

**POST-COITAL CONTRACEPTIVE EFFICACY OF AQUEOUS EXTRACT OF
Curcuma longa RHIZOME IN FEMALE ALBINO RATS**

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Summary

The rhizome of *Curcuma longa*, is known to exhibit a variety of pharmacological effects. In traditional system of medicine also, *Curcuma longa* has been reported to possess antifertility activity. In the present study, an attempt has been made to investigate the anticonceptive activity of aqueous extract of *Curcuma longa* rhizome and its homonal profile in immature bilaterally ovariectomised female rats in order to gain insight into its possible mode of action. Oral administration of the extract induced a significant decline in the quantal pregnancy rate, total number of implantation sites and viable fetuses in a dose dependent manner. In rats, receiving 500 mg/kg b.wt. extract dose a complete blockage of the pregnancy was observed. At 200mg/kg b.wt. dose, out of seven females only one was pregnant having four implantation sites. Further reduction of the dose to 100mg/kg b.wt. exhibited a lesser antifertility effect. In bioassay test, carried out in immature bilaterally ovariectomised female rats the extract (100 mg/kg b.wt.) when administered alone exhibited uterotrophic effect but did not induce premature opening of the vagina. However, when administered conjointly with estradiol valerate, a synergistic effect was obtained, indicating its mild inherent estrogenicity. The histomorphological study of the uterine horns also confirmed these results. Blood sugar and haematological parameters were within normal range. Thus, the results of the present study indicate that the aqueous extract of *Curcuma longa* possesses cent percent postcoital contraceptive efficacy by virtue of anti-implantation activity.

Keywords : Antifertility, anti-implantation, *Curcuma longa*, uterotrophic, female rats.

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Introduction

The use of many plants and herbs for fertility regulation especially among woman has been prevalent in India for many centuries. 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 properties¹. *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 diseases²⁻³. 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 effects⁴⁻⁶.

In traditional system of medicine, *Curcuma longa* has been reported to possess antifertility activity⁷⁻⁸. Various extracts of *Curcuma longa* rhizome have been reported to cause significant decline in pregnancies in female rats⁹. Petroleum ether and aqueous extract exhibit 100% antifertility activity when administered on day 1-7 of pregnancy at a dose of 200 mg/kg b.wt. in rats¹⁰. But the petroleum ether, alcoholic & aqueous extracts did not exhibit any significant effect on the ovulatory activity at different doses in rabbits¹¹. The aqueous and alcoholic extracts of *Curcuma longa* completely inhibit the fertility with reduction of sperm count and motility and germ cell populations in male rats¹².

It has been reported earlier by our group that crude petroleum ether extract and chromatographic fraction of crude petroleum ether extract of *Curcuma longa* possesses potent anti-implantation activity without any detectable estrogenic effect¹³⁻¹⁴. The present study, thus, deals with the anticonceptive activity of aqueous extract of rhizome of this plant and its hormonal profile in immature bilaterally ovariectomised female rats in order to gain insight into its possible mode of action.

Materials and Methods

Plant Collection and Extraction :

The fresh rhizome 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 obtained was then utilized for evaluating antifertility efficacy by suspending in appropriate volume of olive oil.

Experimental Animal

Colony-bred, adult cyclic albino Wistar female rats (weighing 170-200 g) were used for antifertility studies and immature female rats (21-24 days old) for bioassay studies. All the animals were housed in standard laboratory conditions (temp. $22 \pm 3^\circ\text{C}$ 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).

Post-coital antifertility Study :

For the antifertility study, only normal cycling proestrous female rats were caged overnight with males (2:1 ratio) of proven fertility. The 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.

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 b.wt/day (suspended in olive oil) doses, respectively, once a day from day 1-5 *postcoitum* (*pc*). The extract was administered orally by using a curved needle and a tuberculin syringe.

Autopsy :

In order to establish whether implantation occurred following mating, all the control and treated female rats were sacrificed on day 15 *pc* under mild ether anaesthesia and their body weights were recorded. Blood samples for haematological studies were collected directly from the cardiac puncture. During autopsy, both the uterine horns were examined for the number of implantation sites, live or dead/resorbed fetuses. Embryos with bright reddish aspect and clear margins were considered to be normal and those with dull blue colour, no clear margin, smaller in size and with some surrounding exudate were considered to be resorbing. The ovaries were excised and examined for the number of fresh corpora lutea under stereoscopic microscope. The uterine horns were removed and trimmed of fat. These were then, with embryonic contents intact, weighed on an electric single pan-balance to the nearest milligrams. The fetuses were removed from the uterine horns and suitable parts of these horns were fixed in Bouin's fixative for histological observations. The remaining parts of uterine tissues were frozen at -20°C for biochemical estimations.

Hematology :

The counts of RBC and WBC, Haemoglobin and haematocrit values were determined from the blood collected directly from the heart of rats receiving 500mg/kg b.wt. extract at the time of sacrifice¹⁵.

Estrogenic/ antiestrogenic activity :

Estrogenic or antiestrogenic activity of the extract was assessed by uterine wet weight, vaginal cornification and premature vaginal opening in sexually immature bilaterally ovariectomised female rats. Colony-bred immature female albino rats (21-24 days old) were bilaterally ovariectomised by dorsolateral approach, under light ether anaesthesia and semi-sterile conditions. After post-operative care of seven days, these were randomly divided into four groups of seven animals each and treated as follows:

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

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

Group.III : Aqueous extract alone (100 mg/kg b. wt./rat/day) orally;

Group.IV: Extract (100mg/kgb.wt./rat/day; Orally) + EDV (0.1mg/kgb.wt./rat/day; i.m.) conjointly.

All these treatments were given twice daily for three consecutive days. These treated animals were sacrificed 24 hours after the last dose administration. Their body weight were recorded. Uteri were dissected out, freed from adherent tissues and quickly weighed on an electric single pan-balance. The uterine tissues were fixed in Bouin's fluid for histomorphological evaluations. The condition of the vaginal opening was also recorded. Haematoxylin-eosin stained slides were observed microscopically for 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 lengths were measured, averaged and expressed as mean epithelial cell height and were then calibrated with a stage micrometer.

Statistical analysis :

All the results are expressed as the mean value \pm SEM and significance was determined by Student's t-test. A probability level of less than 5% was considered significant.

Results

Post-coital antifertility Study

The results of the reproductive performance of crude aqueous extract of *Curcuma longa* rhizome treated female rats have been shown in Table I. In the control group, all the mated female rats were pregnant, except one. However, oral administration of aqueous extract to the mated female rats from day 1-5 *pc* induced a significant dose dependent pregnancy failure. The number of total uterine implantation sites and viable fetuses showed a dose-dependent decrease by virtue of increase in the percentage of the pre-implantation embryonic loss rate. The number of resorbed / dead fetuses were also increased in females treated with 100 and 200mg/kg b.wt./day extract doses, indicating an increase in percentage of post-implantation embryonic loss. The total number of healthy corpora lutea in control and extract treated rats remained significantly unchanged.

Effect on body and uterine weight

Table II displays the changes in body and uterine weight of female rats sacrificed on day 15 *pc*. Administration of aqueous extract of *Curcuma longa* rhizome at different doses, however, did not produce any significant change in the maternal body weight, but did produce a significant decline in relative uterine weight when compared with controls.

Effect on Haematological studies

A statistically non-significant change in the RBC and WBC counts, haemoglobin and haematocrit values was observed (Data not shown).

Estrogenic activity

The results of uterine bioassay test carried out in bilaterally ovariectomised immature female rats are summarised in Table III. Administration of estradiol valerate (EDV, 0.1mg/kg

Table I : Effect of oral administration of aqueous extract of rhizome of *Curcuma longa* on reproductive performance of mated female rats when fed from day 1-5pc

Treatment Group	Treatment dose (mg/kg b.wt)	No. of pregnant rats (% fertility index ¹)	Number of implantation sites in individual rats	Total no. of resorbing fetuses	Total No. of viable fetuses	Total No. of corpora lutea	Pre-implantation loss (%) ²	Post-implantation loss (%) ³
Control	0.2ml olive oil	6 (85.70)	0,11,10,9,12,13,10	0	65	102	36.27	0
<i>C. longa</i> (Aqueous extract)	100	3 (42.86)	0,13,0,4,0,1,0	12	6	97	81.44	66.67
	200	1 (14.29)	0,4,0,0,0,0,0	3	1	99	95.96	75
	500	0 (0)	0,0,0,0,0,0,0	0	0	98	100	0

No. of animals in each group = 7

¹ % Fertility index = (No. of pregnant females/ No. of mated females) x 100

² Pre-implantation loss(%)=[(No.of corpora lutea- No. of implantations)/ No.of corpora lutea]x100

³ Post-implantation loss(%)=[(No.of implantations-No. of live fetuses)/ No.of implantation]x 100

Table II : Effect of oral administration of aqueous extract of rhizome of *Curcuma longa* from day 1-5 pc on the body and uterine weights of mated female albino rats

Treatment	Dose (mg/kg b.wt./day)	Body weight (gm)		Uterine weight (gm/100 gm b.wt)
		Initial	Final	
Control (0.2ml olive oil/rat)	Vehicle	195 ± 4.13	210 ± 9.25	6.30 ± 0.27
<i>C. longa</i> (Aqueous extract)	100	181 ± 1.87	188 ± 2.55	2.74 ± 1.57 *
	200	187 ± 6.24	190 ± 5.70	2.03 ± 1.11 **
	500	183 ± 3.00	185 ± 2.72	0.27 ± 0.06 ***

(Values are mean ± SEM)

Levels of significance when compared with vehicle treated controls:

*p< 0.05 **p<0.01 ***p<0.001

Table III : Changes in the uterine wet weight, vaginal opening and uterine luminal epithelial cell height of bilaterally ovariectomised immature female rats after treatment with EDV &/or aqueous extract of rhizome of *Curcuma longa*

Group	Treatment (dose)	Uterine weight (mg/100 gm b.wt)	Vaginal opening	Luminal epithelial cell height (µm)
I	Control (Olive oil)	38.36 ± 1.63	Closed	10.45 ± 0.32
II	Estradiol valerate (EDV, 0.1mg/kg b.wt./rat/day)	529.36 ± 23.91 ^{***}	Open	42.50 ± 0.29 ^{***}
III	Aqueous extract (100mg/kg b.wt./rat/day)	55.80 ± 5.68 [*]	Closed	16.15 ± 0.27 ^{***}
IV	Aqueous extract (100mg/kg b.wt./rat/day) + EDV (0.1mg/kg b.wt./rat/day)	672.05 ± 49.46 [#]	Open	46.26 ± 0.16 ^{###}

(Values are mean ± SEM)

Significant differences when

- (i) Group II and III compared with group I :
* P< 0.05, ** p< 0.01, *** p< 0.001
- (ii) Group IV compared with group II
p< 0.05, ### p< 0.001

b.wt./twice daily) alone in ovariectomised immature rats provoked uterine growth as indicated by significant ($p < 0.001$) increase in relative uterine wet weight and height of the luminal epithelium as compared to ovariectomised controls. These rats also showed premature opening of the vagina while the control rats had a closed vagina. A slightly significant ($p < 0.05$) increase in uterine wet weight and a highly significant ($p < 0.001$) increase in uterine luminal epithelial cell height was observed in rats after oral feeding of aqueous extract (100 mg/kg b.wt) of rhizome of *Curcuma longa* alone showing mild uterotrophic activity of the extract. However, it did not induce premature opening of the vagina. But when the extract was administered conjointly with EDV (0.1 mg/kg b.wt) it synergized the estrogen effect ($p < 0.05$) and premature opening of the vagina was also noticed.

Discussion

In our earlier communication¹³, it has been reported that oral administration of crude petroleum ether extract of *Curcuma longa* rhizome during early pregnancy (day 1-5 *pc*) at 100, 200 and 500mg/kg b.wt./day doses caused a significant adverse effect on pregnancy and the extract possesses potent (100%) pregnancy interceptory property at 500mg/kg b.wt./day dose level. The present finding also indicate that oral feeding of aqueous extract of *Curcuma longa* rhizome caused a dose dependent adverse effect on fertility index and number of implantations in female rats at 100, 200 and 500mg/kg b.wt./day doses from day 1-5*pc*, by virtue of an increase in the percentage of the pre-implantation embryonic loss, as a consequence the number of live fetuses decreased. However, a complete (100%) blockage of pregnancy was observed at 500 mg/kg b.wt dose, as none of the mated females showed presence of any implantation site. The present findings correlate well with the findings of Garg *et al.*⁹ who reported that administration of petroleum ether, alcoholic (95%) and aqueous extracts of this plant at the doses of 100 and 200 mg/kg on days 1-7 of pregnancy showed significant antifertility activity. Petroleum ether and aqueous extracts inhibited implantation in all the rats at the dose of 200 mg/kg on days 1-7 of pregnancy. In the present investigation also, aqueous extract at 500 mg/kg b.wt. dose on days 1-5 of pregnancy, showed cent percent inhibition of pregnancy. The difference observed in effective dose amount might be due to the difference in the treatment duration. Further, variation in the sources of plant material and processing techniques may also affect medicinal property of the plants.

There was suppression of uterine decidualization in aqueous treated female rats. The endometrium during a normal pregnancy acquires a potential for decidual cell reaction under the influence of ovarian steroids. Thus, it can be inferred that the aqueous extract interferes with steroid conditioning of the uterus and renders it hostile to ovum implantation¹⁶.

A significant decline in uterine weight of the treated rats was correlated with a decrease in the number of implantation sites and viable fetuses in the uterine horns¹⁷⁻¹⁸. Furthermore, the uterine weight in pregnant rats serves as an index of uterine decidualization and thus a significant decrease in uterine weight indicates suppression of uterine decidualization¹⁹.

In the present investigation, a non-significant change in the total erythrocyte and leucocyte counts, haemoglobin and haematocrit values following oral therapy of aqueous extracts of rhizome of *Curcuma longa* suggests unimpairment of erythropoiesis. These results are in agreement with the report of Mutreja *et al.*²⁰ who reported that oral administration of curcumin at 40 mg/kg body dose for 7 days in rats did not produce any significant changes in total or differential leucocyte counts.

In ovariectomised immature female rats, oral administration of aqueous extract increased the uterine weight and stimulated uterine growth but was unable to induce vaginal opening, suggesting its mild inherent estrogenic activity. And when this extract was administered concomitantly with EDV it synergized the estrogen effect. It is well known that administration of estrogen has uterotrophic effects in ovariectomised immature female rats and mice²¹⁻²², such effects are associated with growth and proliferation of endometrial microvilli on the apical surface as well as an increase in cell number.

A number of plant products exhibiting estrogenic activity and producing antifertility effects are known in literature²³⁻²⁴. It has been demonstrated that there exists a relationship between estrogenicity and antifertility efficacy of compounds²⁵. Estrogens have been reported to accelerate the passage of ova through the uterine horns resulting in premature expulsion of egg from the uterus²⁶⁻²⁷. Thus, the mild estrogenicity of the extract may contribute to its anticonceptive effect²⁸⁻³¹.

Pre-implantation losses can also arise due to disruption of events which are pre-requisite for fertilization or an impairment in the production of cytokines, growth factors and various types of adhesion molecules, either by the developing blastocyst or by the uterine epithelium around the site of implantation³²⁻³³. It is expected that the extract may have a blastotoxic or embryotoxic effect³⁴. Since, successful implantation depends on embryo quality, uterine receptivity and synchronization of embryonic development and endometrial maturity, it is also likely that the component of the extract may act directly on the uterus and make endometrial environment hostile for implantation.

The present study, thus, suggested that aqueous extract of rhizome of *Curcuma longa* possesses anti-implantation activity and the mild estrogenic nature of the extract may be responsible, at least partly, for this anti-conceptive effect. While the hematological studies performed in extract treated rats did not reflect any adverse effect. Although it is very difficult to explain the exact mechanism of antigestational activity of the extract. At present, it can be postulated that its effect is probably due to multiple attributes. Further studies are however needed to establish its mechanism of action and to isolate specific components responsible for it.

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