-hydroxy-3-methoxyphenyl)-1,6- heptadiene-3,5-dione, which is a natural polyphenol alkaloid yellow-orange dye derived from the rhizome of *Curcuma longa*, is known to exhibit a variety of pharmacological effects (Piper *et al.*, 1998; Ramirez-Tortosa *et al.*, 1999). In traditional system of medicine, *Curcuma longa* has been

reported to possess antifertility activity (Chaudhury, 1966). Various extracts of *Curcuma longa* rhizome have been reported to cause significant decline in pregnancies in female rats (Garg *et al.*, 1978).

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 (Yadav and Jain, 2010), it has been reported that post-coital administration of aqueous extract of rhizome of *Curcuma longa* at the dose 500mg/kg body weight/day prevented pregnancy in all the treated female rats by virtue of anti-implantational property with mild estrogenic activity.

With the view to elucidate the possible mode of action of the antifertility effect the present study attempts to analyze the possible modulatory influence of aqueous extract of *C. longa* rhizome on the biochemical constituents of uterus which may play an important role in implantation and foetal development.

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MATERIALS AND METHODS

Plant Collection and Extraction

The fresh rhizomes of *Curcuma longa* were obtained locally from the market and were thoroughly dried in shade and ground to coarse powder (500g) and subjected to soxhlet extraction in distilled water for 36 hours at 100°C. The crude extract thus obtained was concentrated to dryness under low temperature (50-60°C) and reduced pressure to yield a reddish brown viscous residue (55g). The residue thus obtained was then utilized for evaluating antifertility efficacy by suspending in appropriate volume of olive oil.

Animal stock

Colony-bred, adult cyclic albino Wistar female rats (weighing 170-200 g) were used for antifertility studies and bilaterally ovariectomized immature female rats (21-24 days old) for bioassay studies. All the animals were housed in standard laboratory conditions (temp. 22 ± 3°C and 14 hr light/10 hr dark cycle) with free access to 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, 0.2 ml/rat) only and served as control. Animals of Group II, III and IV received crude aqueous extract of *Curcuma longa* at 100, 200 & 500 mg/kg body weight/day (suspended in olive oil) doses, respectively, orally once a day from day 1-5 *postcoitum* (*pc*). The extract was

administered orally by using a curved needle and a tuberculin syringe.

Biochemical estimations

Normal cycling proestrous female rats were caged overnight with males (2:1 ratio) of proven fertility and next morning, insemination was confirmed by the presence of the vaginal plug and spermatozoa in the vaginal smear. This day of mating was designated as day 'Zero' of pregnancy. These mated female rats were isolated, weighed and divided into four groups of seven animals each and were used for further experimentation.

The antinidational properties of the test substance was assessed by oral administration of 100, 200 and 500 mg/kg body weight/day dose of aqueous extract of rhizome of *Curcuma* 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 body weight/day which showed 100% antifertility effect were frozen and used for tissue biochemical analysis.

Quantitative biochemical estimations of glycogen (Montgomery, 1957), cholesterol (Oser, 1965), acid and alkaline phosphatases (Fiske and Subbarow, 1925) and sialic acid (Warren, 1959) 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.

Table 1: Effect of aqueous extract of *Curcuma longa* rhizome on the uterine biochemical parameters of treated female albino rats

Treatment Group	Glycogen (mg/g tissue)	Cholesterol (mg/g tissue)	Sialic acid (mg/g tissue)	Acid phosphatase (mg/ iP/g/hr)	Alkaline phosphatase (mg/ iP/g/hr)
Control (vehicle)	6.32 ± 0.36	6.74 ± 0.21	4.25 ± 0.22	2.12± 0.12	7.22± 0.17
Experimental (<i>C. longa</i> extract)	4.19 ± 0.36**	6.18 ± 0.29ns	3.90 ± 0.18ns	1.02 ± 0.09***	6.53 ± 0.31ns

[Values are mean ± SEM]

Level of Significance in relation to control :

*** $p < 0.001$ (highly significant), ** $p < 0.01$ (significant), ns = non-significant

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RESULTS AND DISCUSSION

Table 1 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 (500mg/kg body weight/day) of aqueous extract of rhizome of *Curcuma longa* showing cent percent antifertility effect, revealed a statistically highly significant decline ($p < 0.001$) in the acid phosphatase activity, a statistically significant decline ($p < 0.01$) in glycogen content and a statistically non-significant decline in alkaline phosphatase activity and sialic acid concentration when compared with control pregnant rats while the cholesterol concentration remained significantly unchanged, in comparison to the controls on day 15 pc.

In our earlier communication (Yadav and Jain, 2010) it has been reported that postcoital administration of aqueous extract of *Curcuma longa* rhizome at the dose 500mg/kg body weight/day from day 1-5pc prevented pregnancy in all the treated female rats by virtue of anti-implantational property with mild estrogenic 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 (Beier *et al.*, 1991). 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 (Denker, 1994).

Endometrial glycogen is one of the most important factor for the development and implantation of blastocyst in early stages of gestation (Christie, 1966). Saldarini and Yochim (1968) have reported that mobilization of glycogen during formation of decidua 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 (Dehadrai *et al.*, 1994; Mutreja *et al.*, 2008)). Inhibition of glycogen content in the uterus is due to the mild estrogenic nature of the extract and may cause a suppression in motor activity of the uterus (Prakash, 1980), 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 (Dhir *et al.*, 1986).

Cholesterol derived from the different sources is the precursor for the steroidogenesis of ovarian endocrine tissue (Strauss *et al.*, 1981). A non-significant change in the uterine cholesterol content of rats treated with aqueous extract of rhizome of *Curcuma longa* 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 (Thakare *et al.*, 2009).

Sialomucoprotein, a derivative of sialic acid, forms mucous in the uterus and vagina (Coppola and Ball, 1966) and sticks around the blastocyst fluid which ultimately helps in the attachment of the blastocyst (Boving, 1963). 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 (Malone, 1960). A high acid phosphatase activity at the time of implantation is associated with its involvement in the preparation of the implantation chamber. A highly 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 (Kabir Syed *et al.*, 1984; Prakash *et al.*, 1988) 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 (Shukla, 1994).

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Therefore, it can be suggested that the pregnancy interceptory effect of aqueous extract of rhizome of *Curcuma longa* 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 (Rao *et al.*, 2005) and thus produce significant antifertility effect.

On the basis of the above observations it may be concluded that aqueous extract of rhizome of *Curcuma longa* owing to its mild estrogenic nature alters the biochemical milieu of the reproductive tract which lead to change the normal status of reproduction in female reproductive tract of rat 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 mild estrogenic 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 *Curcuma longa* rhizome offers itself as a very promising substance for further research in pregnancy interception.

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