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EFFECT OF CHROMATOGRAPHIC FRACTION OF CRUDE PETROLEUM ETHER EXTRACT OF RHIZOME OF *CURCUMA LONGA* ON EARLY PREGNANCY IN FEMALE ALBINO RATS

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Postcoital antifertility activity and hormonal profile of chromatographic fraction (petroleum ether plus benzene, 1:1v/v) of petroleum ether extract of rhizome of *Curcuma longa*, were evaluated in female albino Wistar rats. The chromatographic fraction was administered orally to mated female rats from day 1-5 of pregnancy at 100 and 200mg/kg b.wt./day doses. The status of pregnancy was recorded on day 15. The chromatographic fraction was 100% effective in abolishing pregnancy at 200mg/kg b.wt./day dose level as indicated by complete absence of implantation in treated females. The chromatographic fraction exhibited mild uterotrophic activity when treated alone but when given simultaneously with estradiol valerate it failed to show any significant estrogenic or antiestrogenic activity as compared to estradiol valerate alone treated bilaterally ovariectomised immature female rats. Thus, the results of the present study indicate that the petroleum ether plus benzene fraction of petroleum ether extract of *Curcuma longa* possesses 100% postcoital contraceptive efficacy by virtue of anti-implantation activity.

Keywords: Antifertility; Anti-implantation; *Curcuma longa*; Female rat; Pregnancy.

Introduction

In the recent time population control has assumed great importance in developing countries and attracted the attention of governmental as well as private organizations. In this context, search for orally effective and less expensive agents having minimal side effects for fertility control in human beings has tremendous importance. 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 antiestrogenic properties offer themselves as effective non-conventional sources of contraception with less deleterious side effects.

Curcuma longa Linn.(Family – Zingiberaceae), commonly known as "Haldi" is widely used as a colouring agent, condiment and in prevention and cure of skin, hepatic and inflammatory conditions and many other diseases[1,2]. 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[3].

In traditional system of medicine, *C. longa* has been reported to possess antifertility activity[4]. Various extracts of *C. longa* has been reported to cause significant decline in pregnancies in treated female rats[5]. The aqueous and alcoholic extracts of *C. longa* completely inhibit the fertility with reduction of sperm count and motility and germ cell populations in male rats[6].

It has been reported earlier by our group that crude petroleum ether extract of *C. longa* possesses potent anti-implantation activity without any detectable estrogenic effect[7]. With the aim of isolation of active chromatographic fraction, in this study we have investigated the postcoital antifertility efficacy of petroleum ether plus benzene [1:1 v/v] fraction of crude petroleum ether extract of *C. longa* rhizome in female rats and also investigated its homonal 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 rhizomes of *Curcuma longa* were obtained locally from the market, shade dried, powdered and subjected to soxhlet extraction in petroleum ether for 36 hours at 60-80°C. For chromatographic fractionation of crude petroleum ether extract, the concentrated and dried residue of petroleum ether extract was dissolved in a minimum quantity of petroleum ether and was chromatographed over silica gel. The different fractions from petroleum ether extract were collected by eluting the column successively with petroleum ether; petroleum ether plus benzene [1:1 v/v];

Table 1. Effect of oral administration of petroleum ether plus benzene (1:1 v/v) chromatographic fraction of crude petroleum ether extract of *C. longa* rhizome from day 1-5 *pc* on reproductive performance of female rats.

Treatment Group	Dose (mg/kg b.wt)	Group size	No. of pregnant rats (percent fertility index[1])	No. of implantation sites (mean ±SEM)	No. of resorbing fetuses (mean ± SEM)	No. of viable fetuses (mean±SEM)	No. of corpora lutea (mean ±SEM)	Pre-implantation loss (%)[2]	Post-implantation loss (%)[3]
Control (0.2ml olive oil/rat)		7	6 (85.70)	65 (9.29±1.76)	0 (0)	65 (9.29±1.76)	102 (14.57±0.70	36.27	0
C. longa (Pet.ether+ Benzene, 1:1 v/v) extract	100	7	2 (28.57)	17 (2.43.±1.73*)	14 (2.0±1.41)	3 (0.43±0.32***)	97 (13.86±0.86)	82.47	82.35
	200	7	0 (0)	0 (0***)	0 (0***)	0 (0***)	99 (14±0.78)	100	0

Values are mean ± SEM

Levels of significance when compared with vehicle treated controls:

*p<0.05 , ***p<0.001

[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] x 100

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

Table 2. Effect of oral administration of petroleum ether plus benzene (1:1 v/v) chromatographic fraction of crude petroleum ether extract of *C. longa* rhizome from day 1-5 *pc* on body and relative uterine weight of female albino rats.

Treatments	Dose (mg/kg b.wt/day)	Body weight (g)		Uterine weight (g/100 g b.wt)
		Initial	Final	
Control (0.2ml olive oil/rat)	-	195±4.13	210±9.25	6.30±0.27
C. longa (Pet. ether + Benzene, 1:1v/v) fraction	100	174±8.72	178±9.43	2.31±0.97**
	200	194 ± 5.10	199 ± 5.79	0.25 ± 0.04 ***

Values are mean ± SEM

Levels of significance when compared with vehicle treated controls:
**p<0.01 , ***p<0.001

Table 3. Estrogenic and antiestrogenic activity of petroleum ether plus benzene (1:1 v/v) chromatographic fraction of crude petroleum ether extract of *C. longa* rhizome in bilaterally ovariectomized immature rats

Group	Treatment group	Uterine wt. (mg/100 gm b.wt)	Vaginal opening	Luminal epithelial cell height (μm)
I	Control (0.2ml olive oil/rat)	38.36±1.63	Closed	10.45±0.32
II	Estradiol valerate (0.1mg/kg b.wt.)	529.36±23.91***	Open	42.50±0.29***
III	*C. longa* (Pet.ether+Benzene, 1:1v/v)fraction (100mg/kgb.wt.)	43.30±0.64*	Closed	15.65±0.33*
IV	*C. longa* (Pet.ether+Benzene,1:1v/v) fraction (100mg/kgb.wt.) + Estradiol valerate(0.1mg/kg b.wt.)	534.05±3.71ns	Open	44.59±0.22ns

(values are mean ± SEM)

Levels of significance when compared with vehicle treated controls of group I:
*p< 0.05, ***p< 0.001
Levels of significance when compared with EDV alone treated rats of group II :
ns (Non-significant)

benzene; benzene plus chloroform [1:1 v/v]; chloroform; chloroform plus methanol [1:1 v/v] and methanol by the method of Tomita *et al.*[8]. Out of these chromatographic fraction the petroleum ether plus benzene [1:1 v/v]) fraction thus obtained was concentrated to dryness under low temperature (40°C) and reduced pressure. The viscous extract 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 (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 ± 3°C and 14 hr light/10 hr dark cycle) with free access to pallet food (Lipton India Ltd.) and tap water *ad libitum*.

Antifertility study : Only normal cyclic 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 females were isolated, weighed and divided into four groups of seven animals each. The animals of Group I received vehicle (olive oil, 0.2 ml /rat) and served as control. Animals of Group II and III received chromatographic fraction of crude petroleum ether extract of *C. longa* at 100 and 200 mg/kg b.wt/day (suspended in olive oil), doses, respectively, orally for day 1-5 *postcoitum* (*pc*).

Autopsy : All the control and treated female rats were sacrificed on day 15 *pc* under light ether anesthesia and their body weights were recorded. Blood sample for hematological studies was 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 and quickly weighed on an electric single pan-balance to the nearest milligrams.

Hematology : The counts of RBC and WBC, hemoglobin and hematocrit values were determined from the blood collected directly from the heart of rats receiving 200mg/ kg b.wt. extract, at the time of scarification [9].

Bioassay Study : Estrogenic or antiestrogenic activity of the extract was assessed by uterine wet weight and premature vaginal opening in 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 semisterile conditions. The animals after post-operative care of 7 days, were randomly divided into 4 experimental groups consisting of 7animals in each group and treated as follows:

Group I : Ovariectomized control receiving olive oil only (0.2 ml/rat), orally.

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

Group III : Ovariectomized rats receiving chromatographic fraction alone (100 mg/kg b wt/day), orally.

Group IV: Ovariectomized rats receiving chromatographic fraction (100mg/kg b wt/day; orally) and EDV (0.1mg/kg b wt/day; i.m.), conjointly.

All these rats received treatment twice daily for three consecutive days. These treated rats 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.

Histomorphological study : The uterine tissues of bilaterally ovariectomised rats were dehydrated and embedded in paraffin. Sections were cut at 5 μ and stained with haematoxylin-eosin stain. The height of the uterine luminal epithelium was measured in 25 randomly selected sections using ocular micrometer at x400 and calibrated with a stage micrometer.

Statistical analysis : All the results are expressed as mean ± SEM. Statistical comparisons were made by means of Student's "t" test and $P < 0.05$ was considered as significant.

Results and Discussion

Antifertility activity : The result of the reproductive performance of petroleum ether plus benzene fraction of crude petroleum ether extract of *C. longa* treated female rats have been shown in Table 1. In the control group all the mated female rats, except one, were pregnant (85.70%) with mean number of implantation sites as 9.29 ± 1.76. Oral administration of chromatographic fraction at the dose 100mg/kg b.wt./day from day 1-5 *pc* prevented pregnancy in 71.43% treated female rats. The mean implantation number was significantly reduced ($p<0.05$; 2.43 ± 1.73 versus 9.29±1.76 in control) as a consequence of significant increase in pre-implantation loss as compared to controls ($p<0.001$; 82.47% versus 36.27%). There was a complete blockage of pregnancy in female rats receiving 200mg/kg b.wt./day dose of chromatographic fraction as none of the treated female rats showed the presence of any implantation sites. The mean number of healthy corpora lutea in control and chromatographic fraction treated rats remained significantly unchanged.

Effect on body and uterine weight : Table 2 displays the changes in body and relative uterine weight of the female rats sacrificed on day 15 *pc*. Administration of chromatographic fraction 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.

Hematology : RBC and WBC counts, hemoglobin and hematocrit values in extract treated rats were within normal range (data not shown).

Estrogenic and antiestrogenic activity : Table 3 shows the results obtained in bioassay studies of chromatographic fraction. Administration of estradiol valerate (EDV, 0.1mg/kg b.wt./twice daily) alone in ovariectomized 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 rat had closed vagina. Oral administration of the chromatographic fraction alone (100mg/kg b.wt./twice daily) induced a slightly significant (P<0.05) increase in relative uterine wet weight and luminal epithelium, showing uterotrophic activity when compared to ovariectomized control rats. However, it failed to induce a premature opening of the vagina. Whereas, co-administration of the extract with EDV did not show any significant change in relative uterine wet weight and luminal epithelium, showing neither synergistic nor antagonistic activity when compared with EDV alone primed rats. Further, it did not prevent premature opening of the vagina also.

In our earlier communication[7] it has been reported that oral administration of crude petroleum ether extract of *C. longa* rhizome during early pregnancy (day 1-5*pc*) at 100, 200 and 500mg/kg b.wt./day doses caused a significant decline in the fertility index, number of uterine implants and live fetuses in a dose dependent manner. The extract posses potent (100%) pregnancy interceptory property at 500mg/kg b.wt/day dose level without any significant estrogenic or antiestrogenic activity. In the present study also, oral administration of petroleum ether plus benzene fraction of crude petroleum ether extract of rhizome of *C. longa* from day 1-5 *pc* in mated female rats, showed a significant adverse effect on pregnancy. There was complete (100%) inhibition of implantation in all the treated rats receiving 200 mg/kg b. wt./day dose of the chromatographic fraction of the extract confirming pregnancy interceptory activity even at the low dose. The results also correlate well with the findings of Garg *et al.*[5] who reported that administration of petroleum ether and aqueous extracts results in complete failure of pregnancy in rats at the 200 mg/kg b.wt. dose level during 1-7 days of pregnancy.

The decline in relative uterine weight was correlated with a decrease in the number of implantation sites and viable fetuses in the uterine horns[10,11]. Further, the uterine weight in pregnant rats serve as an index of decidualization and thus a significant decrease in uterine weight indicates suppression of decidualization[12].

In uterine wet weight bioassay test and status of vaginal opening study carried out in bilaterally ovariectomized immature female rats, the extract reflected a mild estrogenic (uterotrophic) effect but in presence of estrogen it did not reflect any estrogen synergistic or antagonistic activity. Thus the observed anti-implantation effect in female rats might be due to mild inherent estrogenic nature of the extract which disturb the delicate hormonal balance responsible for implantation[13]. Pre-implantation embryonic loss might be caused by its mild estrogenic effect on the oviduct which accelerates embryo transport[14].

Successful implantation occurs only when the activated stage of the blastocyst coincides with the receptive stage of the uterus. It is likely that the phytoconstituents present in the chromatographic fraction may elicit modulatory influence on production and/or expression of cytokines, growth factors, prostaglandins and various types of adhesion molecules, either by the developing blastocyst or by the uterine epithelium around the site of implantation[15,16].

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, confirms the postcoital antifertility activity of chromatographic fraction (petroleum ether plus benzene [1:1 v/v]) of crude petroleum ether extract of rhizome of *C. longa* in female rats mainly by virtue of anti-implantation effect. The extract showed a mild estrogenic effect and did not exert any adverse effect on hematological parameters. 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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